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Maternal-Embryo Interaction and Consequences for
Embryo Development in Cattle

Comunicación Materno-Embrionaria y sus Consecuencias
en el Desarrollo Embrionario en Bovino

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List of Abbreviations

AA	Amino acid
AI	Artificial insemination
AIJ	Ampullary-isthmic junction
AUC	Area under the curve
BCS	Body condition score
BHBA	B-hydroxybutyrate
BP	Biological processes
BSA	Bovine serum albumin
CIDR	Controlled internal drug release
CL	Corpus luteum
COC	Cumulus oocyte complex
CP	Crude protein
CR	Conception rate
CV	Coefficients of variation
DEGs	Differentially expressed genes
DF	Dominant follicle
DM	Dry matter
DMI	Dry matter intake
$\mathbf{E_2}$	Estradiol
eCG	Equine chorionic gonadotropin
EGA	Embryonic Genome activation
ESR1	Oestrogen receptor alpha
ET	Embryo transfer
FCS	Fetal calf serum
FF	Follicular fluid
FL	Fetal loss
FR	Fertilization rate
FSH	Follicle stimulating hormone
GH	Growth hormone
GHR	Growth hormone receptor
GnRH	Gonadotropin releasing hormone
GO	Gene ontology
GV	Germinal vesicle
hCG	Human chorionic gonadotropin
HYDC	High yielding dairy cows
ICM	Inner cell mass
IFNT	Interferon τ
IGF-I	Insulin like growth factor-1
IU	International units
IVC	In vitro culture
IVF	*In vitro* fertilization
IVM	*In vitro* maturation
IVP	*In vitro* embryo production
LEL	Late embryo loss
LH	Luteinizing hormone
LHFC	Lactating Holstein Friesian cows
LLC	Large luteal cells
ME	Metabolizable energy
MII	Metaphase II
Mt	Metric tons
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
OF	Oviductal fluid

OPU	Ovum pick up
OT	Oxytocin
P/AI	Pregnancy per artificial insemination
P4	Progesterone
PGF2α	Prostaglandin F2 alpha
PGR	Progesterone receptor
QCs	Quality controls
qRTPCR	Quantitative real-time PCR
RIA	Radioimmunoassay
SAPE	Streptavidin-conjugated phycoerythrin
SCNT	Somatic cell nuclear transfer
SLC	Small luteal cells
SOF	Synthetic oviductal fluid
TE	Trophectoderm
TG	Triglycerides
UTJ	Utero-tubal junction
ZP	Zona pellucida

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Summary

During the last 4-5 decades dairy cows have been selected to yield high amounts of milk. This increase has been achieved due to the improvement of environmental factors, i.e. better nutrition, housing, health and management, and due to the genetic selection carried out. However, this intense genetic selection towards milk yield has been accompanied by a reduction in fertility, characterized by longer postpartum period until first insemination or lower first-service conception rate. This is translated into additional inseminations, more veterinary visits, increased culling rate and higher replacement costs that substantially impact the profitability of the farm. Unlike dairy cows, in dairy heifers fertility has not changed, ranging from 57 to 67% in US Holstein and British Holstein Friesian heifers, respectively.

The difference between dairy cows and heifers is that after calving cows enter in negative energy balance (NEB), while if heifers are fed appropriately are not exposed to this metabolic stress. Negative energy balance is a result of the deficit in energy intake due to decreased dry matter intake, together with the dramatic energy expenditure in milk production after calving. In this situation, as glucose is mainly used by the mammary gland to produce milk, there is a lipid mobilization from the fat reserves, to release non-esterified fatty acids (NEFA). These NEFA are metabolized to ketonic bodies, mainly β-hydroxybutyrate (BHBA), that are used as an alternative energy source in tissues like adipose tissue or skeletal muscle. This mobilization is also accompanied by a loss of weight and body condition score. In addition, there is a certain grade of insulin resistance that favours the utilization of glucose by the mammary gland. Furthermore, in the liver there is an uncoupling of the growth hormone and insulin-like growth factor-1 (IGF-I) axis, resulting in low IGF-I concentrations (~100 ng/ml); IGF-I levels lower than 25 ng/ml a week after calving and 50 ng/ml at first insemination, have been related with a delay returning to ovarian cyclicity. Therefore, the blood metabolic profile of the postpartum lactating dairy cows is characterized by high levels of NEFA and BHBA as well as low levels of glucose, insulin and IGF-I.

Subfertility is considered any condition that leads to failure to establish a pregnancy following completion of uterine involution at 40-50 days postpartum. Given the high percentage of fertilization (90%), it is assumed that low fertility is more related with the quality of the oocyte, the quality of the embryo, the reproductive tract or consequence combination of these factors. In postpartum dairy cows, it has been demonstrated that the systemic metabolic profile is reflected in the follicular fluid; therefore, oocytes developed during the period of NEB may be altered in their capacity to undergo normal development. Studies *in vitro* have demonstrated that the culture of oocytes under conditions of high levels of NEFA, BHBA or low levels of glucose, reduces the fertilization rate and developmental competence of the embryo, and compromises embryo quality, viability and metabolism. Regardless of these results, in a study with dairy cows and ovum pick up after calving (from Day 14 to Day 80 postpartum), no alteration in developmental competence of the recovered oocytes was found *in vitro,* suggesting either the existence of a possible mechanism to protect the oocyte and the granulosa cells or the insufficient sensitivity of *in vitro* production to detect subtle differences in oocyte quality. On the other hand, the quality of the embryo also may contribute to subfertility. Thus, it has been seen that postpartum lactating cows produce embryos of inferior quality compared with heifers or nonlactating cows. Embryos from lactating cows were darker compared with the those from heifers or nonlactating cows and this characteristic is directly related with the content of lipids that in turn has been associated

with embryo survival after cryopreservation. Hence, the darker embryos have higher content of lipids and they are less cryotolerant.

Regardless of all the experiments carried out to study the contribution of the oocyte and the embryo to subfertility, so far there is only one that have evaluated the capacity of the reproductive tract to support early embryo development. In this study lactating cows were compared with heifers knowing that the metabolic situation in both groups was significantly different. For this reason, using postpartum dairy cows that were either dried off immediately at calving (i.e., never milked), or milked twice daily, the first chapter of this thesis was designated to respond the following questions: 1) Is the metabolic profile different in lactating cows than in nonlactating cows after calving? 2) Is the reproductive tract capable of supporting early embryo development up to blastocyst stage? and 3) Is the uterus able to sustain conceptus elongation?

The results from the first chapter showed that, as well as body weight and body condition score, the metabolic profile for all the metabolites measured, i.e. glucose, insulin, IGF-I, NEFA and BHBA was different after calving between lactating and nonlactating cows. Glucose, insulin and IGF-I were lower and NEFA and BHBA were higher in lactating compared to nonlactating cows. In both groups after reaching a nadir, all the metabolites started to recover except IGF-I which remained different between groups for the whole period of the experiment (until Day 95 postpartum). After Day 60 postpartum, following endoscopic transfer of *in vitro* produced zygotes into the oviduct, it was found that the reproductive tract (oviduct and uterus) of lactating cows was less capable of supporting embryo development from Day 2 to 7, as evidenced by the lower number of blastocysts recovered from lactating compared with nonlactating cows (26.3 and 39.6%, respectively) ($P<0.05$). However after Day 90 postpartum it seems that the reproductive tract was fully recovered from NEB as shown by the absence of differences in conceptus elongation between lactating and nonlactating cows (39.8 and 33.3%, respectively).

The conclusion of the first chapter that the reproductive tract of lactating cows was altered after calving raises the question that this may be due to a modification of the oviductal or the uterine environment. Therefore, it is important to understand the relationship between the embryo and the reproductive tract, i.e. between the embryo and the oviduct during the first 3 to 4 days of early embryo development, and between the conceptus and endometrium during the pre-implantation period. The uterus has been extensively studied in relation to maternal recognition of pregnancy and some of the appropriate complex signals to achieve a normal pregnancy have been identified. However, little is known about the interaction between the oviduct and the embryo during the first stages of embryo development. There are very few studies evaluating this communication and only in mice, rats and pigs has it been reported that the presence of embryos results in the upregulation of some genes in the oviduct. Apart from the effect of the embryos, a specific effect of the gametes has been described in the proteome of the oviduct and also differences in the composition and viscosity of the oviductal fluid during the oestrous cycle. Thus, the second chapter was designed to answer the following questions: 1) Does the embryo affect the transcriptome of the oviduct? 2) Does proximity to the *corpus luteum* (CL) affect the transcriptome of the

oviduct? 3) Is there any difference between the transcriptome of the ampulla and isthmus of the ipsilateral oviduct in pregnant animals?

The results of the second chapter indicate that the embryo does not affect the transcriptome of the oviduct. In contrast, the findings in rats, mice and pigs could be related to the number of embryos, considering that these species are poly-ovulatory and therefore these animals support the development of several embryos. In addition, in our experiment the length of the isthmus processed from all animals was approximately 8 cm, while the embryo is ~120 µm; therefore, the specific site of the oviduct where the embryo was located is very small and a possible effect at this point could be missed. Hence, a local effect of the embryo at the specific site where it was located cannot be discounted.

Proximity to the CL, i.e. cells from the oviducts ipsilateral vs. contralateral to the CL, did not affect the transcriptome of the isthmus, irrespective of whether the heifers were cyclic or pregnant. However, site within the oviduct significantly affected the pattern of gene expression. Hence, the comparison between the ampulla and isthmus of the ipsilateral oviduct of pregnant animals revealed 2287 differential expressed genes ($P<0.01$) from which 1132 and 1155 were up- and down-regulated in the isthmus, respectively. Analysis of gene ontology revealed that the main biological processes overrepresented in the isthmus were related with synthesis of compounds like nitrogen, lipids, nucleotides, steroids and cholesterol as well as vesicle-mediated transport, cell cycle, apoptosis, endocytosis and exocytosis; whereas cell motion, motility and migration, DNA repair, calcium ion homeostasis, carbohydrate biosynthetic process and regulation of cilium movement and beat frequency were the biological processes overrepresented in the ampulla. Based on the above we conclude that 1) the presence of an 8-cell embryo in the isthmus does not affect the transcriptome of the oviduct; 2) gene expression of the oviduct in pregnant or cyclic heifers is not modified by proximity to the CL; and 3) in pregnant heifers, major differences exist between the ampulla and isthmus regions of the oviduct ipsilateral to the CL.

Within the factors that may contribute to subfertility it is important to consider that most embryo losses during pregnancy occur in the first two weeks after conception, representing 70% of the total embryo/foetal losses. Progesterone (P4) is the key signal of pregnancy because is responsible for the embryo elongation and this in turn is necessary to synthetize interferon-τ, essential for maternal recognition of pregnancy. Therefore, it has been considered that these embryo losses could be due to an insufficient P4. High P4 concentrations during early embryo development have been associated with more elongated embryos and even better pregnancy rate, while low P4 has been related with a lower ability of the endometrium to support elongation.

Progesterone concentration is directly correlated with the size of the CL. Therefore, one of the strategies to increase P4 during early embryo development is induce an accessory CL or make stimulate the development of the endogenous/native CL. This can be achieved in numerous ways, although it is important to note that too early increase in P4 concentration has been related with a shortening of the oestrous cycle, and also too late increase like Day 7 or 8 does not have any effect on conceptus size.

Human chorionic gonadotrophin (hCG) is a hormone with LH-like activity that has been extensively used between Day 4 and 7 after oestrus, mainly to induce the ovulation of a dominant follicle and the formation of an accessory CL, leading to increased P4. Some studies have reported a positive effect on pregnancy rate while others did not. In other experiments it has been shown that hCG also have an hypertrophic effect on the original CL. Therefore, based on that, together with the fact that the effect of hCG on P4 concentration is not immediate, the third chapter of this thesis was developed to answer the following question: Can a single intramuscular injection of hCG administered on Day 1, 2, 3 or 4 after oestrus increase luteal tissue area of the native CL and P4 concentration?

Crossbreed heifers received a single dose of 3000 IU of hCG on Day 1, 2, 3 or 4 after oestrus. The results revealed that when hCG was used on Day 1 it had no effect on P4 concentration. However, after hCG treatment on Day 2, 3 or 4 there was an increase in the luteal tissue area from: Day 6 to 12, Day 9 to 11 and, Day 9 and 10, respectively ($P<0.05$). Nevertheless, when hCG was used on Day 2 or 4 the increment in luteal tissue area was accompanied by an increase in P4, from Day 6 to 11 and from Day 8 to 13, respectively ($P<0.05$). However, when hCG was injected on Day 4 most of the animals had a double ovulation. Based on the above we concluded that hCG treatment on Day 2 after oestrus increases P4 circulation from Day 6 onwards, that may be beneficial for early embryo development and pregnancy rate.

Resumen

En las últimas 4-5 décadas, las vacas de aptitud lechera han sido seleccionadas para producir grandes cantidades de leche. Este incremento se ha conseguido gracias a la mejora de factores ambientales como la nutrición, el alojamiento, la salud y el manejo, y también debido a la selección genética. Esta selección genética ha sido tan intensa hacia la producción de leche, que se ha visto acompañada de una reducción en la fertilidad, caracterizada por periodos postparto hasta la primera inseminación más largos o tasas de concepción tras el primer servicio inferiores. Esta disminución en la fertilidad conlleva inseminaciones adicionales, más visitas del veterinario, incremento en la tasa de descarte y costes de reemplazo elevados que, en definitiva, afectan considerablemente la rentabilidad de la granja. A diferencia de las vacas adultas, la fertilidad en las novillas no ha cambiado, siendo la tasa de gestación del 57 o 67 % en las razas Holstein americana y Holstein-Friesian británica, respectivamente.

La diferencia entre las vacas y las novillas de leche es que después del parto las vacas entran en balance energético negativo (negative energy balance, NEB), mientras que las novillas, si son alimentadas adecuadamente, no están expuestas a este estrés metabólico. El NEB es el resultado de una deficiencia de energía, debido a una disminución de la materia seca ingerida, junto con el enorme gasto energético que supone la producción láctea después del parto. En esta situación, puesto que la glucosa es usada principalmente por la glándula mamaria, tiene lugar una movilización de los lípidos en las reservas de grasa, liberándose ácidos grados no esterificados (non-esterified fatty acids, NEFA). Estos NEFA son metabolizados a cuerpos cetónicos, fundamentalmente β-hidroxibutirato (β-hydroxybutyrate, BHBA) que es usado como fuente alternativa de energía en el tejido adiposo o esquelético, entre otros. Asimismo, esta movilización está acompañada por una pérdida de peso y de condición corporal. Además, existe cierto grado de resistencia a la insulina que favorece la utilización de glucosa por la glándula mamaria. En el hígado se produce un desacoplamiento del eje conformado por la hormona del crecimiento y el factor de crecimiento insulínico tipo 1 (insulin-like growth factor-1, IGF-I), que da lugar a bajas concentraciones de IGF-I (~100 ng/ml). Niveles de IGF-I inferiores a 25 ng/ml, una semana después del parto, e inferiores a 50 ng/ml, después de la primera inseminación, se han asociado con un retraso en la reanudación de la ciclicidad ovárica. Por tanto, el perfil sanguíneo metabólico en las vacas lactantes durante el postparto se caracteriza por tener niveles altos de NEFA y BHBA así como bajos niveles de glucosa, insulina e IGF-I.

El término subfertilidad se refiere a cualquier condición que lleve a un fallo para establecer la gestación, después de que se haya completado la involución uterina, que tiene lugar entre los días 40-50 postparto. Puesto que el porcentaje de fecundación es elevado (90%), se asume que la baja fertilidad en las vacas lecheras está más relacionada con la calidad del ovocito o del embrión, el tracto reproductivo o una combinación de estos factores. Durante el postparto, se ha demostrado que el perfil metabólico sistémico de las vacas lecheras se encuentra reflejado en el fluido folicular, por tanto, los ovocitos desarrollados durante el periodo de NEB, podrían estar alterados en cuanto a su capacidad para desarrollarse de forma normal. Estudios *in vitro* han demostrado que el cultivo de ovocitos en condiciones de altos niveles de NEFA o BHBA, así como bajos niveles de glucosa, reducen la tasa de fecundación y la capacidad de desarrollo del embrión, comprometiendo la calidad embrionaria, su viabilidad y su metabolismo. A pesar de estos resultados, en un experimento con vacas lecheras en el que se recogieron ovocitos mediante ovum pick up después del parto (desde el día 14 hasta el día 80 postparto), no se

detectó ninguna alteración en la capacidad de desarrollo *in vitro* de los ovocitos recuperados. Esto sugiere la posible existencia de un mecanismo que proteja el ovocito y las células de la granulosa o que la técnica de producción *in vitro* no sea lo suficientemente sensible como para detectar ligeras diferencias en la calidad del ovocito. Como se mencionó anteriormente, la calidad del embrión también podría contribuir a la subfertilidad. De este modo, se ha visto que durante el postparto las vacas lactantes producen embriones de calidad inferior comparados con novillas o vacas no lactantes. Además, los embriones de las vacas lactantes eran más oscuros y esta característica está directamente relacionada con su contenido en lípidos, que a su vez se ha asociado con la supervivencia embrionaria tras la criopreservación. De manera que, los embriones más oscuros tienen mayor contenido en lípidos y son menos criotolerantes.

A pesar de todos los experimentos llevados a cabo para estudiar la contribución del ovocito y del embrión a la subfertilidad, hasta el momento, sólo existe un estudio que ha evaluado la capacidad del tracto reproductivo para mantener el desarrollo embrionario temprano. En este experimento las vacas lactantes se compararon con novillas, considerando que la situación metabólica en ambos grupos era significativamente diferente. Por esta razón, en el primer capítulo de esta tesis se usaron vacas lactantes durante el periodo postparto, que fueron secadas inmediatamente tras el parto, es decir nunca se ordeñaron, u ordeñadas dos veces al día, para responder las siguientes preguntas: 1) ¿Es diferente el perfil metabólico entre las vacas lactantes y no lactantes después del parto? 2) ¿Es capaz el tracto reproductivo de mantener el desarrollo embrionario temprano hasta el estadio de blastocisto? y 3) ¿Es capaz el útero de mantener la elongación del concepto?

Los resultados del primer capítulo mostraron que, al igual que el peso y la condición corporal, el perfil metabólico para todos los metabolitos medidos, es decir glucosa, insulina, NEFA y BHBA fueron diferentes después del parto entre las vacas lactantes y no lactantes. La glucosa, insulina e IGF-I fueron inferiores y los NEFA y BHBA fueron superiores en vacas lactantes comparadas con las no lactantes. En ambos grupos, después de alcanzar sus niveles más bajos, punto denominado nadir, todos los metabolitos empezaron a recuperarse excepto IGF-I que permaneció diferente entre los grupos durante todo el periodo del estudio (hasta el día 95 postparto). Después del día 60 postparto, tras una transferencia endoscópica en el oviducto de cigotos producidos *in vitro*, se observó que el tracto reproductivo (oviducto y útero) de las vacas lactantes fue menos capaz de mantener el desarrollo embrionario desde el día 2 al día 7, debido a que el número de blastocistos recuperados de las vacas lactantes fue inferior comparado con las no lactantes (26.3 y 39.6%, respectivamente) (P<0.05). Sin embargo, después del día 90 postparto aparentemente el tracto reproductivo se había recuperado del NEB, puesto que no se encontraron diferencias en la elongación del concepto entre las vacas lactantes y no lactantes (39.8 y 33.3%, respectivamente).

Tras observar la alteración del tracto reproductivo de las vacas lactantes en el primer capítulo, nos planteamos si la alteración tenía lugar en oviducto o útero. El reconocimiento materno de la gestación ha sido ampliamente estudiado en el útero, identificándose ciertas señalizaciones necesarias para llevar a cabo la gestación. Sin embargo, se conoce poco sobre la interacción entre el oviducto y el embrión durante los primeros estadios del desarrollo embrionario. Algunos estudios han evaluado esta comunicación; en ratones, ratas y cerdos se ha reportado que la presencia de embriones aumenta la

expresión de algunos genes en el oviducto. A parte del efecto de los embriones, se ha descrito que los gametos actúan específicamente en el proteoma del oviducto. Además, la composición y viscosidad del fluido oviductal cambia durante el ciclo estral. Por tanto, el segundo capítulo fue diseñado para contestar a las siguientes preguntas: 1) ¿Ejerce algún efecto la presencia del embrión sobre el transcriptoma del oviducto? 2) ¿Afecta la proximidad del cuerpo lúteo al transcriptoma del oviducto? Y 3) ¿Existe alguna diferencia transcripcional entre el ámpula y el istmo del oviducto ipsilateral de animales gestantes?

Los resultados del segundo capítulo indican que el embrión no afecta el transcriptoma del oviducto. Sin embargo, los hallazgos en ratas, ratones y cerdos podrían estar relacionados con el número de embriones, considerando que estas especies son poliovulatorias, es decir mantienen el desarrollo de varios embriones. Además, en nuestro experimento la longitud del istmo procesado de todos los animales fue aproximadamente de 8 cm, muy superior al tamaño del embrión (~120 µm), por lo que el efecto sobre el punto concreto en el que se encuentra el embrión puede haber pasado desapercibido.

La proximidad del cuerpo lúteo (CL), es decir la comparación entre las células del oviducto ipsilateral y contralateral al CL, no afectó el transcriptoma del istmo, independientemente de si las novillas fueron cíclicas o gestantes. Sin embargo, la porción del oviducto determinó significativamente el patrón de expresión génica, obteniendo 2287 genes expresados diferencialmente ($P<0.01$) entre ámpula e istmo del oviducto ipsilateral de los animales gestantes, de los cuales 1132 se expresaron más y 1155 se expresaron menos en el istmo. El análisis ontológico de estos genes mostró que los procesos biológicos más representados en el istmo estaban relacionados con la síntesis de compuestos como el nitrógeno, lípidos, nucleótidos, esteroides y colesterol, así como transporte mediado por vesículas, ciclo celular, apoptosis, endocitosis y exocitosis. Por otro lado, los procesos biológicos más representados en el ámpula fueron: movimiento celular, motilidad y migración, reparación de ADN, homeostasis del calcio, biosíntesis de carbohidratos y regulación del movimiento ciliar y de la frecuencia del batido ciliar. Por tanto, basándonos en estos resultados concluimos que 1) la presencia de un embrión de 8 células en el istmo no afecta el transcriptoma del oviducto; 2) la expresión génica del oviducto en novillas gestantes o cíclicas no se ve modificada por la proximidad del CL; y 3) en las novillas gestantes, existe una gran diferencia entre el ámpula y el istmo del oviducto ipsilateral al CL.

Dentro de los factores que podrían contribuir a la subfertilidad, es importante considerar que la mayor parte de las pérdidas embrionarias en la gestación tienen lugar durante las dos primeras semanas después de la concepción, representando un 70% de las pérdidas embrionarias/fetales totales. La progesterona (P4) es la señal clave de la gestación porque es necesaria para la elongación del embrión que a su vez es indispensable para la síntesis interferón-τ, fundamental para el reconocimiento materno de la gestación. Por tanto, se ha propuesto que las pérdidas embrionarias podrían deberse a unos niveles insuficientes de P4. Así, la presencia de concentraciones elevadas de P4 durante el desarrollo embrionario temprano se ha asociado con embriones más elongados e incluso mejor tasa de gestación, mientas que unos niveles bajos de P4 se han relacionado con una capacidad reducida del endometrio para mantener la elongación embrionaria.

La concentración de P4 está directamente relacionada con el tamaño del CL. Por tanto, una de las estrategias para incrementar la P4 durante el desarrollo embrionario temprano es inducir un CL accesorio o estimular el desarrollo del CL endógeno/nativo. Esto se puede conseguir de diversas maneras, aunque es importante mencionar que, un incremento de la concentración de P4 demasiado temprano se ha relacionado con un acortamiento del ciclo estral, así como un incremento tardío, como el día 7 u 8 después del celo, no tienen ningún efecto en el tamaño del concepto.

La gonadotropina coriónica humana (hCG) es una hormona con actividad similar a la hormona luteinizante, que ha sido ampliamente usada entre el día 4 y 7 después del celo, principalmente para inducir la ovulación de un folículo dominante y la formación de un CL accesorio, conllevando un incremento de la P4. Algunos estudios han descrito un efecto positivo en la tasa de gestación, aunque otros no han observado mejora. En otros experimentos se ha visto que la hCG también tiene un efecto hipertrófico en el CL original. Por tanto, basándonos en estos datos junto con el hecho de que el efecto de la hCG en la concentración de la P4 no es inmediato, el tercer capítulo de esta tesis se desarrolló para responder a la siguiente pregunta: ¿Puede una sola inyección de hCG, administrada en los días 1, 2, 3 o 4 después del celo, incrementar el área del tejido luteal del CL nativo y la concentración de P4?

Novillas procedentes de cruces recibieron una dosis única de 3000 UI de hCG en los días 1, 2, 3, o 4 después del celo. Los resultados revelaron que cuando la hCG se usa el día 1 no tiene ningún efecto en la concentración de P4. Sin embargo, después del tratamiento con hCG los días 2, 3 o 4 se produjo un incremento en el área del tejido luteal desde el día 6 al 12, desde el día 9 al 11 y, el día 9 y 10, respectivamente (P<0.05). Sólo cuando la hCG se usó el día 2 o 4 el incremento en el área del tejido luteal estuvo acompañado de un incremento en la P4, desde el día 6 al 11 y desde el día 8 al 13, respectivamente (P<0.05). Sin embargo, cuando la hCG fue inyectada el día 4, la mayoría de los animales tuvieron una doble ovulación. Basándonos en estos resultados concluimos que, el tratamiento con hCG el día 2 después del celo incrementa la concentración de P4 desde el día 6 en adelante, que podría ser beneficioso para el desarrollo embrionario y la tasa de gestación.

Literature Review

1. INTRODUCTION

Over the last several decades milk production from dairy cows has dramatically increased while fertility has decreased. This growing yield is interpreted to be the result of the increased demand for animal products as a consequence of the growing global human population. Such is the case that according to the FAO, since 1960 global meat production has more than tripled and milk production has nearly doubled (Speedy 2003). As an example, in The Netherlands from 1992 to 2002 milk production increased from 7000 to 8200kg/305 days (van Knegsel *et al.,* 2005), being even higher in 2012 with 8898 kg/305 days in Holstein-Friesian cows (Buiting 2013) . Meanwhile fertility parameters like pregnancy rate diminished or days postpartum until first insemination rose (Figure 1). This situation is not unique to The Netherlands. Thus, in Spain from 1999 to 2000, pregnancy rate decreased from 42.3 to 33.1% while milk production increased from 7800 to 9900 kg/year, respectively (López-Gatius 2003). In addition, the reduction in first-service conception rate (CR) has also been reported in Ireland (Roche *et al.,* 2000), United Kingdom (Royal *et al.,* 2000b), and USA (Pursley *et al.,* 1998).

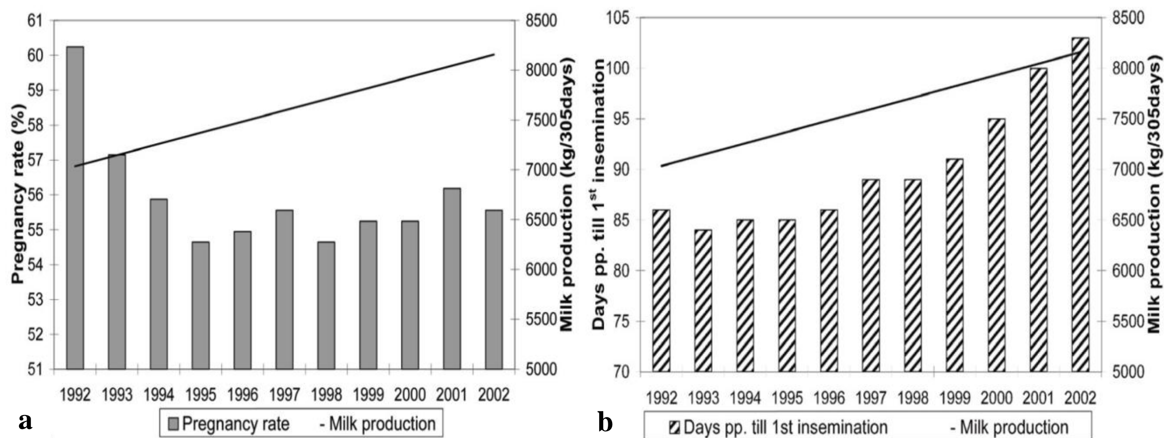

Figure 1. Pregnancy rate and annual milk production of dairy cows in The Netherlands from 1992-2002 (a); Interval postpartum till 1st insemination and annual milk production of dairy cows in The Netherlands from 1992-2002 (b) Data based on > 1 million calving's per year (van Knegsel *et al.* 2005).

Whether or not this increase in milk production is the main cause of low fertility has been extensively discussed (LeBlanc 2010). To understand the actual situation is necessary to know what has been done in the past. During the last decades breeding programs have been focused on genetic improvement of production traits such as milk yield or growth rate (Oltenacu and Broom 2010) mainly because these traits provide more economic benefits within a short time. According to Pryce *et al.,* (2004) 50% of the progress in milk yield can be attributed to environmental factors that have been improved, i.e. better nutrition, housing, health and management, while the other half can be attributed to genetics. Genetic selection was already made since the domestication of cattle [8000 BC (Zeder *et al.,* 2006)], at this time maybe for docility and manageability (Oltenacu and Broom 2010). However, it was not until the use of selection indexes which give appropriate weighting to each trait, that the maximum genetic progress was achieved (Hazel 1943). To use selection indexes it is essential to choose with caution the

traits that are going to be included because, according to the Resource Allocation Theory proposed by Beilharz *et al.,* (1993), the sources that the animals have for their adaptation to different situations are limited. Therefore, if the genetic improvement is highly directed towards increased milk yield, other functions like fertility, immune defence or maintenance will be affected.

The consequences of low fertility include additional inseminations, more veterinary visits, increased culling rate and higher replacement costs that substantially impact the profitability of the farm. For this reason, improving fertility is now a major focus (Höglund *et al.,* 2009). It is important to bear in mind that not only the increase in milk production is contributing to the low fertility but also factors like increasing herd size, greater use of confinement housing, labour shortages, higher inbreeding percentages and global warming may be involved (Lucy 2001).

What is going to happen in the next years and where we need to focus?

It has been estimated that the global production and consumption of meat will rise from 233 million metric tons (Mt) in 2000 to 300 million Mt in 2020, while production of milk will increase from 568 to 700 million Mt over the same period (Delgado *et al.,* 1999). Moreover, in Europe by 2015 the milk quota regime will be abolished (IPTS 2009) and milk production will increase or decrease depending on the country. In those areas were milk yield is going to increase, this will be achieved by increasing herd size (where land area is available) and/or an increase of milk production per cow.

Taking all these aspects together, if we want to enhance fertility it is necessary to adopt some strategies:

a. *To include fertility parameters in selection indexes*. It is well known that the heritability of fertility traits is low, about 5% (Berry *et al.,* 2013). However, since about 1975 the Scandinavian model has proven that total merit indexes, which include not only production but also reproduction (female fertility, calving performance and stillbirths) and health (resistance to mastitis and other diseases) traits, contribute to maintain or improve the results in these traits despite strongly increased production (Philipsson *et al.,* 1994; Philipsson and Lindhé 2003; Refsdal 2007). Similar indexes are used nowadays in other countries like Ireland where in 2000 the relative breeding index (which was based only on traits for milk yield) was replaced with the economic breeding index which takes into account production, fertility and health traits (Parland *et al.,* 2008). Moreover, it is important to include welfare and ecological traits in these selection indexes.
b. *To reduce inbreeding*. In the middle of the genomics era, this new discipline gives us the possibility to implement programs of genomic selection on farms. Genomic selection is based on the use of dense markers, which are spread across the genome and whose effects are estimated and used for the prediction of breeding values (Mc Hugh *et al.,* 2011). This procedure have the potential to increase the accuracy of selection and genetic gain as well as to decrease the rate of inbreeding compared with conventional selection methods (Konig *et al.,* 2009). Some years ago the cost of genotyping was very high although as time goes by this technique is getting cheaper. Thus in 2009 the cost for genotyping was 250€ (Konig *et al.,* 2009) while in 2012 the price for a new bovine low-density SNP array (Boichard *et al.,* 2012) was 29€ (Pryce *et al.,* 2012). Moreover Konig *et al.,* (2009) demonstrated that

the cost associated with genotyping a large number of animals was balanced by the greater monetary genetic gain associated with the implementation of genomic breeding schemes.

c. *To study the physiology of reproduction* and elucidate all the factors that could be involved in low fertility including the oocyte, the sperm, the embryo and embryo-maternal interactions.

d. *To develop treatments to improve fertility* and new reproductive programs.

e. *To care about management conditions and housing*.

Different groups all over the world are currently working on all these issues but we still have further to go. Co-operation of breeding experts, geneticists, epidemiologists, nutritionists, ethologists, veterinaries, farmers and others concerned with the dairy industry, even governments, is crucial to get to the final objective - to have a dairy industry sustainable, i.e. integrating animal welfare (McGlone 2001), efficient production in relation to human requirements and also ecological production by controlling the greenhouse gas production (Oltenacu and Broom 2010).

2. PHYSIOLOGY OF REPRODUCTION IN CATTLE

Cows are polyoestrus animals, i.e. once they reach puberty they have oestrous cycles indefinitely unless they become pregnant. The length of the oestrous cycle is around 21 days in cows and 20 in heifers, within a normal range between 18-24 days. Classically the oestrous cycle is divided into four phases: oestrus (Day 0, sexual receptivity), metoestrus (Day 1 to 3, postovulatory period), dioestrus (Day 5 to 18, active *corpus luteum* (CL) present) and proestrus (Day 18 to 20) (Ball and Peter 2004) (Figure 2). However in cows the cycle is better described in terms of ovarian function, as:

- *Follicular phase (4-6 days)*: comprises follicular development from luteolysis to ovulation and is under oestrogens influence. Close to the end of this phase the oestrus is shown by the animal as willing to be mounted by other cattle, both male and female. The oestrus is shown in a short period of time, on average 7 hours (Ball and Peter 2004).
- *Luteal phase (14-18 days):* comprehends development and maintenance of CL after ovulation. In this case the main hormone is progesterone (P4) secreted by the CL (Ball and Peter 2004).

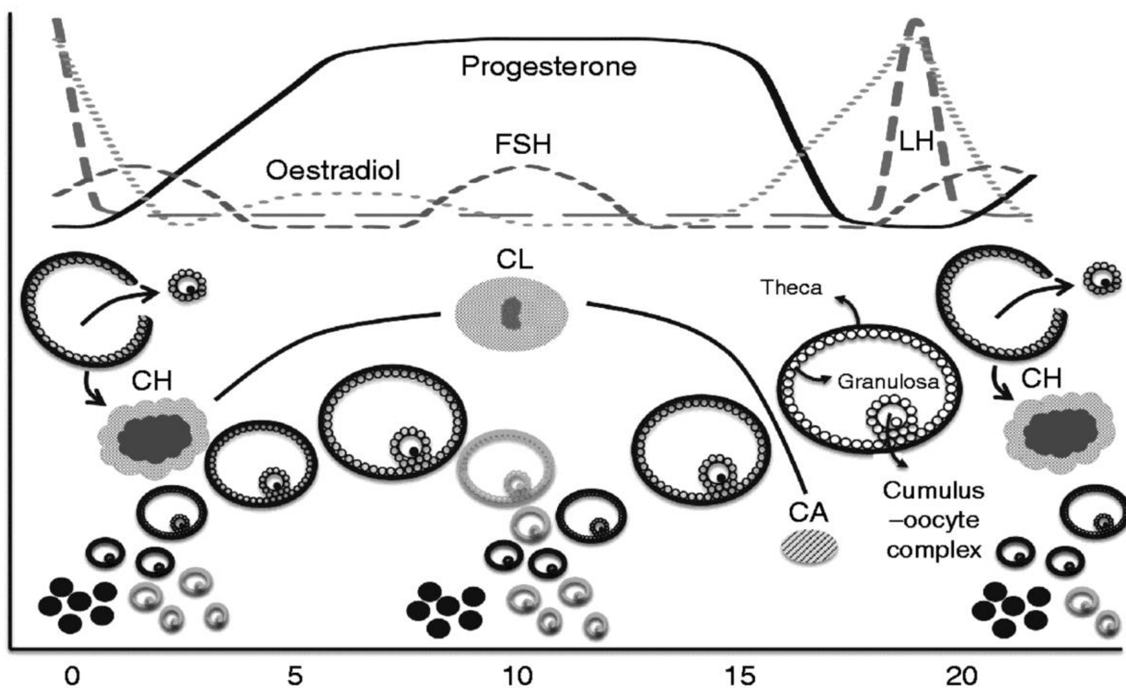

Figure 2. Schematic representation of ovarian events (lower panel) and associated changes in circulating hormone levels (upper panel) during a two wave oestrous cycle (21 days). CH: *corpus haemorrhagicum*; CL: *corpus luteum*; CA: *corpus albicans.* (Donadeu *et al.* 2012).

Follicular growth in cattle is in a wave-like fashion and most of the oestrous cycles consist of two or three waves. Two wave cycles tend to be shorter than 3 wave cycles, 19-20 days vs. 22-23 days, respectively (Adams *et al.,* 2008) (Figure 2 and 3). In addition, when the oestrous cycle has two waves they start around Day 2 and 11 but with three waves start at Day 2, 9 and 16 (Sirois and Fortune 1988). During pregnancy (Ginther *et al.,* 1989) and the pre-pubertal period (Adams *et al.,* 1994) follicular waves also occur. In fact, since birth, waves of primary follicles develop and migrate to the surface of the ovary but without the factors required to mature and ovulate them, they cease to grow and undergone atresia.

The anatomical and hormonal conditions required for regular ovulation is established at puberty (Ball and Peter 2004), i.e. between 6-12 months of age or at a weight of 200-250 kg (Forde *et al.,* 2011b) and thereby puberty is considered the time at which first oestrus occurs, being accompanied by ovulation (Ball and Peter 2004).

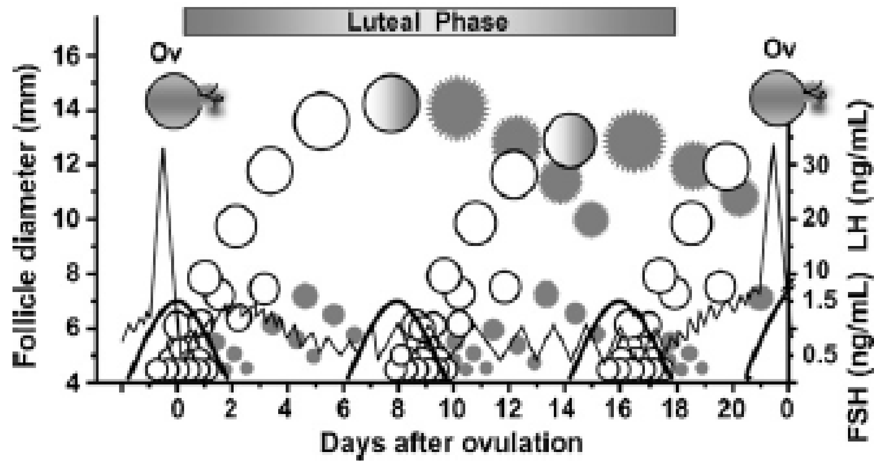

Figure 3. Dynamics of ovarian follicular development and gonadotropin secretion during three wave oestrous cycle in cattle (Adams *et al.* 2008).

2.1 Oogenesis and folliculogenesis

Oogenesis is the formation of oocytes derived from oogonia and is initiated early in fetal development but does not end until months to years later, in the sexually mature adult (Picton *et al.,* 1998). During fetal development, the oogonia penetrates the ovarian stroma and differentiates, becoming primary oocytes (Ball and Peter 2004). The beginning of folliculogenesis occurs around Day 140 of gestation (Russe 1983) when a group of flattened pre-granulosa cells are recruited around the oocyte forming a primordial follicle. Meiosis of oogonia begins by Days 75-80 of gestation (Erickson 1966) but then is arrested in the oocyte at meiotic prophase I, when the chromosomes are decondensed and contained within the nuclear membrane [the germinal vesicle (GV)] and only reenter meiosis or GV breakdown upon ovulation (Picton *et al.,* 1998).

At birth, the ovaries of the female calf have a pool of primordial follicles, that contains all the oocytes she will ever produce, from 200.000 up to half a million, although only a few, 500-1500 will start to grow during the lifespan of the cow and not all of them will ovulate (Hernández-Cerón and Porras-Almeraya 2013). After birth, these primordial follicles are maintained in dormancy or activated to grow into antral follicles. Primordial follicle activation is regulated by close interactive communication with somatic cells and oocytes (Kim 2012). During follicular activation flattened pre-granulosa cells from the primordial follicle become a single layer of cuboidal granulosa cells creating a primary follicle. Then granulosa cells start to proliferate until 2 to 6 layers surround the oocyte (secondary follicle) and finally when more than 6 layers are around the oocyte and a fluid filled antrum is formed constitute the antral or tertiary follicle (Braw-Tal and Yossefi 1997) (Figure 4). During all this time the oocyte undergoes volume expansion and a zona pellucida (ZP) develops between the oocyte and granulosa cells (van Wezel

and Rodgers 1996). The development of antral follicles requires on average 42 days (Lussier *et al.,* 1987) [for a review of follicular dynamics see Aerts and Bols (2010)].

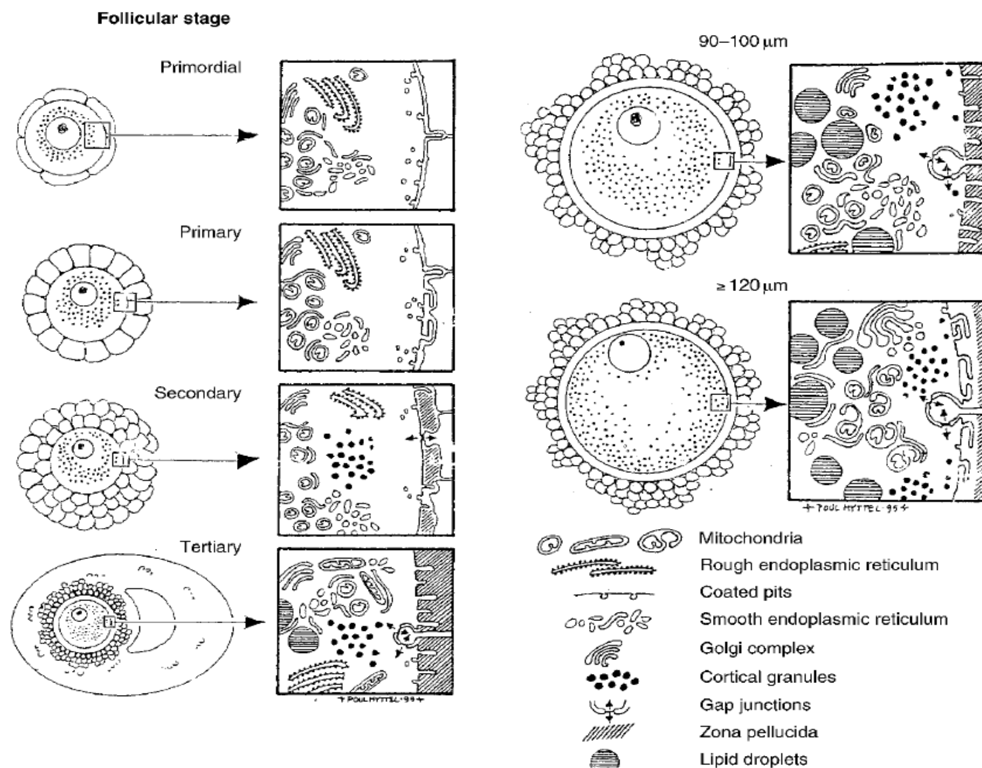

Figure 4. Ultrastructural changes during follicle growth (Fair 1995).

2.2 Follicular development and ovulation

Follicular growth includes two phases. The basal phase, when the follicle grows until reaches a diameter of 3-4 mm without the effect of gonadotrophins, and the tonic phase that involves the growth of follicles in follicular waves until ovulation and is regulated by gonadotrophins (Hernández-Cerón and Porras-Almeraya 2013).

The follicular wave includes: recruitment, selection, dominance and atresia. Recruitment is a process whereby 2-5 follicles (Driancourt 1991)(4mm diameter) are selected for further development (Hodgen 1982). The signal that stimulates recruitment appears to be an elevation in plasma follicle stimulating hormone (FSH) (Fortune 1994) secreted by the anterior pituitary gland due to the effect of gonadotropin releasing hormone (GnRH) (secreted in turn by the hypothalamus). After recruitment, only one follicle will be selected as the dominant follicle (DF) and the others will become atretic (Hernández-Cerón and Porras-Almeraya 2013). During the dominant phase the main gonadotrophin is luteinizing hormone (LH). When the DF reaches a diameter of 9-10 mm (Hernández-Cerón and Porras-Almeraya 2013) it acquires more LH receptors on its granulosa cells than its subordinates (Adams *et al.,* 2008) and therefore is able to shift its gonadotrophin dependence from FSH to LH (Ginther *et al.,* 1996) and to continue growing. In addition, DF secretes oestrogens and inhibin that block the secretion of FSH and therefore subordinate follicles that are dependent on FSH (Ginther *et al.,* 1996) undergo atretic. Oestrogen secretion is achieved

by a coordinated mechanism between theca cells that produce androgens and granulosa cells, which aromatize androgens to estradiol (E_2). The DF can secrete more E_2 because of the increased ability of theca cells to respond to LH by secreting androgen and of granulosa cells to aromatize androgen to E_2 (Fortune 1994). The E_2 rise has 3 functions: to initiate oestrus behaviour, to prepare the reproductive tract for fertilization and to initiate the ovulatory peak of LH (Ball and Peter 2004).

LH secretion occurs in a pulsatile manner. Thus, when there is a CL secreting P4 actively, LH secretion is characterized by high amplitude and low frequency (6-8 pulses each 24 h) (Rahe *et al*. 1980). This pattern makes that the DF undergo atresia, oestrogens and inhibin decrease and FSH increases again, favouring the development of another follicular wave. When the CL regresses, the concentration of P4 decreases, and LH secretion is characterized by low amplitude and high frequency (20-30 pulses/24 h) (Rahe *et al.,* 1980) establishing what it is known as preovulatory LH surge, with a duration of 7-8 h, which final result is trigger the ovulation 24–32 h after the beginning of the surge (Ball and Peter 2004). During this time, 24-32 h, the LH surge is responsible of the oocyte maturation, i.e. when the oocyte acquires its intrinsic ability to support the subsequent stages of development (to see an illustration of gonadotrophin profile during oestrous cycle see Figure 2).

Oocyte maturation involves nuclear and cytoplasmic maturation (Ferreira *et al.,* 2009) and cumulus cell expansion. Firstly, during nuclear maturation, meiosis is resumed, characterized by chromosome condensation, progress from prophase I to metaphase II (MII) with extrusion of the first polar body [8-9 h after LH surge (Ball and Peter 2004; Palma *et al.,* 2012)]. Immature oocytes that have not progressed through meiosis to MII cannot be successfully fertilized (Beall *et al.,* 2010). Secondly, cytoplasmic maturation involves organelle redistribution (mitochondria, ribosomes, endoplasmic reticulum, cortical granules and the Golgi complex), cytoskeleton dynamics and molecular maturation that consists of transcription, storage and processing of maternal mRNA which is stored in a stable, inactive form until translational recruitment (Ferreira *et al.,* 2009). The proteins derived from these mRNAs are involved in maturation, fertilization, pronucleus formation and early embryogenesis. In addition, the cortical granules migrate to the periphery of the oocyte where they contribute to the block of polyspermy after fertilization. Finally, cumulus cells secrete hyaluronic acid that, when it becomes hydrated, causes the spaces between the cumulus cells to enlarge, and the cells to be embedded in a sticky, mucified matrix (Eppig 2001). This process is termed cumulus expansion and when it is suppressed artificially in vivo, ovulation rate is greatly reduced (Chen *et al.,* 1993).

After the completion of oocyte maturation, it has been proposed that the LH surge also stimulates the process of ovulation, by activating an inflammatory reaction, which (1) thins and ruptures the follicle wall (Espey 1980) and (2) initiates luteinisation of the granulosa and theca cells of the follicle, in preparation for the development of the CL.

2.3 Fertilization

Cows can be inseminated by natural service or artificial insemination (AI). In the case of natural service, when the cow is in oestrus or heat, the bull will serve her and sperm reside in the reproductive

tract for several hours prior to the occurrence of ovulation, which happens about 10-12 h after the end of standing oestrus. When freshly ejaculated semen is used, the lifespan in the cow's reproductive oviduct is around 24-48 h while if frozen-thawed semen is employed in AI, the lifespan is reduced to 12-24 h. In comparison, the viable lifespan of the oocyte after ovulation is only 6-12 h (Gordon 1996).

Semen, containing billions of sperm, is deposited in the anterior vagina of the cow but only a few hundreds arrive to the oviduct. After ejaculation, sperm are not immediately capable of fertilizing the oocyte. During their journey through the female tract they have to undergo a further series of maturational changes called capacitation, that requires about 6 h (Ball and Peter 2004). During this transit the sperm encounter different barriers that not only reduce the number that will reach the oviduct but also they will help the sperm to become capacitated and capable of fertilizing the oocyte. The first of these barriers is the cervix and above all the thick mucus (Silva *et al.,* 1995) that ensures that only the vigorously motile sperm pass through it (Kölle *et al.,* 2010). Then, the sperm ascend the uterus by both active and passive processes. Active transport involves activity of the flagellum of the sperm and passive is due to the contraction of uterine smooth muscle contractions (Abramowicz and Archer 1990; Kunz *et al.,* 1996). To reach the oviducts, it is necessary to pass through the second barrier, the utero-tubal junction (UTJ). The UTJ is composed of mucosal folds forming cul-de-sacs that face back towards the uterus (Yániz *et al.,* 2000) to restrict the entry of infectious organism and leukocytes and to regulate the entry of sperm (Suarez 2008). Once the sperm are in the oviduct, they are held in a storage reservoir in the isthmus. This is achieved by a species-specific carbohydrate binding between the sperm head and the ciliated cells of the oviduct epithelium (Kölle *et al.,* 2010) which in case of the cow involves fucose (Lefebvre *et al.,* 1997). This reservoir preserves sperm fertility, reduces the incidence of polyspermy by releasing sperm gradually (Suarez 2008) and constitutes the immediate source of viable sperm at the time of ovulation (Hunter and Wilmut 1984). The sperm that acquire hyperactivated motility are released from the reservoir and progress along the oviduct to the site of fertilization (Demott and Suarez 1992). In a short period of time the spermatozoa undergo the acrosome reaction that involves the formation of gaps between the sperm cell membrane and the acrosome through which the acrosome contents diffuse. This process is necessary to allow penetration of the oocyte by the sperm (Ball and Peter 2004).

After ovulation, the cumulus oocyte complex (COC) is captured by the fimbria of the oviduct. The ciliary beating of the oviductal epithelial cells and the contraction of the oviductal smooth muscle (Halbert *et al.,* 1989; Croxatto 2002) transport the COC to the ampullary-isthmic junction (AIJ). As soon as a vital COC is in the ampulla, the sperm become hyperactivated and released from the epithelium (Kölle *et al.,* 2009). Finally, fertilization takes place in the AIJ of the oviduct.

2.4 *Corpus luteum* formation

Luteinisation comprises a series of morphological, endocrine and enzymatic changes that take place in the pre-ovulatory follicle to form a CL, the main function of which is to secrete P4 to establish and maintain pregnancy.

The LH surge not only triggers ovulation but also initiates luteinisation. This process is characterized by the breakdown of the basement membrane in the preovulatory follicle, the migration of theca cells into

the previous follicular cavity and the development of an extensive vascular network with vessels that invade the follicular antral space (Niswender *et al.,* 1994). During luteinisation, granulosa and theca cells will be differentiated into two luteal cell types, morphologically and biochemically distinct: large luteal cells (LLC) and small luteal cells (SLC), respectively (O'Shea 1987). Besides some SLC can also develop into LLC (Niswender *et al.,* 1985b). Both types of luteal cells secrete P4 but LLC also secrete oxytocin (OT) and are responsive to prostaglandin E while SLC are responsive to LH (Gordon 1996). Morphologically, the CL also comprises endothelial cells and pericytes (from the blood vessels), macrophages, smooth muscle cells and fibrocytes (Rodgers *et al.,* 1984). Unlike the follicular phase when E_2 is the predominant hormone, during the luteal phase P4 is the main hormone.

The intense luteinisation during the first 5–6 days after ovulation results in a progressive increase in plasma P4 concentration from <1 ng/mL at Day 3 after ovulation to approximately 3 ng/mL at Day 6 (Adams *et al.,* 2008). This is accompanied by an increase in the CL volume due to the rise in number and size of luteal cells (Niswender *et al.,* 2000). After the peak of P4, between Day 10 and 14 post ovulation (>4 ng/mL) (Adams *et al.,* 2008), the function of the CL is maintained if the cow becomes pregnant or will be regressed if not. Plasma P4 concentration depends on the blood flow, the capacity of the luteal tissue to synthesize P4 and the amount of luteal tissue that in turn depends on number and size of luteal cells (Niswender *et al.,* 2000).

The luteotrophic hormones, i.e. those that support the growth and/or function of the CL are LH, growth hormone or somatotropin (GH), prolactin, insulin-like growth factor 1 (IGF-I), OT, prostaglandin E_2 and prostaglandin I_2 [(to review their functions, see Niswender *et al.,* (2000)]. LH between Day 2 and 12 is essential for establishing a fully functional CL in the cow though it is not required to maintain its function (Peters *et al.,* 1994).

The main hormone synthetized by the CL is P4, considered the key hormone of pregnancy because it exerts its function at three different levels:

- The hypothalamus-pituitary-axis: In the hypothalamus, P4 blocks surges of GnRH (Kasa-Vubu *et al.,* 1992). In the pituitary, it reduces the number of receptors for GnRH (Laws *et al.,* 1990) by down-regulating its mRNA (Bauer-Dantoin *et al.,* 1995) and decreasing the amount of LH released (Janovick and Conn 1996). These changes suppress the final stages of follicular development and ovulation, allowing the emergence of another follicular wave.
- The endometrium: the default mechanism of P4 is to prepare the endometrium for an expected pregnancy (Forde *et al.,* 2011c) i.e. provide an environment that supports early embryonic development by inducing stromal differentiation, glandular secretion, accumulation of basal vacuoles in the glandular epithelium and changing the pattern of proteins secreted by endometrial cells (Maslar *et al.,* 1986). To carry out its function, the endometrium has to be exposed to the E_2 during the follicular phase, which up-regulates P4 and oestrogen receptor alpha (PGR and ESR1 respectively) (Ing and Tornesi 1997) (Figure 5). P4 regulates conceptus-maternal interactions, pregnancy recognition and uterine receptivity for implantation. In addition, during the mid-luteal phase and early pregnancy, P4 inhibits the expression of ESR1 and down-regulates the expression of its own receptor (PGR) (Spencer and Bazer 1995; Spencer *et al.,* 1995) (Figure 5). This is important in mammals because prior to implantation it is essential that the endometrial epithelia ceases expression

of PGR (Bazer *et al.,* 2010). Thus, it has been shown that elevated P4 concentrations from Day 3 to 7 advances the down-regulation of PGR in the LE (Okumu *et al.,* 2010) as well as advances the expression of some endometrial genes associated with enhanced conceptus development (Forde *et al.,* 2009a). On the contrary, low P4 concentration in serum delayed the expression of genes in the endometrium, inducing a delay in the down-regulation of PGR and reducing the capacity of the uterus to support conceptus development after ET on Day 7 (Forde *et al.,* 2011a).

- The conceptus: it has been well demonstrated that P4 is responsible for conceptus elongation. It seems that P4 induces changes in endometrial gene expression which modify the composition of histotroph required for the conceptus to growth and survive (Spencer *et al.,* 2008). The action of P4 on the embryo is indirect via the endometrium (Clemente *et al.,* 2009). In addition, maternal plasma P4 is correlated with conceptus elongation (Mann *et al.,* 2006) and interferon-τ (IFNT) production by the conceptuses (Kerbler *et al.,* 1997; Mann *et al.,* 2006), suggesting that higher P4 may provide a more sustainable environment for the developing conceptus.

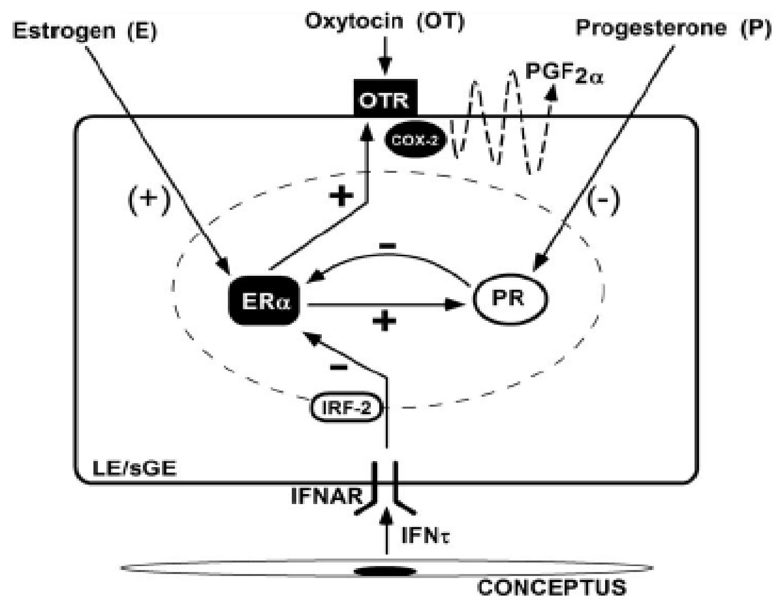

Figure 5. Schematic illustrating hormonal regulation of the endometrial luteolytic mechanism and antiluteolytic effect of the conceptus on the endometrium in the ovine uterus. Legend: COX2, cyclooxygenase 2; E, oestrogen; ERα, oestrogen receptor alpha; IFNτ, interferon tau; IFNAR, type 1 IFN receptor; IRF-2, interferon regulatory factor two; LE, uterine luminal epithelium; OT, oxytocin; OTR, oxytocin receptor; P, progesterone; PGF2α, prostaglandin F2α; PR, progesterone receptor; sGE, superficial ductal glandular epithelium (Spencer and Bazer 2004).

2.5 Luteolysis

Luteolysis is defined as the structural demise of the CL. If around Day 16 after oestrus (Northey and French 1980) there is no viable elongated conceptus in the uterus, the CL regresses allowing the initiation of a new oestrous cycle. When there is no signal for maternal recognition, the CL secretes OT (Wathes and Swann 1982) that binds to its receptor in the endometrium, stimulating the conversion of arachidonic acid to prostaglandin F2α (PGF2α) within the endometrial cell (Ball and Peter 2004). Thanks to the counter current transfer mechanism, PGF2α passes rapidly from the utero-ovarian vein to the ovarian artery (Ginther 1974) (Figure 6), exerting its effect directly on the ovary and avoiding enzymatic

inactivation in the lungs. In the CL, SLC, LLC and endothelial cells express receptors for PGF2α (Mamluk *et al.,* 1998). It is thought that the main consequence of PGF2α is in the endothelial cells of the CL causing its degeneration (Sawyer *et al.,* 1990) that in turn reduces blood flow to the CL (Pharriss *et al.,* 1970; Nett *et al.,* 1976) thereby causing luteolysis by depriving the CL of nutrients, substrates for steroidogenesis, and luteotrophic support. The final result is a progressive regression of the CL together with a reduction in P4 concentrations allowing the gonadotropins LH and FSH to increase up to normal values required to start a new oestrous cycle.

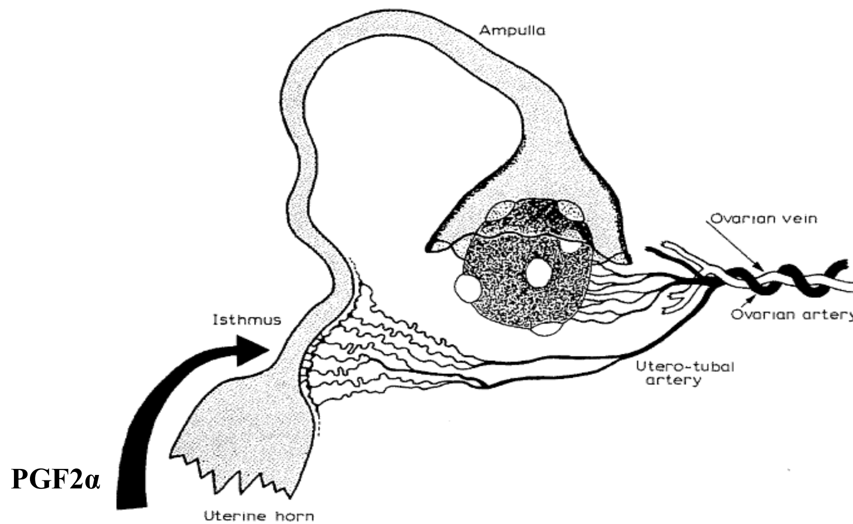

Figure 6. Representation of the arterial blood supply and ovarian vein to the ovary and isthmus of the pig oviduct. This is to demonstrate the counter-current transfer though which PGF2α goes to the ovary and not to the pulmonary system [adapted from Hunter (2005)].

2.6 Early embryo development

After fertilization, the mRNA and proteins that have been synthesized and stored in the oocyte during oogenesis, initiate and support the first stages of embryo development (Memili *et al.,* 1998). Until the embryo reaches the blastocyst stage the most important events are:

- *First cleavage division:* the timing is critical for determining the subsequent development of the embryo. Thus, the sooner the first cleavage occurs, the higher the developmental competence of the embryo (Plante *et al.,* 1994; Lonergan *et al.,* 1999). This morphological difference is reflected in differences in gene expression between early and late cleaved embryos (Lonergan *et al.,* 2000).
- *Embryonic genome activation (EGA):* is characterized by a gradual degradation of maternal RNAs and proteins and the activation of embryonic genes. The aim is to transform the highly differentiated oocyte into a totipotent cell, the zygote [for a review see Kanka (2003)]. Without this activation, differentiation and embryo implantation will not occur (Memili and First 1999; Schultz *et al.,* 1999). In the cow, the major burst of transcriptional activity occurs at the 8-16 cell stage although there are several reports demonstrating that there is a minor gene activation that starts at two cell stage (Crosby *et al.,* 1988; Telford *et al.,* 1990; Plante *et al.,* 1994; Hyttel *et al.,* 1996; Viuff *et al.,* 1996; Memili *et*

al., 1998) (Figure 7). Unlike the importance of major EGA on subsequent development, if the minor activation is inhibited it neither inhibits nor retards development (Plante *et al.,* 1994).

- *Compaction of the morula*: at this stage the first tight junctions between adjacent blastomeres are formed (Boni *et al.,* 1999). This will result in the formation of a communicating polarized epithelium (Schultz *et al.,* 1999).
- *Differentiation of the morula into the blastocyst*: composed of totipotent cells of the inner cell mass (ICM) that will give rise to the embryo, and differentiated cells of the trophectoderm (TE), that will give rise to extra-embryonic tissue (Schultz *et al.,* 1999).

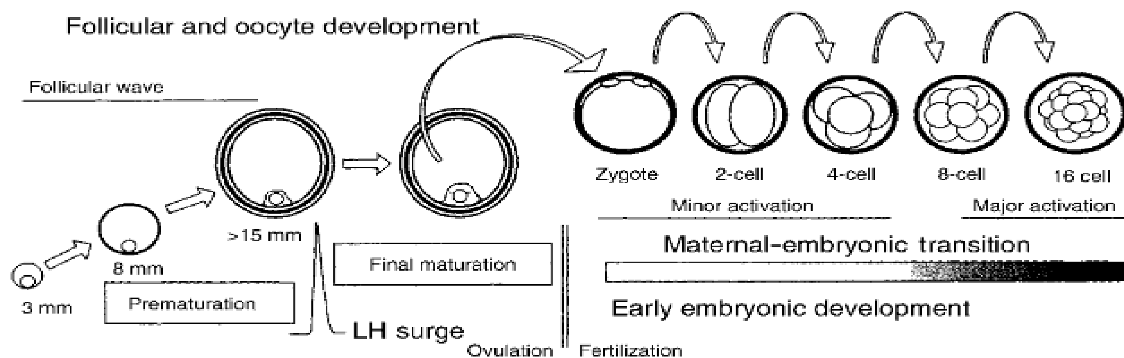

Figure 7. Prematuration for developmental competence and embryonic genome activation (Dieleman *et al.* 2002).

The bovine embryo stays in the oviduct until Day 3-4 when passes into the uterus at the morula stage. At about Day 8 post-fertilization the ZP begins to fragment and the blastocyst 'hatches' (Wolf *et al.,* 2003). After hatching, the blastocyst develops into an ovoid then tubular form and then elongates on Day 15 to form a filamentous conceptus that occupies the entire length of the uterine horn (Spencer *et al.,* 2008) (Figure 8). The elongation is a rapid process where the blastocyst develops from <1 cm (Day 12) to >10 cm (Day 16), essentially because of the rapid trophoblast growth (Robinson *et al.,* 2006). During conceptus elongation, P4 is required to regulate the outgrowth of the TE (Spencer *et al.,* 2007). The elongation initiates IFNT production (Robinson *et al.,* 2008) by TE cells (Roberts *et al.,* 1999; Spencer and Bazer 2004), reaching a maximum level between Day 15 and Day 17 (Wolf *et al.,* 2003).

After that, the establishment of pregnancy includes pregnancy recognition signalling, conceptus implantation and placentation (Spencer *et al.,* 2008).

2.7 Pregnancy recognition

IFNT in cows is the key signal for maternal recognition of pregnancy, i.e. the physiological process whereby the conceptus signals its presence to the maternal system and prolongs the lifespan of the CL (Spencer and Bazer 2004). This is achieved because IFNT prevents the pulsatile release of luteolytic PGF2α by suppressing the transcription of ESR1 and oxytocin receptor genes (Spencer and Bazer 1996) (Figure 5). In the endometrium, IFNT also induces the expression of a variety of IFNT-stimulated genes that together with genes induced by P4, are involved in establishing uterine receptivity to implantation.

Uterine receptivity can be defined as its ability to support conceptus growth and development by different processes that include: changes in expression of genes for attachment of TE to uterine epithelium, modification of uterine stromal cell phenotype, silencing PGR and ESR1 genes in uterine epithelia, signalling for pregnancy recognition, alteration in membrane permeability to enhance conceptus-maternal exchange of factors, increased vascularity of the endometrium and activation of genes for transport of nutrients into the uterine lumen and suppression of genes for immune recognition of the conceptus (embryo/foetus and associated membranes) (Bazer *et al.,* 2008) to avoid harming the embryo/conceptus (Bauersachs *et al.,* 2012).

During the pre-attachment period, nutrition of the conceptus depends on the histotroph, that supports growth and elongation processes (Spencer *et al.,* 2007). The histotroph is mainly synthesized by the endometrial glands and is a complex mixture of amino acids, ions, glucose, enzymes, growth factors, hormones, transport proteins and other substances (Spencer *et al.,* 2008). The importance of the histotroph has been proven in ewes, where the knock out for the endometrial glands resulted in no implantation of the embryos (Gray *et al.,* 2000).

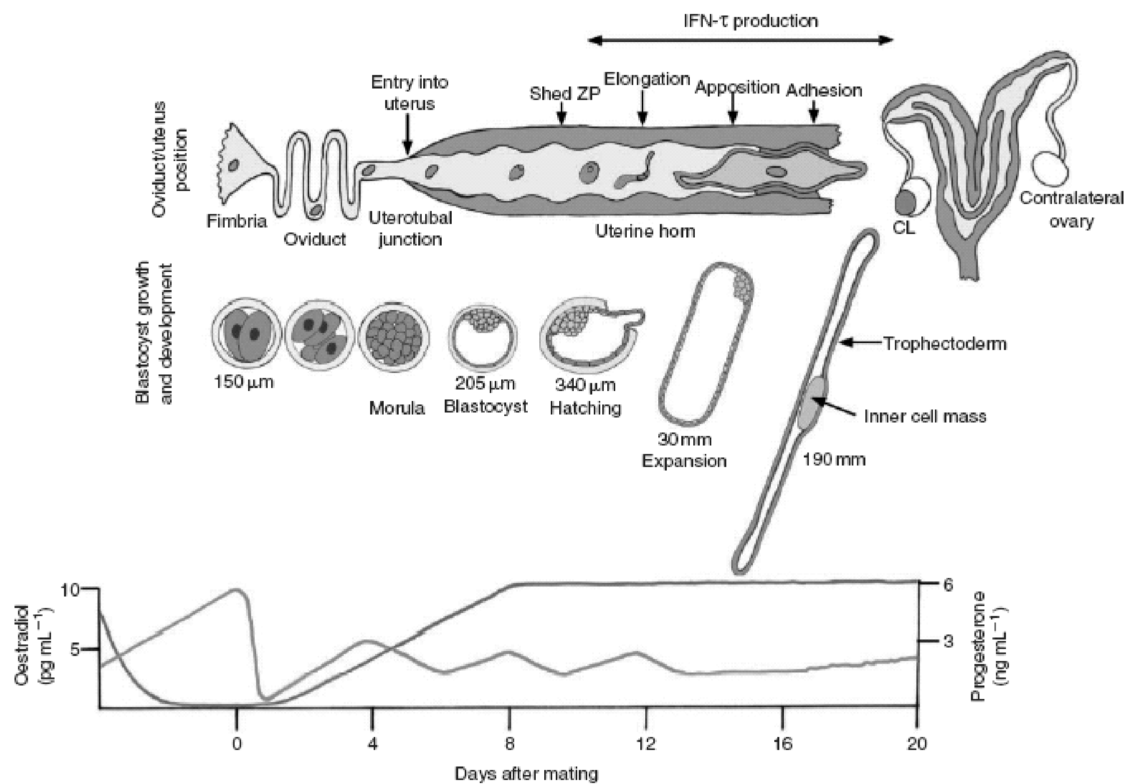

Figure 8. Early embryo development until the beginning of implantation in ovine. Below is the gonadotropic profile, being E_2 high until ovulation while P4 increases from Day 2 onwards (Spencer *et al.* 2007).

2.8 Implantation and placentation

Implantation is the period during which the conceptus acquires a fixed position within the uterine lumen, and leads to the establishment of the placental structures. It is characterized by three main steps: first, pre-attachment during which the conceptus elongates considerably; second, apposition that starts when the conceptus is immobilized in the uterine lumen and cellular contacts are established between the trophoblast and the uterine epithelium (begins at Day 19 in cows); and third, adhesion which ends the process and gives rise to the cellular structure of an epithelio-chorial placenta, characteristic of cattle [for a review see Guillomot (1995)].

3. TRANSITION PERIOD AND NEGATIVE ENERGY BALANCE IN HIGH YIELDING DAIRY COWS

The transition period comprises the interval between late pregnancy and early lactation. In high yielding dairy cows (HYDC) this stage is critical because it involves not only an obvious physical change after calving but also a huge metabolic change the final objective of which is to produce large quantities of milk. If this modification in metabolism is not well regulated, the result will be early postpartum health problems related to: energy metabolism (fatty liver, sub-acute ketosis and acute ruminal acidosis), mineral metabolism (milk fever, subclinical hypocalcaemia, udder edema) or problems related to immune system (retained placenta, metritis and mastitis).

Cow nutrition during the transition phase is crucial. To understand the metabolic situation of the HYDC is necessary to know the nutrient requirements during the transition period [for a review see Bell (1995)], which can be divided in two phases: before and after calving.

The metabolic changes start during late pregnancy when fetal requirements for glucose and amino acids increase. To meet these needs, the cow increases hepatic gluconeogenesis and reduces glucose utilization in peripheral tissues to favour its use by the foetus. In addition, the cow gradually mobilizes non-esterified fatty acids (NEFA) from adipose tissue (Bell 1995) to be used as source of energy in the peripheral tissues. This fat mobilization is facilitated by the diminished ability of insulin to promote lipogenesis (Bell 1995).

There are two main events that trigger metabolic change: decreased dry matter intake (DMI) and milk production. DMI refers to the quantity of feed consumption. According to Bertics *et al.,* (1992) the transition period can be divided in two phases: 7 d prepartum, characterized by a 30% reduction in DMI (compared to the intake during the early dry period), and 0 to 21 days postpartum, during which time DMI should increase rapidly, being more rapid in multiparous than primiparous cows (Block 2010) (Figure 9). The decrease in prepartum DMI has been related to the rapid growth of the foetus taking up abdominal space and displacing rumen volume (Block 2010) as well as other factors like environment, management, feeding system [for a review see Grant and Albright (1995)] or diet characteristics (Robinson 1997).

Milk production increases very quick after calving. Therefore, at this point the cow is in a situation where nutrient requirements for maintenance and lactation exceed its ability to consume energy in the feed (Lucy 2007), bring about a condition called negative energy balance (NEB). This starts a few days before calving, reaches its most negative level (nadir) around 2 weeks later (Butler 2005) and will extend 10–12 weeks until the usual breeding period (Butler 2003). During NEB, a mobilization of body tissue reserves occurs to meet the energy requirements. Usually all lactating cows mobilize body tissue in early lactation (Pryce *et al.,* 2004) but what is important is the duration and severity of the NEB, features related to DMI and its rate of increase during early lactation (Butler 2003).

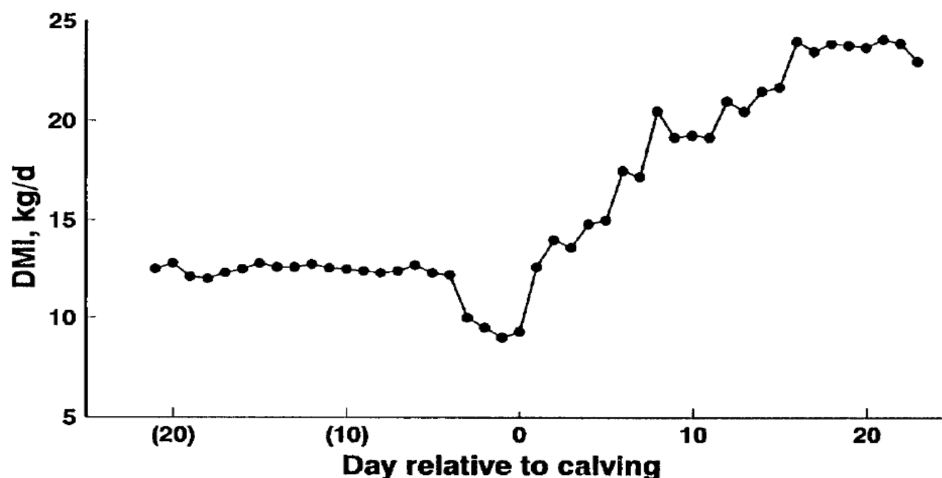

Figure 9. Dry matter intake of cows allowed to experience feed intake decrease before parturition (adapted from (Grant and Albright 1995).

As NEB is associated with mobilization of body tissue, body condition score (BCS) is a parameter used as an indirect measure of NEB which assesses the energy reserves and thereby the nutritional status of dairy cows (Hoedemaker *et al.,* 2009). BCS evaluation is based on observation of the animal and also palpation of certain regions like loin, pelvis, tail and ribs (see Figure 10) (Edmonson *et al.,* 1989) that give information about the quantity of accumulated fat. There are two different scales to record BCS: from 1 to 5 (with quarter point increments) (Lowman *et al.,* 1976; Edmonson *et al.,* 1989) or from 1 to 9 (Herd and Sprott 1986). There is no consensus whether these systems are used differently for dairy or beef cows but the most important thing is that scoring is carried out by the same person (Morris *et al.,* 2002). Considering the 1-5 scale, it has been demonstrated that cows with high condition score at calving (3.5- 4) will exhibit decreased appetite and thereby decreased DMI and will take more time to reach maximum DMI after calving (Garnsworthy and Topps 1982). Therefore, today it is recommended to maintain a moderate BCS between 2.5 and 3 until calving. In addition, high genetic merit cows (normally considered those that produce over 9000 kg of milk per 305-Day lactation) mobilize more body tissue (Pryce *et al.,* 2001); hence, they will experience more severe NEB. Thus, length and depth of NEB vary according to the genetic merit, precalving body condition, milk yield, feed intake and diet [for a review comparing different feeding systems see Grummer (1995)].

This NEB is going to be accompanied by alterations in certain hormones and metabolites to compensate nutrients deficiency. This briefly outlined below.

Glucose

Glucose is the main energy source of the organism and its concentration in the blood is regulated by insulin and other mechanisms. On the day of calving, glucose production is doubled (Paterson and Linzell 1974), likely due to the need of the mammary gland to start to synthetize milk. Following calving, synthesis and production of milk increases so rapidly that glucose requirements by the mammary gland treble those by the foetus during the late pregnancy (Figure 11) causing plasma levels of glucose to decrease drastically.

	SCORE	Spinous processes (SP) (anatomy varies)	Spinous to Transverse processes	Transverse processes	Overhanging shelf (care - rumen fill)	Tuber coxae (hooks) & Tuber ischii (pins)	Between pins and hooks	Between the hooks	Tailhead to pins (anatomy varies)
SEVERE UNDERCONDITIONING (emaciated)	1.00	individual processes distinct, giving a saw-tooth appearance	deep depression	very prominent, > 1/2 length visible	definite shelf, gaunt, tucked	extremely sharp, no tissue cover	severe depression, devoid of flesh	severely depressed	bones very prominent with deep "V" shaped cavity under tail
	1.25								
	1.50								
	1.75			1/2 length of process visible					
FRAME OBVIOUS	2.00	individual processes evident	obvious depression		prominent shelf	prominent	very sunken		bones prominent "U" shaped cavity formed under tail
	2.25			between 1/2 to 1/3 of processes visible					
	2.50	sharp, prominent ridge					thin flesh covering	definite depression	first evidence of fat
	2.75			1/3 - 1/4 visible	moderate shelf				
FRAME & COVERING WELL BALANCED	3.00		smooth concave curve	< 1/4 visible	slight shelf	smooth	depression	moderate depression	bones smooth, cavity under tail shallow & fatty tissue lined
	3.25			appears smooth, TP's just discernable					
	3.50	smooth ridge, the SP's not evident	smooth slope	distinct ridge, no invidual processes discernable		covered	slight depression	slight depression	
	3.75						sloping		
FRAME NOT AS VISIBLE AS COVERING	4.00	flat, no processes discernable	nearly flat	smooth, rounded edge	none	rounded with fat		flat	bones rounded with fat and slight fat-filled depression under tail
	4.25						flat		
	4.50			edge barely discernable		buried in fat			bones buried in fat, cavity filled with fat forming tissue folds
	4.75								
SEVERE OVERCONDITIONING	5.00	buried in fat	rounded (convex)	buried in fat	bulging		rounded	rounded	

Figure 10. Body condition scoring table from 1 to 5 (Edmonson *et al.* 1989).

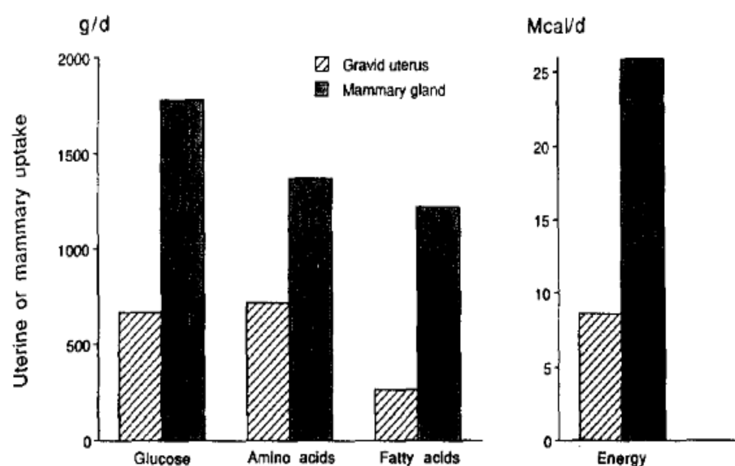

Figure 11. Comparison of estimated values for uterine uptake of specific nutrients and energy at Day 250 of pregnancy, and mammary uptake of these nutrients and energy at Day 4 postpartum, in Holstein cows (Bell 1995).

Growth hormone and non-esterified fatty acids

This is a pituitary hormone that coordinates body fuel utilization. In the liver, GH through binding with its receptor (GHR), induces hepatic IGF-I synthesis (Marshman and Streuli 2002). This relationship forms the basis of the GH-IGF-I axis (Butler *et al.,* 2003). GH regulation is through negative feed-back by IGF-I and GH (Roche *et al.,* 2009).

During early lactation, due to a down-regulation of GHR in the liver [GHR 1A (Kobayashi *et al.,* 1999)], the GH-IGF-I axis uncouples making that the levels of IGF-I will be low and GH high (Butler *et al.,* 2003). This situation promotes gluconeogenesis and lipolysis or mobilization of NEFA from adipose tissue (Rhoads *et al.,* 2004). NEFA are the major component of triglycerides in the fat stores of the body. When glucose supply is not enough, lipolysis of fat releases NEFA to be used as an energy source by many tissues. The concentration of NEFA in blood reflects the degree of adipose tissue mobilization (Pullen *et al.,* 1989). Thus, as NEB increases, more NEFA are released from body fat and their concentration in blood increases (Drackley *et al.,* 2005). Moreover, NEFA seem to be higher in high genetic merit cows than in low genetic merit cows (Hart *et al.,* 1978; Barnes *et al.,* 1985).

Ketone bodies: β-hydroxybutyrate

Ketone bodies are substances produced by the liver from fatty acids during periods of low food intake or carbohydrate restriction. During NEB, due to low levels of glucose, NEFA will be metabolized to ketone bodies, mainly β-hydroxybutyrate (BHBA) to be used as an energy fuel in the skeletal muscle, adipose tissue and fat synthesis in the milk. BHBA is the predominant form of ketone body in blood and its concentration is an index of fatty acid oxidation (Wathes *et al.,* 2007). Therefore, the more severe the NEB the higher the BHBA concentrations.

Insulin

The function of insulin is to regulate lipogenesis and antagonize the lipolytic action of GH through its positive effect on hepatic and adipocyte GHR abundance (Rhoads *et al.,* 2004). In postpartum cows, high GH and NEFA antagonize insulin action and create a state of insulin resistance. Thus, glucose is not

used by non-mammary tissues to conserve it for milk synthesis (Lucy 2007). In addition decreased DMI is associated with low blood concentrations of insulin and IGF-I (Butler *et al.,* 2006), situation found in postpartum dairy cows that in turn favour the effect of GH promoting body tissue mobilization.

Insulin-like growth factor-I

Insulin-like growth factor-I (IGF-I) is mainly produced in the liver in response to GH and its function in the ovary is to regulate the gonadotrophin action at the cellular level and to stimulate granulosa and theca cell proliferation and differentiation (Armstrong and Webb 1997). During NEB due to the uncoupling of the GH-IGF-I axis, IGF-I levels are low. Low levels of IGF-I have been related with longer periods to return to ovarian cyclicity (Taylor *et al.,* 2004).

Urea

To achieve the energy requirements, apart from massive lipid mobilization an important protein catabolism also takes place after calving. During degradation of amino acids, ammonia is produced. This compound is highly toxic in the organism thereby in the liver it has to be transformed into urea that is less toxic and to be eliminated by the urine. Hence protein deamination and detoxification can result in elevated systemic urea concentrations (Leroy *et al.,* 2008a) or ammonia.

To summarize, early lactation is characterized by a certain degree of insulin resistance in adipose tissue and muscle. This favours glucose to be used by the mammary gland and promotes the mobilization of NEFA and amino acids to be used as an alternative energy source by the previous tissues mentioned. This is translated into a plasma metabolic profile of high levels of NEFA, BHBA, GH and urea and low levels of glucose, insulin and IGF-I (Figure 12).

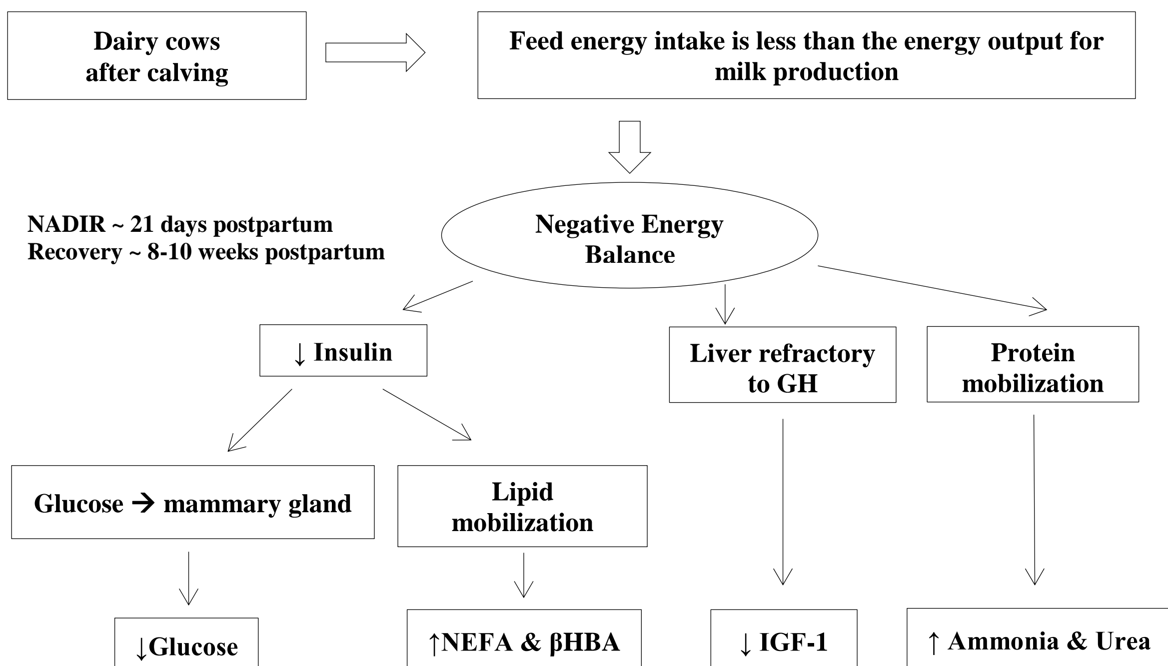

Figure 12. Schematic illustration of the negative energy balance in dairy cows after calving.

3.1 Consequences of NEB….

Metabolites like NEFA or BHBA can contribute to the development of diseases that may affect production, reproduction and the health of the cow. Thus, when large amounts of NEFA are released from adipose tissue into the circulation, cows are predisposed to accumulate NEFA as triglycerides (TG) within the liver (Emery *et al.,* 1992) and develop fatty liver in some cases. This occurs because the liver does not have sufficient capacity to completely dispose of NEFA through export into the blood or catabolism for energy. It is likely that almost all high-producing cows during the first few weeks postpartum develop a certain degree of fatty liver. What is uncertain is the threshold at which fat begins to have detrimental effects on other hepatic processes (Overton and Waldron 2004). However, it is well known that fatty liver is associated with decreased health status, well-being, productivity, and reproductive performance of cows (Wensing *et al.,* 1997) [for a review of fatty liver see Bobe *et al.,* (2004)]. In addition, high concentration of ketones in blood plasma lead to metabolic acidosis (Veerkamp *et al.,* 2003). It is thought that at least 50% of all dairy cows go through a temporary period of subclinical ketosis in the first month of lactation (Wathes *et al.,* 2007).

3.1.1 .... on the oocyte

NEB and BCS have also consequences for fertility. Oocytes recovered from high genetic merit cows that have lower BCS than medium genetic merit cows, had low rates of cleavage and blastocyst formation (Snijders *et al.,* 2000). In addition prolonged periods of NEB were associated with delayed ovulation (Butler *et al.,* 1981; Ducker *et al.,* 1985). This is basically because of the alteration in the hormones and metabolites.

At the ovary level it is crucial to have in mind the role of follicular fluid (FF) during follicular development, oocyte maturation and ovulation. FF is a complex extracellular fluid contained in the follicle and closely related with the oocyte. It is composed partly of secretions from the granulosa and theca interna cells, and partly of exudates from plasma (Edwards 1974). Some studies carried out *in vivo* have demonstrated that biochemical changes that take place during NEB are reflected in the FF relative to glucose, BHBA, urea, total protein, TG, NEFA, total cholesterol (Leroy *et al.,* 2004), insulin (Landau *et al.,* 2000) and IGF-I (Cohick *et al.,* 1996). This could affect the normal physiology of follicular development and oocyte developmental competence and hence could be a reason for low fertility in postpartum dairy cows. In spite of the relation between serum and FF, when glucose an NEFA are low and high respectively in the blood system, their concentration is the opposite in the FF (Leroy *et al.,* 2004; Bender *et al.,* 2010) suggesting the existence of a mechanism to protect the oocyte and the granulosa cells against systemic low glucose and high NEFA concentrations.

To study the effect of metabolic changes in oocyte development competence, *in vitro* models have been designed using different metabolites.

In vitro oocyte maturation with NEFA concentrations similar to that observed in postpartum dairy cows, reduces the fertilization rate (FR), developmental competence (Leroy *et al.,* 2005c) and compromises early embryo quality, viability and metabolism (Van Hoeck *et al.,* 2011; Van Hoeck *et al.,*

2013a; Van Hoeck *et al.,* 2013b). When low glucose and high BHBA were used, the hypoglycaemic conditions seem to be responsible to the hampered developmental competence. Besides when glucose levels are moderately low, BHBA aggravate the toxic effect of low glucose (Leroy *et al.,* 2006).

Changes in systemic levels of IGF-I and IGF binding proteins affect follicular development in heifers (Cohick *et al.,* 1996). In dairy cows, these changes are negatively correlated with milk production in such a way that the higher milk yield the lower IGF-I and the longer periods to return to ovarian cyclicity (Taylor *et al.,* 2004). Besides, low levels of IGF-I in multiparous cows before and after calving are associated with failure to become pregnant after several services (Taylor *et al.,* 2004). These alterations are due to the fact that low circulating concentrations of IGF-I are related to low steroidogenic output of DF in early postpartum cows. This is translated into low peripheral levels of E_2 that may be insufficient to stimulate LH release and this situation has been associated with ovulation failure (Beam and Butler 1997). At the oocyte level, IGF-I stimulates its maturation (Izadyar *et al.,* 1997; Pawshe *et al.,* 1998) and improves blastocyst yield as well as the quality of these embryos (Sirisathien and Brackett 2003). Therefore, low levels of IGF-I also can have a deleterious effect on the quality of the embryo produced.

Hyperinsulinemia is related with hyperandogenism [for a review see Poretsky and Kalin (1987)]; therefore, it is very likely that insulin possesses gonadotropic activity that affects steroidogenesis and the dynamics of the oestrous cycle. Thus, it has been seen that insulin stimulates follicular growth (Simpson *et al.,* 1994; Armstrong *et al.,* 2001) and that after calving, diets inducing high insulin reduces the interval from calving to first ovulation and also tends to reduce the interval from calving to first service and to conception (Gong *et al.,* 2002). In addition, *in vitro* insulin stimulates the proliferation of follicular cells (Spicer *et al.,* 1993) and also cell culture of follicular wall treated with insulin, increases follicular E_2 secretion (Frajblat and Butler 2000). Having these facts in mind low levels of insulin may affect oocyte growth or maturation.

Oocytes cultured under high levels of ammonia and/or urea *in vivo* can compromise the subsequent capacity of oocytes to develop to blastocyst stage *in vitro* (Sinclair *et al.,* 2000). When COCs are maturated with urea, meiosis is impaired and thereby reduces the percentage of oocytes fertilized and embryos that develop to Day 7 or Day 9 (De Wit *et al.,* 2001; Ocon and Hansen 2003). In addition, ammonia alters growth and metabolism of granulosa cells *in vitro* and the ability of these cells to support *in vitro* maturation of oocytes (Rooke *et al.,* 2004).

3.1.2 ….on the embryo

It has been demonstrated that lactating Holstein Friesian cows (LHFC) produce embryos with a significantly reduced quality compared to nonlactating Holstein Friesian heifers and Belgian Blue cows (Leroy *et al.,* 2005b). Morphologically, the embryos coming from LHFC were darker than in the other groups due to the higher content of lipids (Leroy *et al.,* 2005a). This dark aspect was similar to that observed in embryos cultured *in vitro* with serum that also had more lipids (Reis *et al.,* 2003; Leroy *et al.,* 2005a), confirmed as well by transmission electron microscopy (Abe *et al.,* 1999; Rizos *et al.,* 2002a).

Therefore, high content of lipids have a deleterious effect on embryo quality due to the fact that *in vitro* culture conditions without serum (less lipids) produce embryos of better quality in terms of cryotolerance (Yamashita *et al.,* 1999; Rizos *et al.,* 2003). Also, Sartori *et al.,* (2002) confirmed that embryos from lactating cows were of lower quality when compared to heifers or dry cows.

3.1.3 ….on the endometrium

A limited amount of data is available in the literature on the relationship between NEB and the endometrium. In the oviduct mRNA for IGF-I, II and IGF-1R has been detected which could be related with the transport of the embryo though the uterus or in the quality of the produced embryo (Pushpakumara *et al.,* 2002). Wathes *et al.,* (2011) found that under severe NEB the bioavailability of IGF-I and insulin in the endometrium was altered, suggesting that there could be a delay in the endometrial repair processes that contribute to low fertility in these animals. Therefore, it seems that IGF-I plays and important role in the reproductive tract and hence it needs to be studied in depth.

4. SUBFERTILITY IN HIGH YIELDING DAIRY COWS

The concept of fertility refers to the ability of the cow to conceive, maintain pregnancy and finally produce an offspring. It can be measured by different commonly used parameters like: non-return to first service, CR at first service, days from calving to first service or heat, days open and calving interval (Pryce *et al.,* 2004).

Dairy heifers usually calf for the first time at about 24 months of age (Wathes *et al.,* 2007) to maximize the economic benefit. The age at first calving is important because it will affect milk yield, fat and protein percentage, productive life and longevity (Pirlo *et al.,* 2000). It has been considered that cows continue growing until the end of their third lactation, although growth rate slows once the animal reaches about 450 days (Coffey *et al.,* 2006). Hence this fact is important because it could aggravate NEB after calving. In addition, to optimize the lifespan of cows the ideal calving interval has to be nearly 365 days, i.e. one calf per year.

After calving, the voluntary waiting period is between 45-60 days postpartum (Fetrow *et al.,* 2007); thereby farmers usually start to breed cows at Day 60 for having the cow pregnant around Day 85. To achieve this goal it is crucial that at this time the uterine involution will be completed and normal cyclicity restored (Opsomer *et al.,* 2000). Uterine involution normally occurs around 40-50 days postpartum (Gier and Marion 1968; Royal *et al.,* 2000a; Scully *et al.,* 2013).

All these periods are considered the optimal to have a calf per year. However, as it mentioned previously, fertility has decreased in HYDC, prolonging the calving interval. Unlike dairy cows, in dairy heifers fertility has not changed (Sartori *et al.,* 2002), being 67% in British Holstein Friesian heifers and 57% in US Holstein heifers (Kuhn *et al.,* 2006; Brickell *et al.,* 2009). Given that the only difference between AI heifers or cows after calving is lactation, the most likely is that the NEB that cows suffer after calving will be related with this decrease in fertility.

In this context subfertility is considered when any condition leads to failure to establish a pregnancy following completion of uterine involution at 40-50 days postpartum (Royal *et al.,* 2000a). Several factors may contribute to subfertility including production and ovulation of viable oocyte, oocyte transport, expression and detection of oestrus, fertilization, the fertilized oocyte and early embryo development (0-25 days after fertilization), alterations during late embryo/early foetus (36-60 days) or late fetal development (Ball and Peter 2004). Modification in any of these factors may reflect a dysfunction at the hypothalamic, pituitary, ovarian or uterine level and conceptus development (Royal *et al.,* 2000a). Therefore, subfertility is a multifactorial problem and to recognize all the factors implicated could be a difficult task.

In UK dairy farms, subfertility is one of the main problems, together with mastitis and lameness, but it is the one that is associated with the highest economic cost and the most difficult to treat (Royal *et al.,* 2000a). The principal problems related with decreased fertility are:

- *Cystic ovarian follicles*: extend the calving interval (Lee *et al.,* 1988; Borsberry and Dobson 1989; Fourichon *et al.,* 2000) and together with the treatment costs result in economic loss for the dairy farmer (Vanholder *et al.,* 2006b).

- *Delayed oestrus and ovulation postpartum*: may be due to calving season, length of dry period, BCS, puerperal disorders and clinical diseases (Opsomer *et al.,* 2000).
- *Reduced expression of oestrus*: increased level of milk production has a negative effect in the expression of oestrus and is related to decreased E_2 concentration (Lopez *et al.,* 2004).
- *Lowered conception rates*: is due to embryo loss and has enormous economic implications, increasing the number of days open and retarding genetic progress (Wolf *et al.,* 2003). According to the time during gestation when the embryo loss occurs, this can be divided in 3 different types:
    - Early embryo loss: before Day 28.
    - Late embryo loss (LEL): between 28 and 42 days.
    - Fetal loss or abortion (FL): after Day 42 until calving.

The actual calving rate in HYDC is about 40% (Diskin *et al.,* 2006). Taking into account that FR is 90% (Diskin and Sreenan 1980), this means that 50% of the embryos conceived are lost during development. Embryo losses as a consequence of chromosomal abnormalities have been estimated to be about 6% in heifers and 9% in cows (Gayerie de Abreu *et al.,* 1984). The overall loss rates between Days 28 and 84 of gestation is around 7%, from which 3% correspond to LEL and 4% to FL (Silke *et al.,* 2002). Placing all this data together make it clear that the rest and most of the embryo losses, approximately 35% occurs between D8 and D16 (Diskin and Sreenan 1980) or D18 (Roche *et al.,* 1981). This interval coincides with maternal recognition of pregnancy thereby highlighting the importance of the events occurring during this phase.

4.1 Factors that may contribute to low fertility in dairy cows

The establishment and maintenance of pregnancy is a highly complicated process involving the embryo, uterus and cow. There is no single factor that can be manipulated that will consistently improve embryonic survivability. But, by managing genetics, nutrition, parity, stress, and animal health the incidence of embryonic loss can be decreased considerably. Therefore when focusing on the reproductive components, embryo loss could be due to the quality of the oocyte, the quality of the embryo or problems in the reproductive tract (Figure 13).

4.1.1 Oocyte

Follicular fluid reflects more or less the metabolic profile of blood plasma (Cohick *et al.,* 1996; Landau *et al.,* 2000; Leroy *et al.,* 2004). Considering that a follicle needs around 90 days to reach the ovulatory size, Britt (1994)hypothesised that follicles grown during the period of NEB early postpartum could be affected by unfavourable metabolic changes and may contain a developmentally incompetent oocyte. Therefore, although after calving cow breeding may not start until Day 60 the oocyte that will be ovulated may be damaged. Previously it has been shown that oocyte maturation in the presence of high levels of NEFA (Leroy *et al.,* 2005c; Van Hoeck *et al.,* 2011), low glucose and high BHBA or low IGF-I (Izadyar *et al.,* 1997; Pawshe *et al.,* 1998) can alter the oocyte quality and its development. Nevertheless these results have been obtained *in vitro*. *In vivo* studies have seen that in spite of plasma high levels of NEFA (Leroy *et al.,* 2004; Bender *et al.,* 2010) or low levels of glucose (Leroy *et al.,* 2004), their

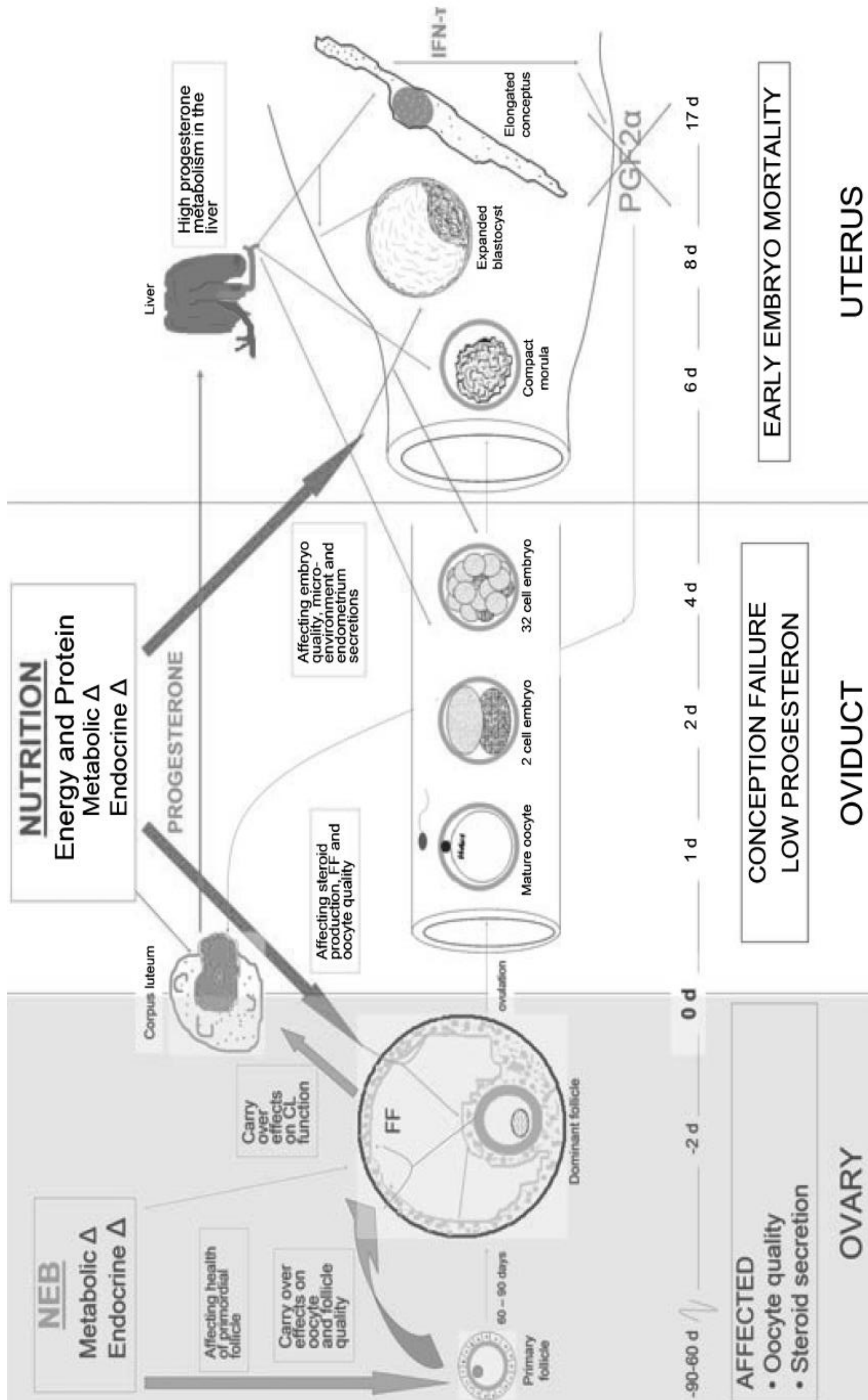

Figure 13. Representation of the major mechanism through which negative energy balance, *corpus luteum* or nutrition can directly influence oocyte and/or embryo quality. Δ stands for “changes”. (Leroy *et al.* 2008b).

concentration in the FF are not exactly the same, keeping the levels of NEFA lower and glucose higher, hence supposing the existence of a mechanism in the follicle to protect the oocyte. This could be one reason for the conclusion in a study with postpartum dairy cows by Matoba *et al.,* (2012), that metabolic changes during postpartum do not affect the quality of the oocytes recovered by ovum pick up (OPU) (oocytes recovered twice per week from Day 14 until Day 80 postpartum) in terms of morphology and to undergo fertilization and reach blastocyst stage *in vitro*. Furthermore, in another experiment with heifers and postpartum dairy cows, the number of oocytes aspirated was higher in heifers although *in vitro* cleavage rate and blastocyst yield did not differ between groups (Rizos *et al.,* 2005). The lack of differences in these two experiments may be because *in vitro* production is not enough sensitive to detect subtle differences in oocyte quality.

4.1.2 Sperm

Nowadays most dairy farms use AI with different protocols of synchronization, depending on the farm. The common factor is that the semen used comes from high fertility bulls, previously tested in reproduction centres. Therefore assuming appropriate oestrus detection, correct time of insemination and good semen quality (Diskin and Morris 2008; Robinson *et al.,* 2008) the actual FR is around 90% (Diskin and Sreenan 1980) and thereby not the main reason of subfertility.

4.1.3 Embryo

The quality of the embryos produced is also important for the subsequent development. As was explained before, lactating dairy cows produce poorer quality embryos compared with dry cows or dairy heifers, in terms of morphology, developmental stage and embryo cell number (Sartori *et al.,* 2002; Leroy *et al.,* 2005b). The disadvantage of these experiments is that it is difficult to know if these embryos are compromised as a consequence of: the quality of the oocyte, or if the reproductive tract was not able to support properly early embryo development. Therefore, it is important to design models where each part can be evaluated separately.

4.1.4 Reproductive tract

Most embryo losses occur between Day 8-16 of pregnancy, an interval that coincides with the signalling of the embryo in the uterus and maternal recognition of pregnancy. Hence, an insufficient communication between the uterus and the embryo may be one of the main reasons for embryo loss.

The first responses of the endometrium transcriptome to the embryo in pregnant animals were detected on Day 15 (Bauersachs *et al.,* 2012) or Day 16 (Forde *et al.,* 2011c) corresponding with maternal recognition of pregnancy. As it was mentioned before, P4 is the key hormone of pregnancy. The mechanism of P4 action during the luteal phase is not directly on the embryo but indirectly through the endometrium. In addition, the embryo does not need to be in the endometrium to benefit from the early increase in P4 (Day 3 after oestrus) (Clemente *et al.,* 2009). Thus, rising P4 levels at early pregnancy (Day 3) changes the uterine gene expression and favour conceptus elongation (Carter *et al.,* 2008; Clemente *et al.,* 2009; Forde *et al.,* 2009a; Forde *et al.,* 2009b). On the other side, it was clearly shown

that low P4 leads to suboptimal uterine environment and reduced its ability to support conceptus elongation (Forde *et al.,* 2011a).

The sooner the embryo starts to elongate and reaches the appropriate size the better its IFNT signal will be at the correct time to complete the maternal recognition. Besides, the endometrium has the capacity to respond in a different way depending on the origin of the embryo*: in vivo-in vitro* (Kues *et al.,* 2008), cloned embryos vs. *in vitro* produced (IVP) embryos (Bauersachs *et al.,* 2009), AI-somatic cell nuclear transfer (SCNT) and IVP-embryo transfer (ET) (Mansouri-Attia *et al.,* 2009). In addition, it is thought that maybe IFNT is not the only signal responsible for maternal recognition (Bauersachs and Wolf 2012) due to other genes detected on Day 13 not controlled by IFNT (Forde *et al.,* 2012). Taking all these facts together it is clear that there is an obvious cross-talk between the embryo and the uterus.

The effect of increased P4 during early pregnancy on blastocyst elongation, which takes place several days after, highlights the importance of the events that occur prior to maternal recognition or even before the arrival of the embryo in the uterus. It is known that the events occurring between the zygote and blastocyst stage determine the quality of the blastocyst (Rizos *et al.,* 2002b). The first stages of embryo development occur in the oviduct, where the embryo spends around 4 days. At the molecular level, the most important occurrence during this time is EGA, at the 8-16 cell stage. At this time the embryo starts to synthetize and use its own mRNA. This is important to ensure normal preimplantation and early fetal development (Niemann and Wrenzycki 2000). In an experiment carried out by Gad *et al.,* (2012) it was demonstrated that if the embryo is cultured *in vivo* before or after EGA, the blastocyst rate is higher than *in vitro* cultured embryos. Other events like first cleavage division and compaction also influence in the subsequent development of the embryo.

At this point the question is: Is there any cross-talk between the oviduct and the embryo? The fact that embryos can be obtained *in vitro* undermines the role of the oviduct. However, it has been demonstrated that when the embryos are cultured in the oviduct of sheep (Enright *et al.,* 2000; Lazzari *et al.,* 2002; Rizos *et al.,* 2002b), cattle (Tesfaye *et al.,* 2007) or mice (Rizos *et al.,* 2007; Rizos *et al.,* 2010b) the embryo quality is better compared to the embryos produced *in vitro*, in terms of morphology, gene expression, cryotolerance and pregnancy rate after transfer. Therefore, this proves that the oviduct is not a mere organ that transports the embryo through the uterus and also that a communication with the embryo exist.

The oviduct is a complex organ that has to provide a suitable microenvironment to capacitate the spermatozoa, to fertilize the oocyte and to support the early stages of embryo development. Thus, if all these events do not happen in the correct way this may have deleterious effects on the subsequent embryo development.

Dealing with the anatomy, the epithelium of the oviduct is made up of ciliary and secretory cells, the latter responsible for the secretion of proteins and other factors that contribute to the formation of the oviductal fluid (OF). The OF is a complex mixture of constituents derived from plasma plus some specific proteins formed by the oviduct epithelium (Leese 1988) and it is responsible for nurturing the early embryo during the early stages of its development. During the oestrous cycle several changes have

been detected in the oviduct. Firstly, during oestrus the volume of OF synthetized is higher (Roberts *et al.,* 1975) and just prior to ovulation is very viscous, perhaps to maintain the sperm in the oviduct reservoir until the follicle ovulates [for a review see Hunter *et al.,* (2011)]. Secondly, the oviduct epithelium changes depending on the phase of the oestrous cycle, the oviductal segment and basal or apical areas within folds (Yániz *et al.,* 2000). Thus, in the ampulla during the follicular phase there are numerous ciliated cells while during the luteal phase the secretory cells predominate, in pigs (Areekijseree 2003) and cows (Abe 1996). However, in the isthmus the proportion of each cell type is approximately the same during the oestrous cycle. Thirdly, the concentration of some amino acids in the OF during the oestrous cycle is higher than in plasma, suggesting that the oviduct epithelium is responsible for this synthesis (Hugentobler *et al.,* 2007b). Finally, there are differences in gene expression of the oviduct between oestrus and dioestrus (Bauersachs *et al.,* 2004).

The oviduct epithelium secretes diverse substances that play a role during fertilization. Until recently it was thought that once the sperm binds to the oocyte the contents of the cortical granules were released inducing a hardening of the ZP which avoids penetration of more spermatozoa. However just a few years ago it was confirmed that the presence in the OF of oviduct-specific glycoprotein together with some heparin protein complex are responsible of the pre-fertilization ZP hardening that is directly related to monospermy levels (Coy *et al.,* 2008; Mondejar *et al.,* 2013). In addition, plasminogen has also been identified in the OF as well as activators of plasminogen in the oolema and the ZP of the oocyte before fertilization (Mondejar *et al.,* 2012). The distribution of the activators of plasminogen after fertilization suggest that after binding the spermatozoa to the ZP these activators are released, transforming plasminogen into its active form plasmin, which has been shown to decrease the number of sperm attached to the ZP and the incidence of polyspermy rates in pigs and cows (Coy *et al.,* 2012; Mondejar *et al.,* 2012; Grullon *et al.,* 2013).

Regarding the effect of the gametes on the oviduct, it has been demonstrated that they can alter in a gamete-specific way the oviductal secretory proteome in sows (Georgiou *et al.,* 2005; Georgiou *et al.,* 2007). In addition, the presence of sperm in mice oviducts triggers the up-regulation of some genes (Fazeli *et al.,* 2004). Furthermore, in an *in vitro* study with bovine oviductal epithelial cells, only motile spermatozoa triggered prostaglandin biosynthesis and secretion which could enhance oviductal motility to facilitate the timely transportation of spermatozoa to the site of fertilization (Kodithuwakku *et al.,* 2007).

Altogether, these findings show that the oviduct adapts its environment to the different periods of the oestrous cycle and the presence of gametes; whether or not it responds to the presence of an embryo is not clear. There is a lack of available data in the literature describing the effect of the embryo on the oviduct. In some studies it was concluded that the presence of embryos in the oviduct up-regulate some genes in mice (Lee *et al.,* 2002), rats (Arganaraz *et al.,* 2007; Arganaraz *et al.,* 2012) and pigs (Almiñana *et al.,* 2012). However, these studies were carried out in poly-ovulatory species and this effect could be due to the presence of several embryos; attempting to find any signal in mono-ovulatory species like cattle could be a difficult task.

The only study carried out in cattle was done with a state-of-the-art technique using a video microscopic system to analyse the cow's oviduct *ex vivo*. In this study it was seen that the presence of the embryo in the ipsilateral oviduct, especially at the site of the embryo, makes the uterine tubal artery twisted, the wall of the oviduct thicker, more oedematous and more transparent than the contralateral, and induces the formation of secretory cells, ensuring optimal microenvironment and nutrition during the first days of embryo's life. In addition, it was demonstrated that the oviduct is able to select vital oocytes and as soon as a vital COC is in the ampulla the sperm become hyperactivated released from the epithelium and after fertilization early embryo down-regulate the speed of transport caused by ciliary beating thus settles down in the depths between the folds and gets in close contact with the oviductal epithelium to establish the first embryo maternal communication (Kölle *et al.,* 2009).

There is a long way to go but the future findings in this area will help us to understand what is happening in the oviduct in relation to the presence of an embryo, and use this information to improve fertility in dairy cattle and to improve *in vitro* production systems.

5. STRATEGIES TO IMPROVE PREGNANCY RATE

Most embryo losses occur between Day 8 and Day 16 of pregnancy coinciding with maternal recognition of pregnancy. As explained earlier, maternal recognition of pregnancy comprises a complex mechanism in which all the signals have to be perfectly synchronized. Thus, the CL has to secrete enough P4 to alter the endometrial transcriptome and ultimately drive conceptus elongation and the conceptus has to produce sufficient IFNT between Day 15 and Day 17 to inhibit the uterine secretion of PGF2α. The principal hormone that drives all these processes is P4 and therefore it is supposed that some of these embryo losses occur because of an insufficient communication between the embryo and the endometrium, i.e. due to insufficient P4. As a consequence, P4 supplementation before the time of maternal recognition could be an adequate therapy to improve pregnancy rate. To achieve an increase of P4 concentration several strategies can be applied in order to: (1) increase the function of the original CL, (2) induce an accessory CL or (3) provide an external source of P4. The aim is to raise P4 after oestrus as soon as possible but taking into account that the sooner P4 increases the worse its effect on luteal lifespan. At this point is important to remember that LH concentration between Day 2-12 is essential for establishing a fully functional CL (Peters *et al.,* 1994). In several studies it has been demonstrated that early P4 injection at Day 0 to 3, Day 1 to 4 (Ginther 1970) or Day 1 to 5 (Burke *et al.,* 1994) after oestrus, reduced the CL lifespan leading to luteolysis and embryo loss. Ginther (1970) described an experiment in which this negative effect was reversed when human chorionic gonadotropin (hCG) was injected together with P4 (Ginther 1970) suggesting that the LH-activity provided by the hCG may be helping in continuing the CL lifespan. In fact Burke *et al.,* (1994) proposed that the shortening cycle may be due to: (1) low levels of LH of the animals treated with P4 and (2) because of high levels of E_2 secreted by the DF that could enhance the release of PGF2α. As it was demonstrated that early P4 advanced the changes in endometrium and enhanced the embryo development (Forde *et al.,* 2009a), a precocious elevation in P4 may also advance synthesis of PGF2α. This mechanism is not fully understood. In conclusion, it is very important to calculate the right day of the treatment to have the maximum increase in P4 without having a negative effect on the CL.

5.1 External source of P4

Examples of external sources of P4 included P4 injections or intravaginal devices that release P4 during a period of time (PRID® or CIDR®). In both cases the increase in plasma of P4 is almost immediate.

The use of 100 mg of P4 on Days 2, 3, 4, 6 and 9 (Johnson 1958) or Days 1, 2, 3 and 4 after oestrus (Garrett *et al.,* 1988a), increased plasma P4 from Day 2 to 5 that was related with larger conceptus on Day 14 (Garrett *et al.,* 1988a) and better pregnancy rate (68% compared to 42% in control) (Johnson 1958).

The use of PRID as early as Day 3 stimulates the development of the elongating conceptuses on Days 13, 14 and 16 (Carter *et al.,* 2008; Clemente *et al.,* 2009) as well as from Day 5 to 12 increases pregnancy rate (Robinson *et al.,* 1989). Besides, its use from Day 3 to 7 increased: peripheral P4, conceptus size and

IFNT secretion. However in some cases it was associated with a reduction in CL lifespan (O'Hara *et al.,* 2014).

5.2 Increase the function of the original CL

To improve the function of the original CL, i.e. to make it bigger and produce more P4, different methods can be used to either manipulate the DF (Wiltbank *et al.,* 2011) or the CL after ovulation.

The effect of preovulatory follicle size on CL function is controversial. Smaller ovulated follicles have been associated with smaller CLs, lower P4 and lower pregnancy rate per AI (Vasconcelos *et al.,* 2001). In contrast, in another study no effect was found between CL size, plasma P4 and pregnancy rate (Spell *et al.,* 2001). Recently, the use of follicle aspiration by OPU before ovulation proved a reduction of the CL size and P4 output compromising the uterine capacity to support conceptus elongation (O'Hara *et al.,* 2012). Equine chorionic gonadotropin (eCG) has been used also to stimulate ovarian follicular growth and ovulation in cattle due to its FSH and LH-like activity (Murphy and Martinuk 1991). This hormone can be used to improve the quality of the DF (400 IU) (Rigoglio *et al.,* 2013). Thus, when eCG is included in protocols of fixed time AI, produces better pregnancy rate in cows with low BCS (Souza *et al.,* 2009), anovulatory anoestrus (Bryan *et al.,* 2010; Garcia-Ispierto *et al.,* 2012), heat stress (Garcia-Ispierto *et al.,* 2012; Garcia-Ispierto *et al.,* 2013), postpartum dairy cows (Pacala *et al.,* 2010; Garcia-Ispierto *et al.,* 2012) and also in buffaloes (Carvalho *et al.,* 2013). In addition, treatment with eCG 6 days after calving enhances the ovulation rate (Rostami *et al.,* 2011). However there are some studies in which this hormone does not improve pregnancy rate (Butler *et al.,* 2011a; Butler *et al.,* 2011b; Ferreira *et al.,* 2013) [for review De Rensis and Lopez-Gatius (2014)].

After ovulation, hCG can be used to improve P4 concentration. hCG is generally used to induce accessory CL, although in the dominant follicle stimulates the differentiation of theca and granulosa cells into small and large luteal cells and the transformation of small into large luteal cells (Donaldson and Hansel 1965). This is translated into an increase of the steroidogenic capacity of the primary CL (De Rensis *et al.,* 2010; Lonergan 2011) and its hypertrophy (Farin *et al.,* 1988; Galvão *et al.,* 2006; Stevenson *et al.,* 2007; Rizos *et al.,* 2012). Thus with early hCG treatment one bigger CL that produces more P4 could be achieved.

5.3 Inducing accessory CL

GnRH, GnRH agonists or hCG can be used to induce ovulation and formation of accessory CLs. It has been demonstrated that both trigger ovulation; however, levels of P4 are higher when hCG is used (Schmitt *et al.,* 1996a; Schmitt *et al.,* 1996b; Stevenson *et al.,* 2007). This can be explained because LH-like activity of hCG is longer than GnRH. The LH activity of hCG was doubled than the control during 10 h (Seguin *et al.,* 1977) while the length of LH activity after an injection of a GnRH agonist was 5 h (Chenault *et al.,* 1990). Also hCG persists in the circulation for a long time, being very high during 30 h after injection and not returning to baseline concentrations even 66 to 72h after treatment (Schmitt *et al.,* 1996b; Nascimento *et al.,* 2013a). The effect of hCG is longer because it contains more sialic acid that reduces the hepatic uptake and therefore increases its half-life (Schmitt *et al.,* 1996b). Besides, the half-

life is longer if the injection is intramuscular compared with intravenous (Rizkallah *et al.,* 1969). For this reason hCG has been used more frequently than GnRH. However, not all studies agree with the fact that this treatment increases pregnancy rate. The formation of accessory luteal structures is greater when hCG is administered during the early luteal phase, i.e. from Day 4 to 7 because induce the ovulation of the first wave dominant follicle (Price and Webb 1989). In addition, in a study carried out by Beltman *et al.,* (2009) a significant relationship was observed between conceptus size on Day 16 and P4 concentration on Day 5 and Day 6. This was demonstrated by Clemente *et al.,* (2009) where high concentrations of P4 between Day 3 and 6 had an indirect effect on embryo elongation though the endometrium. This implies that the rise in P4 should be before D7. Thus, working between Day 4 to 7 after oestrus, several studies have demonstrated that hCG can increase pregnancy rate, regardless of cattle breed (Holsteins or beef cows, and Holsteins and beef heifers), dose of hCG (from 1000IU-3300 IU) or after AI or ET [to see some of them consult table 1; for review Lonergan (2011)]. In a recent study with almost 3000 lactating Holstein cows it has been found that treatment with hCG on Day 5 after oestrus increased pregnancy per artificial insemination (P/AI) by 3.5% compared to not treated cows. Furthermore, it was observed that primiparous cows had greater P/AI after hCG than older cows (49.7% and 35.7%, respectively) (Nascimento *et al.,* 2013a). On the contrary, other studies have not found any differences (See table 1).

These contradictions between studies may be partially explained by the different number of animals used in each treatment, different response of each breed or different management conditions in all the experiments.

Which is the best treatment to increase P4 and improve pregnancy rate?

As shown above, there is no agreement regarding the best treatment or the best day of the cycle to increase P4 and its relation with increased pregnancy rate. Therefore, mimicking the physiology of the cow and having one big and good CL must be the case for possible improvement of pregnancy rate in dairy cattle. Thus, the use of hCG before Day 4 could be an option because: (1) it causes hypertrophy of the original CL (directly correlated with P4 concentration) (Farin *et al.,* 1988; Galvão *et al.,* 2006; Stevenson *et al.,* 2007; Rizos *et al.,* 2012); (2) the P4 increase is not immediate (Rizos *et al.,* 2012); and (3) it will reduce the animal handling from the practical point of view. Only in one old study hCG was injected on Days 2, 3 and 4 after oestrus and it was associated with an increase in P4 (Helmer and Britt 1986) but embryo elongation or pregnancy rate was not evaluated.

<table>
<tr><th colspan="8">TREATMENTS WITH hCG THAT IMPROVE PREGNANCY RATE</th></tr>
<tr><th colspan="2" rowspan="2">Dose (IU)</th><th colspan="2" rowspan="2">Breed</th><th colspan="2" rowspan="2">Day after oestrus</th><th colspan="2">Improved pregnancy rate</th></tr>
<tr><th>After AI</th><th>After ET</th></tr>
<tr><td>1000</td><td>(Sianangama and Rajamahendran 1992; Dahlen *et al.*, 2011; Wallace *et al.*, 2011)</td><td>Lactating Holstein cows</td><td>(Sianangama and Rajamahendran 1992; Santos *et al.*, 2001; Stevenson *et al.*, 2007; Shabankareh *et al.*, 2010; Vasconcelos *et al.*, 2011; Nascimento *et al.*, 2013a; Torres *et al.*, 2013)</td><td>4</td><td>(Breuel *et al.*, 1989; Stevenson *et al.*, 2007)</td><td rowspan="5">(Sianangama and Rajamahendran 1992; Santos *et al.*, 2001; Stevenson *et al.*, 2007; Shabankareh *et al.*, 2010; Dahlen *et al.*, 2011; Rossetti *et al.*, 2011; Nascimento *et al.*, 2013a)</td><td rowspan="5">(Breuel *et al.*, 1989; Nishigai *et al.*, 2002; Chagas e Silva and Lopes da Costa 2005; Vasconcelos *et al.*, 2011; Wallace *et al.*, 2011; Torres *et al.*, 2013)</td></tr>
<tr><td>1500</td><td>(Nishigai *et al.*, 2002; Chagas e Silva and Lopes da Costa 2005; Torres *et al.*, 2013)</td><td>Lactating Nelore cows</td><td>(Rossetti *et al.*, 2011)</td><td>5</td><td>(Santos *et al.*, 2001; Shabankareh *et al.*, 2010; Nascimento *et al.*, 2013a)</td></tr>
<tr><td>2500</td><td>(Rossetti *et al.*, 2011; Vasconcelos *et al.*, 2011)</td><td>Holstein heifers</td><td>(Chagas e Silva and Lopes da Costa 2005)</td><td>6</td><td>(Nishigai *et al.*, 2002)</td></tr>
<tr><td>3000</td><td>(Breuel *et al.*, 1989; Shabankareh *et al.*, 2010)</td><td>Beef cows</td><td>(Nishigai *et al.*, 2002; Dahlen *et al.*, 2011; Wallace *et al.*, 2011)</td><td rowspan="2">7</td><td rowspan="2">(Breuel *et al.*, 1989; Sianangama and Rajamahendran 1992; Chagas e Silva and Lopes da Costa 2005; Rossetti *et al.*, 2011; Vasconcelos *et al.*, 2011; Wallace *et al.*, 2011; Torres *et al.*, 2013)</td></tr>
<tr><td>3300</td><td>(Santos *et al.*, 2001; Stevenson *et al.*, 2007; Nascimento *et al.*, 2013a)</td><td>Beef heifer</td><td>(Breuel *et al.*, 1989)</td></tr>
</table>

<table>
<tr><th colspan="6">TREATMENTS WITH hCG THAT DOES NOT IMPROVE PREGNANCY RATE</th></tr>
<tr><th colspan="2">Dose (IU)</th><th colspan="2">Breed</th><th colspan="2">Day after oestrus</th></tr>
<tr><td>1500</td><td>(Hanlon *et al.*, 2005)</td><td>Lactating Holstein Friesian cows</td><td>(Fischer-Tenhagen *et al.*, 2010)</td><td>4</td><td>(Breuel *et al.*, 1990; Fischer-Tenhagen *et al.*, 2010)</td></tr>
<tr><td>2500</td><td>(Fischer-Tenhagen *et al.*, 2010)</td><td>Lactating Friesian cows</td><td>(Hanlon *et al.*, 2005)</td><td rowspan="2">5</td><td rowspan="2">(Funston *et al.*, 2005; Hanlon *et al.*, 2005; Galvão *et al.*, 2006)</td></tr>
<tr><td>3000</td><td>(Breuel *et al.*, 1990)</td><td>Holstein heifers</td><td>(Galvão *et al.*, 2006)</td></tr>
<tr><td>3300</td><td>(Galvão *et al.*, 2006)</td><td rowspan="2">Beef heifers</td><td rowspan="2">(Breuel *et al.*, 1990; Funston *et al.*, 2005)</td><td rowspan="2">6</td><td rowspan="2">(Funston *et al.*, 2005)</td></tr>
<tr><td>3333</td><td>(Funston *et al.*, 2005)</td></tr>
</table>

Table 1. Different strategies with hCG that improve or not pregnancy rate.

6. *IN VITRO* PRODUCTION AS A TOOL FOR INVESTIGATE INFERTILITY

The first calf obtained by an *in vitro* fertilization (IVF) procedure was born in 1981 (Brackett *et al.,* 1982). More than three decades have passed since then and many improvements have been made in *in vitro* embryo production [for a review see Machaty *et al.,* (2012)]. However, even today IVP systems are not as efficient as *in vivo* embryo production.

The goal of *in vitro* fertilization and embryo culture is to provide high quality embryos capable of continued development and implantation, and resulting in viable births (Menezo *et al.,* 1998). Nowadays, approximately 90% of the oocytes cultured *in vitro* undergo matured (nuclear and cytoplasmic maturation) from which 80% become fertilized and cleave at least once (Lonergan *et al.,* 2003a). Nevertheless only between 30-40% reach the blastocyst stage (Rizos *et al.,* 2008). In addition, after transfer of these embryos into recipient cows, pregnancy rate is between 40-60% compared to about 70% when *in vivo* embryos are transferred (Hasler *et al.,* 1995). It is clear then that more studies are needed to elucidate what is happening *in vivo* to apply this knowledge *in vitro*.

Despite the relatively low efficiency of IVP systems, nowadays is the best way to get low-cost mass production of bovine embryos for transfer, embryo diagnosis, somatic cell and embryo cloning, production of transgenic cows, basic research on the mechanisms of oocyte maturation, fertilization and embryogenesis (Hoshi 2003) as well as being used as a successful model for humans.

In vitro embryo production of bovine embryos is made up by 3 phases: *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC), which are going to be briefly explained.

6.1 IVM

In this step the oocytes, usually aspirated from ovaries recovered in the slaughterhouse, are cultured for 24 h in specific conditions, 38.5 ºC, 5% of CO2 and saturated humidity (Gordon 2003). This time corresponds with the time that the oocyte needs to be fully matured *in vivo* after the LH surge, i.e. to complete the nuclear and cytoplasmic maturation.

The oocytes can be cultured in different media classified as simple or complex. TCM-199 is the complex medium more extendedly used. Media are usually supplemented with macromolecules like those contained in fetal calf serum (FCS) or bovine serum albumin (BSA) (Gordon 2003). Serum can improve oocyte maturation but the problem of its use is that its composition in not fully known and may contain a mixture of amino acids, proteins, growth factors, hormones and other substances that make difficult to know if the response of the oocyte is due to the presence of this unknown components (Gordon 2003).

Some of the main factors that influence the developmental competences of the oocytes are:

- Size of the follicle aspirated: oocytes recovered from follicles >6mm yield a higher proportion of blastocyst (65.3% compared with follicles between 2-6mm, 34.3%) (Lonergan *et al.,* 1994).

- The use of gonadotropins, steroids or growth factors: can improve the oocyte development competence (Lonergan and Fair 2008).
- Origin of the oocyte: determines the subsequent embryo development (Rizos *et al.,* 2002b).

Also IVM could contribute to low efficiency of IVP systems due to the unknown origin of the ovaries in relation to the stage of oestrus cycle or follicular wave, breed, age and nutritional status of the animal. Another important parameter could be the oocyte selection for IVM that are based in morphological characteristics and this could be subjective. The general and common characteristics used to select the oocytes are:

- Presence of cumulus cells: important not only during maturation and fertilization but also for the early embryo development (Zhang *et al.,* 1995).
- Cytoplasm: it has to be homogenous and as less granulations as possible because they have better developmental competence (de Loos *et al.,* 1989).

6.2 IVF

Before IVF, it is essential to select the most active spermatozoa to optimize quality and quantity of the sperm. Selection of sperm allows the elimination of non-motile and dead sperm, seminal plasma, cell debris, prostaglandins and other microorganism as well as to initiate capacitation and to concentrate motile sperm in a small volume of medium to facilitate insemination (Centola *et al.,* 1998). Thus the sperm quality will be improved by enhancing progressive motility and morphological normal spermatozoa (Samardzija *et al.,* 2006).

Nowadays there are different methods to select motile sperm in bovine: swim-up (Parrish *et al.,* 1986), or gradient of Percoll® (Saeki *et al.,* 1991) or Bovipure™. In 1996, Percoll® was removed from use in the human clinical setting, presumably because of possible endotoxin contamination of the product (Svalander *et al.,* 1995). Although in a subsequent study the endotoxins were found in the media for washing the sperm but not in Percoll® (Scott and Smith 1997). Nevertheless, this triggered the emergence of new products with low endotoxin content like Bovipure™ or its homologous in human Puresperm® which have the same results as Percoll® [compared with: Puresperm® by Centola *et al.,* (1998) and Chen and Bongso (1999); or with Bovipure™ by Samardzija *et al.,* (2006)] with the advantage that are less toxic.

After sperm selection, the concentration is adjusted normally to 1,000,000 sperm /ml and co-incubated with the matured oocytes for 18 h, at 38.5 ºC, 5% CO_2 and saturated humidity. The IVF media usually contains heparin that capacitates the sperm and prepares it for the acrosome reaction to have a successful fertilization (Parrish *et al.,* 1985). It is important to know that sperm collected from different bulls react different in *in vitro* conditions. The day of IVF is considered as Day 0.

6.3 IVC

Finally the presumptive zygotes obtained after IVF are selected based on their morphological characteristics like homogeneity of the ZP, perivitelline space and cytoplasm and put into the culture at

38.5 ºC, 5% of CO2 and 5% O2, depending on the culture system/media used and saturated humidity. The embryos can be cultured in defined or semi-defined media; co-culture with oviductal, granulosa or Vero cells; or with conditioned media. Today, the most used media is synthetic oviductal fluid (SOF) that is usually supplemented with 5% of FCS and/or BSA (Tervit *et al.,* 1972; Holm *et al.,* 1999). The use of serum during IVC has been related with an increase in the speed of development and early blastocyst (Gutierrez-Adan *et al.,* 2001; Rizos *et al.,* 2003). However, FCS has a negative effect on embryo quality (Lazzari *et al.,* 2002; Rizos *et al.,* 2003).

Usually, zygotes will be under *in vitro* culture conditions until Day 8 or 9. Day 7 is the day, which coincides with ET *in vivo* and is normally used in *in vitro* conditions for measuring the embryo development and embryo quality. The ideal situation for measuring the quality of the *in vitro* produced embryos would be transfer them into recipients previously synchronized, and see pregnancy and/or calving rates. However, this is not always possible based on the big number of *in vitro* produced embryos in laboratory conditions through different experimental designs and the high cost of live animals used in farm conditions. Thereby a number of invasive and non-invasive methods are used in the laboratory to measure the quality of the *in vitro* produced embryos. An example of widely used invasive methods is: differential staining of embryos, which provides the relation between the number of cells from the ICM and the TE; cryotolerance of the embryos-survival rate after vitrification and warming; and also relative abundance of genes related with embryo quality (i.e. apoptosis, cell connections, antioxidant stress, metabolism, implantation etc.).

How can IVP help in deciphering the causes that could be involved in decreasing fertility in dairy cows?

It was mentioned before that one of the strategies to increase fertility in HYDC is to study basic science, i.e. the normal physiology of the reproductive tract. Knowing all the mechanisms involved in fertilization and embryo development will guide us to know what aspects are altered in the HYDC. Therefore, to study the physiology of the reproductive tract the ideal situation is to design *in vivo* experiments. However, experiments involving live animals in bovine are not easy to accomplish because on the one hand they are costly; animals, facilities, personnel, specific equipment and professionals are needed and on the other hand, it is not the optimal way to study subfertility due to its difficulty to discriminate the individual steps involved like follicular growing and oocyte quality, failure of fertilization or early embryonic loss. Assuming that the failure in fertilization is low, the other two main factors that could be related with subfertility are: the oocyte and the reproductive tract. Therefore in these cases if the oocyte or the reproductive tract wants to be studied independently, OPU or embryo transfer, are the appropriate techniques, respectively, together with IVM/IVF/IVC.

- OPU-IVM-IVF-IVC: with this methodology, oocytes from dairy heifers (Roth *et al.,* 2008) or lactating dairy cows (Roth *et al.,* 2008; Matoba *et al.,* 2012) can be isolated, matured, fertilized and cultivated *in vitro* to study their development competence. OPU has also been used to assess the impact of dietary energy and stage of lactation on follicular development (Kendrick *et al.,* 1999; Gwazdauskas *et al.,* 2000).

- IVM-IVF-IVC-ET: the specific effect of the reproductive tract on embryo development and quality can be evaluated by transferring early stage embryos originated in the same conditions. The effect of the homologous oviduct on embryo development and quality can be studied thanks to the development of endoscopic ET, a state-of-the-art technique in which the embryo is transferred directly into the oviduct and recovered after a few days by oviductal/utero flushing (Besenfelder and Brem 1998). To examine the effect of the uterus, classical ET of blastocyst of the same origin on Day 7 can be applied. Following that, early ovoid or elongated conceptus can be recovered and studied between Day 12 and 16.

Justification and Objectives

Low fertility in high yielding dairy cows (HYDC) has resulted in the development of different research lines to elucidate the possible causes with the goal of improving pregnancy rate. As stated in the introduction of this thesis, there is evidence that reproductive performance has been decreasing in HYDC. During the postpartum period, animals enter a variable period of negative energy balance (NEB) during which body reserves are mobilised to meet the combined demands of maintenance and lactation. Also, an embryo loss of 35% occurs between Days 8 and 16 (Diskin *et al.,* 2012). Therefore, subfertility is a multifactorial problem and to recognize all the factors involved is challenging. Thus, the main research has been focus on the oocyte and the early embryo while few trials have been dealt with the uterus. It has been demonstrated that the modifications in circulating metabolic hormones during NEB is reflected in the composition of the follicular fluid (FF) (Cohick *et al.,* 1996; Landau *et al.,* 2000; Leroy *et al.,* 2004) and this could therefore affect not only the maturation of the oocyte but also the subsequent embryo development. *In vitro*, the culture of oocytes with some metabolites like glucose, β-hydroxybutyrate and non-esterified fatty acids (NEFA) that are altered during NEB, affects their quality and developmental competence (Leroy *et al.,* 2005c; Leroy *et al.,* 2006; Van Hoeck *et al.,* 2011). However *in vivo*, other studies have failed to show a relationship between NEB and oocyte quality (Rizos *et al.,* 2005; Matoba *et al.,* 2012). These experiments together with others that have shown that NEFA and glucose plasma levels are not exactly reflected in the FF (Leroy *et al.,* 2004; Bender *et al.,* 2010), suggest a mechanism in the follicle to protect the oocyte, although further investigations are needed in this area. On the other hand, it has been seen that HYDC produce darker and poorer embryos compared with heifers or beef cattle (Sartori *et al.,* 2002; Leroy *et al.,* 2005b). This dark appearance has been related with a major content of lipids (Leroy *et al.,* 2005a) that *in vitro* has been associated with poorer embryo cryotolerance (Yamashita *et al.,* 1999; Rizos *et al.,* 2002b). Having these aspects in mind, it is clear that the quality of the oocyte and the embryo could be responsible for subfertility. However, the ability of the reproductive tract of the HYDC in the early postpartum period to support embryo development cannot be ruled out as evidenced by the differences found in a recent study from our group between lactating cows and heifers (Rizos *et al.,* 2010a).

In the uterus mechanisms involved in maternal recognition of pregnancy have been extensively studied (Farin *et al.,* 2010); however, not much attention has been paid to the oviduct. The oviduct is the place where sperm become hyperactivated, fertilization, early embryo development and major developmental events occurs like embryonic genome activation (EGA). EGA is crucial for the subsequent development of the embryo (Niemann and Wrenzycki 2000) and it has been shown that when it occurs *in vitro* as opposed to the oviduct many molecular mechanisms and pathways are altered, decreasing the quality of the resulting blastocysts (Gad *et al.,* 2012). Nowadays, it is clear that the embryos cultured in the oviducts *in vivo* of different intermediate hosts, have better quality in terms of morphology, gene expression, cryotolerance and pregnancy rate after transfer, compared to those cultured *in vitro* (Enright *et al.,* 2000; Rizos *et al.,* 2002a; Rizos *et al.,* 2007; Tesfaye *et al.,* 2007; Rizos *et al.,* 2010b). Therefore, it is important to know the mechanisms that take place in the oviduct during early embryo development and design new strategies to improve pregnancy rate and apply this knowledge to in *in vitro* embryo production.

In order to improve pregnancy rate in dairy cattle, different treatments have been used with different results. Given that most of embryo losses occur between Day 8 and 16 it has been demonstrated that increasing progesterone (P4) before these days improves conceptus elongation (Carter *et al.,* 2008; Clemente *et al.,* 2009; Forde *et al.,* 2009a; Forde *et al.,* 2009b). The timing of exogenous P4 supplementation is crucial because if it is too early after oestrus it could have a shortening effect on *corpus luteum* (CL) lifespan (Ginther 1970; Burke *et al.,* 1994; O'Hara *et al.,* 2014). A rapid increase in P4 can be obtained by using P4 injections (Ginther 1970; Burke *et al.,* 1994) or intravaginal devices (Robinson *et al.,* 1989; Carter *et al.,* 2008; Clemente *et al.,* 2009; O'Hara *et al.,* 2014). However, moderate rise and long period can be achieved by different hormone treatments. Human chorionic gonadotropin (hCG), which has LH-like activity, has been used between Day 4 and 7 showing a positive or neutral effect on pregnancy rate, depending on the breed, dose and time of treatment (Lonergan 2011). Furthermore, hCG has the ability to (1) increase P4 but not immediately (Rizos *et al.,* 2012) and (2) causes hypertrophy of the original CL (Farin *et al.,* 1988; Galvão *et al.,* 2006; Stevenson *et al.,* 2007; Rizos *et al.,* 2012). For these reasons hCG could be a good strategy to improve pregnancy rate in the dairy cows.

Therefore, the main objective of this thesis was to study embryo-maternal interaction and its consequences on embryo development in cattle.

To achieve this general objective, specific objectives have been discussed in three experimental chapters that comprise this report:

➢ Chapter 1

1. To characterize the direct effects of lactation on postpartum metabolic profiles.
2. To study the ability of the reproductive tract to support embryo development to the blastocyst stage.
3. To study the ability of the reproductive tract to support elongation of the conceptus following transfer of a blastocyst at Day 7.

➢ Chapter 2

4. To examine the effect of the presence of an embryo (versus an unfertilized oocyte) on the oviduct transcriptome.
5. To compare gene expression between ipsilateral and contralateral isthmus tissue in pregnant and cyclic animals.
6. To compare gene expression in the ampulla and isthmus of the ipsilateral oviduct in pregnant animals.

➢ Chapter 3

7. To examine the effect of early administration of hCG (on Day 1, 2, 3, or 4 after oestrus) on development and function in terms of P4 secretion in beef heifers.

Chapter 1

Influence of lactation on metabolic characteristics and embryo development in postpartum Holstein dairy cows

ABSTRACT

The aim of this study was to examine the direct effect of lactation on the ability of the reproductive tract of postpartum dairy cows to support early embryo development. Twenty-one primiparous Holstein heifers were used. Immediately after calving, half of the cows were dried off (i.e., never milked), and the other half entered the milking herd and were milked twice daily. Jugular blood samples were taken twice per week from 15 d before calving to approximately 100 d postpartum to measure nonesterified fatty acids, β-hydroxybutyrate, glucose insulin and insulin-like growth factor-I. At the same time, body weight and body condition score were recorded for each cow. At approximately 60 d postpartum (experiment 1), approximately 65 two- to four-cell embryos, produced by *in vitro* maturation and fertilization, were endoscopically transferred to the oviduct ipsilateral to the *corpus luteum* of all cows on Day 2 of the estrous cycle. Five days later (Day 7), the oviduct and uterus were flushed nonsurgically and the number of embryos developing to the blastocyst stage was recorded. At approximately 90 d postpartum (experiment 2), the estrous cycles of the same cows were resynchronized and 15 to 20 *in vitro*-produced blastocysts were transferred to the uterus of each recipient on Day 7. All cows were slaughtered on Day 14 to assess embryo survival and dimensions. Body weight and body condition score were significantly different between groups for the entire postpartum period of the study. Concentrations of nonesterified fatty acids and β-hydroxybutyrate were higher and concentrations of glucose, insulin and insulin-like growth factor-I were lower in lactating compared to nonlactating cows. Embryo recovery rates from lactating and nonlactating cows were similar. In experiment 1, fewer embryos developed to the blastocyst stage in the lactating cows compared with the nonlactating cows. In experiment 2, embryo survival and conceptus dimensions were not different between lactating and nonlactating cows. In conclusion, the data indicate that the reproductive tract of the lactating dairy cow is compromised in its ability to support early embryo development compared with that of matched nonlactating cows and this may contribute to early embryo mortality observed in such animals.

INTRODUCTION

The physiological changes associated with high milk production are associated with poor reproductive inefficiency in commercial dairy herds (Lucy 2001; Pryce *et al.,* 2004). Decreasing [glucose, insulin, insulin like growth factor-1 (IGF-I)] or increasing (non-esterified fatty acids (NEFA), ketone bodies) circulating metabolites during nutrient partitioning associated with low body condition score (BCS) undoubtedly play a role in determining reproductive outcome. However, understanding the causes of infertility in dairy cattle is complex and may be attributable to compromised oocyte quality, a suboptimal reproductive tract environment incapable of supporting normal development, or a combination of both (Leroy *et al.,* 2008a; 2008b; Walsh *et al.,* 2011).

Evidence for a contribution of poor oocyte quality to infertility comes from a variety of sources. First, data on nonsurgical flushing of unstimulated dairy cows [reviewed by Sartori *et al.,* (2010)] suggest that a significant proportion of embryos degenerate before the blastocyst stage. For example, in 3 studies by Cerri *et al.,* (2009a; 2009b; 2009c) the proportion of viable embryos recovered on Day 6 to 7 was approximately 50%. Given that fertilization rate is estimated at 85-95%, this suggests that a significant proportion of embryos are lost as early as Day 7. Second, several studies have reported higher pregnancy rate in lactating dairy cows after embryo transfer compared with artificial insemination (AI) (Putney *et al.,* 1989; Ambrose *et al.,* 1999; Drost *et al.,* 1999; Rutledge 2001; Al-Katanani *et al.,* 2002; Vasconcelos *et al.,* 2006; Demetrio *et al.,* 2007). Third, exposure of oocytes *in vitro* to NEFA at physiological concentrations consistent with those measured in the preovulatory follicle of postpartum lactating cows is detrimental to oocyte development (Leroy *et al.,* 2004; 2005c).

In vitro studies examining the effect of lactation on oocyte quality have led to equivocal results. For example, Snijders *et al.,* (2000) found that a lower proportion of oocytes recovered from dairy cows with a higher genetic merit for milk production underwent cleavage or developed to the blastocyst stage *in vitro* compared with those from cows of average genetic merit. Rizos *et al.,* (2005) reported no difference in the proportion of good quality oocytes undergoing fertilization and development to the blastocyst stage between lactating cows and heifers. Several studies from Virginia (Kendrick *et al.,* 1999; Gwazdauskas *et al.,* 2000; Walters *et al.,* 2002) demonstrated that conditions related to early lactation have a negative effect on oocyte quality and endocrine measures in dairy cattle; however, in these papers, oocyte quality was assessed based solely on morphology, which may be of limited value. A recent study from our group (Matoba *et al.,* 2012) failed to demonstrate an effect of metabolic status postpartum on oocyte ability to undergo *in vitro* fertilization and develop to the blastocyst stage *in vitro*.

The reproductive tract (oviduct/uterus) clearly also plays a crucial role in providing an appropriate environment conducive to normal embryo development leading up to maternal recognition of pregnancy, a period around which a substantial part of embryo loss occurs (Diskin and Morris 2008). Several studies from our group have emphasized the important role of progesterone in the first week after conception in establishing an optimum uterine milieu to support conceptus elongation around the time of maternal recognition (Clemente *et al.,* 2009; Forde *et al.,* 2009a; Forde *et al.,* 2011a; Rizos *et al.,* 2012). Embryo transfer studies allow us to test the ability of the reproductive tract to support development

without the confounding effect of the cow's own, potentially compromised, oocyte. We recently reported that embryo development to Day 7 in the reproductive tract of postpartum lactating cows was compromised compared with that in the tract of nulliparous heifers (Rizos *et al.,* 2010a), consistent with the data reviewed by Sartori *et al.,* (2010). One justifiable criticism of that model is that a nulliparous heifer is not the same as a metabolically-stressed postpartum cow in early lactation. To overcome this criticism, in the current study we used age-matched postpartum primiparous dairy cows that were either milked post calving (i.e. lactating) or were dried off immediately at calving (i.e., never milked, nonlactating) to directly test the effects of lactation on postpartum fertility characteristics. The specific objectives of this study were to characterize the direct effects of lactation on postpartum metabolic profiles and the ability of the reproductive tract to (1) support embryo development to the blastocyst stage and (2) support elongation of the conceptus following transfer of a blastocyst at Day 7.

MATERIALS AND METHODS

Animal Management

All experimental procedures involving animals were licensed by the Department of Health and Children, Ireland, in accordance with the Cruelty to Animals Act, 1897 and the European Community Directive 86/609/EC. All procedures were sanctioned by the University College Dublin, Ireland Animals Research Ethics Committee.

Holstein-Friesian, primiparous, autumn-calving cows were used (n = 21, mean age 3.9 ± 0.1 years). The cows had been part of a previous fertility trial which involved four cycles of AI, pregnancy diagnosis and prostaglandin-induced abortion and were known to be of high fertility [~70% of 118 heifers pregnant at each cycle of AI; Parr *et al.,* (2012)]. All cows were pregnant to a synchronised oestrus following insemination with semen from the same bull. At calving, cows were randomly assigned to 1 of 2 treatment groups: lactating (n=11) or nonlactating (n=10). All cows were housed indoors on a slatted floor for the duration of the experiment. Lactating cows were offered a diet that consisted of 50:50 maize silage (Dry Matter (DM) 344 g/kg, Crude Protein (CP) 76 g/kg and Metabolizable Energy (ME) 11.8 MJ/kg DM):grass forage (DM 239 g/kg, CP 101 g/kg and ME 11.1 MJ/kg DM) ad libitum plus 8 kg concentrates (DM 883 g/kg, CP 281 g/kg and ME 12.9 MJ/kg DM) per day at milking (twice daily at 07:00 and 16:00 h). The nonlactating cows were offered the standard forage diet on an ad libitum basis. In the lactating group, milk production was recorded twice daily at each milking. A vaginal mucus score was taken at Day 28 postpartum because evaluation of the character of vaginal mucus at this time has been shown to reflect the bacterial load within the uterine lumen (Williams *et al.,* 2005); all cows scored 0 or 1 on a scale of 0 to 3, indicative of a normal bacterial load.

Body weight and BCS were recorded twice a week at approximately 2 weeks before expected calving date, at calving and then twice per week until the end of the experiment (approximately 95 d postpartum). Body condition score was assessed by the same person based on a scale of 1 to 5 (with 1 being extremely thin and 5 being extremely fat) with increments of 0.25, based on the scoring described by Lowman *et al.,* (1976) and Prendiville *et al.,* (2009).

Plasma Hormone and Metabolite Analysis

To fully characterize the metabolic status of the cows, blood plasma samples were collected twice weekly, starting 2 weeks before the expected calving date and continuing until the end of the experiment, and were analyzed for NEFA, β-hydroxybutyrate (BHBA), IGF-I, insulin and glucose. Circulating progesterone concentrations were monitored following embryo transfer (see below).

Insulin concentrations in serum were measured using a solid-phase ^{125}I radioimmunoassay (RIA) Insulin Coat-A-Count kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA) with a sensitivity of 1.2 µL U/mL. Briefly, 200 µL of serum was pipette into an antibody-coated tube in duplicate and 1 mL of iodinated insulin tracer was subsequently added. All samples were incubated at room temperature (15 to 18 ºC) for 18 h, decanted and counted for 90 s using the Wallac 1470 gamma counter (Wallac/Perkin Elmer, Waltham, MA). The inter- and intraassay coefficients of variation (CV) for insulin were 12.0 and 12.6%, 14.2 and 13.6%, and 8.4 and 8.8% for low, medium and high quality controls respectively.

Serum IGF-I concentrations were measured using a RIA as previously described (Beltman *et al.,* 2010). Serum samples (100 µL) were extracted with 400 µL of ethanol, acetone and acetic acid in a 60:30:10 ratio at 4 °C for 16 h. All samples were spun at 1,500 x *g* for 30 min, and 100 µL of supernatant removed, neutralized with 100 µl of Tris (0.855 *Molar*) and centrifuged again at 1,500 x *g* for a further 30 min. This supernatant was diluted 1:10 with 900 µL of assay buffer in polypropylene tubes. Fifty microliters of a 1:750,000 dilution of the primary antibody (anti-human IGF-I, National Hormone and Peptide Program, Torrance, CA) was added to each tube and incubated at room temperature for 1 h. Following addition of 100 µL of iodinated IGF-I (approximately 10,000 counts per minute) to each tube, samples were incubated overnight at 4 °C and 50 µL of secondary antibody (anti-rabbit IgG, Immunodiagnostic Systems, Bolden, UK) added for a further 30-min incubation at room temperature. Finally, 250 µl of distilled water was added to each tube, samples spun at 1,500 x *g* for 15 min and counted on the Wallac 1470 gamma counter (Wallac/Perkin Elmer). Intraassay CV were 12.9, 5.2, and 9.2% for low, medium, and high standards, respectively. Interassay CV were 6.6, 4.2, and 8.1% for low, medium, and high standards, respectively.

Serum BHBA concentrations were measured using the RANBUT D-3-Hydroxybutyrate kit (Randox Laboratories Ltd, Crumlin, UK) using a kinetic enzymatic reaction with a sensitivity of 0.1 nmol/L. Twenty-five microliters of serum was added to each tube, 1000 µL of buffer mixed with enzyme/coenzyme was incubated for 60 s at 37 °C and the first reading was taken. Subsequent readings were taken after 1 and 2 min and mean absorbance change per minute was calculated. The inter- and intraassay CV were 1.9 and 1.9% for the low and 0.7 and 0.8% for the high quality controls (QCs) respectively.

Circulating NEFA concentrations were measured using the Randox NEFA enzyme assay with a sensitivity of 0.072 mmol/L. Serum samples (10 µL) were incubated with 200 µL of assay reagent 1 for 5 min at 37 °C and 400 µL of reagent 2 incubated for a further 5 min at 37 °C, and absorbance readings

were taken. Inter- and intraassay CV for the medium and high NEFA QCs were 1.0 and 0.8 (interassay CV) and 1.6 and 1.4 (intraassay CV), respectively.

Plasma glucose concentrations were measured using the automated Randox Glucose (Gluc-HK) hexokinase enzymatic method (sensitivity 0.662 mmol/L). Interassay CV were 1.1 and 0.7% for the low and high standards, and the intraassay CV were 0.8 and 0.7% for the low and high standards, respectively.

Serum progesterone concentrations were measured using a competitive binding ^{125}I RIA Progesterone Coat-A-Count kit (Siemens Medical Solutions Diagnostics) as previously described (Forde *et al.,* 2011a). Briefly, 100 μL of serum was dispensed into antibody coated tubes in duplicate. One milliliter of iodinated P4 tracer was added to each tube incubated for 3 h at room temperature (15 to 18 ºC) and counted for 90 s using the Wallac 1470 gamma counter (Wallac/Perkin Elmer). The low, medium, and high progesterone CV were 13.4, 3.6, and 5.4% for the interassay CV and 18.9, 8.8 and 7.5% for the intraassay CV, respectively.

In vitro Production of Bovine Embryos

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co. (Poole, UK). The techniques for producing embryos in vitro were described previously (Rizos *et al.,* 2002b). Immature cumulus oocyte complexes (COC) were obtained by aspirating follicles on bovine ovaries collected at slaughter. The COC were matured for 24 h in Tissue Culture Medium-199 supplemented with 10% (vol/vol) fetal calf serum and 10 ng/mL epidermal growth factor at 39 ºC under an atmosphere of 5% CO2 in air with maximum humidity. For in vitro fertilization, matured COC were inseminated with frozen-thawed, Percoll-separated bull sperm at a concentration of 1 x 106 spermatozoa/mL. Gametes were co-incubated at 39 ºC under an atmosphere of 5% CO2 in air with maximum humidity. At approximately 20 h post insemination, presumptive zygotes were denuded, divided in groups of 50, and transferred to 500-μL culture wells. The basal medium for all embryo culture was synthetic oviduct fluid (SOF) supplemented with 5% FCS. Cleavage rate was recorded at 48 h post insemination; only cleaved embryos were used for transfer for experiment 1 and only blastocysts on Day 7 were used for transfer for experiment 2.

Experiment 1. Effect of Lactation on the Ability of the Reproductive Tract to Support Embryo Development from Day 2 to Day 7

At approximately Day 60 postpartum (60.9 ± 2.32), the estrous cycles of the cows (n = 21) were synchronized using a controlled internal drug release device (CIDR; Pfizer, Sandwich, UK) for 8 d. One day before device removal, all animals received 0.5 mg of a PGF2α analog (Estrumate, Shering-Plough Animal Health, Hertfordshire, UK). Standing oestrus was defined as Day 0. Cleaved embryos produced *in vitro* were transferred (n = 60-65 embryos per recipient) to the oviduct ipsilateral to the *corpus luteum* on Day 2 of the estrous cycle as described previously (Havlicek *et al.,* 2005; Rizos *et al.,* 2010a). For transfer, recipients were restrained and received an epidural anaesthesia (3.5 mL of 2% lidocaine solution, Selectavet, Weyarn-Holzolleng, Germany). The tip of a bitubular endoscopic set (Storz, Tuttlingen, Germany) containing the endoscope and the transfer system was placed in the peritoneal cavity via the

fornix of the vagina. After passive air movement into the cavity, the reproductive organs were assessed for suitability for embryo transfer. The transfer system consisted of a 1 mL syringe embedded into a manual dosimeter (IVFETflex.com, Graz, Austria) and connected to a perfusion tube (no. 701908, Braun, Melsungen, Germany). A fire polished and curved 50 µL glass capillary (Brand, Wertheim, Germany) was attached to the end of the perfusion tube. Embryos were loaded in SOF into the tip of the glass capillary and transferred via the infundibulum into the ampulla.

Five days later, on Day 7 of the estrous cycle, embryos were recovered by endoscopic flushing of the oviduct and uterine horns. Accessing the oviduct was performed as described above. The glass capillary used for transfer was replaced by a silicon covered flushing metal tube. Forty to 60 mL of PBS supplemented with 1% fetal calf serum were flushed through the oviducts and uterine horns and collected via an embryo flushing catheter in an embryo filter (Em Con, no. 04135; Immuno Systems Inc., Spring Valley, WI). A further 300 to 400 mL was used for additional flushing of the uterine horns. Embryos were located under a stereo microscope. The number of embryos developing to the blastocyst stage was recorded immediately after recovery and following overnight culture in SOF medium. A representative number (n = 24 per group) of Day 7 blastocysts recovered from both groups were fixed in ethanol overnight and stained with Hoechst 33342 to assess cell number per blastocyst. A daily blood sample was taken from all animals by jugular venipuncture from d 0 to d 7 to establish the recipient endogenous P4 concentrations.

Experiment 2. Effect of Lactation on the Ability of the Reproductive Tract to Support Embryo Development from Day 7 to Day 14

At approximately Day 90 postpartum (93.9 ± 1.95), the same cows were resynchronized as described above. A total of 15 to 20 *in vitro*-produced blastocysts were transferred to each recipient on Day 7 and all recipients were slaughtered on Day 14 to assess embryo survival as described previously (Clemente *et al.,* 2009). A daily blood sample was taken from all animals by jugular venipuncture from d 0 to d 14 to establish the recipient endogenous P4 concentrations. Following slaughter, the reproductive tract was removed, sealed in a plastic bag and placed in a sealed polystyrene box for transportation to the laboratory (within 60 min). After removal of the ovaries and the oviducts the uterine horns were trimmed free of excess tissue before being flushed with 40 mL PBS. Embryos were located under a stereomicroscope, measured, and then snap frozen individually in liquid nitrogen and stored at -80 ºC. The weight and dimensions of the corpora lutea (CL) were also recorded.

Statistical Analysis

Data were checked for normality and homogeneity of variance using histograms, qplots, and formal statistical tests in the UNIVARIATE procedure (version 9.1.3; SAS Institute Inc., Cary, NC). Data that were not normally distributed were transformed by raising the variable to the power of lambda. The appropriate lambda value was obtained by conducting a Box-Cox transformation analysis using the TRANSREG procedure of SAS. The transformed data were used to calculate *P*-values. The corresponding least squares means and SE of the non-transformed data are presented in the results for

clarity. For all analyses, cow was the experimental unit. Characterization data (Body weight, BCS and metabolic profiles) were analyzed using repeated measures with the MIXED procedure of SAS. Fixed effects included experimental treatment (lactating or nonlactating), day, and their interaction. The interaction term if not statistically significant ($P > 0.10$), was subsequently excluded from the final model. Actual calving day was used as a linear covariate. Animal within treatment was included as a random effect in the model, with the most appropriate covariance structure between records within cow determined by minimizing the Akaike Information Criterion (AIC). Models were run under compound symmetry, unstructured, autoregressive, or Toeplitz variance-covariance structures. The model with the least AIC value was selected. Embryo-related data (for experiments 1 and 2) were analyzed using the PROC MIXED procedure of SAS. The model had experimental treatment as a fixed effect and animal within treatment was included as a random effect. Differences between treatments were determined by *F*-tests using Type III sums of squares. The PDIFF command incorporating the Tukey test was applied to evaluate pairwise comparisons between treatment means.

RESULTS

Animal Characterization

Body weight, BCS and metabolite profiles across the entire experimental period are shown in Figure 1. Cows in the lactating group had a mean milk yield across the study period of 25.04 ± 0.19 kg/d. Mean BW across the entire study period was 640.2 ± 15.2 kg and 688.3 ± 15.9 kg for lactating and nonlactating cows, respectively. Lactating and nonlactating cows had a similar BW before calving and for up to 2 weeks postcalving. Subsequently, the BW of lactating cows declined and remained significantly lower ($P<0.001$) than of the nonlactating group throughout the remainder of the experimental period (Figure 1).

Mean BCS across the entire study period was 3.0 ± 0.05 and 3.6 ± 0.05 for lactating and nonlactating cows, respectively; BCS declined from approximately Day 7 prepartum to Day 7 postpartum in both groups. The BCS diverged significantly ($P<0.001$) between lactating and nonlactating cows from d 7 onwards, reaching a nadir at approximately Day 32 in the lactating group. In the nonlactating groups, BCS gradually increased from d 7 (3.0 ± 0.03) up to d 95 postpartum (4.2 ± 0.09), whereas in the lactating group, BCS remained low up to d 95 (3.0±0.1).

Concentrations of NEFA diverged between lactating and nonlactating cows from Day 7 postpartum and were higher ($P<0.05$) in lactating cows from Day 14 to 49 postpartum compared with in the nonlactating group, for which concentrations fell from a peak at calving to a nadir at approximately Day 21, after which they remained relatively constant. Concentrations of BHBA were higher in lactating cows from calving throughout the experimental period compared to their nonlactating counterparts.

Concentrations of IGF-I declined dramatically from Day 14 prepartum to a nadir on Day 4 in lactating cows after which they remained constant to Day 95. In contrast, after an initial pre-calving decline from Day -14 to calving, IGF-I concentrations increased markedly in nonlactating cows and maintained concentrations greater than double those in lactating cows throughout the study period.

Similarly, insulin concentrations were significantly higher in nonlactating cows from calving throughout the experimental period.

We observed a dramatic spike in glucose concentrations coincident with calving in both groups, followed by a precipitous decline immediately postpartum. The extent of the postpartum decrease was less in lactating animals, resulting in divergent glucose concentrations between experimental groups from Day 3 to approximately Day 49 postpartum.

Experiment 1

Of the 11 cows in the lactating group, 7 exhibited standing oestrus confirmed by the presence of an appropriately sized CL on Day 2 at embryo transfer and on Day 7 at embryo recovery; only data from these cows were included. In contrast, all 10 cows from the nonlactating group showed standing oestrus, confirmed by the presence of a CL on the day of embryo transfer (Day 2) and embryo recovery (Day 7). Mean progesterone concentrations were similar between groups from Day 0 to Day 7 (Figure 2).

For cows from which embryonic structures were recovered at Day 7 (all except one), the recovery rate was similar for the lactating and nonlactating groups (65.6 ± 8.6 vs 63. 9 ± 7.2, respectively). Of the structures recovered, 26.3 ± 4.1% had developed to the blastocyst stage in the lactating cows compared to 39.6 ± 3.6% in the nonlactating groups ($P < 0.05$; Table 1). Following overnight culture *in vitro*, these values increased to 32.6 ± 4.4% and 49.3 ± 3.8% for lactating and nonlactating cows, respectively (P=0.03). Blastocysts were recovered from all cows in both groups (range: 12.8 to 36.4% per cow in the lactating group; range: 18.4 to 58.9% per cow in the nonlactating group). We found no evidence of a difference in blastocyst quality, as evidenced by total cell number in the blastocysts (63.1 ± 1.9 vs 62.6 ± 1.9, for lactating and nonlactating cows, respectively).

Table 1. Recovery and development of bovine embryos following endoscopic transfer to the oviducts of Holstein lactating or dry dairy cows.

Cows	Embryos transferred, (no)	Recovery (no; mean % ± SEM)	Day 7 Blastocysts, (no; mean % ± SEM)	Total Blastocysts, (no; mean % ± SEM)*
Lactating	435	289 (65.6±8.6)	75 (26.3±4.1) [a]	97 (32.6±4.4) [a]
Nonlactating	627	403 (63.9±7.2)	165 (39.6±3.6) [b]	203 (49.3±3.8) [b]
P-value		0.88	0.05	0.03

[a, b]: values in the same column with different letters differ significantly
*Following overnight culture

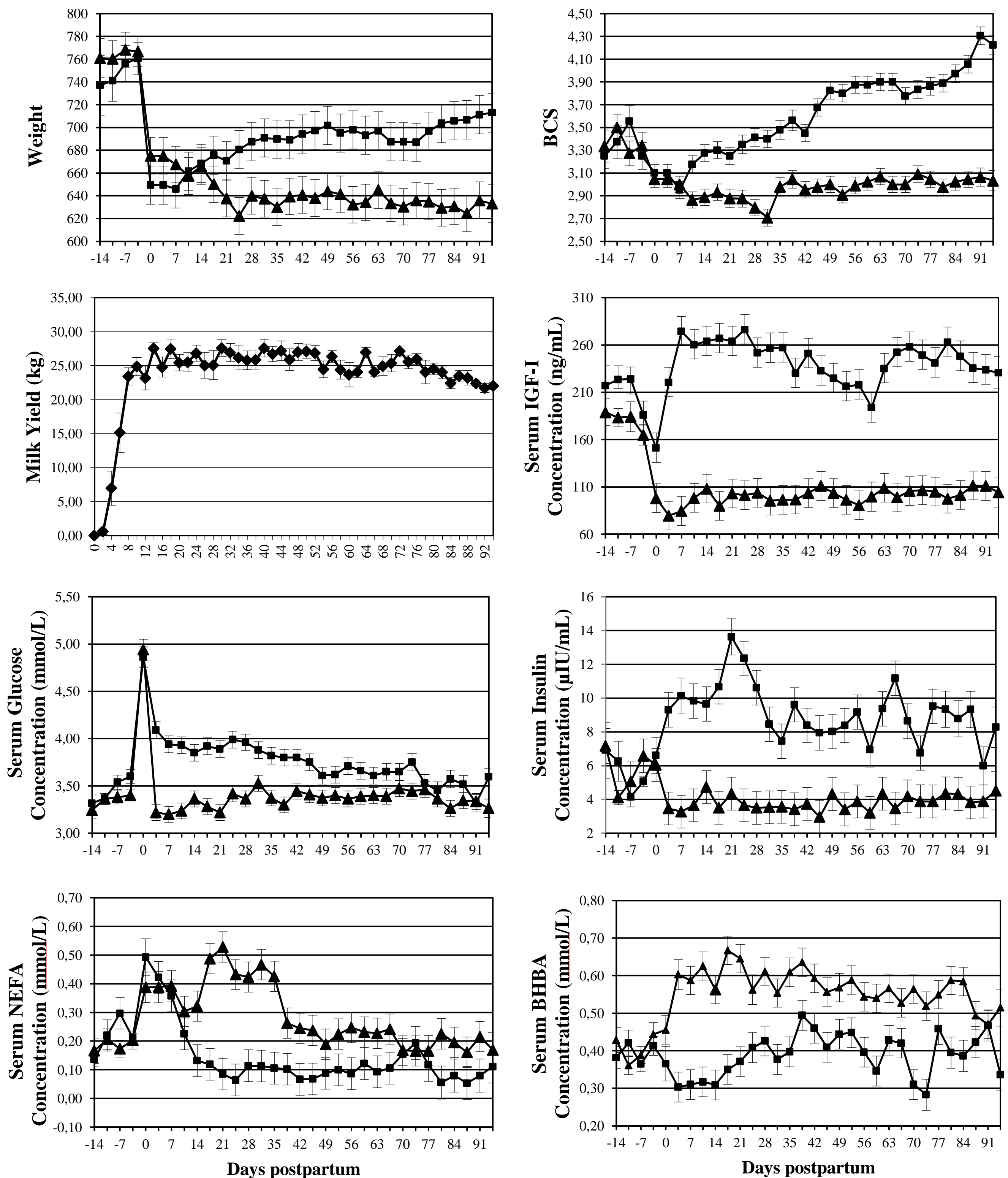

Figure 1. Characterization of postpartum lactating (▲) and nonlactating (■) dairy cows used in the study. Body weight and BCS (on a scale of 1 to 5) were recorded approximately 2 weeks before expected calving date, at calving, and then weekly until the end of the experiment (approximately 95 d postpartum). Milk yield was recorded daily for each cow during the experimental period. Blood plasma samples were collected weekly, starting 2 weeks before the expected calving date and continuing until the end of the experiment and were analyzed for NEFA, BHBA, insulin, IGF-I, and glucose. Values refer to means ± SEM.

Experiment 2

All animals in both groups showed standing oestrus following CIDR removal (Day 0), which was confirmed by the presence of an active CL at embryo transfer on Day 7 and at slaughter on Day 14. One animal from the lactating group was excluded from the analysis because the elongated embryos recovered on Day 14 were tangled and impossible to separate for measurement. Thus, 10 cows from both groups were used in the analysis. The recovery rate of embryos on d 14 was similar for both groups (lactating: 39.8 ± 9.6 %; nonlactating: 33.3 ± 9.6%; Table 2). Embryo dimensions (length, width, area) did not differ between groups (length: 1.6 ±0.5 mm vs 1.2 ± 0.5 mm; width: 0.7 ± 0.1 mm vs 0.5 ± 0.1 mm; area: 1.5 ± 0.6 mm^2 vs 0.9 ± 0.7 mm^2, for lactating and nonlactating, respectively; Table 2).

Lactating cows had significantly greater progesterone concentrations from Day 9 until slaughter at Day 14 compared to nonlactating cows (Figure 2). That was consistent with a greater CL weight recorded at slaughter on Day 14 (10.4 ± 1.1 g vs 7.0 ± 0.8 g, for lactating and nonlactating, respectively; Table 2).

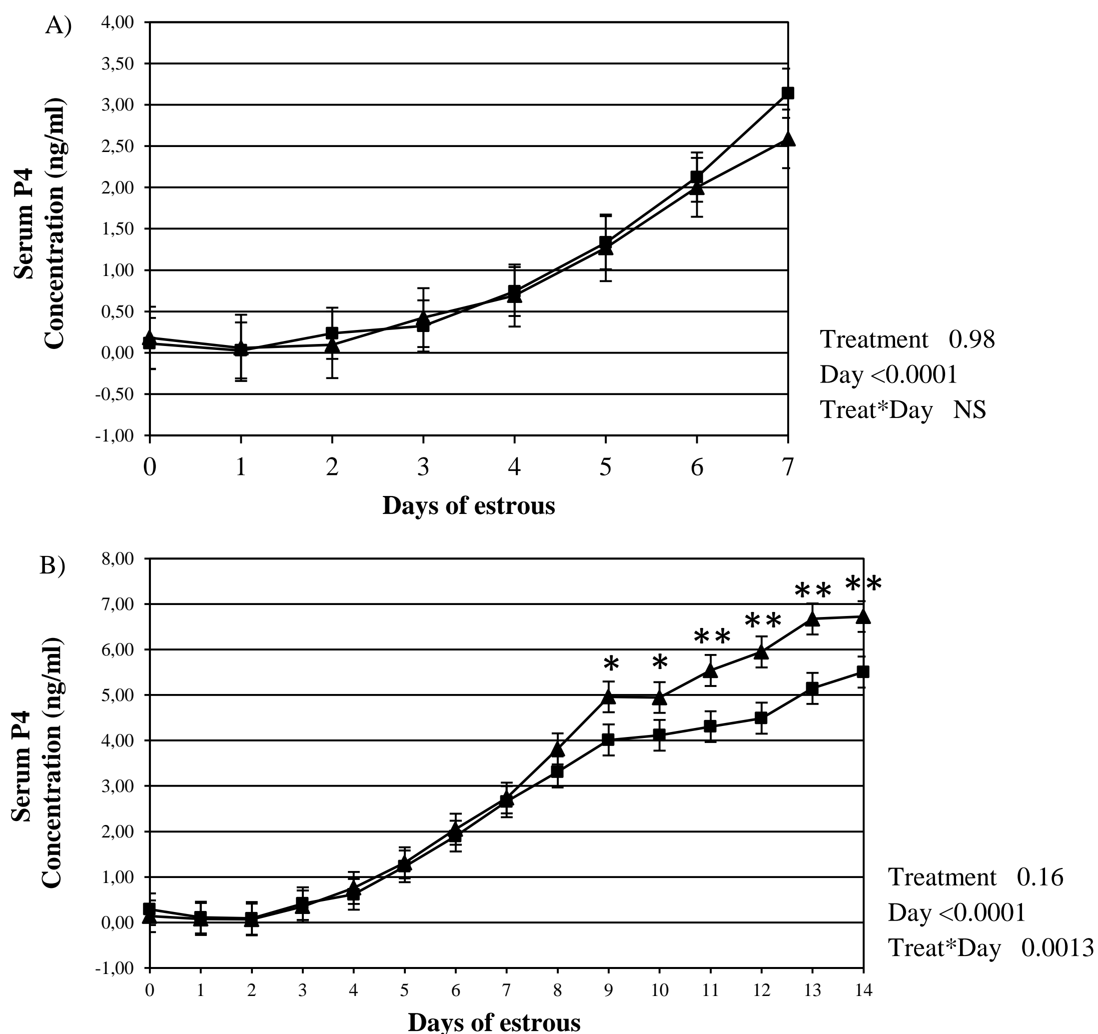

Figure 2. Progesterone concentrations (means ± SEM) in postpartum lactating (▲) and nonlactating (■) dairy cows used in the study during experiments 1 and 2. (A) Experiment 1: At approximately 60 d postpartum, approximately 65 two- to four-cell embryos, produced by in vitro maturation and fertilization of oocytes, were endoscopically transferred to the oviduct ipsilateral to the corpus luteum on Day 2 of the estrous cycle. Five days later, on Day 7, embryos were recovered by nonsurgically flushing the oviduct and uterus. (B) Experiment 2: At approximately 90 d postpartum, 15 to 20 in vitro-produced blastocysts were transferred to the uterus on Day 7. All cows were slaughtered on Day 14 to assess embryo survival and conceptus dimensions.

Table 2. Recovery rate and measurements of Day 14 embryos, recovered after the transfer of blastocysts on Day 7 and corpus luteum diameter and weight at Day 14.

Cows	Embryos transferred (no)	Recovery (no; mean % ± SEM)	Length(mm), mean±SEM	Width(mm), mean±SEM	Area(mm^3), mean±SEM	CL diameter (mm)	CL Weight (g)
Lactating	175	67 (39.8±9.6)	1.6±0.5	0.7±0.1	1.5±0.6	26.4±1.5	10.4±1.1[a]
Nonlactating	175	65 (33.3±9.6)	1.2±0.5	0.5±0.1	0.9±0.7	24.1±1.3	7.0±0.8[b]

[a, b] : values in the same column with different letters differ significantly (one way ANOVA, $P<0.05$)

DISCUSSION

By comparing postpartum lactating and nonlactating (i.e., immediately dried off at calving) Holstein cows, this study is one of the first to address directly the influence of lactation and associated metabolic perturbance on embryo development in postpartum dairy cows. The main findings from the study were that (1) lactation induces a significant alteration in the pattern of many key metabolites associated with fertility in postpartum cows; (2) this is associated with an impairment in the ability of the reproductive tract of the postpartum lactating dairy cow to support early embryo development to the blastocyst stage around Day 60 postpartum; and (3) by Day 90 postpartum, despite some latent differences in metabolic profiles between groups, we did not find evidence for a deleterious effect of lactation on the ability of the uterus to support conceptus elongation.

Lactating dairy cows typically enter a state of NEB postpartum when the combined energy requirements for maintenance and milk production exceed dietary energy intake. The requirement for cows to conceive when they are in peak lactation in a 300-d lactation associated with a seasonally concentrated calving pattern, such as that in Ireland, often coincides with this period of NEB. Both the duration and severity of early postpartum NEB, and the associated reduced circulating concentrations of insulin, IGF-I and glucose and elevated concentrations of NEFA and BHBA, have been linked to impaired reproductive performance (Butler and Smith 1989; Lucy *et al.,* 1991). The current study is one of the first to compare the metabolic profiles of lactating and nonlactating (immediately dried off at calving) cows and avoids the justifiable criticism of studies, including some of our own (Rizos *et al.,* 2005; 2010a), that compared lactating cows to nulliparous heifers. The metabolite data from the lactating cows in the present study are entirely consistent with those from a recent study from our group (Matoba *et al.,* 2012).

Associations of glucose, NEFA and BHBA with energy balance in early lactation are well established, and they reflect enhanced mobilization of body reserves and partitioning of nutrients toward milk production. Negative energy balance is typically associated with a decrease in circulating concentrations of insulin, glucose and IGF-I and increased concentrations of NEFA and BHBA, a product of tissue fatty acid catabolism (Grummer 1995). Consistent with the literature, in both groups, NEFA concentrations rose in the 2 weeks before calving. However, after an initial decline post calving, concentrations were elevated in lactating cows from Day 14 to 49 postpartum compared with those in nonlactating cows, for which concentrations fell from a peak at calving to a nadir at approximately Day

21, after which they remained relatively constant. Concentrations of BHBA were higher in lactating cows from calving throughout the experimental period compared to their nonlactating counterparts. It has been suggested that metabolic alterations associated with postpartum NEB affect the oocyte; some studies have indicated that increased concentrations of NEFA and BHBA in follicular fluid adversely affect oocyte quality (Leroy *et al.,* 2004; 2005c) . In addition, NEFA have been shown to reduce steroidogenesis and proliferation in follicular thecal cells (Vanholder *et al.,* 2006a). Leroy *et al.,* (2005c) determined the NEFA concentration and composition in follicular fluid of high-yielding dairy cows in relation to serum early and late postpartum and subsequently added the 3 predominant NEFA (oleic, palmitic, stearic) in follicular fluid during oocyte maturation *in vitro*. Both palmitic and stearic acid had a negative effect on meiotic maturation, fertilization and blastocyst formation. In agreement, Bender *et al.,* (2010) compared the metabolomic profiles of preovulatory follicular fluid from heifers and postpartum dairy cows and found higher concentrations of saturated fatty acids (palmitic and stearic) in follicular fluid from postpartum cows compared to heifers. Recent data suggest that elevated NEFA in the follicle can result in compromised early embryo quality, viability and metabolism (Van Hoeck *et al.,* 2011). Data on NEFA concentrations in oviduct fluid in the postpartum period have not been reported but it is likely that they are elevated there also and may partly contribute to the reduced embryo development observed in lactating cows in the current study.

Glucose concentrations increased slightly during the prepartum period, increased dramatically at calving and then decreased immediately postpartum, consistent with the published literature (Grummer 1995; Butler *et al.,* 2006; Patton *et al.,* 2007). The extent of the postpartum decrease in glucose was less in lactating animals resulting in divergent glucose concentrations between lactating and nonlactating cows from Day 3 to approximately Day 49 postpartum, presumably reflective of the dramatic increase in mammary glucose requirements associated with the onset of lactation. An increase in net glucose uptake, usually at the expense of pyruvate, is a feature of preimplantation embryo metabolism in all mammals studied including cattle; glucose consumption is relatively low during early preimplantation development but increases dramatically at blastocyst formation, after which glucose is a major energy source (Tiffin *et al.,* 1991; Rieger *et al.,* 1992a). The concentrations of glucose in oviduct (1.87 to 3.17 mM) and uterine fluid (3.78 to 4.54 mM) of nonlactating beef heifers have been reported to remain relatively stable throughout the estrous cycle and to be approximately half than those in plasma (Hugentobler *et al.,* 2008). The peripheral glucose concentrations reported in the current study are consistent with these values; whether lactation-induced changes in oviduct fluid glucose concentrations exist which may directly affect embryo development is unknown.

The temporal pattern of plasma IGF-I concentration in lactating cows in the present study was similar to that in previous reports, with a decline at parturition and a gradual increase thereafter (McGuire *et al.,* 1995; Patton *et al.,* 2008; Matoba *et al.,* 2012). In the current study, IGF-I concentrations declined dramatically from Day -14 to a nadir on Day 4 in lactating cows, after which they remained constant to Day 95. In contrast, after a similar initial precalving decline, IGF-I concentrations increased markedly in nonlactating cows and maintained concentrations greater than double those in lactating cows throughout the study period. Similarly, insulin concentrations were significantly higher in nonlactating cows from

calving throughout the experimental period. These data are consistent with the suggestion that plasma IGF-I concentrations in early lactation may be useful indicators of reproductive efficiency in dairy cattle (Patton *et al.,* 2007). Addition of IGF-I to culture medium increases the proportion of bovine embryos that develop to the blastocyst stage *in vitro* and increases embryo survival following transfer to heat-stressed, lactating dairy cows (Block and Hansen 2007), likely through alteration of gene expression in the embryo (Block *et al.,* 2008). In addition to lower circulating IGF-I after calving in lactating cows, NEB may also influence IGF availability in the oviduct indirectly through changes in specific insulin-like growth factor binding protein expression (Fenwick *et al.,* 2008).

As mentioned earlier, several studies have reported the outcome of embryo transfer at Day 7 in dairy cows, thereby testing the ability of the reproductive tract to support development from d 7 onward independent of the oocyte (Putney *et al.,* 1989; Ambrose *et al.,* 1999; Drost *et al.,* 1999; Rutledge 2001; Al-Katanani *et al.,* 2002; Vasconcelos *et al.,* 2006; Demetrio *et al.,* 2007). The consistently higher pregnancy rate following embryo transfer in those studies would suggest that impaired oocyte quality is a contributory factor to low fertility in dairy cows, but does not rule out a role for the embryo or reproductive tract environment. The recovery rate of approximately 65% in Experiment 1 is entirely consistent with our previous study in postpartum dairy cows (Rizos *et al.,* 2010a) and is lower than that typically achieved with nulliparous heifers [~80%; Tesfaye *et al.,* (2007); Rizos *et al.,* (2010a)]. The lower proportion of embryos developing to the blastocyst stage in lactating cows is also consistent with our previous study, in which lactating cows were compared with nulliparous heifers (Rizos *et al.,* 2010a), and suggests an impairment in the ability of the tract to support development, likely associated with the altered metabolic status as described above.

Using a previously validated multiple embryo transfer model (Clemente *et al.,* 2009; Carter *et al.,* 2010; Forde *et al.,* 2011a), the current study is the first to examine the ability of the postpartum reproductive tract of the dairy cow to support embryo development in the period encompassing the events between fertilization and Day 7 and between Day 7 and 14 independent of the confounding factors potentially associated with the endogenous oocyte. Somewhat surprisingly, we observed no difference in conceptus dimensions between lactating and nonlactating cows; indeed, the numerically greater conceptus length in the lactating group was associated with significantly higher progesterone concentrations from Day 9 to 14 in that group. It is likely that by Day 90, the deleterious influence of the lactation-induced perturbed metabolic status is significantly reduced compared with earlier postpartum time points.

Early luteal (Day 4 to 5) concentrations of progesterone are a reasonable predictor of concentrations on d 7 and may provide the potential to identify animals at risk of early embryo loss because of low concentrations of P4 and to selectively supplement such animals (Parr *et al.,* 2012). Enhanced embryo development has been associated with elevated concentration of progesterone during the first week post conception (Carter *et al.,* 2008; Clemente *et al.,* 2009). Interestingly, progesterone concentrations from Day 0 to d 7 were not different in experiment 1, which was carried out at approximately 60 d postpartum, between lactating and nonlactating cows, contrasting with the hypothesis that lactation is associated with depressed steroid concentrations due to greater metabolic clearance associated with greater in DMI (Sangsritavong *et al.,* 2002). However, the lack of an association between

progesterone concentrations and blastocyst development is consistent with our previous data involving embryo recovery from inseminated beef heifers (Carter *et al.,* 2008) and those following endoscopic transfer of 2- to 4-cell embryos to the oviducts of beef heifers with normal or elevated progesterone concentrations (Carter *et al.,* 2010) but contrasts with a similar study comparing postpartum dairy cows and nulliparous heifers (Rizos *et al.,* 2010a). Consistent with experiment 1, in experiment 2 (carried out at approximately 90 d postpartum), progesterone concentrations were similar between lactating and nonlactating cows from Day 0 to 8 but were significantly higher in lactating cows from Day 9 to 14. These greater progesterone concentrations were associated with a significantly larger CL assessed at slaughter on Day 14.

CONCLUSION

This study is one of the first to characterize the metabolic profile of postpartum dairy cows specifically induced by lactation and confirms our previous findings (Rizos *et al.,* 2010a) that lactation is associated with a compromised ability of the early postpartum reproductive tract to support early development to the blastocyst stage. These results are consistent with the results of several published studies indicating that a significant proportion (almost 50%) of embryos are not viable by Day 6 to 7 (Sartori *et al.,* 2010) and highlight the fact that although the oocyte is clearly a key player in explaining infertility in dairy cows, an important role for the embryo and for the reproductive tract and their interaction must also be considered.

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Chapter 2

Oviduct-embryo interactions: two-way traffic or a one-way street? Transcriptomic response of the bovine oviduct to the presence of an embryo

ABSTRACT

Despite clear evidence of a two-way interaction between the developing conceptus and the uterine endometrium in early pregnancy, there is limited evidence for reciprocal cross-talk between the oviduct and the early embryo during its transit to the uterus. The aims of this study were (1) to examine the effect of the presence of an embryo (versus an unfertilized oocyte) on the oviduct transcriptome, (2) to compare gene expression between ipsilateral and contralateral isthmus tissue in pregnant and cyclic animals, and (3) to compare gene expression in the ampulla and isthmus of the ipsilateral oviduct in pregnant animals.Cross-bred beef heifers were synchronized and those in standing oestrus (=Day 0) were randomly assigned to cyclic (non bred, n=6), or pregnant (artificially inseminated, n=11) groups. All heifers were slaughtered on Day 3 and both oviducts from each animal were isolated, straightened and cut in half (ampulla and isthmus). Each portion was flushed with 500 µl of PBS to confirm the presence of an oocyte/embryo and was then opened longitudinally and scraped to obtain epithelial cells. Cells were snap-frozen in liquid nitrogen for microarray analysis. All recovered oocytes and embryos were located in the isthmus of the oviduct ipsilateral to the *corpus luteum*. Microarray analysis of oviductal cells revealed that the presence of an embryo did not affect the oviduct transcriptome. However, major differences existed between the ampulla and isthmus regions of the oviduct ipsilateral to the *corpus luteum*. Thus, 2287 genes were differentially expressed ($P<0.01$) of which 1132 and 1155 were up- and down-regulated in the isthmus, respectively. Analysis of gene ontology revealed that the main of the biological processes overrepresented in the isthmus were: synthesis of compounds like nitrogen, lipids, nucleotides, steroids and cholesterol as well as vesicle-mediated transport, cell cycle, apoptosis, endocytosis and exocytosis; whereas cell motion, motility and migration, DNA repair, calcium ion homeostasis, carbohydrate biosynthetic process and regulation of cilium movement and beat frequency were overrepresented in the ampulla. In conclusion, while large differences in gene expression were observed between the isthmus and ampulla, data suggest that the presence of an 8-cell embryo does not alter the transcriptome of the cells of the isthmus, although a local effect at the precise position of the embryo cannot be ruled out.

INTRODUCTION

Following ovulation, the bovine oocyte undergoes fertilisation and spends the first 3 to 4 days of life in the oviduct, during which time morphologically it undergoes the first mitotic cell divisions and transcriptionally it undergoes embryonic genome activation (at the 8- to 16-cell stage). The developing embryo then enters the uterus where it soon forms a blastocyst, hatches from the zona pellucida and forms an ovoid, then tubular form before undergoing a dramatic elongation to form a filamentous conceptus which initiates implantation around Day 19.

There is clear evidence of a two-way interaction between the uterus and developing conceptus. For example, it is well accepted that circulating progesterone (P4) concentrations directly regulate uterine gene expression which, in turn, drives conceptus elongation (Carter *et al.,* 2008; Clemente *et al.,* 2009; Forde *et al.,* 2009a; Forde *et al.,* 2009b; Forde *et al.,* 2011a; Mamo *et al.,* 2012). Up to the time of maternal recognition of pregnancy, the temporal changes that occur in the endometrial transcriptome are similar between pregnant and cyclic animals (Forde *et al.,* 2011c). However by Day 15 (Bauersachs *et al.,* 2012) to Day 16 (Forde *et al.,* 2011c) the first responses of the endometrium to the embryo can be detected largely, but perhaps not exclusively (Bauersachs *et al.,* 2012), due to the conceptus secretion of interferon-tau (IFNT). Indeed, not only does the endometrium respond to the embryo but the response elicited is related to the type of embryo (e.g., IVF, cloned) and the likely developmental outcome (Bauersachs *et al.,* 2009; Mansouri-Attia *et al.,* 2009).

Despite this demonstration of an interaction between the developing conceptus and the uterine endometrium in early pregnancy, the evidence for reciprocal cross-talk during the transit of the early embryo through the oviduct is less clear. On the one hand, there is very convincing evidence for a positive influence of the oviduct on the quality of the early embryo. For example, short term culture of *in vitro* produced bovine zygotes in the oviducts of cattle (Tesfaye *et al.,* 2007; Gad *et al.,* 2012), sheep (Enright *et al.,* 2000; Lazzari *et al.,* 2002; Rizos *et al.,* 2002b) or even mice (Rizos *et al.,* 2007; Rizos *et al.,* 2010b) has been shown to improve embryo quality measured in terms of morphology, gene expression, cryotolerance and pregnancy rate after transfer. In contrast, relatively little evidence exists of an effect of the early embryo on the oviduct. The limited data reporting an effect of gametes on the oviduct come from litter-bearing species, where any effect is likely to be amplified (Lee *et al.,* 2002; Fazeli *et al.,* 2004; Georgiou *et al.,* 2005; Georgiou *et al.,* 2007; Almiñana *et al.,* 2012). However, tangible evidence that embryo-oviduct interaction is reciprocal comes from the investigation of differential transport of fertilized and unfertilized eggs into the uterus in the mare; it has been suggested that the embryo produces prostaglandin E2 that favours its oviductal transport to the uterus (Weber *et al.,* 1991a; Weber *et al.,* 1991b) while non-fertilized oocytes are retained in the oviduct (Van Niekerk and Gerneke 1966).

It is known that temporal changes in the transcriptome of the oviduct epithelium occur during the oestrous cycle [bovine: (Gabler *et al.,* 1999; Lapointe and Bilodeau 2003; Bauersachs *et al.,* 2004; Swangchan-Uthai *et al.,* 2011) and mice: (Jeoung and Bridges 2011)] or ovarian cycle [humans: (Horne *et al.,* 2008)]. Furthermore, transcriptome differences have been described between the oviducts

ipsilateral and contralateral to the *corpus luteum* (CL) (Bauersachs *et al.,* 2003). However, the physiological relevance of studying such changes in the oviduct after Day 4 is questionable as the embryo has entered the uterus at this time.

Anatomically, the oviductal epithelium changes depending on the phase of the oestrous cycle, the oviductal segment and basal or apical location within folds (Yániz *et al.,* 2000). These changes reflect the variable environment of the oviduct during the oestrous cycle. The aims of this study were (1) to examine the effect of the presence of an embryo (versus an unfertilized oocyte) on the oviduct transcriptome, (2) to compare gene expression between ipsilateral and contralateral isthmus tissue in pregnant and cyclic animals, and (3) to compare gene expression in the ampulla and isthmus of the ipsilateral oviduct in pregnant animals.

MATERIALS AND METHODS

Animals and treatments

All experimental procedures involving animals were licensed by the Department of Health and Children, Ireland. Protocols were in accord with the Cruelty to Animals Act (Ireland 1897) and the European Community Directive 86/609/EC and were sanctioned by the Institutional Animal Research Ethics Committee.

For the duration of the experiment, all animals were housed indoors on a slatted floor and were fed a diet consisting of grass and maize silage supplemented with a standard beef ration. The oestrous cycles of cross-bred beef heifers (n = 19, predominantly Charolais and Limousin cross; mean ± SEM 23.00 ± 0.74 months old; mean ± SEM 583.26 ± 12.45 kg weight) were synchronized using a 7-day Controlled Internal Drug Release (CIDR 1.38g; Pfizer, Sandwich, UK) insert combined with a dose of 0.02 mg of a GnRH agonist (buserelin, Receptal; Intervet, Dublin, Ireland) and administration of 15 mg of a prostaglandin F2α analogue (Prosolvin; Intervet, Dublin, Ireland) given on the day before CIDR removal. Heifers were observed for signs of oestrus four times per day commencing 30 h after CIDR withdrawal and only those recorded in standing oestrus (=Day 0; n=17) were used. Heifers were randomly allocated to one of two groups: (a) cyclic group, non-bred (n=6) or (b) pregnant group (n=11), artificially inseminated 12 and 24 h after first sign of oestrus, with frozen-thawed semen from a bull of proven fertility.

Samples Collection

Animals were slaughtered in a commercial abattoir 3 d after oestrus (mean ± SEM, 3.09±0.04 days). Following slaughter, the reproductive tract was removed, sealed in a plastic bag, transported to the laboratory on ice and processed approximately 3.5 h after slaughter (mean ± SEM, 3.60±0.25 h). Both oviducts were trimmed free of tissue and cut in half to separate ampulla and isthmus regions. The ampulla and isthmus of both the ipsilateral and contralateral oviduct were flushed with 500 µl of PBS. The presence of an unfertilized oocyte or an embryo was verified under a microscope. After flushing each part, the ampulla and isthmus sections of both oviducts were opened longitudinally and gently scraped

with a blade to recover epithelial cells. The cells obtained were snap frozen and stored at -80 °C. Samples from 5 heifers with a confirmed non-fertilized oocyte or 5 heifers with an 8-cell stage embryo were used for microarray and quantitative real-time PCR analysis (qRTPCR) (see Figure 1).

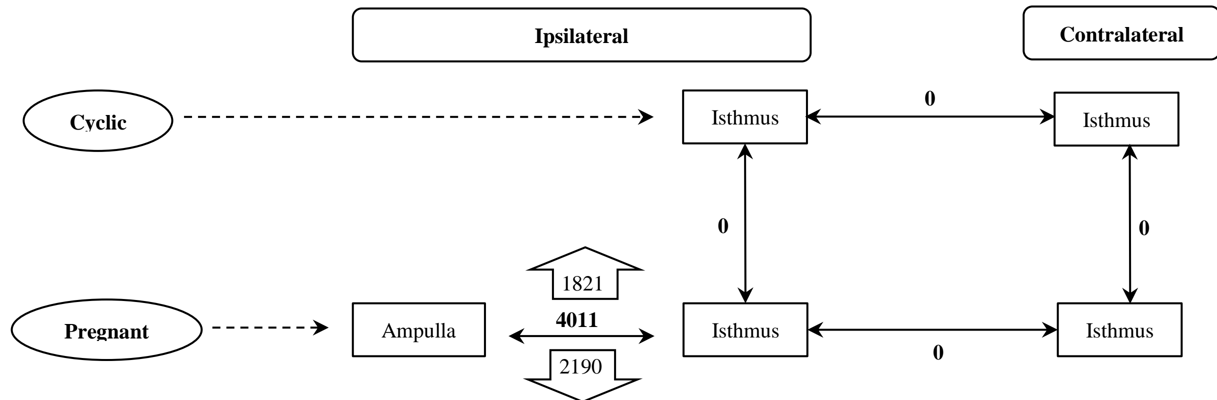

Figure 1. Overview of the groups compared in the microarray. Numbers represent the differentially expressed genes in each comparison. Numbers in arrows refer to up- or down-regulated genes in the isthmus of pregnant heifers ($P<0.05$). See text for further details.

RNA extraction and microarray hybridisation

Total RNA was extracted from oviductal cell samples by the Trizol method as per manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Following on column DNAse digestion and RNA clean up, (Qiagen, Crawley, West Sussex, UK) both the quality and quantity of the RNA was determined using the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and the NanoDrop 1000 (Thermo Fischer Scientific Inc. Wilmington, DE, USA), respectively. Only samples with an RNA Integrity Number of greater than 8.0 were used for microarray analysis. Transcriptomic analysis was carried out using the Bovine Gene ST 1.0 microarray (Affymetrix, Santa Clara, CA, USA). One hundred and fifty nanograms of Total RNA were used for reverse transcription using the Ambion WT Expression Kit (Life Technologies, Carlsbad, CA, USA) while the rest was stored for microarray validation analysis by qRTPCR. All samples were processed with the appropriate amount of Poly-A RNA controls from the Affymetrix GeneChip Poly-A RNA Control Kit (Affymetrix, Santa Clara, CA, USA) as specified within the Ambion user manual. Five point five micrograms of the purified cDNA was fragmented and labelled using the GeneChip WT Terminal Labelling kit and fragmentation was verified using the Agilent 2100 bioanalyzer. Hybridization was performed according to the Affymetrix user manual. Briefly, fragmented, biotin-labelled cDNA was hybridized to the Affymetrix Bovine Gene ST 1.0 microarray as described within the Encore Biotin Module user's guide appendix. Samples were hybridized for 16 h at 45 °C in a GeneChip Hybridization Oven 640 while rotating at 45 rpm. Microarrays were processed using the Affymetrix GeneChip Fluidic Station 450. Staining was carried out with streptavidin-conjugated phycoerythrin (SAPE) followed by amplification with a biotinylated anti-streptavidin antibody and by a second round of SAPE prior to scanning using a GeneChip Scanner 3000 (Affymetrix) and GeneChip Command Console software.

Quantitative real-time PCR (qRTPCR)

Validation of the microarray results was performed by quantitative real-time PCR (qRTPCR) analysis of 12 of the top up- and down-regulated genes selected from the list of differentially expressed genes (DEGs) obtained from the comparison between the ipsilateral isthmus and ampulla of pregnant animals. Total RNA (1000 ng) from the samples used for microarray analysis was reverse transcribed into cDNA using the high capacity reverse transcription kit as per manufacturer's instruction (Applied Biosystems, Carlsbad, CA). All primers were designed using Primer-BLAST software (www.ncbi.nlm.nih.gov/tools/primersblast/) to span exon-exon boundaries when possible. All qRTPCR reactions were carried out in duplicate on the Rotorgene 6000 Real Time Cycler TM (Corbett Research, Sydney, Australia) by adding 5 ng of each sample to the PCR mix (GoTaq® qPCR Master Mix, Promega Corporation, Madison, USA) containing the specific primers selected to amplify gastrin-releasing peptide (*GRP*), ribonuclease, RNase A family, 1 (pancreatic) (*RNASE1*), neuropilin (NRP) and tolloid (TLL)-like 1 (*NETO1*), aldo-keto reductase family 1, member B1 (aldose reductase) (*AKR1B1*), low density lipoprotein receptor-related protein 2 (*LRP2*), glycoprotein M6B (*GPM6B*), connective tissue growth factor (*CTGF*), cyclin B1 (*CCNB1*), lysozyme 1 (*LYZ1*), prostaglandin D2 synthase 21kDa (brain) (*PTGDS*), pleckstrin homology domain containing, family G (with RhoGef domain) member 7 (*PLEKHG7*) and flavin containing monooxygenase 2 (non-functional) (*FMO2*). Primer sequences and the approximate sizes of the amplified fragments of all transcripts are shown in Supplemental Table 1. Cycling conditions were 94º C for 3 min followed by 35 cycles of 94 ºC for 15 sec, 56 ºC for 30 sec, 72 ºC for 10 sec and 10 sec of fluorescence acquisition. Each pair of primers were tested to achieve efficiencies close to 1 and then the comparative cycle threshold (CT) method was used to quantify expression levels as described by (Schmittgen and Livak 2008). To avoid primer dimer artefacts, fluorescence was acquired in each cycle at a temperature higher than the melting temperature of primer dimmers (specific for each product, 76–86 °C). Then, the threshold cycle or the cycle during the log-linear phase of the reaction at which fluorescence increased above background was determined for each sample. The ΔCT value was determined by subtracting the endogenous control (an average of *H2AZ, ACTB* and *18S*) CT value for each sample from each gene CT value of the sample. Calculation of ΔΔCT involved using the highest sample ΔCT value (i.e., the sample with the lowest target expression) as a constant to subtract from all other ΔCT sample values. Fold-changes in the relative gene expression of the target were determined using the equation $2^{-\Delta\Delta CT}$.

Data analysis

For microarray results, the raw signal intensities were read into R and pre-processed using functions of both Affy and GCRMA packages of the BioConductor project (Gentleman *et al.,* 2004). Hierarchical clustering analysis was performed to determine the greatest source of variation in the tissue samples. Lists of DEGs were determined by the Limma package (Smyth 2005) employing linear modeling and an empirical Bayes framework to shrink the variance of measurements on each probe set. A modified *t*-test was then carried out and all *P*-values were adjusted for multiple testing using the Benjamini and Hochberg false discovery rate method. Lists of DEGs were selected on the basis of an

adjusted *P*-value of <0.05. Given the large number of DEGs obtained with this *P*-value, a more stringent level of *P*<0.01 was used to generate the list of differentially expressed probe sets inputted for the gene ontology (GO) overrepresentation analysis.

Data obtained by qRTPCR was analyzed using the Sigma Stat (Jandel Scientific, San Rafael, CA) software package. Student's *t*-test was performed to study the differences in expression values between isthmus and ampulla regions.

Gene ontology analysis

Gene ontology analysis was carried out using DAVID website (Huang da *et al.,* 2009). The list of DEGs with a more stringent level of *P*<0.01 was used to limit the input to DAVID to achieve meaningful overrepresented data. From the output obtained by DAVID, GO FAT terms were used instead of GO ALL, because the FAT category filters out the very broad GO terms based on a measured specificity of each term to yield more specific terms. Using these data differences in biological process (BP), cellular component (CC), molecular function (MF) and KEGG pathways were analysed.

RESULTS

Oocyte and embryo recovery

Of the 19 animals synchronised, 17 exhibited standing oestrus (89.48%) and were used for the experiment. In the inseminated group, 8 of the 11 heifers yielded an embryo (72.72% recovery rate), which was located in all cases in the isthmus of the oviduct ipsilateral to the CL. Of the eight embryos recovered, one was at the 4-cell stage of development, five were at the on 8-cell stage and while embryos recovered from two animals were between the 8-and 16-cell stage of development. In the cyclic group, an unfertilized oocyte was found also in the ipsilateral isthmus of the oviduct in all animals (n=6; 100% recovery rate).

Changes in oviduct gene expression

Correspondence analysis was performed to identify the factor the contributed most to gene expression in the oviduct epithelial cells. This analysis revealed that oviduct region was the factor that contributed most to the transcriptional profile of the oviduct i.e. all samples recovered from the isthmus, irrespective of pregnancy status or site of the CL clustered together, whereas samples analysed from the ampulla clearly segregated from the rest (Figure 2).

Factors that affect the transcriptome of the oviduct.

Under our experimental conditions, the presence of an 8-cell stage embryo did not significantly affect the transcriptome of the oviduct, as evidenced by the absence of DEGs between the ipsilateral isthmus of pregnant and cyclic heifers. In addition, proximity to the CL i.e. cells from the oviducts ipsilateral vs contralateral to the CL, did not affect the transcriptome of the isthmus, irrespective of whether the heifer was cyclic or pregnant. However, site within the oviduct significantly affected the

pattern of gene expression; when the ampulla and isthmus of the oviducts ipsilateral to the CL in pregnant animals were compared, 4011 DEGs were identified (*P*<0.05) (Figure 1) and when a more stringent level of *P*<0.01 the number of DEGs was 2287 (Figure 3).

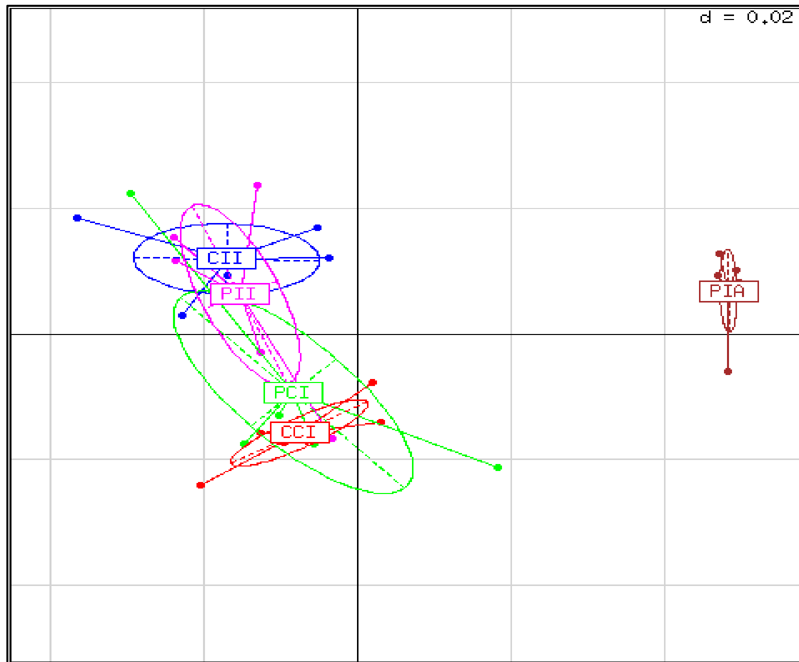

Figure 2. Correspondence analysis demonstrating the source of greatest variation in the oviduct transcriptional profile. Each dot represents all the transcripts expressed on one microarray representing one tissue site from one animal. CII – Cyclic Ipsi Isthmus, PII – Pregnant Ipsi Isthmus, CCI – Cyclic Contra Isthmus, PCI – Pregnant Contra Isthmus, PIA – Pregnant Ipsi Ampulla.

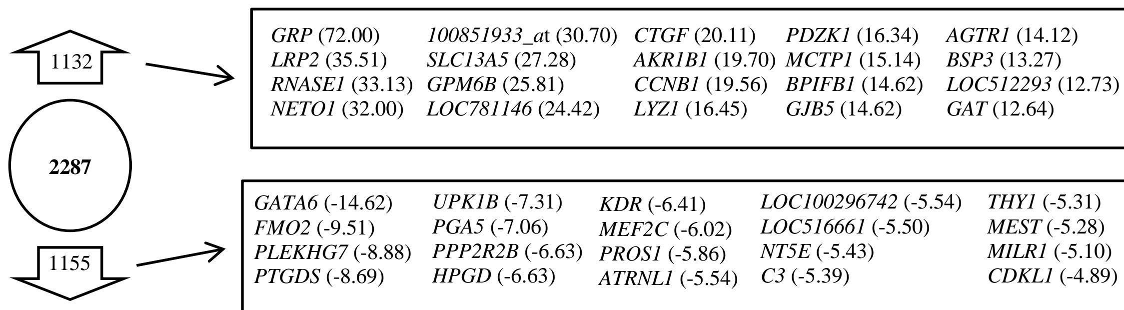

Figure 3. Illustration of differentially expressed genes between isthmus and ampulla of the ipsilateral oviduct in pregnant heifers showing the top 40 up- and down-regulated genes in the isthmus (1132 and 1155, respectively) (*P*<0.01). Values in parentheses indicate the fold-change difference for each gene. Complete gene lists can be found in Supplemental Table 2.

Of the 2287 DEGs recognized between the isthmus and ampulla, 1132 genes were up-regulated in the isthmus region including gastrin-releasing peptide (*GRP*: fold change 72.00), low density lipoprotein receptor-related protein 2 (*LRP2*: 35.51), ribonuclease, RNase A family, 1 (pancreatic) (*RNASE1*: 33.13), neuropilin (NRP) and tolloid (TLL)-like 1 (*NETO*1, 32.00), solute carrier family 13 (sodium-dependent citrate transporter), member 5 (*SLC13A5*: 27.28), glycoprotein M6B (*GPM6B*: 25.81), lysozyme (*LOC781146*: 24.42), connective tissue growth factor (*CTGF*: 20.11), aldo-keto reductase family 1, member B1 (aldose reductase) (*AKR1B1*: 19.70) and cyclin B1 (*CCNB1*: 19.56). Of the 1155 genes down-regulated in the isthmus region, the expression of myocyte enhancer factor 2C (*MEF2C*: -

6.02), kinase insert domain receptor (a type III receptor tyrosine kinase) (*KDR*: -6.41), hydroxyprostaglandin dehydrogenase 15-(NAD) (*HPGD*: -6.63), protein phosphatase 2, regulatory subunit B, beta (*PPP2R2B*: -6.63), pepsinogen 5, group I (pepsinogen A) (*PGA5*: -7.06), uroplakin 1B (*UPK1B*: -7.31), prostaglandin D2 synthase 21kDa (brain) (*PTGDS*: -8.69), pleckstrin homology domain containing, family G (with RhoGef domain) member 7 (*PLEKHG7*: -8.88), flavin containing monooxygenase 2 (non-functional) (*FMO2*: -9.51) and GATA binding protein 6 (*GATA6*: -14.62) changed to the greatest degree (Fig. 3). In addition, of the 1132 genes up-regulated in the isthmus, 403 had a fold change equal or greater than 2, and 30 exhibited more than a 10-fold change, while of the 1155 down-regulated in the isthmus, 245 had a fold change equal or greater than 2 with only one gene changing more than 10-fold (Supplemental Table 2).

Analysis of the GO terms associated with those genes, indicated that 206 and 129 biological processes (BP) (containing a minimum of two genes) were overrepresented in the isthmus and the ampulla, respectively. Of these BP, 26 were common between isthmus and ampulla including 1) phosphate metabolic process (47 and 44 genes in isthmus and ampulla, respectively), 2) intracellular signalling cascade (42 and 32 genes), 3) ion transport (38 and 33 genes), 4) phosphorylation (38 and 36 genes) and 5) regulation of cell proliferation (26 and 23 genes). From the different BP in each region, in the isthmus, synthesis of nitrogen compounds (24), lipids (17), nucleotides (15), steroids (10) and cholesterol (10) as well as vesicle-mediated transport (31), cell cycle (17), apoptosis (16), endocytosis (13) and exocytosis (7) were considered as possible candidates to provide an optimal environment to support early embryo development (Supplemental Table 3.1.). In the ampulla, cell motion (19), motility (17) and migration (15), DNA repair (11), calcium ion homeostasis (9), carbohydrate biosynthetic process (7) and regulation of cilium movement (2) and beat frequency (2) involved in ciliary motility were taking into consideration for oocyte transport and maintenance (Supplemental Table 3.2.).

In the KEGG pathway analysis, 24 pathways were present in the isthmus and 11 in the ampulla with WNT- and VEGF-signalling pathways overrepresented in the isthmus and MAPK- and calcium-signalling overrepresented in the ampulla (Supplemental Table 3.3 and 3.4., respectively).

qRTPCR microarray validation

The expression pattern of twelve genes (9 top up-regulated: *GRP, RNASE1, NETO1, AKR1B1, LRP2, GPM6B, CTGF, CCNB1*, *LYZ1*; and 3 down-regulated: *PTGDS, PLEKHG7* and *FMO2* in the isthmus) was confirmed by qRTPCR and was consistent with the results from the microarray analysis (Figure 4).

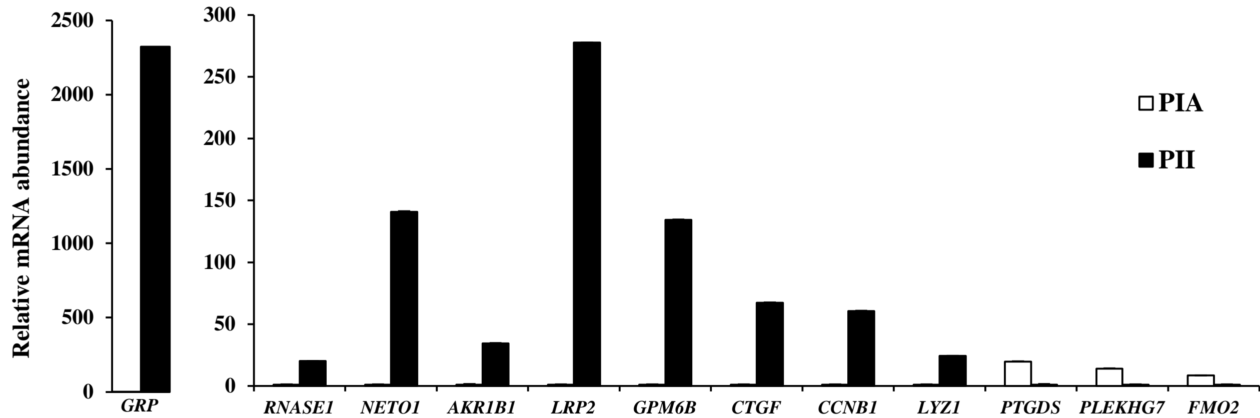

Figure 4. Quantitative real-time PCR (qRTPCR) analysis of selected genes for microarray validation across 24 comparisons (12 genes x 2 groups) between pregnant ipsilateral isthmus (PII) and pregnant ipsilateral ampulla (PIA). Quantification was normalized to the endogenous control (an average of *H2AZ, ACTB* and *18S*) (P<0.001).

DISCUSSION

The main findings of this study are that 1) the presence of an 8-cell embryo in the isthmus does not affect the transcriptome of the oviduct; 2) gene expression of the oviduct in pregnant or cyclic heifers is not modified by proximity to the CL; and 3) in pregnant heifers, major differences exist between the ampulla and isthmus regions of the oviduct ipsilateral to the CL.

Under the conditions of this study, we failed to detect an effect of the presence of an 8-cell embryo on the transcriptome of the oviduct. This is in contrast to observations in mice, rats and pigs were a variable number of genes were up-regulated in the presence of embryos (Lee *et al.,* 2002; Arganaraz *et al.,* 2007; Almiñana *et al.,* 2012; Arganaraz *et al.,* 2012). However, it is important to point out that these are litter-bearing species in which the observed changes are a result of an amplification effect induced by the presence of multiple embryos. In addition, the total length of the oviduct in the cow is approximately 20 cm (Crisman *et al.,* 1980; Bello *et al.,* 2012) and the isthmus flushed in our experiment was about 8 cm. Therefore, in cattle where there is normally only one embryo, the possibility that the embryo (~120 µm) could have an effect at the precise point where it was located cannot be dismissed.

In mice, sperm-induced modulation of the oviduct transcriptome has been reported as early as 6 h after mating (Fazeli *et al.,* 2004). Similar findings were detected *in vitro,* 24 h after co-incubating sperm with oviductal cells (Li *et al.,* 2010). In our study we did not find evidence for an effect of insemination on the oviduct, but given that we collected the samples 3 days after insemination, this is not surprising.

Proximity of the oviduct region to the CL (i.e. ipsilateral versus contralateral oviduct) did not affect the gene expression in the isthmus in either pregnant or cyclic animals. In contrast, Bauersachs *et al.,* (2003) found a small number DEGs (35) between the ipsilateral and contralateral oviduct in cyclic animals. In that study, suppressive subtractive hybridization and cDNA microarray hybridization were used and also the cells were taken from the entire oviduct epithelium, as opposed to where the embryo

was located. Thus, the differences in the technique and the origin of the samples may explain the discrepancy between the two studies.

It is well known that different regions of the oviduct are both anatomically and functionally distinct (Abe 1996; Yániz *et al.,* 2000). This study clearly demonstrates that the transcriptome of the oviduct differs significantly between ampulla and isthmus regions. The isthmus and ampulla are both composed of ciliary and secretory cells but the predominant form changes throughout the oestrous cycle. During the follicular phase the cells in the ampulla are predominantly ciliated while in the luteal phase the secretory cells prevail. In the isthmus, the proportion of both cells is similar through the oestrous cycle (Abe 1996).

The composition of bovine oviductal fluid (OF) has been well characterised in recent years. Glycine, alanine and glutamate are the predominant amino acids (Hugentobler *et al.,* 2007a). Furthermore, oviductal amino acid concentrations are modulated by progesterone; 9 of 20 amino acids increased following supplementation with progesterone, with glycine showing the largest increase of approximately two-fold (Hugentobler *et al.,* 2010). Partridge and Leese (1996) reported that individual amino acids (AAs) are depleted at different rates by bovine preimplantation embryos, being higher at later developmental stages implying an increase in AA requirement as development progresses. Threonine was the only AA to be depleted at every stage; glutamine was depleted at the zygote and 4-cell stage but not subsequently. Alanine was the only AA to appear consistently and its production increased with development. Aspartate, glutamate, threonine and lysine were depleted significantly by blastocysts (Partridge and Leese 1996).

Oviductal fluid from different regions of the bovine oviduct, differentially facilitate sperm binding to the oocyte and fertilization *in vitro* (Way *et al.,* 1997). Recently, it has been demonstrated the presence of oviduct-specific glycoprotein which is responsible of the pre-fertilization zona hardening, is directly related to monospermy levels (Coy *et al.,* 2008; Mondejar *et al.,* 2013). Furthermore, plasminogen and activators of plasminogen have been detected in the OF, and oolema and zona, respectively, being associated with decreased number of attached sperm and decreased incidence of polyspermy rates in pigs and cows (Coy *et al.,* 2012; Mondejar *et al.,* 2012; Grullon *et al.,* 2013).

The expression of antioxidants during the oestrous cycle also differs between the ampulla and isthmus, with more *GPX2* expressed in the ampulla but increased *GPX3* observed in the isthmus (Lapointe and Bilodeau 2003), similar to what was observed in this study. These antioxidants belong to the family of glutathione peroxidases, which are responsible for metabolizing H_2O_2, one of the reactive oxygen species (ROS). It is likely that a balance must exist in the oviduct between ROS and antioxidants as it has been found that ROS decrease the motility of bovine sperm *in vitro* (Bilodeau *et al.,* 2000) and also reduce the ability of sperm to fuse with the egg plasma membrane (Mammoto *et al.,* 1996). However, sperm binding to the zona pellucida is promoted by low levels of ROS and is inhibited by antioxidants (Aitken *et al.,* 1989). Therefore, the production of ROS controlled by the reproductive tract could be a key factor in successful fertilization and subsequent implantation (Lapointe and Bilodeau 2003).

In line with the changes in the oviduct morphology and AA composition of the OF, the energy requirements of the developing embryo change as it develops from a one-cell zygote through the early cleavage divisions to form a multicellular blastocyst. In general, embryos throughout pre-elongation development are reliant on oxidative phosphorylation via oxidation of pyruvate and amino acids for the generation of ATP for embryo development (Javed and Wright 1991; Rieger *et al.,* 1992a; 1992b; Gardner *et al.,* 1993; Thompson *et al.,* 1996; 2000). However, there is a switch to an increased contribution of glycolysis during compaction and blastulation (Gardner *et al.,* 1993; Thompson *et al.,* 1996; 2000).

Early embryonic development is probably the most critical period of mammalian development. In this short time (1-6 days) various morphological and biochemical changes occur and are affected by the culture environment. Among these changes, the bovine embryo at the eight-cell stage (Day 3 after oestrus *in vivo* or after IVF *in vitro*) switches from using the mRNA derived from the maternal genome to that resulting from embryonic genome activation (EGA) (Memili and First 2000). EGA is considered to be the most critical event for viability during early development (Meirelles *et al.,* 2004), and is associated with early differentiation events, successful embryo implantation, and fetal development (Niemann and Wrenzycki 2000). Gad *et al*. (2012) in a very comprehensive study showed that changing the culture conditions from *in vivo* to *in vitro* or vice versa, either before or after the time of EGA critically influenced the gene expression patterns of the resulting blastocysts. Similarly, we have shown that bovine embryos show temporal sensitivity to the culture environment after fertilization, which is manifested in terms of the quality of the blastocysts produced (Lonergan *et al.,* 2003b).

The most up-regulated gene in the isthmus was *GRP* with a fold change of 72 compared to the ampulla region. *GRP* as the name denotes is a peptide that stimulates gastrin release. In the reproductive tract, GRP has been found in pregnant uterus of humans and sheep (Fraser *et al.,* 1992; Giraud *et al.,* 1993; Fraser *et al.,* 1994; Giraud *et al.,* 1994; Shulkes *et al.,* 1996; Whitley *et al.,* 1996; Xiao *et al.,* 1996a; Xiao *et al.,* 1996b). In sheep, it is highly expressed in the glandular epithelium of the uterus during pregnancy as well as during the oestrous cycle and in several foetal tissues (Whitley *et al.,* 1998; Whitley *et al.,* 2002). In addition, Song *et al.,* (2008) described that *GRP* expression in the ovine uterus was modified by P4 and IFNT. In cattle, *GRP* was up-regulated during oestrus compared to dioestrus (Bauersachs *et al.,* 2004) suggesting a role during the early stages of embryo development.

Connective tissue growth factor *(CTGF)* is a cysteine-rich protein that is expressed in multiple tissues and has been linked to embryo development, cell proliferation, mitosis, migration, adhesion, matrix production, differentiation and maintenance of normal cell and connective tissue function (Brigstock *et al.,* 1997; De Winter *et al.,* 2008). In the uterus of mice and humans, during the luteal phase, *CTGF* is expressed in the luminal and glandular epithelium (Surveyor *et al.,* 1998; Uzumcu *et al.,* 2000). During early pregnancy, this gene is up-regulated in the uterus of mice from Day 1.5 to 3.5 compared to Day 4.5 (Surveyor *et al.,* 1998) and in the endometrium of pregnant compared to non-pregnant cattle at Day 13, 16 and 18 post oestrus (Klein *et al.,* 2006; Forde *et al.,* 2010). In murine embryos, *CTGF* expression was higher at Day 5.5 and 6.5 compared to Day 4.5 after implantation, particularly in the

embryonic ectoderm cells, but was also differentially distributed throughout the various embryonic structures (Surveyor *et al.,* 1998). CTGF protein has also been detected in uterine flushings of mice and pigs (Brigstock *et al.,* 1997; Surveyor *et al.,* 1998). Therefore, *CTGF* may play an important role in cell proliferation of the oviduct epithelium, which could have an effect on the embryo.

The aldose reductase gene (*AKR1B1*) encodes the enzyme aldolase reductase responsible for metabolizing progesterone, and is involved in the production of prostaglandin $F_{2\alpha}$ by the endometrium in cattle and humans (Madore *et al.,* 2003; Bresson *et al.,* 2011). This gene was up-regulated in biopsies derived from blastocysts which failed to establish a pregnancy after transfer (El-Sayed *et al.,* 2006). In contrast, in another study with the use of bovine OF before fertilization *in vitro,* it was found that expression of *AKR1B1* was greater in grade 1 (excellent) than in grade 2 (fair) blastocysts (Cebrian-Serrano *et al.,* 2013). However, the differentiation between "excellent" and "fair" blastocysts was based on morphological parameters, which is subject to operator bias. The protein encoded by *AKR1B1* has also been found in the uterine luminal fluid of pregnant heifers on Day 16 (Forde *et al.,* 2014). In addition, high glucose in culture media could lead to up-regulation of aldose reductase and subsequent accumulation of sorbitol in cytoplasm and activation apoptotic pathways (Wirtu *et al.,* 2003). Therefore, as glucose requirements are very low during the first stages of embryo development, *AKR1B1* may be involved in maintaining low glucose levels in the oviduct at this time.

Cyclin B1 *(CCNB1)* is involved in the generation of maturation promoting factor which drives oocytes into and through meiosis (Marangos and Carroll 2004). This gene is accumulated and stored during oogenesis (Rekik *et al.,* 2011) until it is activated to complete the maturation of the oocyte. Transcripts for *CCNB1* are abundant in bovine oocytes and embryos up to 4-cell stage, after which they decrease to very low levels in the 8-cell embryo, when embryonic genome activation begins, and remain low up to the blastocyst stage (Tremblay *et al.,* 2005). Apart from the specific effect of *CCNB1* on the maturation of the oocyte, the accumulation of CCNB1 is necessary for the cell cycle progression through G_2 to mitosis, i.e. to start mitosis (Scaife 2004). Therefore, taking into account the above, *CCNB1* expression in the embryo is due to maternally-derived mRNA up to 4-cell stage, while in later stages the appropriate CCNB1 requirements can be provided by the oviduct.

Prostaglandin D2 synthase 21 kDa (*PTGDS* or *PGDS*) synthetises PGD2 and induces sleep, allergic responses, inhibition of platelet aggregation, and relaxation of vascular and non-vascular smooth muscle (Kengni *et al.,* 2007). There are two types of PGD2 synthase: lipocalin type PGDS (*L-PGDS*) and hematopoietic PGDS (*H-PGDS*). *H-PGDS* is highly expressed in the rat oviduct and also in human uterine epithelial cells, endometrial gland cells and trophoblast (Kanaoka *et al.,* 1997; Michimata *et al.,* 2002). *PTGDS* is up-regulated during pregnancy in rats and it has been suggested that its expression is dependent on the presence of an embryo (Kengni *et al.,* 2007). In addition, in humans PGD2 may contribute to the maintenance of pregnancy by suppressing antigen presentation (Michimata *et al.,* 2002). The up-regulation of *PTGDS* in the ipsilateral ampulla of pregnant animals in the current study may suggest an immune role for this gene at the time of zygote formation.

According to gene ontology (GO) analysis, in the ampulla some of the genes from the over-represented categories were related with cell motion, motility and migration, ciliary motility and beat frequency, consistent with the greater population of ciliated cells there facilitating the transport of the oocyte to the site of fertilization (Halbert *et al.,* 1989). In the isthmus, genes were detected which were related with vesicle-mediated transport, endocytosis, exocytosis, cell cycle and apoptosis, likely involved in the provision of an optimal environment to support early embryo development.

CONCLUSION

Under the conditions of the current study, no evidence was detected for embryo-induced alterations of oviduct gene expression, although a local effect at the precise position of the embryo cannot be ruled out. The data provide a comprehensive description of the transcriptional differences present between the isthmus and ampulla regions of the oviduct at the time when the embryo is exposed to these environments. These alterations in gene expression reflect morphological and functional differences between these two distinct regions of the oviduct.

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Supplemental Table 1. Details of primers used for qRTPCR.

Entrez Gene Symbol	Gene name	Gene Bank Accesion no.	Forward primer (5'-3')	Reverse primer (5'-3')	Product length
ACTB	Actin, beta	AF191490.1	GAGAAGCTCTGCTACGTCG	CCAGACAGCACCGTGTTGG	264
AKR1B1	Aldo-keto reductase family 1, member B1 (aldose reductase)	NM_001012519.1	GTGGCAATCGACCTTGGGTA	ACCACAGCTTGCTGACGATG	142
CCNB1	Cyclin B1	NM_001045872.1	CCCGAGCCTATTTTGGTCGAT	TTTGGATCCGCTCCGTCTTC	146
CTGF	Connective tissue growth factor	NM_174030.2	TGTGCACCGCTAAAGATGGT	TTGCAGCTGCTCTGGAAAGA	82
FMO2	Flavin containing monooxygenase 2 (non-functional)	NM_001075162.2	GTTTTCAAAGGCTTATGTACCTTGC	CAGCTAGGTGATTCTTGTGAGC	148
GPM6B	Glycoprotein M6B	NM_001104981.1	GGATGGTATGAAGCCAGCCA	AAGCAGCCTTTTCTTTCTCGG	75
GRP	Gastrin-releasing peptide	NM_001101239.1	TCAAAGACACAGGTCCTCAGC	ACTGATGCCCATAGAACGCA	122
H2AFZ	H2A histone family, member Z	NM_174809	AGGACGACTAGCCATGGACGTGTG	CCACCACCAGCAATTGTAGCCTTG	209
LRP2	Low density lipoprotein receptor-related protein 2	XM_002685308.3	ACTGCGTCGATTTTGACGAT	TGGCCAATTCGGTCTTCACA	70
LYZ1	Lysozyme 1	NM_001077829.1	GAGGGTTGTCAGAGATCCACA	AGCTGAAGACGAAAACTCCAC	126
NETO1	Neuropilin (NRP) and tolloid (TLL)-like 1	NM_001192694.1	CGTGGACAAAACATGCAGAGG	CTGTCTTGGGGCAGCTTCTAT	107
PLEKHG7	Pleckstrin homology domain containing, family G (with RhoGef domain) member 7	XM_005206126.1	AGGCTGACTCGATACCCCTT	CCTTCAAGATCCTGGATTGCCT	122
PTGDS	Prostaglandin D2 synthase 21kDa (brain)	NM_174791.4	TCCTCAGGAAAGACCAGTGTG	GTCTCTGCCACTGACACCTC	121
RNA18S	18S ribosomal RNA gene	AF176811.1	AGAAACGGCTACCACATCCAA	CCTGTATTGTTATTTTTCGTCACTACCT	91
RNASE1	Ribonuclease, Rnase A family, 1 (pancreatic)	NM_001014386.4	GACCCAGGTTTCTCCAGGGGAGTGC	AGCAGCACCAGGACCAACAGC	82

Supplemental Table 2. Differentially expressed genes (n=2287) in the ipsilateral oviduct of pregnant heifers between isthmus and ampulla. A positive value indicates up-regulation in the isthmus and a negative value indicates up-regulation in the ampulla.

Symbol	Affymetrix Probe ID	Description	Fold change	Symbol	Affymetrix Probe ID	Description	Fold change
GRP	615323_at	Gastrin-releasing peptide	72,00	*SPRY3*	539402_at	Sprouty homolog 3 (Drosophila)	11,47
LRP2	100337021_at	Low density lipoprotein receptor-related protein 2	35,51	*CFTR*	281067_at	Cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7)	10,85
RNASE1	282340_at	Ribonuclease, rnase A family, 1 (pancreatic)	33,13	*IHH*	522714_at	Indian hedgehog	10,78
NETO1	530407_at	Neuropilin (NRP) and tolloid (TLL)-like 1	32,00	*MBOAT2*	785489_at	Membrane bound O-acyltransferase domain containing 2	10,56
	100851933_at		30,70	*NDRG4*	515033_at	NDRG family member 4	10,13
SLC13A5	507000_at	Solute carrier family 13 (sodium-dependent citrate transporter), member 5	27,28	*SCN3A*	534223_at	Sodium channel, voltage-gated, type III, alpha subunit	9,85
GPM6B	516689_at	Glycoprotein M6B	25,81	*AGPAT1*	282137_at	1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase, alpha)	9,51
LOC781146	781146_at	Lysozyme	24,42	*DSC3*	281129_at	Desmocollin 3	9,45
CTGF	281103_at	Connective tissue growth factor	20,11	*NPPC*	281356_at	Natriuretic peptide C	9,45
AKR1B1	317748_at	Aldo-keto reductase family 1, member B1 (aldose reductase)	19,70	*SLC27A2*	535727_at	Solute carrier family 27 (fatty acid transporter), member 2	9,38
CCNB1	327679_at	Cyclin B1	19,56	*MIR95*	100313090_at	Microrna mir-95	9,32
LYZ1	281287_at	Lysozyme 1	16,45	*LIX1*	535033_at	Lix1 homolog (chicken)	8,88
PDZK1	534439_at	PDZ domain containing 1	16,34	*CA10*	535917_at	Carbonic anhydrase X	8,82
MCTP1	533635_at	Multiple C2 domains, transmembrane 1	15,14	*SLC31A2*	526609_at	Solute carrier family 31 (copper transporters), member 2	8,51
BPIFB1	282643_at	BPI fold containing family B, member 1	14,62	*KSR2*	617325_at	Kinase suppressor of ras 2	8,22
GJB5	524030_at	Gap junction protein, beta 5, 31.1kda	14,62	*UGT1A1*	751790_at	UDP glucuronosyltransferase 1 family, polypeptide A1	8,06
AGTR1	281607_at	Angiotensin II receptor, type 1	14,12	*MRPS36*	613835_at	Mitochondrial ribosomal protein S36	7,89
BSP3	317695_at	Binder of sperm 3	13,27	*ARHGAP44*	509257_at	Rho gtpase activating protein 44	7,84
LOC512293	512293_at	G2/mitotic-specific cyclin-B1-like	12,73	*LOC507891*	507891_at	Ankyrin repeat domain 26-like	7,78
GAT	280801_at	Glycine-N-acyltransferase-like	12,64	*BMP5*	507682_at	Bone morphogenetic protein 5	7,78
CPE	280753_at	Carboxypeptidase E	12,47	*TFF3*	517889_at	Trefoil factor 3 (intestinal)	7,62
CWH43	785528_at	Cell wall biogenesis 43 C-terminal homolog (S. Cerevisiae)	12,04	*THBS1*	281530_at	Thrombospondin 1	7,62
ATP13A5	509596_at	Atpase type 13A5	11,79	*SLC18A2*	282471_at	Solute carrier family 18 (vesicular monoamine), member 2	7,52
BLNK	510393_at	B-cell linker	11,71	*SMOC1*	508379_at	SPARC related modular calcium binding 1	7,26
NPL	507597_at	N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)	11,63	*DPP10*	617222_at	Dipeptidyl-peptidase 10 (non-functional)	7,21

LGR5	520189_at	Leucine-rich repeat containing G protein-coupled receptor 5	7,11	*FHOD1*	787862_at	Formin homology 2 domain containing 1	5,43
TFPI	508763_at	Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	7,11	*CYYR1*	768230_at	Cysteine/tyrosine-rich 1	5,28
LOC100850808	100850808_at	Elafin-like	6,92	*SLITRK5*	781586_at	SLIT and NTRK-like family, member 5	5,28
SUSD2	510458_at	Sushi domain containing 2	6,82	*MYO10*	281935_at	Myosin X	5,24
LOC515128	515128_at	Major facilitator superfamily domain-containing protein 4-like	6,59	*KIAA1644*	789734_at	KIAA1644 ortholog	5,13
AKAP5	281612_at	A kinase (PRKA) anchor protein 5	6,59	*CGREF1*	507586_at	Cell growth regulator with EF-hand domain 1	5,13
LOC100337001	100337001_at	Ankyrin repeat domain 26-like	6,54	*RTN4RL2*	529030_at	Reticulon 4 receptor-like 2	5,13
GRM8	538360_at	Glutamate receptor, metabotropic 8	6,50	*AK5*	613448_at	Adenylate kinase 5	5,06
SLC43A3	516840_at	Solute carrier family 43, member 3	6,50	*PHGDH*	505103_at	Phosphoglycerate dehydrogenase	4,99
CLDN8	538761_at	Claudin 8	6,41	*HEY1*	408005_at	Hairy/enhancer-of-split related with YRPW motif 1	4,96
SALL1	514467_at	Sal-like 1 (Drosophila)	6,41	*MYO1A*	281936_at	Myosin IA	4,96
MOCOS	281226_at	Molybdenum cofactor sulfurase	6,41	*LOC526200*	526200_at	Absent in melanoma 1 protein-like	4,96
MEGF10	539136_at	Multiple EGF-like-domains 10	6,32	*SULT1A1*	282485_at	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	4,89
NRCAM	534500_at	Neuronal cell adhesion molecule	6,15	*GM2A*	504524_at	GM2 ganglioside activator	4,86
CD44	281057_at	CD44 molecule (Indian blood group)	6,15	*FBN2*	540017_at	Fibrillin 2	4,86
MDK	280852_at	Midkine (neurite growth-promoting factor 2)	6,11	*KCNH7*	534542_at	Potassium voltage-gated channel, subfamily H (eag-related), member 7	4,76
C27H8orf4	617047_at	Chromosome 27 open reading frame, human c8orf4	6,11	*MUC15*	337919_at	Mucin 15, cell surface associated	4,76
LTF	280846_at	Lactotransferrin	6,06	*SEL1L3*	535060_at	Sel-1 suppressor of lin-12-like 3 (C. Elegans)	4,69
SLC26A3	512856_at	Solute carrier family 26, member 3	6,02	*LOC100337293*	100337293_at	Ankyrin repeat domain-containing protein 26-like	4,66
IGFBP3	282261_at	Insulin-like growth factor binding protein 3	5,94	*LOC508486*	508486_at	Serine/threonine-protein kinase/endoribonuclease IRE2-like	4,56
ANG	777597_at	Angiogenin, ribonuclease, rnase A family, 5	5,94	*PRR5L*	505048_at	Proline rich 5 like	4,56
OMG	407186_at	Oligodendrocyte myelin glycoprotein	5,94	*CXCL14*	511771_at	Chemokine (C-X-C motif) ligand 14	4,56
TRIM9	767615_at	Tripartite motif containing 9	5,86	*DAGLA*	523665_at	Diacylglycerol lipase, alpha	4,50
PROK2	387602_at	Prokineticin 2	5,78	*BPIFA1*	281989_at	BPI fold containing family A, member 1	4,47
FOLR1	539750_at	Folate receptor 1 (adult)	5,74	*SLC12A2*	286845_at	Solute carrier family 12 (sodium/potassium/chloride transporters), member 2	4,44
NKAIN1	618218_at	Na+/K+ transporting atpase interacting 1	5,66	*ELF5*	539420_at	E74-like factor 5 (ets domain transcription factor)	4,44
CSTB	512805_at	Cystatin B (stefin B)	5,50	*CLIC5*	281696_at	Chloride intracellular channel 5	4,44
SFRP2	510821_at	Secreted frizzled-related protein 2	5,43	*ABLIM2*	618227_at	Actin binding LIM protein family, member 2	4,35

GJB4	100140553_at	Gap junction protein, beta 4, 30.3kda	4,35	*GNAL*	100124520_at	Guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type	3,84
S100A4	282343_at	S100 calcium binding protein A4	4,35	*FAM105A*	534389_at	Family with sequence similarity 105, member A	3,84
IGF2BP2	519028_at	Insulin-like growth factor 2 mrna binding protein 2	4,32	*CEL*	280748_at	Carboxyl ester lipase (bile salt-stimulated lipase)	3,84
CLEC3B	515783_at	C-type lectin domain family 3, member B	4,29	*WIPF3*	786606_at	WAS/WASL interacting protein family, member 3	3,78
CHST4	539063_at	Carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 4	4,29	*PIK3R3*	286865_at	Phosphoinositide-3-kinase, regulatory subunit 3 (gamma)	3,76
NOSTRIN	521834_at	Nitric oxide synthase trafficker	4,26	*KIF5C*	538771_at	Kinesin family member 5C	3,73
UGT8	281566_at	UDP glycosyltransferase 8	4,20	*ATP1B2*	282562_at	Atpase, Na+/K+ transporting, beta 2 polypeptide	3,71
PDZK1IP1	613915_at	PDZK1 interacting protein 1	4,14	*MSX1*	286872_at	Msh homeobox 1	3,71
SEMA6A	516019_at	Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A	4,08	*MYH11*	530050_at	Myosin, heavy chain 11, smooth muscle	3,68
GPX3	281210_at	Glutathione peroxidase 3 (plasma)	4,08	*BCAT1*	505926_at	Branched chain amino-acid transaminase 1, cytosolic	3,66
GSTT3	516190_at	Glutathione S-transferase, theta 3	4,06	*ATP13A4*	521728_at	Atpase type 13A4	3,58
GNA14	281789_at	Guanine nucleotide binding protein (G protein), alpha 14	4,03	*ADCY7*	281603_at	Adenylate cyclase 7	3,58
MGAT3	520087_at	Mannosyl (beta-1,4-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase	4,03	*CAMK2B*	525416_at	Calcium/calmodulin-dependent protein kinase II beta	3,58
GAL3ST2	523830_at	Galactose-3-O-sulfotransferase 2	4,03	*FBLN1*	514588_at	Fibulin 1	3,56
TMEM45B	510305_at	Transmembrane protein 45B	4,03	*JAK3*	538276_at	Janus kinase 3	3,56
SVOPL	518832_at	SVOP-like	3,97	*KIAA0922*	505156_at	KIAA0922 ortholog	3,53
MKNK1	525647_at	MAP kinase interacting serine/threonine kinase 1	3,97	*HPN*	508148_at	Hepsin	3,51
PLA2G10	613966_at	Phospholipase A2, group X	3,97	*KCNIP3*	513316_at	Kv channel interacting protein 3, calsenilin	3,51
ATP2A3	512313_at	Atpase, Ca++ transporting, ubiquitous	3,94	*FAT4*	781683_at	FAT tumor suppressor homolog 4 (Drosophila)	3,51
LOC100295004	100295004_at	Uncharacterized LOC100295004	3,94	*RHOJ*	540619_at	Ras homolog gene family, member J	3,51
CACNA1A	282648_at	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	3,92	*CDK7*	515462_at	Cyclin-dependent kinase 7	3,48
BAI3	100337167_at	Brain-specific angiogenesis inhibitor 3	3,86	*GFPT2*	530101_at	Glutamine-fructose-6-phosphate transaminase 2	3,48
ABAT	280969_at	4-aminobutyrate aminotransferase	3,86	*SLC29A2*	531564_at	Solute carrier family 29 (nucleoside transporters), member 2	3,48

SLC7A5	282369_at	Solute carrier family 7 (amino acid transporter light chain, L system), member 5	3,48	*ENPP5*	512304_at	Ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative)	3,25
MSI1	527436_at	Musashi homolog 1 (Drosophila)	3,46	*DLC1*	511433_at	Deleted in liver cancer 1	3,25
GRIN2A	524212_at	Glutamate receptor, ionotropic, N-methyl D-aspartate 2A	3,46	*FGFR1*	281768_at	Fibroblast growth factor receptor 1	3,25
ACTG2	281595_at	Actin, gamma 2, smooth muscle, enteric	3,43	*FADS2*	521822_at	Fatty acid desaturase 2	3,23
PRG3	783660_at	Proteoglycan 3	3,43	*LOC100849501*	100849501_at	Uronyl 2-sulfotransferase-like	3,18
ABRACL	505914_at	ABRA C-terminal like	3,41	*PEBP4*	513254_at	Phosphatidylethanolamine-binding protein 4	3,16
SLC1A1	282353_at	Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	3,41	*SCP2*	508918_at	Sterol carrier protein 2	3,16
SAA3	281474_at	Serum amyloid A 3	3,41	*LOC512464*	512464_at	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2-like	3,16
B3GNT3	784997_at	UDP-glcnac:betagal beta-1,3-N-acetylglucosaminyltransferase 3	3,39	*GLUD1*	281785_at	Glutamate dehydrogenase 1	3,14
LOC100296081	100296081_at	Uncharacterized LOC100296081	3,36	*MTHFR*	497032_at	Methylenetetrahydrofolate reductase (NAD(P)H)	3,14
ATF7IP2	529852_at	Activating transcription factor 7 interacting protein 2	3,36	*TSKU*	529661_at	Tsukushi small leucine rich proteoglycan homolog (Xenopus laevis)	3,12
SERTM1	100297762_at	Serine-rich and transmembrane domain containing 1	3,36	*RHOBTB3*	530930_at	Rho-related BTB domain containing 3	3,10
HOXB8	785855_at	Homeobox B8	3,34	*PLCB1*	287026_at	Phospholipase C, beta 1 (phosphoinositide-specific)	3,10
ATP6V0A4	518974_at	Atpase, H+ transporting, lysosomal V0 subunit a4	3,32	*LOC100302389*	100302389_at	Uncharacterized LOC100302389	3,10
SNTB1	617927_at	Syntrophin, beta 1 (dystrophin-associated protein A1, 59kda, basic component 1)	3,29	*ALK*	536642_at	Anaplastic lymphoma receptor tyrosine kinase	3,07
NSG1	523110_at	Neuron specific gene family member 1	3,29	*ITGB3*	282642_at	Integrin, beta 3 (platelet glycoprotein iiia, antigen CD61)	3,07
FBN1	281154_at	Fibrillin 1	3,29	*ZNF385D*	789528_at	Zinc finger protein 385D	3,03
PDXK	514168_at	Pyridoxal (pyridoxine, vitamin B6) kinase	3,27	*KCNH8*	100336609_at	Potassium voltage-gated channel, subfamily H (eag-related), member 8	3,01
ENPP1	615535_at	Ectonucleotide pyrophosphatase/phosphodiesterase 1	3,27	*ADAMTS18*	518395_at	ADAM metallopeptidase with thrombospondin type 1 motif, 18	2,99
TOX	525888_at	Thymocyte selection-associated high mobility group box	3,27	*TMEM213*	510137_at	Transmembrane protein 213	2,99
ST8SIA5	497020_at	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 5	3,25	*ATP1B1*	519758_at	ATPase, Na+/K+ transporting, beta 1 polypeptide	2,97

PKHD1	537895_at	Polycystic kidney and hepatic disease 1 (autosomal recessive)	2,97	*SLC1A4*	326577_at	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	2,81
ENPP4	538583_at	Ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative)	2,97	*SEMA3D*	536417_at	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D	2,81
SLC28A3	508028_at	Solute carrier family 28 (sodium-coupled nucleoside transporter), member 3	2,95	*SERTAD4*	614583_at	SERTA domain containing 4	2,79
WIF1	533672_at	WNT inhibitory factor 1	2,95	*TXNDC17*	404159_at	Thioredoxin domain containing 17	2,79
RNASE4	616089_at	Ribonuclease, rnase A family, 4	2,95	*SLC30A4*	540869_at	Solute carrier family 30 (zinc transporter), member 4	2,79
LOC528262	528262_at	Intestinal alkaline phosphatase VI	2,93	*CDC25A*	520188_at	Cell division cycle 25 homolog A (S. Pombe)	2,77
GBP4	613313_at	Guanylate binding protein 4	2,93	*SLC24A6*	508887_at	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 6	2,75
OR9Q2	510573_at	Olfactory receptor, family 9, subfamily Q, member 2	2,93	*SERPINF1*	281386_at	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	2,75
CDKN1C	510972_at	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	2,91	*CABYR*	510319_at	Calcium binding tyrosine-(Y)-phosphorylation regulated	2,73
ACVR1C	536380_at	Activin A receptor, type IC	2,91	*FBLN5*	535185_at	Fibulin 5	2,73
FOLR1	516067_at	Folate receptor 1 (adult)	2,91	*BPIFA2C*	618389_at	BPI fold containing family A, member 2C	2,73
C10H5orf13	100125763_at	Chromosome 10 open reading frame, human c5orf13	2,89	*EFHD1*	522462_at	EF-hand domain family, member D1	2,73
AKAP12	513774_at	A kinase (PRKA) anchor protein 12	2,89	*SPTBN2*	100336865_at	Spectrin, beta, non-erythrocytic 2	2,71
FAM131B	617268_at	Family with sequence similarity 131, member B	2,87	*ALOX12*	407169_at	Arachidonate 12-lipoxygenase	2,69
LDLR	281276_at	Low density lipoprotein receptor	2,87	*SPON2*	513844_at	Spondin 2, extracellular matrix protein	2,69
RASEF	513223_at	RAS and EF-hand domain containing	2,85	*RAB25*	506482_at	RAB25, member RAS oncogene family	2,69
MGAT4C	539661_at	Mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme C (putative)	2,85	*RGNEF*	616969_at	190 kda guanine nucleotide exchange factor	2,68
GHR	280805_at	Growth hormone receptor	2,85	*CLDN4*	414921_at	Claudin 4	2,66
LRRC3	506054_at	Leucine rich repeat containing 3	2,83	*TMEM35*	533337_at	Transmembrane protein 35	2,66
SLC5A9	526890_at	Solute carrier family 5 (sodium/glucose cotransporter), member 9	2,83	*OGDH*	534599_at	Oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	2,66
GPR64	100299135_at	G protein-coupled receptor 64	2,83	*ACOX3*	510065_at	Acyl-coa oxidase 3, pristanoyl	2,64
38231	538801_at	Septin 4	2,83	*GRHL1*	617248_at	Grainyhead-like 1 (Drosophila)	2,64

ARHGDIG	613745_at	Rho GDP dissociation inhibitor (GDI) gamma	2,64	*PCSK6*	524684_at	Proprotein convertase subtilisin/kexin type 6	2,48
CYB5B	506370_at	Cytochrome b5 type B (outer mitochondrial membrane)	2,64	*SPRY1*	507095_at	Sprouty homolog 1, antagonist of FGF signaling (Drosophila)	2,48
FLRT2	539905_at	Leucine-rich repeat transmembrane protein FLRT2-like	2,64	*SETD7*	515928_at	SET domain containing (lysine methyltransferase) 7	2,48
MPND	512718_at	MPN domain containing	2,64	*RGMB*	540954_at	RGM domain family, member B	2,48
BCL2L15	509786_at	BCL2-like 15	2,64	*SLC34A2*	282484_at	Solute carrier family 34 (sodium phosphate), member 2	2,48
CLIP4	515213_at	CAP-GLY domain containing linker protein family, member 4	2,64	*ANG2*	783907_at	Angiogenin 2	2,48
TMEM51	514936_at	Transmembrane protein 51	2,62	*KLK12*	618448_at	Kallikrein-related peptidase 12	2,48
SDC1	529759_at	Syndecan 1	2,62	*SNAP91*	516917_at	Synaptosomal-associated protein, 91kda homolog (mouse)	2,48
SGK2	517909_at	Serum/glucocorticoid regulated kinase 2	2,62	*CDA*	616377_at	Cytidine deaminase	2,46
ARSJ	540514_at	Arylsulfatase family, member J	2,60	*CYP51A1*	505060_at	Cytochrome P450, family 51, subfamily A, polypeptide 1	2,46
SYTL5	536666_at	Synaptotagmin-like 5	2,60	*APOBEC3B*	504505_at	Apolipoprotein B mrna editing enzyme, catalytic polypeptide-like 3B	2,45
TM7SF2	282384_at	Transmembrane 7 superfamily member 2	2,60	*BICC1*	537799_at	Bicaudal C homolog 1 (Drosophila)	2,45
NOS1	536132_at	Nitric oxide synthase 1 (neuronal)	2,60	*ACP5*	517002_at	Acid phosphatase 5, tartrate resistant	2,45
ABHD1	510774_at	Abhydrolase domain containing 1	2,58	*MPPED2*	540914_at	Metallophosphoesterase domain containing 2	2,43
ARHGDIB	327676_at	Rho GDP dissociation inhibitor (GDI) beta	2,58	*PMVK*	513533_at	Phosphomevalonate kinase	2,43
DHRS4	281360_at	Dehydrogenase/reductase (SDR family) member 4	2,57	*RFFL*	530263_at	Ring finger and FYVE-like domain containing 1	2,43
COL6A6	530102_at	Collagen, type VI, alpha 6	2,57	*GRB14*	497029_at	Growth factor receptor-bound protein 14	2,43
ELMOD1	768233_at	ELMO/CED-12 domain containing 1	2,57	*VIM*	280955_at	Vimentin	2,43
A2ML1	516769_at	Alpha-2-macroglobulin-like 1	2,57	*TMTC2*	100337258_at	Transmembrane and tetratricopeptide repeat containing 2	2,41
TACC1	507012_at	Transforming, acidic coiled-coil containing protein 1	2,53	*MFSD2A*	512633_at	Major facilitator superfamily domain containing 2A	2,41
LOC783893	783893_at	Ankyrin repeat domain-containing protein 26-like	2,51	*TMPRSS2*	511037_at	Transmembrane protease, serine 2	2,41
CITED4	504742_at	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 4	2,50	*SIK1*	100337254_at	Salt-inducible kinase 1	2,41
BCL2	281020_at	B-cell CLL/lymphoma 2	2,50	*MIF*	280858_at	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	2,41
GUCY1A3	281216_at	Guanylate cyclase 1, soluble, alpha 3	2,48	*C6H4orf19*	511424_at	Chromosome 6 open reading frame, human c4orf19	2,39

CD320	505043_at	CD320 molecule	2,39	*CENPH*	505284_at	Centromere protein H	2,30
TRIB1	521857_at	Tribbles homolog 1 (Drosophila)	2,36	*FMNL2*	788312_at	Formin-like 2	2,30
GJB3	539935_at	Gap junction protein, beta 3, 31kda	2,36	*DCTPP1*	614103_at	Dctp pyrophosphatase 1	2,28
LOC100125412	100125412_at	Differential display clone 8	2,36	*OR6Q1*	511777_at	Olfactory receptor, family 6, subfamily Q, member 1	2,27
RARRES2	508990_at	Retinoic acid receptor responder (tazarotene induced) 2	2,36	*PROM2*	520936_at	Prominin 2	2,27
TMED6	533277_at	Transmembrane emp24 protein transport domain containing 6	2,36	*LOC100335642*	100335642_at	Zinc finger protein 177-like	2,27
SLC43A1	614153_at	Solute carrier family 43, member 1	2,35	*VOPP1*	767861_at	Vesicular, overexpressed in cancer, prosurvival protein 1	2,27
ITGB4	506995_at	Integrin, beta 4	2,35	*ZNF821*	532668_at	Zinc finger protein 821	2,27
RGS11	521040_at	Regulator of G-protein signaling 11	2,35	*CDCA7*	614893_at	Cell division cycle associated 7	2,27
ANAPC4	514572_at	Anaphase promoting complex subunit 4	2,35	*RABEP1*	504785_at	Rabaptin, RAB gtpase binding effector protein 1	2,25
SEMA5A	506636_at	Sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A	2,35	*ADAMTS16*	520133_at	ADAM metallopeptidase with thrombospondin type 1 motif, 16	2,25
PTGFRN	538209_at	Prostaglandin F2 receptor negative regulator	2,35	*ABCA1*	535379_at	ATP-binding cassette, sub-family A (ABC1), member 1	2,25
COL12A1	359712_at	Collagen, type XII, alpha 1	2,33	*PHLDA2*	618810_at	Pleckstrin homology-like domain, family A, member 2	2,25
INSIG1	511899_at	Insulin induced gene 1	2,33	*FAM3D*	514459_at	Family with sequence similarity 3, member D	2,23
PRDM1	538384_at	PR domain containing 1, with ZNF domain	2,33	*FAM13C*	540918_at	Family with sequence similarity 13, member C	2,23
GATSL3	506974_at	GATS protein-like 3	2,33	*ZSWIM5*	540778_at	Zinc finger, SWIM-type containing 5	2,23
STAP2	505456_at	Signal transducing adaptor family member 2	2,31	*FDFT1*	281767_at	Farnesyl-diphosphate farnesyltransferase 1	2,23
PRRX1	540901_at	Paired related homeobox 1	2,31	*TCF7*	782690_at	Transcription factor 7 (T-cell specific, HMG-box)	2,22
GSN	535077_at	Gelsolin	2,31	*EFCAB4B*	525377_at	EF-hand calcium binding domain 4B	2,22
KIAA1456	510845_at	Putative methyltransferase KIAA1456 homolog	2,31	*SLC38A7*	513110_at	Solute carrier family 38, member 7	2,22
RGS14	532605_at	Regulator of G-protein signaling 14	2,31	*AFMID*	518864_at	Arylformamidase	2,22
RASA4	521224_at	RAS p21 protein activator 4	2,30	*GCA*	507139_at	Grancalcin, EF-hand calcium binding protein	2,22
FAM35A	508335_at	Family with sequence similarity 35, member A	2,30	*LOC100139888*	100139888_at	Heterogeneous nuclear ribonucleoproteins C1/C2-like	2,22
APOPT1	617441_at	Apoptogenic 1, mitochondrial	2,30	*SCNN1A*	282348_at	Sodium channel, non-voltage-gated 1 alpha subunit	2,20

FREM3	781161_at	FRAS1 related extracellular matrix 3	2,20	*NXPE3*	532838_at	Neurexophilin and PC-esterase domain family, member 3	2,11
LOC100335918	100335918_at	Autism susceptibility gene 2 protein-like	2,19	*SQLE*	526535_at	Squalene epoxidase	2,11
ST6GALNAC2	511690_at	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	2,17	*ROBO1*	536815_at	Roundabout, axon guidance receptor, homolog 1 (Drosophila)	2,11
HYAL1	515397_at	Hyaluronoglucosaminidase 1	2,17	*MYCN*	616888_at	V-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	2,11
BAIAP2L2	617924_at	BAI1-associated protein 2-like 2	2,17	*FIGN*	540478_at	Fidgetin	2,10
PDE8B	100337124_at	Phosphodiesterase 8B	2,17	*CAP2*	515190_at	CAP, adenylate cyclase-associated protein, 2 (yeast)	2,10
UPK3BL	617471_at	Uroplakin 3B-like	2,17	*UNC5B*	524942_at	Unc-5 homolog B (C. Elegans)	2,10
WNT5A	530005_at	Wingless-type MMTV integration site family, member 5A	2,17	*IDI1*	514293_at	Isopentenyl-diphosphate delta isomerase 1	2,10
SLC30A5	508169_at	Solute carrier family 30 (zinc transporter), member 5	2,16	*DAAM2*	783665_at	Dishevelled associated activator of morphogenesis 2	2,10
ACSL3	100138312_at	Acyl-coa synthetase long-chain family member 3	2,16	*C29H11orf75*	614071_at	Chromosome 29 open reading frame, human c11orf75	2,08
POLE2	518653_at	Polymerase (DNA directed), epsilon 2 (p59 subunit)	2,16	*ECI2*	505355_at	Enoyl-coa delta isomerase 2	2,08
SIDT1	508259_at	SID1 transmembrane family, member 1	2,16	*LOC537017*	537017_at	Cytidine monophosphate-N-acetylneuraminic acid hydroxylase-like	2,08
MECOM	532209_at	MDS1 and EVI1 complex locus	2,14	*GUCY1B3*	282433_at	Guanylate cyclase 1, soluble, beta 3	2,07
LIFR	539504_at	Leukemia inhibitory factor receptor alpha	2,14	*ZFYVE9*	613428_at	Zinc finger, FYVE domain containing 9	2,07
TCF7L1	515303_at	Transcription factor 7-like 1 (T-cell specific, HMG-box)	2,14	*LGALS4*	614804_at	Lectin, galactoside-binding, soluble, 4	2,07
VIPR2	790124_at	Vasoactive intestinal peptide receptor 2	2,14	*AKAP2*	614497_at	A kinase (PRKA) anchor protein 2	2,07
CREB3L1	513105_at	Camp responsive element binding protein 3-like 1	2,14	*CPNE2*	782388_at	Copine II	2,07
SEMA4D	785942_at	Sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D	2,13	*CHAD*	281069_at	Chondroadherin	2,07
CAPN1	281661_at	Calpain 1, (mu/I) large subunit	2,13	*CNRIP1*	539715_at	Cannabinoid receptor interacting protein 1	2,07
RNF180	540391_at	Ring finger protein 180	2,13	*PCDH1*	509388_at	Protocadherin 1	2,06
CXHXorf69	100302527_at	Uncharacterized LOC100302527	2,13	*SAT1*	508861_at	Spermidine/spermine N1-acetyltransferase 1	2,06
AP1S3	540693_at	Adaptor-related protein complex 1, sigma 3 subunit	2,11	*PKDCC*	539467_at	Protein kinase domain containing, cytoplasmic homolog (mouse)	2,06
HMGCS1	407767_at	HMGCS1 protein-like	2,11	*ORAI2*	511233_at	ORAI calcium release-activated calcium modulator 2	2,06

GLT1D1	516510_at	Glycosyltransferase 1 domain containing 1	2,06	*FZD5*	538536_at	Frizzled family receptor 5	2,00
SH2D4A	506242_at	SH2 domain containing 4A	2,06	*VAV3*	521961_at	Vav 3 guanine nucleotide exchange factor	1,99
POR	532512_at	P450 (cytochrome) oxidoreductase	2,06	*MARK2*	535197_at	MAP/microtubule affinity-regulating kinase 2	1,99
GCNT2	613924_at	Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group)	2,04	*GCNT2*	520336_at	Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group)	1,99
GALNT10	787200_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10 (galnac-T10)	2,04	*LOC785630*	785630_at	Zinc finger protein 480-like	1,99
ESR2	281146_at	Estrogen receptor 2 (ER beta)	2,04	*MMP23B*	527590_at	Matrix metallopeptidase 23B	1,99
NET1	507365_at	Neuroepithelial cell transforming 1	2,03	*CAPG*	353121_at	Capping protein (actin filament), gelsolin-like	1,99
NRTN	525562_at	Neurturin	2,03	*HMCN1*	521326_at	Hemicentin 1	1,99
RAPGEFL1	618568_at	Rap guanine nucleotide exchange factor (GEF)-like 1	2,03	*NUPR1*	614673_at	Nuclear protein, transcriptional regulator, 1	1,99
AFAP1	534032_at	Actin filament associated protein 1	2,03	*SLC1A5*	282355_at	Solute carrier family 1 (neutral amino acid transporter), member 5	1,97
MSMO1	504481_at	Methylsterol monooxygenase 1	2,03	*LOC510078*	510078_at	Zinc finger protein 354A-like	1,97
PCK2	282856_at	Phosphoenolpyruvate carboxykinase 2 (mitochondrial)	2,03	*GRN*	767942_at	Granulin	1,97
KCTD15	512578_at	Potassium channel tetramerisation domain containing 15	2,01	*WIPI1*	528410_at	WD repeat domain, phosphoinositide interacting 1	1,97
ZNF235	504436_at	Zinc finger protein 235	2,01	*KIAA1671*	533883_at	KIAA1671 ortholog	1,97
BAMBI	530147_at	BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)	2,01	*MATN1*	512059_at	Matrilin 1, cartilage matrix protein	1,97
NLGN3	511251_at	Neuroligin 3	2,01	*C18H16orf74*	613483_at	Chromosome 18 open reading frame, human c16orf74	1,96
RNF122	510037_at	Ring finger protein 122	2,01	*PLD1*	514554_at	Phospholipase D1, phosphatidylcholine-specific	1,96
ALS2	535750_at	Amyotrophic lateral sclerosis 2 (juvenile)	2,01	*ARHGEF26*	531741_at	Rho guanine nucleotide exchange factor (GEF) 26	1,96
GALE	523154_at	UDP-galactose-4-epimerase	2,00	*SH3PXD2A*	100299286_at	SH3 and PX domains 2A	1,96
ANKIB1	505204_at	Ankyrin repeat and IBR domain containing 1	2,00	*SYT7*	540850_at	Synaptotagmin VII	1,96
SLC16A3	510085_at	Solute carrier family 16, member 3 (monocarboxylic acid transporter 4)	2,00	*C8H9orf64*	767897_at	Chromosome 8 open reading frame, human c9orf64	1,95
C28H10orf116	613941_at	Chromosome 28 open reading frame, human c10orf116	2,00	*ADCK1*	533372_at	Aarf domain containing kinase 1	1,95

SPARCL1	507537_at	SPARC-like 1 (hevin)	1,95	*RNF43*	784035_at	Ring finger protein 43	1,88
GADD45B	618405_at	Growth arrest and DNA-damage-inducible, beta	1,95	*COL5A1*	537387_at	Collagen, type V, alpha 1	1,88
C6H4orf32	767842_at	Chromosome 6 open reading frame, human c4orf32	1,95	*TSTA3*	513158_at	Tissue specific transplantation antigen P35B	1,87
CCDC101	510859_at	Coiled-coil domain containing 101	1,93	*SETX*	534284_at	Senataxin	1,87
CRTC1	510465_at	CREB regulated transcription coactivator 1	1,93	*PPFIA3*	505162_at	Protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 3	1,87
FER1L6	527366_at	Fer-1-like 6 (C. Elegans)	1,93	*FAM134B*	540068_at	Family with sequence similarity 134, member B	1,87
ARMC12	540812_at	Armadillo repeat containing 12	1,93	*AGT*	527114_at	Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	1,87
SLC15A1	521181_at	Solute carrier family 15 (oligopeptide transporter), member 1	1,93	*SH3PXD2B*	518356_at	SH3 and PX domains 2B	1,87
LOC784650	784650_at	Uncharacterized LOC784650	1,92	*KIAA2013*	506454_at	Kiaa2013	1,87
TTC39C	532895_at	Tetratricopeptide repeat protein 39C-like	1,92	*ENPP2*	532663_at	Ectonucleotide pyrophosphatase/phosphodiesterase 2	1,85
LOC783195	783195_at	Ribonuclease 4-like	1,92	*PTCH2*	507948_at	Patched 2	1,85
CFI	513197_at	Complement factor I	1,92	*MRPL19*	510957_at	Mitochondrial ribosomal protein L19	1,85
CLN3	504799_at	Ceroid-lipofuscinosis, neuronal 3	1,91	*PLLP*	613446_at	Plasmolipin	1,85
CDK2AP2	517206_at	Cyclin-dependent kinase 2 associated protein 2	1,91	*PTPRS*	537480_at	Protein tyrosine phosphatase, receptor type, S	1,85
CHKA	514865_at	Choline kinase alpha	1,91	*PDZRN3*	509083_at	PDZ domain containing ring finger 3	1,85
RAI2	539414_at	Retinoic acid induced 2	1,91	*FANCL*	614512_at	Fanconi anemia, complementation group L	1,84
GDPD1	615890_at	Glycerophosphodiester phosphodiesterase domain containing 1	1,91	*ARHGEF38*	618404_at	Rho guanine nucleotide exchange factor (GEF) 38	1,84
ANKRD5	788870_at	Ankyrin repeat domain 5	1,91	*PLD2*	522159_at	Phospholipase D2	1,84
LOC534520	534520_at	Spermine synthase-like	1,91	*CLCN4*	511699_at	Chloride channel 4	1,84
GCK	616576_at	Glucokinase (hexokinase 4)	1,89	*DSG3*	529902_at	Desmoglein 3	1,84
ACSF2	768237_at	Acyl-coa synthetase family member 2	1,89	*FRYL*	511059_at	FRY-like	1,83
IQSEC1	521541_at	IQ motif and Sec7 domain 1	1,89	*BAG5*	522854_at	BCL2-associated athanogene 5	1,83
CEACAM1	404118_at	Carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)	1,89	*NIPA1*	539162_at	Non imprinted in Prader-Willi/Angelman syndrome 1	1,83
VSTM2A	782902_at	V-set and transmembrane domain containing 2A	1,89	*LIMK2*	513539_at	LIM domain kinase 2	1,83
XXYLT1	533703_at	Xyloside xylosyltransferase 1	1,88	*CLDN23*	514634_at	Claudin 23	1,83

HEXIM1	539696_at	Hexamethylene bis-acetamide inducible 1	1,83	*ATAT1*	786491_at	Alpha tubulin acetyltransferase 1	1,78
TIMP2	282093_at	TIMP metallopeptidase inhibitor 2	1,83	*LCP1*	540990_at	Lymphocyte cytosolic protein 1 (L-plastin)	1,78
AMACR	540376_at	Alpha-methylacyl-coa racemase	1,83	*SATB1*	516952_at	SATB homeobox 1	1,78
PPM1L	541235_at	Protein phosphatase, Mg2+/Mn2+ dependent, 1L	1,82	*RWDD4*	509865_at	RWD domain containing 4	1,78
SUN2	618392_at	Sad1 and UNC84 domain containing 2	1,82	*SNED1*	514207_at	Sushi, nidogen and EGF-like domains 1	1,78
ROBO2	534842_at	Roundabout, axon guidance receptor, homolog 2 (Drosophila)	1,82	*UCK2*	541028_at	Uridine-cytidine kinase 2	1,78
HSD17B10	281809_at	Hydroxysteroid (17-beta) dehydrogenase 10	1,82	*LOC618369*	618369_at	Lactosylceramide 4-alpha-galactosyltransferase-like	1,78
NEK6	515816_at	NIMA (never in mitosis gene a)-related kinase 6	1,82	*KCNN2*	404177_at	Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2	1,78
KIAA2022	512493_at	KIAA2022 ortholog	1,82	*MIR2470*	100313445_at	Microrna mir-2470	1,78
IGIP	404059_at	Iga regulatory protein	1,80	*ABTB2*	528597_at	Ankyrin repeat and BTB (POZ) domain containing 2	1,78
MAPK3	531391_at	Mitogen-activated protein kinase 3	1,80	*RIN2*	537459_at	Ras and Rab interactor 2	1,77
SH3BP2	617344_at	SH3-domain binding protein 2	1,80	*PSEN1*	282705_at	Presenilin 1	1,77
GAA	280798_at	Glucosidase, alpha; acid	1,80	*ARHGEF15*	512021_at	Rho guanine nucleotide exchange factor (GEF) 15	1,77
BCAR1	527550_at	Breast cancer anti-estrogen resistance 1	1,80	*SLC19A2*	532860_at	Solute carrier family 19 (thiamine transporter), member 2	1,77
ITFG3	507493_at	Integrin alpha FG-GAP repeat containing 3	1,80	*PTPRB*	505696_at	Protein tyrosine phosphatase, receptor type, B	1,77
SLC35A3	520918_at	Solute carrier family 35 (UDP-N-acetylglucosamine (UDP-glcnac) transporter), member A3	1,80	*BCL2L1*	282152_at	BCL2-like 1	1,77
GINS1	523427_at	GINS complex subunit 1 (Psf1 homolog)	1,79	*CELSR2*	538194_at	Cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila)	1,77
CDC34	616156_at	Cell division cycle 34 homolog (S. Cerevisiae)	1,79	*MYCL1*	540350_at	V-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian)	1,77
SMPDL3B	518699_at	Sphingomyelin phosphodiesterase, acid-like 3B	1,79	*USP54*	100336042_at	Ubiquitin specific peptidase 54-like	1,77
HMGA1	618849_at	High mobility group AT-hook 1	1,79	*COL27A1*	513668_at	Collagen, type XXVII, alpha 1	1,77
TPCN1	510830_at	Two pore segment channel 1	1,79	*PPARGC1A*	338446_at	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	1,77
TACR3	404136_at	Tachykinin receptor 3	1,79	*FAM125B*	617416_at	Family with sequence similarity 125, member B	1,77
ARAF	540421_at	V-raf murine sarcoma 3611 viral oncogene homolog	1,79	*KLF2*	520939_at	Kruppel-like factor 2 (lung)	1,77

DLG5	535699_at	Discs, large homolog 5 (Drosophila)	1,75	*MIR2471*	100313238_at	Microrna mir-2471	1,73
PMEPA1	617469_at	Prostate transmembrane protein, androgen induced 1	1,75	*RASAL1*	512872_at	RAS protein activator like 1 (GAP1 like)	1,73
TMLHE	535630_at	Trimethyllysine hydroxylase, epsilon	1,75	*LOC512271*	512271_at	Protein tweety homolog 3-like	1,73
PLEKHM1	523424_at	Pleckstrin homology domain containing, family M (with RUN domain) member 1	1,75	*GAS2*	614840_at	Growth arrest-specific 2	1,73
EPHB2	535137_at	EPH receptor B2	1,75	*PTCH1*	520994_at	Patched 1	1,72
HECW1	514243_at	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1	1,75	*ATP8A1*	317692_at	Atpase, aminophospholipid transporter (APLT), class I, type 8A, member 1	1,72
SWAP70	533720_at	SWAP switching B-cell complex 70kda subunit	1,75	*FASN*	281152_at	Fatty acid synthase	1,72
FHOD3	785433_at	Formin homology 2 domain containing 3	1,75	*YPEL3*	787498_at	Yippee-like 3 (Drosophila)	1,71
CSRP2BP	541019_at	CSRP2 binding protein	1,74	*LYN*	534996_at	V-yes-1 Yamaguchi sarcoma viral related oncogene homolog	1,71
KRTCAP3	508550_at	Keratinocyte associated protein 3	1,74	*EAF1*	507577_at	ELL associated factor 1	1,71
SOX17	534010_at	SRY (sex determining region Y)-box 17	1,74	*TMEM54*	509773_at	Transmembrane protein 54	1,71
APBA1	515571_at	Amyloid beta (A4) precursor protein-binding, family A, member 1	1,74	*SLC37A1*	511558_at	Solute carrier family 37 (glycerol-3-phosphate transporter), member 1	1,71
CDC42EP5	618745_at	CDC42 effector protein (Rho gtpase binding) 5	1,74	*ABCC1*	281588_at	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	1,71
RNF183	539200_at	Ring finger protein 183	1,74	*PLXNB1*	616798_at	Plexin B1	1,69
MIEN1	505710_at	Migration and invasion enhancer 1	1,74	*TRPM4*	100295930_at	Transient receptor potential cation channel, subfamily M, member 4	1,69
FAM78A	506709_at	Family with sequence similarity 78, member A	1,74	*CNOT1*	533968_at	CCR4-NOT transcription complex, subunit 1	1,69
SEPHS2	512060_at	Selenophosphate synthetase 2	1,74	*SYVN1*	508358_at	Synovial apoptosis inhibitor 1, synoviolin	1,69
MIR2406	100313357_at	Microrna mir-2406	1,74	*AGAP1*	522241_at	Arfgap with gtpase domain, ankyrin repeat and PH domain 1	1,69
HOXA2	524150_at	Homeobox A2	1,74	*TMEM204*	615464_at	Transmembrane protein 204	1,69
TTC39A	527172_at	Tetratricopeptide repeat domain 39A	1,73	*GADD45A*	505463_at	Growth arrest and DNA-damage-inducible, alpha	1,69
LLGL2	539545_at	Lethal giant larvae homolog 2 (Drosophila)	1,73	*TRPV4*	540259_at	Transient receptor potential cation channel, subfamily V, member 4	1,69
TMEM79	513599_at	Transmembrane protein 79	1,73	*SEPX1*	618441_at	Selenoprotein X, 1	1,69
NFAT5	538523_at	Nuclear factor of activated T-cells 5, tonicity-responsive	1,73	*FOXA3*	503622_at	Forkhead box A3	1,69
CRYBG3	516526_at	Beta-gamma crystallin domain containing 3	1,73	*SCUBE1*	523518_at	Signal peptide, CUB domain, EGF-like 1	1,69
SLMO1	616292_at	Slowmo homolog 1 (Drosophila)	1,73	*PROM1*	618054_at	Prominin 1	1,69

OSBPL10	507708_at	Oxysterol binding protein-like 10	1,68	*LOC614741*	614741_at	Formin-2-like	1,66
NAV2	100139508_at	Neuron navigator 2	1,68	*RPAIN*	618324_at	RPA interacting protein	1,65
KBTBD3	524207_at	Kelch repeat and BTB (POZ) domain containing 3	1,68	*SORBS1*	504625_at	Sorbin and SH3 domain containing 1	1,65
SIRPA	327666_at	Signal-regulatory protein alpha	1,68	*PPAP2C*	504545_at	Phosphatidic acid phosphatase type 2C	1,65
TMC7	785640_at	Transmembrane channel-like 7	1,68	*ABHD2*	508717_at	Abhydrolase domain containing 2	1,65
ELL2	782605_at	Elongation factor, RNA polymerase II, 2	1,68	*RSPRY1*	538571_at	Ring finger and SPRY domain containing 1	1,65
NXNL2	530279_at	Nucleoredoxin-like 2	1,68	*AUTS2*	615936_at	Autism susceptibility candidate 2	1,65
ALOX12B	504803_at	Arachidonate 12-lipoxygenase, 12R type	1,68	*NUAK1*	519892_at	NUAK family, SNF1-like kinase, 1	1,65
DSC2	281128_at	Desmocollin 2	1,67	*VAMP1*	513621_at	Vesicle-associated membrane protein 1 (synaptobrevin 1)	1,65
TMEM38B	615646_at	Transmembrane protein 38B	1,67	*C4H7orf41*	615685_at	Chromosome 4 open reading frame, human c7orf41	1,65
RMI1	614063_at	RMI1, recq mediated genome instability 1, homolog (S. Cerevisiae)	1,67	*SIPA1*	508234_at	Signal-induced proliferation-associated 1	1,65
PLAGL1	539761_at	Pleiomorphic adenoma gene-like 1	1,67	*QPRT*	614254_at	Quinolinate phosphoribosyltransferase	1,64
LBH	616148_at	Limb bud and heart development homolog (mouse)	1,67	*LOC100138354*	100138354_at	Uncharacterized LOC100138354	1,64
LPCAT3	515361_at	Lysophosphatidylcholine acyltransferase 3	1,67	*WWP2*	512457_at	WW domain containing E3 ubiquitin protein ligase 2	1,64
SMARCC1	522045_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 1	1,66	*AMDHD2*	521401_at	Amidohydrolase domain containing 2	1,64
ECHDC2	513795_at	Enoyl coa hydratase domain containing 2	1,66	*SULT1C4*	783020_at	Sulfotransferase family, cytosolic, 1C, member 4	1,64
FAM111B	509351_at	Family with sequence similarity 111, member B	1,66	*COL6A3*	530657_at	Collagen, type VI, alpha 3	1,64
SEC14L1	513449_at	SEC14-like 1 (S. Cerevisiae)	1,66	*SLC5A6*	516021_at	Solute carrier family 5 (sodium-dependent vitamin transporter), member 6	1,64
TMEM8A	508215_at	Transmembrane protein 8A	1,66	*HDAC4*	517559_at	Histone deacetylase 4	1,62
BSG	508716_at	Basigin (Ok blood group)	1,66	*FAM69C*	517668_at	Family with sequence similarity 69, member C	1,62
PLCG1	281987_at	Phospholipase C, gamma 1	1,66	*LAMTOR1*	614849_at	Late endosomal/lysosomal adaptor, MAPK and MTOR activator 1	1,62
MUM1L1	539380_at	Melanoma associated antigen (mutated) 1-like 1	1,66	*MERTK*	504429_at	C-mer proto-oncogene tyrosine kinase	1,62
TOR1B	533928_at	Torsin family 1, member B (torsin B)	1,66	*CAPN11*	527966_at	Calpain 11	1,62
C10H14orf105	614197_at	Chromosome 10 open reading frame, human c14orf105	1,66	*NUDCD3*	533678_at	Nudc domain containing 3	1,62
OSBPL8	533350_at	Oxysterol binding protein-like 8	1,66	*CUBN*	523202_at	Cubilin (intrinsic factor-cobalamin receptor)	1,62

IL6R	507359_at	Interleukin 6 receptor	1,62	*GIPC1*	519617_at	GIPC PDZ domain containing family, member 1	1,60
IGFBP4	282262_at	Insulin-like growth factor binding protein 4	1,62	*PLXNC1*	518168_at	Plexin C1	1,60
FAM108C1	520956_at	Family with sequence similarity 108, member C1	1,61	*LOC510723*	510723_at	Probable phospholipid-transporting atpase VA-like	1,60
ZNF238	538793_at	Zinc finger protein 238	1,61	*GIPC2*	518246_at	GIPC PDZ domain containing family, member 2	1,60
ARHGEF37	526631_at	Rho guanine nucleotide exchange factor (GEF) 37	1,61	*MS4A8B*	415111_at	Membrane-spanning 4-domains, subfamily A, member 8B	1,59
AKAP8	522905_at	A kinase (PRKA) anchor protein 8	1,61	*ARHGAP27*	789296_at	Rho gtpase activating protein 27	1,59
RDH11	505995_at	Retinol dehydrogenase 11 (all-trans/9-cis/11-cis)	1,61	*C1QTNF5*	614671_at	C1q and tumor necrosis factor related protein 5	1,59
C13H20orf151	515877_at	Chromosome 13 open reading frame, human c20orf151	1,61	*LOC523454*	523454_at	Protein WWC3-like	1,59
FAIM	616795_at	Fas apoptotic inhibitory molecule	1,61	*RAB3D*	100139105_at	RAB3D, member RAS oncogene family	1,59
REPS1	536155_at	RALBP1 associated Eps domain containing 1	1,61	*TMEM173*	533661_at	Transmembrane protein 173	1,59
PTAR1	784830_at	Protein prenyltransferase alpha subunit repeat containing 1	1,61	*B3GNT2*	515585_at	UDP-glcnac:betagal beta-1,3-N-acetylglucosaminyltransferase 2	1,59
C3H1orf110	617478_at	Coiled-coil domain-containing protein c1orf110 homolog	1,61	*LDOC1L*	786696_at	Leucine zipper, down-regulated in cancer 1-like	1,59
TMEM141	514305_at	Transmembrane protein 141	1,61	*BRSK1*	538009_at	BR serine/threonine kinase 1	1,59
SLC6A6	282366_at	Solute carrier family 6 (neurotransmitter transporter, taurine), member 6	1,61	*C20H5orf28*	780867_at	Chromosome 20 open reading frame, human c5orf28	1,59
CEP85L	537625_at	Centrosomal protein 85kda-like	1,61	*GGT5*	787326_at	Gamma-glutamyltransferase 5	1,59
GSTT1	517724_at	Glutathione S-transferase theta 1	1,61	*OCLN*	512405_at	Occludin	1,58
NTN1	522767_at	Netrin 1	1,61	*COBLL1*	532067_at	COBL-like 1	1,58
CLDN10	506545_at	Claudin 10	1,61	*PAPOLG*	529071_at	Poly(A) polymerase gamma	1,58
FAM213A	534049_at	Chromosome 28 open reading frame, human c10orf58	1,61	*LOC100335177*	100335177_at	Rex1, RNA exonuclease 1 homolog (S. Cerevisiae)-like	1,58
PITPNC1	782067_at	Cytoplasmic phosphatidylinositol transfer protein 1-like	1,61	*PLXNA3*	782382_at	Plexin A3	1,58
LRRC59	532659_at	Leucine rich repeat containing 59	1,60	*GAS2L1*	518935_at	Growth arrest-specific 2 like 1	1,58
TMEM145	513015_at	Transmembrane protein 145	1,60	*CREB3L4*	529566_at	Camp responsive element binding protein 3-like 4	1,58
TRIM14	522632_at	Tripartite motif containing 14	1,60	*OSBPL5*	532690_at	Oxysterol binding protein-like 5	1,58

FUCA1	509522_at	Fucosidase, alpha-L- 1, tissue	1,58	*ARHGEF10L*	529043_at	Rho guanine nucleotide exchange factor (GEF) 10-like	1,56
NLK	507204_at	Nemo-like kinase	1,58	*NXF1*	512136_at	Nuclear RNA export factor 1	1,56
MMP11	539109_at	Matrix metallopeptidase 11 (stromelysin 3)	1,58	*FLNB*	613533_at	Filamin B, beta	1,56
ST14	767617_at	Suppression of tumorigenicity 14 (colon carcinoma)	1,58	*ALCAM*	281614_at	Activated leukocyte cell adhesion molecule	1,56
ME3	525813_at	Malic enzyme 3, NADP(+)-dependent, mitochondrial	1,58	*DDHD2*	513116_at	DDHD domain containing 2	1,56
GALNT1	282241_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (galnac-T1)	1,58	*CAMSAP3*	616206_at	Calmodulin regulated spectrin-associated protein family, member 3	1,56
ALDOA	509566_at	Aldolase A, fructose-bisphosphate	1,58	*BAG3*	782633_at	BCL2-associated athanogene 3	1,56
PARVA	615430_at	Parvin, alpha	1,58	*LOC100335346*	100335346_at	Protein shisa-5-like	1,56
NDRG1	504499_at	N-myc downstream regulated 1	1,58	*GCNT1*	281778_at	Glucosaminyl (N-acetyl) transferase 1, core 2	1,56
IL20RB	534581_at	Interleukin 20 receptor beta	1,58	*EMB*	785366_at	Embigin	1,56
SNX33	511561_at	Sorting nexin 33	1,57	*ZNF398*	525559_at	Zinc finger protein 398	1,56
MOB3B	540817_at	MOB kinase activator 3B	1,57	*NR2E1*	528156_at	Nuclear receptor subfamily 2, group E, member 1	1,56
CYP27A1	511960_at	Cytochrome P450, family 27, subfamily A, polypeptide 1	1,57	*PLEKHA1*	513040_at	Pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1	1,55
MAPKAPK5	535625_at	Mitogen-activated protein kinase-activated protein kinase 5	1,57	*TRIO*	538292_at	Triple functional domain (PTPRF interacting)	1,55
KIAA1274	524694_at	KIAA1274 ortholog	1,57	*INPPL1*	783833_at	Inositol polyphosphate phosphatase-like 1	1,55
SLC4A11	532407_at	Solute carrier family 4, sodium borate transporter, member 11	1,57	*SOCS6*	615146_at	Suppressor of cytokine signaling 6	1,55
PATZ1	532416_at	POZ (BTB) and AT hook containing zinc finger 1	1,57	*GDPD3*	767913_at	Glycerophosphodiester phosphodiesterase domain containing 3	1,55
MARVELD3	533131_at	MARVEL domain containing 3	1,57	*SLC38A1*	527491_at	Solute carrier family 38, member 1	1,55
DBN1	505406_at	Drebrin 1	1,57	*CYB5R3*	515773_at	Cytochrome b5 reductase 3	1,54
MMRN2	512308_at	Multimerin 2	1,57	*ARFGAP3*	532778_at	ADP-ribosylation factor gtpase activating protein 3	1,54
NSDHL	616694_at	NAD(P) dependent steroid dehydrogenase-like	1,57	*PLEKHB1*	511885_at	Pleckstrin homology domain containing, family B (evectins) member 1	1,54
CA5B	514494_at	Carbonic anhydrase VB, mitochondrial	1,57	*CLCN6*	520210_at	Chloride channel 6	1,54
ODZ3	511615_at	Odz, odd Oz/ten-m homolog 3 (Drosophila)	1,56	*CTSS*	327711_at	Cathepsin S	1,54
VMAC	515212_at	Vimentin-type intermediate filament associated coiled-coil protein	1,56	*RNF148*	538888_at	Ring finger protein 148	1,54

VSTM5	100141246_at	V-set and transmembrane domain containing 5	1,54	*TMC5*	513865_at	Transmembrane channel-like 5	1,52
TMEM184B	514220_at	Transmembrane protein 184B	1,53	*GOLIM4*	538532_at	Golgi integral membrane protein 4	1,51
POLR2G	526320_at	Polymerase (RNA) II (DNA directed) polypeptide G	1,53	*BCR*	789892_at	Breakpoint cluster region	1,51
HOXB4	768240_at	Homeobox B4	1,53	*GRAMD1A*	507027_at	GRAM domain containing 1A	1,51
CHD3	532673_at	Chromodomain helicase DNA binding protein 3	1,53	*LOC510369*	510369_at	Hypoxanthine phosphoribosyltransferase 1-like	1,51
FAM174B	614841_at	Family with sequence similarity 174, member B	1,53	*UTP18*	505846_at	UTP18, small subunit (SSU) processome component, homolog (yeast)	1,51
TAF4	789854_at	TAF4 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 135kda	1,53	*OCIAD2*	505877_at	OCIA domain containing 2	1,51
NEDD9	504967_at	Neural precursor cell expressed, developmentally down-regulated 9	1,53	*CLDN3*	404153_at	Claudin 3	1,49
REPIN1	511510_at	Replication initiator 1	1,53	*PTPRM*	536092_at	Protein tyrosine phosphatase, receptor type, M	1,49
GCH1	286815_at	GTP cyclohydrolase 1	1,53	*SETD6*	539651_at	SET domain containing 6	1,49
FAM169A	519307_at	Family with sequence similarity 169, member A	1,53	*SMPDL3A*	505300_at	Sphingomyelin phosphodiesterase, acid-like 3A	1,49
ACYP1	507844_at	Acylphosphatase 1, erythrocyte (common) type	1,53	*ARHGAP26*	538219_at	Rho gtpase activating protein 26	1,49
GLI4	518201_at	GLI family zinc finger 4	1,53	*HHIPL2*	533766_at	HHIP-like 2	1,49
SIPA1L1	787248_at	Signal-induced proliferation-associated 1 like 1	1,52	*GYG2*	505258_at	Glycogenin 2	1,49
FNIP2	100138353_at	Folliculin interacting protein 2	1,52	*CABIN1*	530023_at	Calcineurin binding protein 1	1,49
EYA3	514364_at	Eyes absent homolog 3 (Drosophila)	1,52	*DNAJC25*	535430_at	Dnaj (Hsp40) homolog, subfamily C , member 25	1,49
GPS2	518494_at	G protein pathway suppressor 2	1,52	*MAB21L3*	518597_at	Mab-21-like 3 (C. Elegans)	1,49
FGFR1OP2	532881_at	FGFR1 oncogene partner 2	1,52	*SLC3A2*	507107_at	Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	1,49
MIR449D	100313194_at	Microrna mir-449d	1,52	*GDF10*	539510_at	Growth differentiation factor 10	1,49
MYADM	506295_at	Myeloid-associated differentiation marker	1,52	*RAD18*	514440_at	RAD18 homolog (S. Cerevisiae)	1,49
C6H4orf34	614774_at	Chromosome 6 open reading frame, human c4orf34	1,52	*INPP5A*	615232_at	Inositol polyphosphate-5-phosphatase, 40kda	1,49
SEPP1	282066_at	Selenoprotein P, plasma, 1	1,52	*SYNGR1*	534995_at	Synaptogyrin 1	1,48
37316	508949_at	Membrane-associated ring finger (C3HC4) 2	1,52	*ABCG1*	510745_at	ATP-binding cassette, sub-family G (WHITE), member 1	1,48
ARL2	511349_at	ADP-ribosylation factor-like 2	1,52	*ZFHX2*	539758_at	Zinc finger homeobox 2	1,48
TINAGL1	509642_at	Tubulointerstitial nephritis antigen-like 1	1,52	*DPH5*	508904_at	DPH5 homolog (S. Cerevisiae)	1,48

FUT5	338077_at	Fucosyltransferase 5 (alpha (1,3) fucosyltransferase)	1,48	*STARD13*	538697_at	Star-related lipid transfer (START) domain containing 13	1,46
MET	280855_at	Met proto-oncogene (hepatocyte growth factor receptor)	1,48	*RAD54L*	100140639_at	RAD54-like (S. Cerevisiae)	1,46
C21H14orf2	767909_at	Chromosome 21 open reading frame, human c14orf2	1,48	*COBL*	613554_at	Cordon-bleu homolog (mouse)	1,46
PPARA	281992_at	Peroxisome proliferator-activated receptor alpha	1,48	*PHB2*	515363_at	Prohibitin 2	1,46
RNF157	507697_at	Ring finger protein 157	1,47	*LRCH1*	505325_at	Leucine-rich repeats and calponin homology (CH) domain containing 1	1,46
ARL15	534329_at	ADP-ribosylation factor-like 15	1,47	*FUT10*	360195_at	Fucosyltransferase 10 (alpha (1,3) fucosyltransferase)	1,45
HIVEP2	540396_at	Human immunodeficiency virus type I enhancer binding protein 2	1,47	*NUMA1*	513091_at	Nuclear mitotic apparatus protein 1	1,45
PPM1M	510238_at	Protein phosphatase, Mg2+/Mn2+ dependent, 1M	1,47	*RCE1*	539539_at	RCE1 homolog, prenyl protein protease (S. Cerevisiae)	1,45
PATL1	537453_at	Protein associated with topoisomerase II homolog 1 (yeast)	1,47	*CBX6*	513830_at	Chromobox homolog 6	1,45
PER1	516318_at	Period homolog 1 (Drosophila)	1,47	*ACSL1*	537161_at	Acyl-coa synthetase long-chain family member 1	1,45
SHKBP1	512402_at	SH3KBP1 binding protein 1	1,47	*LPIN2*	514448_at	Lipin 2	1,45
SEMA4G	524002_at	Sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4G	1,47	*MLEC*	515309_at	Malectin	1,45
FAM65A	100336872_at	Family with sequence similarity 65, member A	1,47	*PRKAG2*	504219_at	Protein kinase, AMP-activated, gamma 2 non-catalytic subunit	1,45
CDH1	282637_at	Cadherin 1, type 1, E-cadherin (epithelial)	1,46	*TLL2*	504975_at	Tolloid-like 2	1,45
EPS8	538419_at	Epidermal growth factor receptor pathway substrate 8	1,46	*DUS1L*	617998_at	Dihydrouridine synthase 1-like (S. Cerevisiae)	1,45
GLIS3	524909_at	GLIS family zinc finger 3	1,46	*SLC17A5*	530164_at	Solute carrier family 17 (anion/sugar transporter), member 5	1,44
PURA	782746_at	Purine-rich element binding protein A	1,46	*ATN1*	513125_at	Atrophin 1	1,44
RGL2	504334_at	Ral guanine nucleotide dissociation stimulator-like 2	1,46	*NFIB*	538474_at	Nuclear factor I/B	1,44
ADAM10	282132_at	ADAM metallopeptidase domain 10	1,46	*TOM1L1*	513303_at	Target of myb1 (chicken)-like 1	1,44
FURIN	281374_at	Furin (paired basic amino acid cleaving enzyme)	1,46	*STX3*	513275_at	Syntaxin 3	1,44
NPAS2	614049_at	Neuronal PAS domain protein 2	1,46	*POLR2E*	512971_at	Polymerase (RNA) II (DNA directed) polypeptide E, 25kda	1,44

DHRS1	528832_at	Dehydrogenase/reductase (SDR family) member 1	1,44	*APC*	533233_at	Adenomatous polyposis coli	1,42
ALDH18A1	514759_at	Aldehyde dehydrogenase 18 family, member A1	1,44	*TBC1D30*	541051_at	TBC1 domain family, member 30	1,42
FGFR2	404193_at	Fibroblast growth factor receptor 2	1,44	*TRIM2*	538617_at	Tripartite motif containing 2	1,42
PYGB	505560_at	Phosphorylase, glycogen; brain	1,44	*GORAB*	614920_at	Golgin, RAB6-interacting	1,42
WSB1	614851_at	WD repeat and SOCS box containing 1	1,44	*ATP6V0D1*	282148_at	Atpase, H+ transporting, lysosomal 38kda, V0 subunit d1	1,42
OVOL2	532761_at	Ovo-like 2 (Drosophila)	1,44	*MTMR2*	536810_at	Myotubularin related protein 2	1,42
AES	505375_at	Amino-terminal enhancer of split	1,44	*FAM83G*	100139162_at	Family with sequence similarity 83, member G	1,42
TJP3	407100_at	Tight junction protein 3 (zona occludens 3)	1,44	*NBEAL2*	788207_at	Neurobeachin-like 2	1,42
LPAR2	509748_at	Lysophosphatidic acid receptor 2	1,44	*WSCD1*	788123_at	WSC domain containing 1	1,42
SYNJ1	282087_at	Synaptojanin 1	1,44	*PXN*	517456_at	Paxillin	1,42
PAX2	100297382_at	Paired box 2	1,44	*C21H15orf38*	613997_at	UPF0552 protein c15orf38 homolog	1,41
HMGCR	407159_at	3-hydroxy-3-methylglutaryl-coa reductase	1,44	*B3GALT6*	522406_at	UDP-Gal:betagal beta 1,3-galactosyltransferase polypeptide 6	1,41
PPP1R13B	511414_at	Protein phosphatase 1, regulatory subunit 13B	1,44	*PPARD*	353106_at	Peroxisome proliferator-activated receptor delta	1,41
NDUFB4	327706_at	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kda	1,44	*LIMS1*	540281_at	LIM and senescent cell antigen-like domains 1	1,41
PIK3CB	517948_at	Phosphoinositide-3-kinase, catalytic, beta polypeptide	1,43	*PLCB3*	515669_at	Phospholipase C, beta 3 (phosphatidylinositol-specific)	1,41
POLD2	281991_at	Polymerase (DNA directed), delta 2, regulatory subunit 50kda	1,43	*PRRC1*	510272_at	Proline-rich coiled-coil 1	1,41
CNPPD1	507473_at	Cyclin Pas1/PHO80 domain containing 1	1,43	*ESRP1*	538640_at	Epithelial splicing regulatory protein 1	1,41
PINK1	510683_at	PTEN induced putative kinase 1	1,43	*SIRT7*	505662_at	Sirtuin 7	1,41
CDC42SE2	789618_at	CDC42 small effector 2	1,43	*ERRFI1*	516303_at	ERBB receptor feedback inhibitor 1	1,41
CRIM1	506264_at	Cysteine rich transmembrane BMP regulator 1 (chordin-like)	1,43	*DECR2*	768256_at	2,4-dienoyl coa reductase 2, peroxisomal	1,41
PRRC2B	505073_at	Proline-rich coiled-coil 2B	1,43	*ATP13A3*	523889_at	Atpase type 13A3	1,41
C17H22orf13	517135_at	Chromosome 17 open reading frame, human c22orf13	1,43	*EYA2*	615264_at	Eyes absent homolog 2 (Drosophila)	1,41
CASP3	408016_at	Caspase 3, apoptosis-related cysteine peptidase	1,43	*AMMECR1L*	539958_at	AMME chromosomal region gene 1-like	1,41
PTAFR	518283_at	Platelet-activating factor receptor	1,43	*NAMPT*	520472_at	Nicotinamide phosphoribosyltransferase	1,41
FKBP9	534182_at	FK506 binding protein 9, 63 kda	1,43	*NUMB*	512187_at	Numb homolog (Drosophila)	1,40
RBM7	515307_at	RNA binding motif protein 7	1,42	*PGM2*	506980_at	Phosphoglucomutase 2	1,40

SLC41A1	533907_at	Solute carrier family 41, member 1	1,40	*GRB10*	407210_at	Growth factor receptor-bound protein 10	1,39
C25H7orf43	511902_at	Chromosome 25 open reading frame, human c7orf43	1,40	*TOP1*	534799_at	Topoisomerase (DNA) I	1,39
SCRN1	534933_at	Secernin 1	1,40	*SHANK1*	518970_at	SH3 and multiple ankyrin repeat domains 1	1,39
MARVELD2	541110_at	MARVEL domain containing 2	1,40	*BAZ2A*	509799_at	Bromodomain adjacent to zinc finger domain, 2A	1,39
SOX5	533829_at	SRY (sex determining region Y)-box 5	1,40	*FNDC3A*	508840_at	Fibronectin type III domain containing 3A	1,39
PRKD1	533270_at	Protein kinase D1	1,40	*NCOA1*	525346_at	Nuclear receptor coactivator 1	1,39
TWF2	282024_at	Twinfilin, actin-binding protein, homolog 2 (Drosophila)	1,40	*SEC31A*	531964_at	SEC31 homolog A (S. Cerevisiae)	1,39
SPTSSA	615641_at	Serine palmitoyltransferase, small subunit A	1,40	*SMC1A*	282370_at	Structural maintenance of chromosomes 1A	1,39
OTUD7B	525579_at	OTU domain containing 7B	1,40	*GGT7*	615929_at	Gamma-glutamyltransferase 7	1,39
C8H9orf152	614478_at	Chromosome 8 open reading frame, human c9orf152	1,40	*LOC785007*	785007_at	Uncharacterized LOC785007	1,39
C2H1orf172	540019_at	Chromosome 2 open reading frame, human c1orf172	1,40	*GARS*	408010_at	Glycyl-trna synthetase	1,39
AMOT	535509_at	Angiomotin	1,40	*MICALL2*	510644_at	MICAL-like 2	1,39
GOT2	286886_at	Glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)	1,40	*PPP4C*	540398_at	Protein phosphatase 4, catalytic subunit	1,39
B4GALT3	515771_at	UDP-Gal:betaglcnac beta 1,4-galactosyltransferase, polypeptide 3	1,39	*TAF8*	539938_at	TAF8 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 43kda	1,39
ZFAND2B	508203_at	Zinc finger, AN1-type domain 2B	1,39	*HYAL2*	281838_at	Hyaluronoglucosaminidase 2	1,39
DSG2	508151_at	Desmoglein 2	1,39	*KLF5*	535702_at	Kruppel-like factor 5 (intestinal)	1,39
SLC39A1	530352_at	Solute carrier family 39 (zinc transporter), member 1	1,39	*SLC6A9*	282368_at	Solute carrier family 6 (neurotransmitter transporter, glycine), member 9	1,39
LRWD1	100138861_at	Leucine-rich repeats and WD repeat domain containing 1	1,39	*PRR14*	533235_at	Proline rich 14	1,39
PLEKHN1	520081_at	Pleckstrin homology domain containing, family N member 1	1,39	*ARHGEF16*	514453_at	Rho guanine nucleotide exchange factor (GEF) 16	1,39
LNPEP	521633_at	Leucyl/cystinyl aminopeptidase	1,39	*ARHGEF11*	511220_at	Rho guanine nucleotide exchange factor (GEF) 11	1,39
PFKL	508683_at	Phosphofructokinase, liver	1,39	*RIPK2*	534407_at	Receptor-interacting serine-threonine kinase 2	1,39
LOC100297221	100297221_at	Uncharacterized LOC100297221	1,39	*SLC25A42*	504608_at	Solute carrier family 25, member 42	1,39
SHROOM3	100139141_at	Shroom family member 3	1,39	*CBY1*	282859_at	Chibby homolog 1 (Drosophila)	1,39
	100850549_at		1,39	*MTA2*	515389_at	Metastasis associated 1 family, member 2	1,39

FLVCR1	533317_at	Feline leukemia virus subgroup C cellular receptor 1	1,39	*INPP1*	281869_at	Inositol polyphosphate-1-phosphatase	1,37
PDLIM4	515410_at	PDZ and LIM domain 4	1,39	*FAM59A*	507330_at	Family with sequence similarity 59, member A	1,37
PRSS8	613506_at	Protease, serine, 8	1,38	*SNAPC2*	516078_at	Small nuclear RNA activating complex, polypeptide 2, 45kda	1,37
SCAND1	513983_at	SCAN domain containing 1	1,38	*ARAP2*	512010_at	Arfgap with rhogap domain, ankyrin repeat and PH domain 2	1,37
NMT1	281351_at	N-myristoyltransferase 1	1,38	*EIF4G1*	444858_at	Eukaryotic translation initiation factor 4 gamma, 1	1,37
STXBP2	515618_at	Syntaxin binding protein 2	1,38	*ADCY6*	509936_at	Adenylate cyclase 6	1,36
LSR	508651_at	Lipolysis stimulated lipoprotein receptor	1,38	*ORAI1*	517688_at	ORAI calcium release-activated calcium modulator 1	1,36
SGSH	535442_at	N-sulfoglucosamine sulfohydrolase	1,38	*DIAPH1*	786565_at	Diaphanous homolog 1 (Drosophila)	1,36
MAPKAPK2	788091_at	Mitogen-activated protein kinase-activated protein kinase 2	1,38	*ABL1*	540876_at	C-abl oncogene 1, non-receptor tyrosine kinase	1,36
NR2F6	100296331_at	Nuclear receptor subfamily 2, group F, member 6	1,38	*MAP3K9*	538340_at	Mitogen-activated protein kinase kinase kinase 9	1,36
EEF2K	521730_at	Eukaryotic elongation factor-2 kinase	1,38	*STX17*	534304_at	Syntaxin 17	1,36
GCFC2	521363_at	GC-rich sequence DNA-binding factor 2	1,38	*ARHGAP35*	540310_at	Rho gtpase activating protein 35	1,36
LMAN1	511649_at	Lectin, mannose-binding, 1	1,38	*CALR*	281036_at	Calreticulin	1,36
IGSF9	504209_at	Immunoglobulin superfamily, member 9	1,38	*CDC42SE1*	614042_at	CDC42 small effector 1	1,36
PANK3	510749_at	Pantothenate kinase 3	1,38	*CBLB*	525906_at	Cas-Br-M (murine) ecotropic retroviral transforming sequence b	1,36
PLEKHG5	615910_at	Pleckstrin homology domain containing, family G (with rhogef domain) member 5	1,38	*MLXIP*	783217_at	MLX interacting protein	1,36
SLC10A7	613859_at	Solute carrier family 10 (sodium/bile acid cotransporter family), member 7	1,38	*THOC2*	507738_at	THO complex 2	1,35
DOCK1	537203_at	Dedicator of cytokinesis 1	1,37	*ACADVL*	282130_at	Acyl-coa dehydrogenase, very long chain	1,35
SETBP1	617265_at	SET binding protein 1	1,37	*ICA1*	535346_at	Islet cell autoantigen 1, 69kda	1,35
UBE2J1	539754_at	Ubiquitin-conjugating enzyme E2, J1, U	1,37	*LOC783807*	783807_at	Methyl-cpg-binding domain protein 6-like	1,35
RNF187	618753_at	Ring finger protein 187	1,37	*TMEM39A*	615128_at	Transmembrane protein 39A	1,35
INO80C	533426_at	INO80 complex subunit C	1,37	*FAM83H*	524974_at	Family with sequence similarity 83, member H	1,35
RAB4B	616314_at	RAB4B, member RAS oncogene family	1,37	*LOC100335608*	100335608_at	Uncharacterized LOC100335608	1,35
EHBP1	100300164_at	EH domain binding protein 1	1,37	*VAMP2*	282116_at	Vesicle-associated membrane protein 2 (synaptobrevin 2)	1,35
TBL1XR1	614346_at	Transducin (beta)-like 1 X-linked receptor 1	1,37	*PEX7*	533077_at	Peroxisomal biogenesis factor 7	1,35
SPIRE1	519030_at	Spire homolog 1 (Drosophila)	1,37	*ARHGEF2*	505940_at	Rho/Rac guanine nucleotide exchange factor (GEF) 2	1,35

METTL22	509540_at	Methyltransferase like 22	1,35	*GALNT7*	524529_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (galnac-T7)	1,33
LOC100299043	100299043_at	Uncharacterized LOC100299043	1,35	*WDR6*	526884_at	WD repeat domain 6	1,33
FAM3A	614075_at	Family with sequence similarity 3, member A	1,35	*PORCN*	540373_at	Porcupine homolog (Drosophila)	1,33
SRP14	512792_at	Signal recognition particle 14kda (homologous Alu RNA binding protein)	1,35	*CELF2*	777790_at	CUGBP, Elav-like family member 2	1,33
AQP9	516762_at	Aquaporin 9	1,35	*TOM1*	504912_at	Target of myb1 (chicken)	1,33
MED24	504613_at	Mediator complex subunit 24	1,35	*RELL1*	768210_at	RELT-like 1	1,33
SNRPA	509802_at	Small nuclear ribonucleoprotein polypeptide A	1,35	*ADIPOR2*	407234_at	Adiponectin receptor 2	1,33
SNX24	614112_at	Sorting nexin 24	1,35	*LOC100335379*	100335379_at	Uncharacterized LOC100335379	1,33
DLGAP4	520521_at	Discs, large (Drosophila) homolog-associated protein 4	1,35	*GID4*	509503_at	GID complex subunit 4, VID24 homolog (S. Cerevisiae)	1,33
MED27	525389_at	Mediator complex subunit 27	1,35	*GLTPD1*	505009_at	Glycolipid transfer protein domain containing 1	1,32
ASS1	280726_at	Argininosuccinate synthase 1	1,35	*JHDM1D*	521504_at	Jumonji C domain containing histone demethylase 1 homolog D (S. Cerevisiae)	1,32
BEND7	504404_at	BEN domain containing 7	1,34	*ATF7*	541204_at	Activating transcription factor 7	1,32
OSBPL7	508936_at	Oxysterol binding protein-like 7	1,34	*RUSC1*	100125592_at	RUN and SH3 domain containing 1	1,32
MACF1	506730_at	Microtubule-actin crosslinking factor 1	1,34	*MPV17*	505763_at	Mpv17 mitochondrial inner membrane protein	1,32
PYGO2	540401_at	Pygopus homolog 2 (Drosophila)	1,34	*EMG1*	515362_at	EMG1 nucleolar protein homolog (S. Cerevisiae)	1,32
SYMPK	100337373_at	Symplekin	1,34	*MRPL41*	506521_at	Mitochondrial ribosomal protein L41	1,32
STK19	508320_at	Serine/threonine kinase 19	1,34	*ALDH3A2*	513967_at	Aldehyde dehydrogenase 3 family, member A2	1,32
ZNF454	525942_at	Zinc finger protein 454	1,34	*RHOG*	538559_at	Ras homolog gene family, member G (rho G)	1,32
PIK3AP1	100138311_at	Phosphoinositide-3-kinase adaptor protein 1	1,34	*EFNA4*	615879_at	Ephrin-A4	1,32
KIAA0664	531049_at	KIAA0664 ortholog	1,34	*MRPL43*	282277_at	Mitochondrial ribosomal protein L43	1,32
FAM50A	515539_at	Family with sequence similarity 50, member A	1,34	*ACP2*	535407_at	Acid phosphatase 2, lysosomal	1,32
SNX15	507751_at	Sorting nexin 15	1,34	*SNW1*	326578_at	SNW domain containing 1	1,32
DDX39B	540191_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39B	1,33	*FBXL12*	617304_at	F-box and leucine-rich repeat protein 12	1,32
EMC8	510727_at	COX4 neighbor	1,33	*ABHD14B*	615289_at	Abhydrolase domain containing 14B	1,31
PI4K2B	521790_at	Phosphatidylinositol 4-kinase type 2 beta	1,33	*COQ10B*	514221_at	Coenzyme Q10 homolog B (S. Cerevisiae)	1,31
STX5	510312_at	Syntaxin 5	1,33	*CADPS2*	519444_at	Ca++-dependent secretion activator 2	1,31
PEX26	537878_at	Peroxisomal biogenesis factor 26	1,33	*ATXN7L3*	525252_at	Ataxin 7-like 3	1,31

EPHB4	515756_at	EPH receptor B4	1,31	*ZDHHC5*	533250_at	Zinc finger, DHHC-type containing 5	1,29
TNKS1BP1	783548_at	Tankyrase 1 binding protein 1, 182kda	1,31	*TJP1*	407102_at	Tight junction protein 1	1,29
MAN2A2	527449_at	Mannosidase, alpha, class 2A, member 2	1,31	*COPG1*	338055_at	Coatomer protein complex, subunit gamma	1,29
CBFB	614678_at	Core-binding factor, beta subunit	1,31	*PTPRF*	512072_at	Protein tyrosine phosphatase, receptor type, F	1,29
MGRN1	616130_at	Mahogunin ring finger 1, E3 ubiquitin protein ligase	1,31	*FAM100B*	618617_at	Family with sequence similarity 100, member B	1,29
VPS37A	513985_at	Vacuolar protein sorting 37 homolog A (S. Cerevisiae)	1,31	*HSPB1*	516099_at	Heat shock 27kda protein 1	1,29
LOC100851604	100851604_at	Solute carrier family 41 member 3-like	1,31	*GGA2*	517031_at	Golgi-associated, gamma adaptin ear containing, ARF binding protein 2	1,29
CRAT	512902_at	Carnitine O-acetyltransferase	1,31	*WWC1*	520730_at	WW and C2 domain containing 1	1,29
MANSC1	512999_at	MANSC domain containing 1	1,31	*NDST1*	514172_at	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1	1,29
SRC	535742_at	V-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	1,30	*TBKBP1*	785603_at	TBK1 binding protein 1	1,29
ELMO3	525427_at	Engulfment and cell motility 3	1,30	*ARMCX1*	504577_at	Armadillo repeat containing, X-linked 1	1,29
PLBD2	514347_at	Phospholipase B domain containing 2	1,30	*SRSF2*	508312_at	Serine/arginine-rich splicing factor 2	1,29
RRM1	505537_at	Ribonucleotide reductase M1	1,30	*LAMP2*	529148_at	Lysosomal-associated membrane protein 2	1,28
PXDC1	613986_at	Chromosome 23 open reading frame, human c6orf145	1,30	*RGP1*	539393_at	RGP1 retrograde golgi transport homolog (S. Cerevisiae)	1,28
SLMAP	529366_at	Sarcolemma associated protein	1,30	*AP1G2*	100126076_at	Adaptor-related protein complex 1, gamma 2 subunit	1,28
EED	404183_at	Embryonic ectoderm development	1,30	*SSU72*	614837_at	SSU72 RNA polymerase II CTD phosphatase homolog (S. Cerevisiae)	1,28
FAM107B	535023_at	Family with sequence similarity 107, member B	1,30	*SH3BP4*	520462_at	SH3-domain binding protein 4	1,28
CHRNE	281688_at	Cholinergic receptor, nicotinic, epsilon (muscle)	1,30	*LUC7L*	535131_at	LUC7-like (S. Cerevisiae)	1,28
RNF185	524459_at	Ring finger protein 185	1,30	*ZC3H3*	515615_at	Zinc finger CCCH-type containing 3	1,28
PCSK7	515398_at	Proprotein convertase subtilisin/kexin type 7	1,30	*TCF12*	509039_at	Transcription factor 12	1,28
TOM1L2	616315_at	Target of myb1-like 2 (chicken)	1,29	*SBNO2*	512682_at	Strawberry notch homolog 2 (Drosophila)	1,28
FAM53B	615557_at	Family with sequence similarity 53, member B	1,29	*ACAD8*	512070_at	Acyl-coa dehydrogenase family, member 8	1,28
TSEN54	511091_at	Trna splicing endonuclease 54 homolog (S. Cerevisiae)	1,29	*SLC35E2*	527591_at	Solute carrier family 35, member E2	1,28
MANBAL	787482_at	Mannosidase, beta A, lysosomal-like	1,29	*KRTCAP2*	540389_at	Keratinocyte associated protein 2	1,28
WNK1	506433_at	WNK lysine deficient protein kinase 1	1,29	*GK5*	616031_at	Glycerol kinase 5 (putative)	1,28

ATXN2L	539507_at	Ataxin 2-like	1,27	*EIF2B4*	521926_at	Eukaryotic translation initiation factor 2B, subunit 4 delta, 67kda	1,26
GTF3C2	782752_at	General transcription factor IIIC, polypeptide 2, beta 110kda	1,27	*RPS6KA1*	533908_at	Ribosomal protein S6 kinase, 90kda, polypeptide 1	1,26
SYNE2	540504_at	Spectrin repeat containing, nuclear envelope 2	1,27	*NIN*	510366_at	Ninein (GSK3B interacting protein)	1,26
LCOR	507668_at	Ligand dependent nuclear receptor corepressor	1,27	*MLLT4*	504856_at	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4	1,26
TMEM131	540861_at	Transmembrane protein 131	1,27	*SRSF6*	507828_at	Serine/arginine-rich splicing factor 6	1,25
CNNM4	522382_at	Cyclin M4	1,27	*EZR*	281574_at	Ezrin	1,25
FKBP15	783009_at	FK506 binding protein 15, 133kda	1,27	*ATG4D*	615657_at	ATG4 autophagy related 4 homolog D (S. Cerevisiae)	1,25
RABGGTA	516619_at	Rab geranylgeranyltransferase, alpha subunit	1,27	*OSBP*	530000_at	Oxysterol binding protein	1,25
LRRC1	506753_at	Leucine rich repeat containing 1	1,27	*AVL9*	534141_at	AVL9 homolog (S. Cerevisiase)	1,25
MIR2443	100313439_at	Microrna mir-2443	1,27	*GMIP*	533029_at	GEM interacting protein	1,25
PPP1R39	540718_at	Protein phosphatase 1, regulatory subunit 39	1,27	*SUCLA2*	511090_at	Succinate-coa ligase, ADP-forming, beta subunit	1,25
MKLN1	508844_at	Muskelin 1, intracellular mediator containing kelch motifs	1,27	*PIAS2*	533403_at	Protein inhibitor of activated STAT, 2	1,25
C13H20orf196	615129_at	Chromosome 13 open reading frame, human c20orf196	1,27	*PICALM*	513579_at	Phosphatidylinositol binding clathrin assembly protein	1,24
VAMP7	613984_at	Vesicle-associated membrane protein 7	1,27	*ARID1A*	540181_at	AT rich interactive domain 1A (SWI-like)	1,24
WDR91	540606_at	WD repeat domain 91	1,27	*CCS*	515022_at	Copper chaperone for superoxide dismutase	1,24
RAB11B	532723_at	RAB11B, member RAS oncogene family	1,27	*LYPLA2*	784764_at	Lysophospholipase II	1,24
TRIM3	534510_at	Tripartite motif containing 3	1,27	*ZDHHC3*	506728_at	Zinc finger, DHHC-type containing 3	1,23
LGR4	505423_at	Leucine-rich repeat containing G protein-coupled receptor 4	1,26	*PRDM2*	789222_at	PR domain containing 2, with ZNF domain	1,23
LMTK2	512290_at	Serine/threonine-protein kinase LMTK2-like	1,26	*SRF*	533039_at	Serum response factor (c-fos serum response element-binding transcription factor)	1,23
DIP2B	512064_at	DIP2 disco-interacting protein 2 homolog B (Drosophila)	1,26	*UHRF1BP1*	534225_at	UHRF1 binding protein 1	1,23
TP53BP2	514474_at	Tumor protein p53 binding protein, 2	1,26	*MOB3A*	505007_at	MOB kinase activator 3A	1,23
DONSON	522248_at	Downstream neighbor of SON	1,26	*ETV6*	504512_at	Ets variant 6	1,23
DOLPP1	504908_at	Dolichyl pyrophosphate phosphatase 1	1,26	*SLC25A3*	282477_at	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3	1,23
LRP6	536328_at	Low density lipoprotein receptor-related protein 6	1,26	*CSNK1E*	768234_at	Casein kinase 1, epsilon	1,23

JAK1	537201_at	Janus kinase 1	1,22	*CLASP1*	523441_at	Cytoplasmic linker associated protein 1	-1,17
ZMYND11	506325_at	Zinc finger, MYND-type containing 11	1,22	*DENND1A*	514134_at	DENN/MADD domain containing 1A	-1,18
SKIL	527910_at	SKI-like oncogene	1,22	*ZNF292*	541264_at	Zinc finger protein 292	-1,19
DCP1A	783258_at	DCP1 decapping enzyme homolog A (S. Cerevisiae)	1,22	*XRN1*	540834_at	5'-3' exoribonuclease 1	-1,19
GTF3C5	783869_at	General transcription factor IIIC, polypeptide 5, 63kda	1,22	*SMARCA5*	537503_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	-1,19
DYNC1H1	537748_at	Dynein, cytoplasmic 1, heavy chain 1	1,22	*REEP5*	617543_at	Receptor accessory protein 5	-1,19
ZC3H7B	534109_at	Zinc finger CCCH-type containing 7B	1,22	*SPR*	533836_at	Sepiapterin reductase (7,8-dihydrobiopterin:NADP+ oxidoreductase)	-1,20
PABPN1	282298_at	Poly(A) binding protein, nuclear 1	1,21	*FAM45A*	534370_at	Family with sequence similarity 45, member A	-1,21
PIP4K2A	533289_at	Phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	1,21	*KIAA1279*	527433_at	KIAA1279 ortholog	-1,21
ACVR2B	282131_at	Activin A receptor, type IIB	1,21	*MGC152353*	617443_at	Uncharacterized LOC617443	-1,21
CPSF1	282703_at	Cleavage and polyadenylation specific factor 1, 160kda	1,21	*MFSD8*	515944_at	Major facilitator superfamily domain containing 8	-1,21
CUTA	508956_at	Cuta divalent cation tolerance homolog (E. Coli)	1,21	*MIB1*	533735_at	Mindbomb homolog 1 (Drosophila)	-1,21
PDLIM5	503621_at	PDZ and LIM domain 5	1,21	*RBM5*	534216_at	RNA binding motif protein 5	-1,21
PCNXL3	536942_at	Pecanex-like 3 (Drosophila)	1,21	*SLC39A3*	505294_at	Solute carrier family 39 (zinc transporter), member 3	-1,21
GOLGA3	533600_at	Golgin A3	1,21	*C1H3orf38*	511707_at	Chromosome 1 open reading frame, human c3orf38	-1,21
UFL1	515894_at	UFM1-specific ligase 1	1,21	*MYH10*	317655_at	Myosin, heavy chain 10, non-muscle	-1,21
SRA1	780787_at	Steroid receptor RNA activator 1	1,20	*MOCS2*	507986_at	Molybdenum cofactor synthesis 2	-1,21
NFIX	536348_at	Nuclear factor I/X (CCAAT-binding transcription factor)	1,20	*THAP10*	785266_at	THAP domain containing 10	-1,21
HEATR7A	515055_at	HEAT repeat containing 7A	1,20	*SHISA6*	539499_at	Shisa homolog 6 (Xenopus laevis)	-1,21
CD9	280746_at	CD9 molecule	1,19	*LOC100137763*	100137763_at	Uncharacterized LOC100137763	-1,21
PRRG2	511235_at	Proline rich Gla (G-carboxyglutamic acid) 2	1,19	*ENG*	615844_at	Endoglin	-1,21
UBR3	537932_at	Ubiquitin protein ligase E3 component n-recognin 3 (putative)	1,17	*PIP4K2B*	539211_at	Phosphatidylinositol-5-phosphate 4-kinase, type II, beta	-1,21
AP2B1	282183_at	Adaptor-related protein complex 2, beta 1 subunit	-1,16	*TAB2*	540203_at	TGF-beta activated kinase 1/MAP3K7 binding protein 2	-1,21
VPS13B	512656_at	Vacuolar protein sorting 13 homolog B (yeast)	-1,16	*DDB2*	519357_at	Damage-specific DNA binding protein 2, 48kda	-1,21
ZNF638	517669_at	Zinc finger protein 638	-1,16	*PCM1*	525337_at	Pericentriolar material 1	-1,21
WRAP53	509631_at	WD repeat containing, antisense to TP53	-1,17	*NTHL1*	535203_at	Nth endonuclease III-like 1 (E. Coli)	-1,21

FEM1C	541180_at	Fem-1 homolog c (C. Elegans)	-1,22	*LLGL1*	781865_at	Lethal giant larvae homolog 1 (Drosophila)	-1,24
TRMT1L	540872_at	Trna methyltransferase 1 homolog (S. Cerevisiae)-like	-1,22	*ALPK1*	524375_at	Alpha-kinase 1	-1,24
IMPACT	517248_at	Impact homolog (mouse)	-1,22	*GTF2E1*	540525_at	General transcription factor IIE, polypeptide 1, alpha 56kda	-1,24
NEK4	511455_at	NIMA (never in mitosis gene a)-related kinase 4	-1,22	*LOC536267*	536267_at	Kinase D-interacting substrate of 220 kda-like	-1,24
LRIG2	535493_at	Leucine-rich repeats and immunoglobulin-like domains 2	-1,22	*TDP1*	517053_at	Tyrosyl-DNA phosphodiesterase 1	-1,24
UROD	504914_at	Uroporphyrinogen decarboxylase	-1,22	*EIF3E*	534165_at	Eukaryotic translation initiation factor 3, subunit E	-1,24
MYO15B	508470_at	Myosin XVB pseudogene	-1,22	*SMYD3*	616050_at	SET and MYND domain containing 3	-1,24
UBN2	540792_at	Ubinuclein 2	-1,22	*POLA1*	534848_at	Polymerase (DNA directed), alpha 1, catalytic subunit	-1,25
TMEM231	511832_at	Transmembrane protein 231	-1,22	*ZNF644*	539923_at	Zinc finger protein 644	-1,25
ZMYM1	527379_at	Zinc finger, MYM-type 1	-1,23	*DDX50*	534331_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 50	-1,25
TTLL5	538422_at	Tubulin tyrosine ligase-like family, member 5	-1,23	*ULK2*	618601_at	Unc-51-like kinase 2 (C. Elegans)	-1,25
FARP1	531927_at	FERM, rhogef (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)	-1,23	*FBXW9*	532538_at	F-box and WD repeat domain containing 9	-1,25
CDC40	514003_at	Cell division cycle 40 homolog (S. Cerevisiae)	-1,23	*ANKRD13C*	528021_at	Ankyrin repeat domain 13C	-1,25
TEKT4	510343_at	Tektin 4	-1,23	*HDAC2*	407223_at	Histone deacetylase 2	-1,25
UBXN11	515112_at	UBX domain protein 11	-1,23	*TBCK*	528650_at	TBC1 domain containing kinase	-1,26
SLC25A14	513415_at	Solute carrier family 25 (mitochondrial carrier, brain), member 14	-1,23	*SLC9A3R2*	768005_at	Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 2	-1,26
WDR59	526642_at	WD repeat domain 59	-1,24	*SRSF4*	614310_at	Serine/arginine-rich splicing factor 4	-1,26
FBXO7	508235_at	F-box protein 7	-1,24	*ZNF474*	781423_at	Zinc finger protein 474	-1,26
PAPD4	533862_at	PAP associated domain containing 4	-1,24	*C22H3orf37*	530527_at	Chromosome 22 open reading frame, human c3orf37	-1,26
ATP11B	614392_at	Atpase, class VI, type 11B	-1,24	*XRRA1*	369019_at	X-ray radiation resistance associated 1	-1,26
KLHDC10	505844_at	Kelch domain containing 10	-1,24	*AIMP1*	505126_at	Aminoacyl trna synthetase complex-interacting multifunctional protein 1	-1,26
WDR1	533223_at	WD repeat domain 1	-1,24	*TMEM67*	506762_at	Transmembrane protein 67	-1,26
TSPYL4	508104_at	TSPY-like 4	-1,24	*LDLRAP1*	511199_at	Low density lipoprotein receptor adaptor protein 1	-1,26
PARP11	539763_at	Poly (ADP-ribose) polymerase family, member 11	-1,24	*TMEM163*	534678_at	Transmembrane protein 163	-1,26
SPATA6	534169_at	Spermatogenesis associated 6	-1,24	*IRAK1BP1*	782235_at	Interleukin-1 receptor-associated kinase 1 binding protein 1	-1,26

WDSUB1	783784_at	WD repeat, sterile alpha motif and U-box domain containing 1	-1,26	*PC*	338471_at	Pyruvate carboxylase	-1,27
KIAA1429	782848_at	KIAA1429 ortholog	-1,27	*RCBTB2*	513632_at	Regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2	-1,27
NCF4	507859_at	Neutrophil cytosolic factor 4, 40kda	-1,27	*ADHFE1*	507711_at	Alcohol dehydrogenase, iron containing, 1	-1,27
GNG12	286850_at	Guanine nucleotide binding protein (G protein), gamma 12	-1,27	*PPIP5K1*	510684_at	Diphosphoinositol pentakisphosphate kinase 1	-1,27
MNAT1	534176_at	Menage a trois homolog 1, cyclin H assembly factor (Xenopus laevis)	-1,27	*TTC8*	615652_at	Tetratricopeptide repeat domain 8	-1,27
LMBR1	519337_at	Limb region 1 homolog (mouse)	-1,27	*PREPL*	533479_at	Prolyl endopeptidase-like	-1,28
RABL5	516107_at	RAB, member RAS oncogene family-like 5	-1,27	*C7H5orf15*	514781_at	Chromosome 7 open reading frame, human c5orf15	-1,28
FAF1	531770_at	Fas (TNFRSF6) associated factor 1	-1,27	*HMGN3*	515652_at	High mobility group nucleosomal binding domain 3	-1,28
ALG9	504346_at	Asparagine-linked glycosylation 9, alpha-1,2-mannosyltransferase homolog (S. Cerevisiae)	-1,27	*ZBTB20*	508864_at	Zinc finger and BTB domain containing 20	-1,28
EIF4E3	616906_at	Eukaryotic translation initiation factor 4E family member 3	-1,27	*OSCP1*	504974_at	Organic solute carrier partner 1	-1,28
CARS	515715_at	Cysteinyl-trna synthetase	-1,27	*RBL2*	533294_at	Retinoblastoma-like 2 (p130)	-1,28
CNIH4	508912_at	Cornichon homolog 4 (Drosophila)	-1,27	*UHRF2*	613759_at	Ubiquitin-like with PHD and ring finger domains 2	-1,28
FAM92B	768078_at	Family with sequence similarity 92, member B	-1,27	*FAM81A*	538402_at	Family with sequence similarity 81, member A	-1,28
ELOF1	100125945_at	Elongation factor 1 homolog (S. Cerevisiae)	-1,27	*ORC6*	515476_at	Origin recognition complex, subunit 6	-1,28
ARL2BP	613462_at	ADP-ribosylation factor-like 2 binding protein	-1,27	*ELF2*	613439_at	E74-like factor 2 (ets domain transcription factor)	-1,28
SRD5A1	614612_at	Steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1)	-1,27	*TUBG2*	540216_at	Tubulin, gamma 2	-1,28
TEAD1	536560_at	TEA domain family member 1 (SV40 transcriptional enhancer factor)	-1,27	*LOC787225*	787225_at	Keratin associated protein-like	-1,28
PARL	514191_at	Presenilin associated, rhomboid-like	-1,27	*LOC100335242*	100335242_at	Zinc finger protein 292-like	-1,28
RNF139	788471_at	Ring finger protein 139	-1,27	*C15H11orf58*	616182_at	Chromosome 15 open reading frame, human c11orf58	-1,29
EFCAB11	617365_at	EF-hand calcium binding domain 11	-1,27	*SRGAP3*	512892_at	SLIT-ROBO Rho gtpase activating protein 3	-1,29
ANGEL1	508197_at	Angel homolog 1 (Drosophila)	-1,27	*PHF17*	509264_at	PHD finger protein 17	-1,29
PHKB	511783_at	Phosphorylase kinase, beta	-1,27	*IFT52*	513908_at	Intraflagellar transport 52 homolog (Chlamydomonas)	-1,29

IFT46	505579_at	Intraflagellar transport 46 homolog (Chlamydomonas)	-1,29	*CARM1*	784795_at	Coactivator-associated arginine methyltransferase 1	-1,31
LETMD1	514595_at	LETM1 domain containing 1	-1,29	*KATNAL2*	514354_at	Katanin p60 subunit A-like 2	-1,31
EEA1	516259_at	Early endosome antigen 1	-1,29	*POLI*	515909_at	Polymerase (DNA directed) iota	-1,31
SCRG1	515382_at	Stimulator of chondrogenesis 1	-1,29	*C11H9orf171*	617847_at	Chromosome 11 open reading frame, human c9orf171	-1,31
SLC25A28	538529_at	Solute carrier family 25, member 28	-1,29	*SASS6*	504467_at	Spindle assembly 6 homolog (C. Elegans)	-1,31
POGLUT1	511862_at	Protein O-glucosyltransferase 1	-1,29	*PTBP2*	537341_at	Polypyrimidine tract binding protein 2	-1,31
SEC22A	532730_at	SEC22 vesicle trafficking protein homolog A (S. Cerevisiae)	-1,29	*DYNC2H1*	512287_at	Dynein, cytoplasmic 2, heavy chain 1	-1,31
CCDC60	614062_at	Coiled-coil domain containing 60	-1,29	*GLCCI1*	541048_at	Glucocorticoid induced transcript 1	-1,31
EXOC1	504440_at	Exocyst complex component 1	-1,30	*FBXO15*	505376_at	F-box protein 15	-1,31
MKS1	530761_at	Meckel syndrome, type 1	-1,30	*SCAMP1*	535352_at	Secretory carrier membrane protein 1	-1,31
ZNF451	529894_at	Zinc finger protein 451	-1,30	*CCDC11*	540193_at	Coiled-coil domain containing 11	-1,31
CPSF3	281712_at	Cleavage and polyadenylation specific factor 3, 73kda	-1,30	*LOC513496*	513496_at	Uncharacterized LOC513496	-1,31
ZMYM6	618234_at	Zinc finger, MYM-type 6	-1,30	*GFRA3*	540009_at	GDNF family receptor alpha 3	-1,31
CLDN25	522679_at	Claudin 25	-1,30	*ICAM1*	281839_at	Intercellular adhesion molecule 1	-1,31
PAPSS1	504439_at	3'-phosphoadenosine 5'-phosphosulfate synthase 1	-1,30	*WDR27*	788957_at	WD repeat domain 27	-1,31
EIF3D	515226_at	Eukaryotic translation initiation factor 3, subunit D	-1,30	*VPS41*	528654_at	Vacuolar protein sorting 41 homolog (S. Cerevisiae)	-1,32
CLYBL	533186_at	Citrate lyase beta like	-1,30	*XRCC1*	616905_at	X-ray repair complementing defective repair in Chinese hamster cells 1	-1,32
AKD1	504511_at	Adenylate kinase domain containing 1	-1,30	*BTBD9*	505504_at	BTB (POZ) domain containing 9	-1,32
POC5	533555_at	POC5 centriolar protein homolog (Chlamydomonas)	-1,30	*MAPKBP1*	788366_at	Mitogen-activated protein kinase binding protein 1	-1,32
RAC2	327671_at	Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	-1,30	*SEC23A*	533994_at	Sec23 homolog A (S. Cerevisiae)	-1,32
IKZF2	786795_at	IKAROS family zinc finger 2 (Helios)	-1,30	*BBS4*	532120_at	Bardet-Biedl syndrome 4	-1,32
ZHX1	782847_at	Zinc fingers and homeoboxes 1	-1,30	*ARMC2*	520151_at	Armadillo repeat containing 2	-1,32
WDR60	767895_at	WD repeat domain 60	-1,30	*MPP2*	509346_at	Membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2)	-1,32
REEP3	512704_at	Receptor accessory protein 3	-1,30	*ADGB*	528523_at	Androglobin	-1,32
C8H9orf3	531757_at	Chromosome 8 open reading frame, human c9orf3	-1,30	*WDR78*	535410_at	WD repeat domain 78	-1,32
NLRX1	539974_at	NLR family member X1	-1,31	*COQ6*	511624_at	Coenzyme Q6 homolog, monooxygenase (S. Cerevisiae)	-1,32

ECE1	281133_at	Endothelin converting enzyme 1	-1,32	*ITGAV*	281875_at	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	-1,34
WDR65	521560_at	WD repeat domain 65	-1,32	*MORN1*	617176_at	MORN repeat containing 1	-1,34
PSMD4	282016_at	Proteasome (prosome, macropain) 26S subunit, non-atpase, 4	-1,32	*ABI2*	537711_at	Abl-interactor 2	-1,34
ERN1	524719_at	Endoplasmic reticulum to nucleus signaling 1	-1,32	*LOC528767*	528767_at	Kinesin family member 19-like	-1,34
C8H9orf24	540767_at	Chromosome 8 open reading frame, human c9orf24	-1,32	*ACAT1*	511082_at	Acetyl-coa acetyltransferase 1	-1,34
MAK	536048_at	Male germ cell-associated kinase	-1,32	*PARD6A*	524653_at	Par-6 partitioning defective 6 homolog alpha (C. Elegans)	-1,34
FAM221B	783189_at	Family with sequence similarity 221, member B	-1,32	*ZNF365*	521207_at	Zinc finger protein 365	-1,34
UTS2R	286969_at	Urotensin 2 receptor	-1,32	*THRA*	532621_at	Thyroid hormone receptor, alpha	-1,34
DCAF6	524645_at	DDB1 and CUL4 associated factor 6	-1,32	*RFWD2*	519896_at	Ring finger and WD repeat domain 2, E3 ubiquitin protein ligase	-1,34
IFT140	100139697_at	Intraflagellar transport 140 homolog (Chlamydomonas)	-1,33	*DZANK1*	512107_at	Double zinc ribbon and ankyrin repeat domains 1	-1,34
MGST2	615552_at	Microsomal glutathione S-transferase 2	-1,33	*CAMTA1*	532321_at	Calmodulin-binding transcription activator 1	-1,34
CCDC155	617651_at	Coiled-coil domain containing 155	-1,33	*ZMPSTE24*	538104_at	Zinc metallopeptidase (STE24 homolog, S. Cerevisiae)	-1,34
NAP1L4	513028_at	Nucleosome assembly protein 1-like 4	-1,33	*C1QTNF1*	511774_at	C1q and tumor necrosis factor related protein 1	-1,34
C17H5orf52	787653_at	Chromosome 17 open reading frame, human c5orf52	-1,33	*LFNG*	516209_at	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	-1,34
L2HGDH	514230_at	L-2-hydroxyglutarate dehydrogenase	-1,33	*RP1*	280916_at	Retinitis pigmentosa 1 (autosomal dominant)	-1,34
ANKRD32	520250_at	Ankyrin repeat domain 32	-1,33	*LOC100295019*	100295019_at	Transmembrane protein 232-like	-1,34
MORN3	531482_at	MORN repeat containing 3	-1,33	*XRCC5*	531945_at	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining)	-1,34
CERS1	615296_at	Ceramide synthase 1	-1,33	*FAM184B*	523874_at	Family with sequence similarity 184, member B	-1,34
SLC2A1	282356_at	Solute carrier family 2 (facilitated glucose transporter), member 1	-1,33	*IL17RE*	783335_at	Interleukin 17 receptor E	-1,34
SLC22A7	407224_at	Solute carrier family 22 (organic anion transporter), member 7	-1,33	*CYP8B1*	538964_at	Cytochrome P450, family 8, subfamily B, polypeptide 1	-1,34
BRS3	539011_at	Bombesin-like receptor 3	-1,33	*RNF216*	534455_at	Ring finger protein 216	-1,35
ANKMY1	538124_at	Ankyrin repeat and MYND domain containing 1	-1,33	*RIBC1*	512894_at	RIB43A domain with coiled-coils 1	-1,35
EPHA2	512798_at	EPH receptor A2	-1,33	*DFFA*	507981_at	DNA fragmentation factor, 45kda, alpha polypeptide	-1,35

DYX1C1	527393_at	Dyslexia susceptibility 1 candidate 1	-1,35	*NGRN*	508115_at	Neugrin, neurite outgrowth associated	-1,36
HYDIN	504406_at	HYDIN, axonemal central pair apparatus protein	-1,35	*NUDT4*	614183_at	Nudix (nucleoside diphosphate linked moiety X)-type motif 4	-1,36
LOC521764	521764_at	Myosin-7B-like	-1,35		100851473_at		-1,36
CYB5D1	507171_at	Cytochrome b5 domain containing 1	-1,35	*ZNF167*	539552_at	Zinc finger protein 167	-1,36
LOC530264	530264_at	Leucine-rich repeat-containing protein LOC400891-like	-1,35	*DNAJB13*	520270_at	Dnaj (Hsp40) homolog, subfamily B, member 13	-1,36
ZNF512	533884_at	Zinc finger protein 512	-1,35	*STK31*	781749_at	Serine/threonine kinase 31	-1,36
IQCD	513272_at	IQ motif containing D	-1,35	*CELSR1*	522422_at	Cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo homolog, Drosophila)	-1,36
SLC35F5	615480_at	Solute carrier family 35, member F5	-1,35	*ABHD10*	515563_at	Abhydrolase domain containing 10	-1,36
OSBP2	510311_at	Oxysterol binding protein 2	-1,35	*ZC3HAV1*	614589_at	Zinc finger CCCH-type, antiviral 1	-1,36
VWA3A	522712_at	Von Willebrand factor A domain-containing protein 3A-like	-1,35	*BRCA1*	353120_at	Breast cancer 1, early onset	-1,36
KIF9	508574_at	Kinesin family member 9	-1,35	*ZSWIM6*	538841_at	Zinc finger, SWIM-type containing 6	-1,37
CCDC65	535207_at	Coiled-coil domain containing 65	-1,35	*C11H2orf56*	504290_at	Chromosome 11 open reading frame, human c2orf56	-1,37
DYSF	508157_at	Dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive)	-1,35	*NUBPL*	614641_at	Nucleotide binding protein-like	-1,37
SCLT1	534830_at	Sodium channel and clathrin linker 1	-1,35	*LOC509109*	509109_at	Mitogen-activated protein kinase kinase kinase kinase 4-like	-1,37
CCDC19	617350_at	Coiled-coil domain containing 19	-1,35	*BBS12*	516376_at	Bardet-Biedl syndrome 12	-1,37
KCNJ2	281883_at	Potassium inwardly-rectifying channel, subfamily J, member 2	-1,35	*CASC1*	526444_at	Cancer susceptibility candidate 1	-1,37
LASP1	532851_at	LIM and SH3 protein 1	-1,36	*MGMT*	616091_at	O-6-methylguanine-DNA methyltransferase	-1,37
MAPKAP1	533861_at	Mitogen-activated protein kinase associated protein 1	-1,36	*TCTN1*	510513_at	Tectonic family member 1	-1,37
CDH3	281063_at	Cadherin 3, type 1, P-cadherin (placental)	-1,36	*HSD17B4*	493643_at	Hydroxysteroid (17-beta) dehydrogenase 4	-1,37
CNTRL	520186_at	Centriolin	-1,36	*FBXL2*	616212_at	F-box and leucine-rich repeat protein 2	-1,37
IPMK	615135_at	Inositol polyphosphate multikinase	-1,36	*HGSNAT*	511607_at	Heparan-alpha-glucosaminide N-acetyltransferase	-1,37
ARL6	519014_at	ADP-ribosylation factor-like 6	-1,36	*YIPF7*	522186_at	Yip1 domain family, member 7	-1,37
IVNS1ABP	514940_at	Influenza virus NS1A binding protein	-1,36	*EEPD1*	511767_at	Endonuclease/exonuclease/phosphatase family domain containing 1	-1,37
ARMCX3	516747_at	Armadillo repeat containing, X-linked 3	-1,36	*LRRC46*	780880_at	Leucine rich repeat containing 46	-1,37
METAP1D	533042_at	Methionyl aminopeptidase type 1D (mitochondrial)	-1,36	*DCLRE1A*	504377_at	DNA cross-link repair 1A	-1,37
RAI14	525869_at	Retinoic acid induced 14	-1,36	*NSMCE1*	534249_at	Non-SMC element 1 homolog (S. Cerevisiae)	-1,37
PL-5283	784845_at	PL-5283 protein	-1,36	*EML6*	516921_at	Echinoderm microtubule associated protein like 6	-1,37

EHD4	505206_at	EH-domain containing 4	-1,37	*ANXA8L1*	281627_at	Annexin A8-like 1	-1,39
C13H20orf26	616648_at	Chromosome 13 open reading frame, human c20orf26	-1,37	*SGK3*	504480_at	Serum/glucocorticoid regulated kinase family, member 3	-1,39
EML4	540187_at	Echinoderm microtubule associated protein like 4	-1,38	*NFE2L1*	534582_at	Nuclear factor (erythroid-derived 2)-like 1	-1,39
TCP11	523297_at	T-complex 11 homolog (mouse)	-1,38	*MGST3*	507346_at	Microsomal glutathione S-transferase 3	-1,39
CEP112	617266_at	Centrosomal protein 112kda	-1,38	*LOC781401*	781401_at	Methyltransferase-like protein 7A-like	-1,39
CBL	527418_at	Cas-Br-M (murine) ecotropic retroviral transforming sequence	-1,38	*PRRG4*	616767_at	Proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)	-1,39
SCMH1	540926_at	Sex comb on midleg homolog 1 (Drosophila)	-1,38	*UBE2L6*	509471_at	Ubiquitin-conjugating enzyme E2L 6	-1,39
TYMS	507631_at	Thymidylate synthetase	-1,38	*SRR*	525340_at	Serine racemase	-1,39
IQCH	519277_at	IQ motif containing H	-1,38	*ACOXL*	100336467_at	Acyl-coa oxidase-like	-1,39
ACVR2A	281598_at	Activin A receptor, type IIA	-1,38	*TEX2*	513262_at	Testis expressed 2	-1,39
KIF3C	777770_at	Kinesin family member 3C	-1,38	*BBS2*	533611_at	Bardet-Biedl syndrome 2	-1,39
ZSWIM7	514094_at	Zinc finger, SWIM-type containing 7	-1,38	*EIF3J*	539052_at	Eukaryotic translation initiation factor 3, subunit J	-1,39
DNAH3	786654_at	Dynein, axonemal, heavy chain 3	-1,38	*WASF1*	531488_at	WAS protein family, member 1	-1,39
MEFV	529195_at	Mediterranean fever	-1,38	*HS6ST1*	518563_at	Heparan sulfate 6-O-sulfotransferase 1	-1,39
RBMS1	526135_at	RNA binding motif, single stranded interacting protein 1	-1,38	*ZNF627*	505918_at	Zinc finger protein 627	-1,39
MNS1	532884_at	Meiosis-specific nuclear structural 1	-1,38	*SDCCAG8*	616342_at	Serologically defined colon cancer antigen 8	-1,39
ATP11C	504969_at	Atpase, class VI, type 11C	-1,38	*SLC22A23*	614701_at	Solute carrier family 22, member 23	-1,39
LOC516579	516579_at	Probable phospholipid-transporting atpase IIA-like	-1,38	*ABCG8*	508829_at	ATP-binding cassette, sub-family G (WHITE), member 8	-1,39
LOC539231	539231_at	Kinesin-like protein KIFC3-like	-1,38	*LRRN4CL*	788567_at	LRRN4 C-terminal like	-1,39
RSPH10B	510922_at	Radial spoke head 10 homolog B (Chlamydomonas)	-1,39	*TEKT1*	519068_at	Tektin 1	-1,39
LSM11	785718_at	LSM11, U7 small nuclear RNA associated	-1,39	*LOC782834*	782834_at	Anthrax toxin receptor-like	-1,39
RAD51B	617007_at	RAD51 homolog B (S. Cerevisiae)	-1,39	*C19H17orf109*	100302388_at	Chromosome 19 open reading frame, human c17orf109	-1,39
TMEM17	514758_at	Transmembrane protein 17	-1,39	*RASSF1*	510276_at	Ras association (ralgds/AF-6) domain family member 1	-1,39
PCNXL4	539495_at	Pecanex-like 4 (Drosophila)	-1,39	*ARPM1*	509723_at	Actin related protein M1	-1,39
WDR66	536738_at	WD repeat domain 66	-1,39	*SLC45A4*	520630_at	Solute carrier family 45, member 4	-1,39
GPD2	504948_at	Glycerol-3-phosphate dehydrogenase 2 (mitochondrial)	-1,39	*DIXDC1*	541211_at	DIX domain containing 1	-1,40
	100852137_at		-1,39	*DUSP14*	507294_at	Dual specificity phosphatase 14	-1,40

HEATR5B	540503_at	HEAT repeat containing 5B	-1,40	*PBX3*	539222_at	Pre-B-cell leukemia homeobox 3	-1,41
WDR17	783416_at	WD repeat domain 17	-1,40	*GALK2*	536133_at	Galactokinase 2	-1,41
KCTD1	784587_at	Potassium channel tetramerisation domain containing 1	-1,40	*LCORL*	540095_at	Ligand dependent nuclear receptor corepressor-like	-1,41
PTPLB	613886_at	Protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	-1,40	*AOAH*	768208_at	Acyloxyacyl hydrolase (neutrophil)	-1,41
ARHGAP19	526945_at	Rho gtpase activating protein 19	-1,40	*RAB26*	515675_at	RAB26, member RAS oncogene family	-1,41
FAM154B	538044_at	Family with sequence similarity 154, member B	-1,40	*ADAMTS14*	510214_at	ADAM metallopeptidase with thrombospondin type 1 motif, 14	-1,41
TMEM209	510567_at	Transmembrane protein 209	-1,40	*ALDOB*	515263_at	Aldolase B, fructose-bisphosphate	-1,41
BBS7	100138953_at	Bardet-Biedl syndrome 7	-1,40	*TMEM185A*	541149_at	Transmembrane protein 185A	-1,41
LOC100295193	100295193_at	Swi5-dependent recombination DNA repair protein 1 homolog	-1,40	*LRGUK*	529920_at	Leucine-rich repeats and guanylate kinase domain containing	-1,41
PTPN14	518798_at	Protein tyrosine phosphatase, non-receptor type 14	-1,40	*POU3F2*	783320_at	POU class 3 homeobox 2	-1,41
IFT81	519602_at	Intraflagellar transport 81 homolog (Chlamydomonas)	-1,40	*LRRC71*	511219_at	Leucine rich repeat containing 71	-1,41
HPCAL1	513870_at	Hippocalcin-like 1	-1,40	*RBBP8*	512977_at	Retinoblastoma binding protein 8	-1,41
SLC4A9	509031_at	Solute carrier family 4, sodium bicarbonate cotransporter, member 9	-1,40	*SPATA6L*	613587_at	Spermatogenesis associated 6-like	-1,41
SIGLEC14	614923_at	Sialic acid binding Ig-like lectin 14	-1,40	*LOC782076*	782076_at	Uncharacterized LOC782076	-1,41
NT5C2	281951_at	5'-nucleotidase, cytosolic II	-1,40	*RSPH1*	514556_at	Radial spoke head 1 homolog (Chlamydomonas)	-1,41
LRRC36	512403_at	Leucine rich repeat containing 36	-1,40	*CROCC*	530641_at	Ciliary rootlet coiled-coil, rootletin	-1,41
FAM166B	507810_at	Family with sequence similarity 166, member B	-1,40	*CIDEB*	528834_at	Cell death-inducing DFFA-like effector b	-1,41
NEK10	522335_at	NIMA (never in mitosis gene a)- related kinase 10	-1,40	*PLSCR4*	539290_at	Phospholipid scramblase 4	-1,41
VEPH1	100337421_at	Ventricular zone expressed PH domain homolog 1 (zebrafish)	-1,40	*DDR1*	534092_at	Discoidin domain receptor tyrosine kinase 1	-1,41
AKAP3	281610_at	A kinase (PRKA) anchor protein 3	-1,40	*LOC100851354*	100851354_at	SCAN domain-containing protein 3-like	-1,41
HES4	507480_at	Hairy and enhancer of split 4 (Drosophila)	-1,40	*SCLY*	790815_at	Selenocysteine lyase	-1,41
CPNE7	515424_at	Copine VII	-1,40	*EGFL6*	781765_at	EGF-like-domain, multiple 6	-1,41
AGBL5	538585_at	ATP/GTP binding protein-like 5	-1,41	*DBT*	280759_at	Dihydrolipoamide branched chain transacylase E2	-1,42
MAP4K5	781335_at	Mitogen-activated protein kinase kinase kinase kinase 5	-1,41	*TOR3A*	789634_at	Torsin family 3, member A	-1,42

DAPK1	540873_at	Death-associated protein kinase 1	-1,42	*VRK1*	618880_at	Vaccinia related kinase 1	-1,43
BTBD17	789790_at	BTB (POZ) domain containing 17	-1,42		100851789_at		-1,43
CDC14B	520428_at	CDC14 cell division cycle 14 homolog B (S. Cerevisiae)	-1,42	*MORN5*	614076_at	MORN repeat containing 5	-1,43
C27H4orf47	523361_at	Chromosome 27 open reading frame, human c4orf47	-1,42	*ZMYND15*	539735_at	Zinc finger, MYND-type containing 15	-1,43
COX7A1	338086_at	Cytochrome c oxidase subunit viia polypeptide 1 (muscle)	-1,42	*TSGA10*	536433_at	Testis specific, 10	-1,43
TOX2	519845_at	TOX high mobility group box family member 2	-1,42	*SAMD15*	530413_at	Sterile alpha motif domain containing 15	-1,43
SPAG1	530104_at	Sperm associated antigen 1	-1,42	*KRT14*	404111_at	Keratin 14	-1,43
WDR63	520702_at	WD repeat domain 63	-1,42	*UPB1*	504557_at	Ureidopropionase, beta	-1,43
DGKH	537533_at	Diacylglycerol kinase, eta	-1,42	*IL6ST*	522155_at	Interleukin 6 signal transducer (gp130, oncostatin M receptor)	-1,43
RNF182	518996_at	Ring finger protein 182	-1,42	*MAMDC2*	788176_at	MAM domain containing 2	-1,43
GPRC5C	535664_at	G protein-coupled receptor, family C, group 5, member C	-1,42	*LOC538699*	538699_at	TAF5-like RNA polymerase II p300/CBP-associated factor-associated factor 65 kda subunit 5L-like	-1,43
GEN1	785690_at	Gen homolog 1, endonuclease (Drosophila)	-1,42	*GLRB*	281198_at	Glycine receptor, beta	-1,43
MYB	317776_at	V-myb myeloblastosis viral oncogene homolog (avian)	-1,42	*CELA1*	281139_at	Chymotrypsin-like elastase family, member 1	-1,43
CAMKK2	509084_at	Calcium/calmodulin-dependent protein kinase kinase 2, beta	-1,42	*SOD2*	281496_at	Superoxide dismutase 2, mitochondrial	-1,43
WDR69	530118_at	WD repeat domain 69	-1,43	*MR1*	506206_at	Major histocompatibility complex, class I-related	-1,43
C1H3orf15	100300968_at	Chromosome 1 open reading frame, human c3orf15	-1,43	*NARS2*	504824_at	Asparaginyl-trna synthetase 2, mitochondrial (putative)	-1,44
ZBTB25	538889_at	Zinc finger and BTB domain containing 25	-1,43	*FANCI*	522442_at	Fanconi anemia, complementation group I	-1,44
TTC26	508011_at	Tetratricopeptide repeat domain 26	-1,43	*TLN2*	528252_at	Talin 2	-1,44
PKD2	530393_at	Polycystic kidney disease 2 (autosomal dominant)	-1,43	*THG1L*	507084_at	Trna-histidine guanylyltransferase 1-like (S. Cerevisiae)	-1,44
DNAI1	524709_at	Dynein, axonemal, intermediate chain 1	-1,43	*KIAA1841*	538151_at	KIAA1841 ortholog	-1,44
C2H2orf62	514380_at	Chromosome 2 open reading frame, human c2orf62	-1,43	*BACH2*	538296_at	BTB and CNC homology 1, basic leucine zipper transcription factor 2	-1,44
MSI2	505542_at	Musashi homolog 2 (Drosophila)	-1,43	*ANKRD50*	527956_at	Ankyrin repeat domain 50	-1,44
ZNHIT2	539138_at	Zinc finger, HIT-type containing 2	-1,43	*SNN*	615361_at	Stannin	-1,44
ROPN1	527583_at	Rhophilin associated tail protein 1	-1,43	*MAP3K8*	535622_at	Mitogen-activated protein kinase kinase kinase 8	-1,44
LCMT2	538825_at	Leucine carboxyl methyltransferase 2	-1,43	*WDR96*	518801_at	WD repeat domain 96	-1,44
ZNF697	540379_at	Zinc finger protein 697	-1,43	*SMYD2*	615229_at	SET and MYND domain containing 2	-1,44

RAB37	613954_at	RAB37, member RAS oncogene family	-1,44	*LOC787810*	787810_at	Olfactory receptor, family 56, subfamily B, member 2 pseudogene-like	-1,45
GREB1L	535053_at	Growth regulation by estrogen in breast cancer-like	-1,44	*CCDC147*	536914_at	Coiled-coil domain containing 147	-1,45
RNF32	520408_at	Ring finger protein 32	-1,44	*METTL4*	521222_at	Methyltransferase like 4	-1,46
ADAM32	520297_at	ADAM metallopeptidase domain 32	-1,44	*ZNF215*	529157_at	Zinc finger protein 215	-1,46
DLX2	528490_at	Distal-less homeobox 2	-1,44	*TTC25*	788831_at	Tetratricopeptide repeat domain 25	-1,46
HAS2	281220_at	Hyaluronan synthase 2	-1,44	*WDR93*	515609_at	WD repeat domain 93	-1,46
SMARCA2	540904_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	-1,44	*LOC100336046*	100336046_at	Protocadherin gamma-B6-like	-1,46
PIK3C2A	537790_at	Phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha	-1,45	*SALL4*	541170_at	Sal-like 4 (Drosophila)	-1,46
C19H17orf108	615250_at	Chromosome 19 open reading frame, human c17orf108	-1,45	*FBXO47*	613655_at	F-box protein 47	-1,46
CD97	338066_at	CD97 molecule	-1,45	*CCDC114*	617306_at	Coiled-coil domain containing 114	-1,46
SPEF1	512410_at	Sperm flagellar 1	-1,45	*IFNGR2*	514889_at	Interferon gamma receptor 2 (interferon gamma transducer 1)	-1,46
SFMBT1	511984_at	Scm-like with four mbt domains 1	-1,45	*LOC781667*	781667_at	Kin of IRRE like	-1,46
TMEM164	524183_at	Transmembrane protein 164	-1,45	*LAP3*	781648_at	Leucine aminopeptidase 3	-1,46
ATF3	515266_at	Activating transcription factor 3	-1,45	*SMTNL2*	532143_at	Smoothelin-like 2	-1,46
ANXA4	281625_at	Annexin A4	-1,45	*EPB41L4A*	519867_at	Erythrocyte membrane protein band 4.1 like 4A	-1,47
GAS2L2	539169_at	Growth arrest-specific 2 like 2	-1,45	*RFX3*	538070_at	Regulatory factor X, 3 (influences HLA class II expression)	-1,47
PFKM	506544_at	Phosphofructokinase, muscle	-1,45	*TPK1*	788066_at	Thiamin pyrophosphokinase 1	-1,47
MLF1	533379_at	Myeloid leukemia factor 1	-1,45	*DCDC2B*	514470_at	Doublecortin domain containing 2B	-1,47
LRRC10B	520410_at	Leucine rich repeat containing 10B	-1,45	*FOXJ1*	505891_at	Forkhead box J1	-1,47
CCDC135	504736_at	Coiled-coil domain containing 135	-1,45	*F2R*	526585_at	Coagulation factor II (thrombin) receptor	-1,47
SRPX	337918_at	Sushi-repeat containing protein, X-linked	-1,45	*CACHD1*	536020_at	Cache domain containing 1	-1,47
CCDC42B	515855_at	Coiled-coil domain containing 42B	-1,45	*DCDC1*	616832_at	Doublecortin domain containing 1	-1,47
HDAC11	519899_at	Histone deacetylase 11	-1,45	*LOC100297243*	100297243_at	Coiled-coil domain-containing protein 30-like	-1,47
CAPSL	507306_at	Calcyphosine-like	-1,45	*EFCAB12*	514973_at	EF-hand calcium binding domain 12	-1,47
ZNFX1	539807_at	Zinc finger, NFX1-type containing 1	-1,45	*ARNT2*	533445_at	Aryl-hydrocarbon receptor nuclear translocator 2	-1,47
GLB1L	532551_at	Galactosidase, beta 1-like	-1,45	*ABHD6*	505283_at	Abhydrolase domain containing 6	-1,48
PFKFB4	534928_at	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	-1,45	*SLC35D1*	613734_at	Solute carrier family 35 (UDP-glucuronic acid/UDP-N-acetylgalactosamine dual transporter), member D1	-1,48

EFHC1	510124_at	EF-hand domain (C-terminal) containing 1	-1,48	*ANKRD35*	514513_at	Ankyrin repeat domain 35	-1,49
FGF10	326285_at	Fibroblast growth factor 10	-1,48	*CDC25C*	507731_at	Cell division cycle 25 homolog c (s. Pombe)	-1,49
PLXDC2	515731_at	Plexin domain containing 2	-1,48	*MAP6*	518794_at	Microtubule-associated protein 6	-1,49
EFCAB1	505272_at	EF-hand calcium binding domain 1	-1,48	*PACRG*	767959_at	Park2 co-regulated	-1,49
RIIAD1	767988_at	Regulatory subunit of type II PKA R-subunit (riia) domain containing 1	-1,48	*SLC9A11*	787966_at	Solute carrier family 9, member 11	-1,49
LOC100336936	100336936_at	Phosphatidate cytidylyltransferase 1-like	-1,48	*ZNF624*	508931_at	Zinc finger protein 624	-1,49
TGFBR2	535376_at	Transforming growth factor, beta receptor II (70/80kda)	-1,48	*LOC614881*	614881_at	Histone cluster 3, h2a-like	-1,49
FILIP1	514193_at	Filamin A interacting protein 1	-1,48	*USP27X*	781718_at	Ubiquitin specific peptidase 27, x-linked	-1,49
GALNT3	535458_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (galnac-T3)	-1,48	*FUT8*	281177_at	Fucosyltransferase 8 (alpha (1,6) fucosyltransferase)	-1,49
KPNA5	783596_at	Karyopherin alpha 5 (importin alpha 6)	-1,48	*LOC784846*	784846_at	Cytohesin-2-like	-1,49
	100851094_at		-1,48	*TRHR*	281549_at	Thyrotropin-releasing hormone receptor	-1,49
LOC618012	618012_at	Histone H2B type 1-like	-1,48	*CCDC171*	538331_at	Coiled-coil domain containing 171	-1,49
IFT27	617147_at	Intraflagellar transport 27 homolog (Chlamydomonas)	-1,48	*TNFRSF1B*	338033_at	Tumor necrosis factor receptor superfamily, member 1b	-1,51
MIR16A	100313007_at	Microrna mir-16a	-1,48	*LOC505843*	505843_at	Uncharacterized loc505843	-1,51
EXT1	538602_at	Exostosin 1	-1,48	*SMPD3*	514201_at	Sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase ii)	-1,51
VSIG8	520493_at	V-set and immunoglobulin domain containing 8	-1,49	*FAM216B*	614187_at	Chromosome 12 open reading frame, human c13orf30	-1,51
DFNB59	618114_at	Deafness, autosomal recessive 59	-1,49	*LAMC2*	511043_at	Laminin, gamma 2	-1,51
CETN4	505789_at	Centrin 4	-1,49	*ATP11C*	529689_at	Atpase, class vi, type 11c	-1,51
IQUB	536449_at	IQ motif and ubiquitin domain containing	-1,49	*IFT57*	531436_at	Intraflagellar transport 57 homolog (chlamydomonas)	-1,51
DCUN1D4	538195_at	DCN1, defective in cullin neddylation 1, domain containing 4 (S. Cerevisiae)	-1,49	*DNAH11*	497208_at	Dynein, axonemal, heavy chain 11	-1,51
B3GNT5	767899_at	UDP-glcnac:betagal beta-1,3-N-acetylglucosaminyltransferase 5	-1,49	*RPS6KA5*	504408_at	Ribosomal protein s6 kinase, 90kda, polypeptide 5	-1,51
WDR52	513653_at	WD repeat domain 52	-1,49	*LRRC66*	516507_at	Leucine rich repeat containing 66	-1,51
LOC785478	785478_at	Hypothetical LOC785478	-1,49	*SLC25A21*	513423_at	Solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	-1,51
RSPH4A	509632_at	Radial spoke head 4 homolog A (Chlamydomonas)	-1,49	*C17H4orf29*	530484_at	Chromosome 17 open reading frame, human c4orf29	-1,51
RHOV	538143_at	Ras homolog gene family, member V	-1,49	*TEK*	280939_at	Tek tyrosine kinase, endothelial	-1,51
C3H1orf88	615099_at	Chromosome 3 open reading frame, human c1orf88	-1,49	*KL*	784635_at	Klotho	-1,51

IL15	281248_at	Interleukin 15	-1,51	*TMEM154*	510523_at	Transmembrane protein 154	-1,53
PRSS44	100140621_at	Protease, serine, 44	-1,51	*DCDC5*	529596_at	Uncharacterized loc529596	-1,53
ISYNA1	509394_at	Inositol-3-phosphate synthase 1	-1,51	*C6H4orf48*	615222_at	Chromosome 6 open reading frame, human c4orf48	-1,53
DUPD1	616082_at	Dual specificity phosphatase and pro isomerase domain containing 1	-1,51	*CXCR4*	281736_at	Chemokine (c-x-c motif) receptor 4	-1,53
SNTN	768326_at	Sentan, cilia apical structure protein	-1,51	*SMO*	539308_at	Smoothened, frizzled family receptor	-1,53
WWTR1	614786_at	WW domain containing transcription regulator 1	-1,52	*MAPK10*	537631_at	Mitogen-activated protein kinase 10	-1,53
NPHP1	505421_at	Nephronophthisis 1 (juvenile)	-1,52	*IKZF1*	541154_at	Ikaros family zinc finger 1 (ikaros)	-1,53
ANP32E	507203_at	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member E	-1,52	*MAP1A*	515593_at	Microtubule-associated protein 1a	-1,54
FRRS1	516522_at	Ferric-chelate reductase 1	-1,52	*HNMT*	613413_at	Histamine n-methyltransferase	-1,54
ZNF462	515561_at	Zinc finger protein 462	-1,52	*GAS6*	504526_at	Growth arrest-specific 6	-1,54
LOC100296849	100296849_at	Protein BEX3-like	-1,52	*SLC9A3R1*	505242_at	Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	-1,54
ALOX5	404074_at	Arachidonate 5-lipoxygenase	-1,52	*HIST1H2BL*	506306_at	Histone cluster 1, h2bl	-1,54
DPY19L2	524676_at	Dpy-19-like 2 (C. Elegans)	-1,52	*CAMK2G*	282162_at	Calcium/calmodulin-dependent protein kinase ii gamma	-1,54
LOC614376	614376_at	Histone cluster 2, h2be-like	-1,52	*ZMYND10*	528799_at	Zinc finger, mynd-type containing 10	-1,54
NFKBIZ	282713_at	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	-1,52	*EPHB6*	529800_at	Eph receptor b6	-1,54
FBXO2	512589_at	F-box protein 2	-1,52	*GPR87*	785030_at	G protein-coupled receptor 87	-1,54
FZD6	445418_at	Frizzled family receptor 6	-1,52	*ELK3*	541125_at	Elk3, ets-domain protein (srf accessory protein 2)	-1,54
PLEKHA7	528261_at	Pleckstrin homology domain containing, family A member 7	-1,53	*LOC509884*	509884_at	Olfactory receptor, family 4, subfamily x, member 2-like	-1,54
IFT80	513583_at	Intraflagellar transport 80 homolog (Chlamydomonas)	-1,53	*EZH2*	509106_at	Enhancer of zeste homolog 2 (drosophila)	-1,55
ABCG4	508443_at	ATP-binding cassette, sub-family G (WHITE), member 4	-1,53	*ST3GAL2*	444879_at	St3 beta-galactoside alpha-2,3-sialyltransferase 2	-1,55
PLXND1	781625_at	Plexin D1	-1,53	*AGTRAP*	508521_at	Angiotensin ii receptor-associated protein	-1,55
CEP350	534896_at	Centrosomal protein 350kda	-1,53	*CYP4F3*	534967_at	Cytochrome p450, family 4, subfamily f, polypeptide 3	-1,55
IGFBP2	282260_at	Insulin-like growth factor binding protein 2, 36kda	-1,53	*CCT6B*	538090_at	Chaperonin containing tcp1, subunit 6b (zeta 2)	-1,55
CCDC13	538960_at	Coiled-coil domain containing 13	-1,53	*ENO4*	767880_at	Enolase family member 4	-1,55
UNC5C	533256_at	Unc-5 homolog C (C. Elegans)	-1,53	*RHOBTB1*	540513_at	Rho-related btb domain containing 1	-1,55
GNB3	513340_at	Guanine nucleotide binding protein (G protein), beta polypeptide 3	-1,53	*TPP2*	526052_at	Tripeptidyl peptidase ii	-1,55

C1H21orf63	516536_at	Chromosome 1 open reading frame, human c21orf63	-1,55	*GALNT6*	506903_at	Udp-n-acetyl-alpha-d-galactosamine:polypeptide n-acetylgalactosaminyltransferase 6 (galnac-t6)	-1,56
WFDC3	505523_at	WAP four-disulfide core domain 3	-1,55	*ZNF703*	782419_at	Zinc finger protein 703	-1,56
TDP2	507579_at	Tyrosyl-DNA phosphodiesterase 2	-1,55	*TBC1D16*	512099_at	Tbc1 domain family, member 16	-1,56
PLA1A	515900_at	Phospholipase A1 member A	-1,55	*C5H12orf63*	787705_at	Chromosome 5 open reading frame, human c12orf63	-1,56
TMC1	538406_at	Transmembrane channel-like 1	-1,55	*ST3GAL3*	444859_at	St3 beta-galactoside alpha-2,3-sialyltransferase 3	-1,56
FHDC1	784913_at	FH2 domain containing 1	-1,55	*TNFRSF19*	768037_at	Tumor necrosis factor receptor superfamily, member 19	-1,56
BCL11A	538680_at	B-cell CLL/lymphoma 11A (zinc finger protein)	-1,55	*FAM216A*	616613_at	Chromosome 17 open reading frame, human c12orf24	-1,56
CYFIP2	518833_at	Cytoplasmic FMR1 interacting protein 2	-1,55	*MDH1B*	527943_at	Malate dehydrogenase 1b, nad (soluble)	-1,56
PLEKHA5	532887_at	Pleckstrin homology domain containing, family A member 5	-1,56	*SCML2*	523477_at	Sex comb on midleg-like 2 (drosophila)	-1,56
GTF2IRD1	507792_at	GTF2I repeat domain containing 1	-1,56	*LOC516849*	516849_at	Probable phospholipid-transporting atpase feta-like	-1,56
ZCCHC14	528171_at	Zinc finger, CCHC domain containing 14	-1,56	*DZIP1L*	512800_at	Daz interacting protein 1-like	-1,57
IGFBP7	616368_at	Insulin-like growth factor binding protein 7	-1,56	*RFX2*	534475_at	Regulatory factor x, 2 (influences hla class ii expression)	-1,57
ORC3	523714_at	Origin recognition complex, subunit 3	-1,56	*AGL*	517397_at	Amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase	-1,57
DSP	514360_at	Desmoplakin	-1,56	*HEPACAM2*	513430_at	Hepacam family member 2	-1,57
TTLL9	529246_at	Tubulin tyrosine ligase-like family, member 9	-1,56	*ACOT13*	504870_at	Acyl-coa thioesterase 13	-1,57
SPATA17	618365_at	Spermatogenesis associated 17	-1,56	*CCDC170*	787062_at	Coiled-coil domain containing 170	-1,57
C13H20orf194	519774_at	Chromosome 13 open reading frame, human c20orf194	-1,56	*PCDHGA10*	539554_at	Protocadherin gamma subfamily a, 10	-1,57
GPX2	533088_at	Glutathione peroxidase 2 (gastrointestinal)	-1,56	*LOC537248*	537248_at	Acid phosphatase-like protein 2-like	-1,57
DNAH6	538058_at	Dynein, axonemal, heavy chain 6	-1,56	*SLC39A10*	521004_at	Solute carrier family 39 (zinc transporter), member 10	-1,57
ZDHHC11	617224_at	Zinc finger, DHHC-type containing 11-like	-1,56	*PDGFC*	613787_at	Platelet derived growth factor c	-1,57
SYNE1	353348_at	Spectrin repeat containing, nuclear envelope 1	-1,56	*LOC537580*	537580_at	Leucine-rich repeat-containing protein 9-like	-1,57
ZMYND12	512257_at	Zinc finger, MYND-type containing 12	-1,56	*SRCIN1*	535629_at	Src kinase signaling inhibitor 1	-1,57
USP28	508902_at	Ubiquitin specific peptidase 28	-1,56	*CAPRIN2*	536187_at	Caprin family member 2	-1,58
GLIPR1	767905_at	GLI pathogenesis-related 1	-1,56	*ANKS6*	530846_at	Ankyrin repeat and sterile alpha motif domain containing 6	-1,58

ESYT1	520669_at	Extended synaptotagmin-like protein 1	-1,58	*ACN9*	783805_at	Acn9 homolog (s. Cerevisiae)	-1,60
CC2D2A	517240_at	Coiled-coil and C2 domain containing 2A	-1,58	*LOC786510*	786510_at	Putative zinc finger cchc domain-containing protein 18-like	-1,60
HLTF	539633_at	Helicase-like transcription factor	-1,58	*TEKT2*	514463_at	Tektin 2 (testicular)	-1,60
C23H6orf228	100140337_at	Uncharacterized LOC100140337	-1,58	*LOC515517*	515517_at	La-related protein 1b-like	-1,60
PDE1B	281970_at	Phosphodiesterase 1B, calmodulin-dependent	-1,58	*MIR1251*	100313398_at	Microrna mir-1251	-1,60
EPHX1	535293_at	Epoxide hydrolase 1, microsomal (xenobiotic)	-1,58	*LEKR1*	785790_at	Leucine, glutamate and lysine rich 1	-1,60
SPATA18	615729_at	Spermatogenesis associated 18 homolog (rat)	-1,58	*OSBPL3*	537304_at	Oxysterol binding protein-like 3	-1,60
TSNAXIP1	527504_at	Translin-associated factor X interacting protein 1	-1,58	*KIAA1257*	785686_at	Kiaa1257 ortholog	-1,60
LOC782350	782350_at	Histone cluster 2, h2be-like	-1,58	*HSD17B11*	527592_at	Hydroxysteroid (17-beta) dehydrogenase 11	-1,60
TSPAN33	539284_at	Tetraspanin 33	-1,58	*EHD2*	538348_at	Eh-domain containing 2	-1,60
PDE11A	524446_at	Phosphodiesterase 11A	-1,58	*LOC100296943*	100296943_at	Uncharacterized loc100296943	-1,60
SLC25A13	615470_at	Solute carrier family 25, member 13 (citrin)	-1,59	*TCEA2*	507729_at	Transcription elongation factor a (sii), 2	-1,61
CLDND1	515537_at	Claudin domain containing 1	-1,59	*CAMK1D*	526873_at	Calcium/calmodulin-dependent protein kinase id	-1,61
RARS2	525894_at	Arginyl-trna synthetase 2, mitochondrial	-1,59	*C23H6orf141*	100271839_at	Chromosome 23 open reading frame, human c6orf141	-1,61
SLC38A6	520243_at	Solute carrier family 38, member 6	-1,59	*LOC506634*	506634_at	Uncharacterized loc506634	-1,61
ARMCX6	768308_at	Armadillo repeat containing, X-linked 6	-1,59	*S1PR5*	517533_at	Sphingosine-1-phosphate receptor 5	-1,61
NSUN7	533295_at	NOP2/Sun domain family, member 7	-1,59	*TGFBR3*	784894_at	Transforming growth factor, beta receptor iii	-1,61
DPYSL5	100126171_at	Dihydropyrimidinase-like 5	-1,59	*LOC789715*	789715_at	Zinc finger protein 548-like	-1,61
SOSTDC1	523184_at	Sclerostin domain containing 1	-1,59	*DEGS1*	507290_at	Degenerative spermatocyte homolog 1, lipid desaturase (drosophila)	-1,61
ALS2CR12	784225_at	Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 12	-1,59	*NCOA3*	523707_at	Nuclear receptor coactivator 3	-1,62
LOC787122	787122_at	ADP/ATP translocase 1-like	-1,59	*CCDC113*	528348_at	Coiled-coil domain containing 113	-1,62
DYRK2	514916_at	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	-1,59	*GNPTAB*	509610_at	N-acetylglucosamine-1-phosphate transferase, alpha and beta subunits	-1,62
NFASC	512796_at	Neurofascin	-1,59	*KIF2C*	533161_at	Kinesin family member 2c	-1,62
HS6ST2	517768_at	Heparan sulfate 6-O-sulfotransferase 2	-1,59	*ARMC3*	525665_at	Armadillo repeat containing 3	-1,62
ST8SIA1	282352_at	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1	-1,59	*KIAA0195*	512110_at	Kiaa0195 ortholog	-1,62
LTA4H	507130_at	Leukotriene A4 hydrolase	-1,60	*GPR39*	100139476_at	G protein-coupled receptor 39	-1,62
SHROOM4	519045_at	Shroom family member 4	-1,60	*KLF12*	100140477_at	Kruppel-like factor 12	-1,62

RSPH9	523327_at	Radial spoke head 9 homolog (Chlamydomonas)	-1,62	*PTPLA*	615191_at	Protein tyrosine phosphatase-like (proline instead of catalytic arginine), member A	-1,66
DBP	503577_at	D site of albumin promoter (albumin D-box) binding protein	-1,62		100851137_at		-1,66
GPT2	618400_at	Glutamic pyruvate transaminase (alanine aminotransferase) 2	-1,62	*HIST1H2BB*	614958_at	Histone cluster 1, h2bn	-1,66
MCF2L2	100139125_at	MCF.2 cell line derived transforming sequence-like 2	-1,64	*NAB1*	516781_at	NGFI-A binding protein 1 (EGR1 binding protein 1)	-1,66
CCDC78	511835_at	Coiled-coil domain containing 78	-1,64	*DNAAF1*	523187_at	Dynein, axonemal, assembly factor 1	-1,66
TTC18	529891_at	Tetratricopeptide repeat domain 18	-1,64	*GPR155*	538798_at	G protein-coupled receptor 155	-1,66
GTPBP10	613957_at	GTP-binding protein 10 (putative)	-1,64	*DOCK4*	534227_at	Dedicator of cytokinesis 4	-1,66
TTC6	517396_at	Tetratricopeptide repeat domain 6	-1,64	*SIRT2*	504463_at	Sirtuin 2	-1,66
OSMR	514720_at	Oncostatin M receptor	-1,64	*CYP7B1*	529552_at	Cytochrome P450, family 7, subfamily B, polypeptide 1	-1,66
FZD8	616913_at	Frizzled family receptor 8	-1,64	*LOC100296257*	100296257_at	Uncharacterized LOC100296257	-1,66
ITGA1	535951_at	Integrin, alpha 1	-1,64	*RASA2*	533491_at	RAS p21 protein activator 2	-1,67
ENTPD3	506087_at	Ectonucleoside triphosphate diphosphohydrolase 3	-1,64	*PREX2*	520704_at	Phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2	-1,67
FRMPD2	520665_at	FERM and PDZ domain containing 2	-1,64	*C7H19orf71*	768023_at	Chromosome 7 open reading frame, human c19orf71	-1,67
STYXL1	513107_at	Serine/threonine/tyrosine interacting-like 1	-1,64	*P2RX6*	618262_at	Purinergic receptor P2X, ligand-gated ion channel, 6	-1,67
LOC100295410	100295410_at	Retinoic acid receptor, beta-like	-1,64	*LOC100851861*	100851861_at	Cadherin-11-like	-1,67
CCDC7	616662_at	Coiled-coil domain containing 7	-1,64	*PLEKHD1*	100141172_at	Pleckstrin homology domain containing, family D (with coiled-coil domains) member 1	-1,67
IRF4	506141_at	Interferon regulatory factor 4	-1,65	*CHST9*	525909_at	Carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 9	-1,67
CEP128	529746_at	Centrosomal protein 128kda	-1,65	*YPEL2*	616180_at	Yippee-like 2 (Drosophila)	-1,68
ASNS	514209_at	Asparagine synthetase (glutamine-hydrolyzing)	-1,65	*RRAGD*	541106_at	Ras-related GTP binding D	-1,68
EFHB	530549_at	EF-hand domain family, member B	-1,65	*C16H1orf116*	539441_at	Chromosome 16 open reading frame, human c1orf116	-1,68
FUT11	539329_at	Fucosyltransferase 11 (alpha (1,3) fucosyltransferase)	-1,65	*SAP30*	781150_at	Sin3A-associated protein, 30kda	-1,68
SLC2A9	100337051_at	Solute carrier family 2 (facilitated glucose transporter), member 9	-1,65	*EPSTI1*	614555_at	Epithelial stromal interaction 1 (breast)	-1,68
LRRIQ3	523789_at	Leucine-rich repeats and IQ motif containing 3	-1,65	*TMEM55A*	616641_at	Transmembrane protein 55A	-1,68
CORO1C	515798_at	Coronin, actin binding protein, 1C	-1,66	*CDCA7L*	514631_at	Cell division cycle associated 7-like	-1,68

CD276	508656_at	CD276 molecule	-1,69	*DNAI2*	534479_at	Dynein, axonemal, intermediate chain 2	-1,72
TMEM212	100335300_at	Transmembrane protein 212	-1,69	*HESX1*	781811_at	HESX homeobox 1	-1,72
CADM1	529873_at	Cell adhesion molecule 1	-1,69	*LOC783300*	783300_at	Uncharacterized LOC783300	-1,72
BASP1	286842_at	Brain abundant, membrane attached signal protein 1	-1,69	*EID3*	507232_at	EP300 interacting inhibitor of differentiation 3	-1,73
AAED1	616897_at	Ahpc/TSA antioxidant enzyme domain containing 1	-1,69	*CAPS2*	524599_at	Calcyphosine 2	-1,73
LRRC49	511727_at	Leucine rich repeat containing 49	-1,69	*C20H5orf41*	513587_at	Chromosome 20 open reading frame, human c5orf41	-1,73
CHRNA5	282177_at	Cholinergic receptor, nicotinic, alpha 5	-1,69	*CEP135*	509483_at	Centrosomal protein 135kda	-1,73
CCDC151	517994_at	Coiled-coil domain containing 151	-1,69	*CADPS*	534328_at	Ca++-dependent secretion activator	-1,73
SPINLW1	768002_at	Serine peptidase inhibitor-like, with Kunitz and WAP domains 1 (eppin)	-1,69	*GPRC5B*	516334_at	G protein-coupled receptor, family C, group 5, member B	-1,73
LOC523214	523214_at	Histone cluster 1, h3a-like	-1,69	*PROCA1*	510175_at	Protein interacting with cyclin A1	-1,73
NOVA1	790874_at	RNA-binding protein Nova-1-like	-1,69	*NCAPG*	531234_at	Non-SMC condensin I complex, subunit G	-1,73
GATM	414732_at	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	-1,69	*FBXL21*	505624_at	F-box and leucine-rich repeat protein 21	-1,73
ANO5	100140158_at	Anoctamin 5	-1,69	*MIR218-2*	791016_at	Microrna mir-218-2	-1,73
C15H11orf16	504224_at	Chromosome 15 open reading frame, human c11orf16	-1,71	*NHSL2*	513680_at	NHS-like 2	-1,73
GPR110	512637_at	G protein-coupled receptor 110	-1,71	*SRGAP1*	539452_at	SLIT-ROBO Rho gtpase activating protein 1	-1,73
PION	615147_at	Pigeon homolog (Drosophila)	-1,71	*SSPN*	613989_at	Sarcospan (Kras oncogene-associated gene)	-1,73
LURAP1L	616371_at	Leucine rich adaptor protein 1-like	-1,71	*TMEM246*	786832_at	Transmembrane protein 246	-1,74
MEIG1	617353_at	Meiosis expressed gene 1 homolog (mouse)	-1,71		100849357_at		-1,74
C11H9orf117	617116_at	Chromosome 11 open reading frame, human c9orf117	-1,71	*NR3C1*	281946_at	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	-1,74
LYPD6	100337491_at	LY6/PLAUR domain containing 6	-1,71	*ANKRD65*	788407_at	Ankyrin repeat domain 65	-1,74
40238	540263_at	Membrane-associated ring finger (C3HC4) 10	-1,71	*PVALB*	538603_at	Parvalbumin	-1,74
BTBD19	615438_at	BTB (POZ) domain containing 19	-1,71	*RACGAP1*	514618_at	Rac gtpase activating protein 1	-1,74
AASS	520865_at	Aminoadipate-semialdehyde synthase	-1,71	*C1H21orf91*	540784_at	Chromosome 1 open reading frame, human c21orf91	-1,74
ANKRD6	516065_at	Ankyrin repeat domain 6	-1,72	*HIST1H2AC*	524808_at	Histone cluster 1, h2ac	-1,74
CMTM3	787512_at	CKLF-like MARVEL transmembrane domain containing 3	-1,72	*MEGF9*	533820_at	Multiple epidermal growth factor-like domains protein 9-like	-1,74
PIM2	508424_at	Pim-2 oncogene	-1,72	*RGL1*	522344_at	Ral guanine nucleotide dissociation stimulator-like 1	-1,74
SPON1	282866_at	Spondin 1, extracellular matrix protein	-1,72	*SVIL*	281509_at	Supervillin	-1,74

C13H10orf68	786891_at	Chromosome 13 open reading frame, human c10orf68	-1,74	*PLN*	100125240_at	Phospholamban	-1,78
TCF23	616841_at	Transcription factor 23	-1,74	*PLA2G2D4*	503625_at	Calcium-dependent phospholipase A2 PLA2G2D4	-1,78
LOC100295767	100295767_at	Uncharacterized LOC100295767	-1,74	*MSH5*	100139295_at	Muts homolog 5 (E. Coli)	-1,78
TMEM52B	618567_at	Transmembrane protein 52B	-1,74	*COL4A5*	511602_at	Collagen, type IV, alpha 5	-1,79
FAM118A	505415_at	Family with sequence similarity 118, member A	-1,75	*RBFOX3*	511773_at	RNA binding protein, fox-1 homolog (C. Elegans) 3	-1,79
CACNA1H	282412_at	Calcium channel, voltage-dependent, T type, alpha 1H subunit	-1,75	*RETSAT*	614455_at	Retinol saturase (all-trans-retinol 13,14-reductase)	-1,79
ASB14	509431_at	Ankyrin repeat and SOCS box containing 14	-1,75	*CNGA1*	281700_at	Cyclic nucleotide gated channel alpha 1	-1,79
PDE1C	526211_at	Phosphodiesterase 1C, calmodulin-dependent 70kda	-1,75	*TM9SF2*	509946_at	Transmembrane 9 superfamily member 2	-1,79
LDLRAD3	525908_at	Low density lipoprotein receptor class A domain containing 3	-1,75	*ITPR1*	317697_at	Inositol 1,4,5-trisphosphate receptor, type 1	-1,79
ARID5A	524118_at	AT rich interactive domain 5A (MRF1-like)	-1,77	*MIR505*	100313062_at	Microrna mir-505	-1,79
ZNF391	513014_at	Zinc finger protein 391	-1,77	*RAMP1*	617017_at	Receptor (G protein-coupled) activity modifying protein 1	-1,79
MIR2292	100313119_at	Microrna mir-2292	-1,77	*TLR7*	493686_at	Toll-like receptor 7	-1,79
CTSC	352958_at	Cathepsin C	-1,77	*N4BP2L1*	616069_at	NEDD4 binding protein 2-like 1	-1,79
PTPDC1	519311_at	Protein tyrosine phosphatase domain containing 1	-1,77	*TYRO3*	788224_at	TYRO3 protein tyrosine kinase	-1,79
DBNDD2	507590_at	Dysbindin (dystrobrevin binding protein 1) domain containing 2	-1,77	*GPLD1*	287025_at	Glycosylphosphatidylinositol specific phospholipase D1	-1,79
ESYT3	530157_at	Extended synaptotagmin-like protein 3	-1,78	*TMEM218*	616789_at	Transmembrane protein 218	-1,80
AHCYL2	532836_at	Adenosylhomocysteinase-like 2	-1,78	*STON2*	785664_at	Stonin 2	-1,80
ARHGAP25	534994_at	Rho gtpase activating protein 25	-1,78	*LRRC48*	527539_at	Leucine rich repeat containing 48	-1,80
CCDC8	616838_at	Coiled-coil domain containing 8	-1,78	*IGDCC4*	541098_at	Immunoglobulin superfamily, DCC subclass, member 4	-1,80
ACSBG2	526688_at	Acyl-coa synthetase bubblegum family member 2	-1,78	*PCSK5*	528098_at	Proprotein convertase subtilisin/kexin type 5	-1,80
PLCZ1	497026_at	Phospholipase C, zeta 1	-1,78	*GLT8D2*	523294_at	Glycosyltransferase 8 domain containing 2	-1,80
CCDC36	520504_at	Coiled-coil domain containing 36	-1,78	*GCNT4*	782825_at	Glucosaminyl (N-acetyl) transferase 4, core 2	-1,80
ENC1	617091_at	Ectodermal-neural cortex 1 (with BTB-like domain)	-1,78	*OTUD7A*	789946_at	OTU domain containing 7A	-1,82
GJA8	524042_at	Gap junction protein, alpha 8, 50kda	-1,78	*NEK11*	614924_at	NIMA (never in mitosis gene a)- related kinase 11	-1,82
F2	280685_at	Coagulation factor II (thrombin)	-1,78	*C3H1orf168*	615569_at	Chromosome 3 open reading frame, human c1orf168	-1,82

KIAA1324L	518313_at	KIAA1324-like ortholog	-1,82	*KCNA5*	508960_at	Potassium voltage-gated channel, shaker-related subfamily, member 5	-1,89
RTKN2	539797_at	Rhotekin 2	-1,82	*PPM1E*	532050_at	Protein phosphatase, Mg2+/Mn2+ dependent, 1E	-1,91
CKAP2L	507498_at	Cytoskeleton associated protein 2-like	-1,82	*FGFR4*	317696_at	Fibroblast growth factor receptor 4	-1,91
ZMAT1	504576_at	Zinc finger, matrin-type 1	-1,83	*PLTP*	505640_at	Phospholipid transfer protein	-1,91
HSPA1A	282254_at	Heat shock 70kda protein 1A	-1,83	*SELENBP1*	510154_at	Selenium binding protein 1	-1,91
LRIG3	506574_at	Leucine-rich repeats and immunoglobulin-like domains 3	-1,83	*RNF217*	541287_at	Ring finger protein 217	-1,92
CD36	281052_at	CD36 molecule (thrombospondin receptor)	-1,83	*PDZRN4*	511963_at	PDZ domain containing ring finger 4	-1,92
PNMAL1	532062_at	PNMA-like 1	-1,84	*PECAM1*	282303_at	Platelet/endothelial cell adhesion molecule	-1,92
LOC100297857	100297857_at	Uncharacterized LOC100297857	-1,84	*SLC23A2*	783536_at	Solute carrier family 23 (nucleobase transporters), member 2	-1,92
KCNIP4	614299_at	Kv channel interacting protein 4	-1,85	*RNF149*	506267_at	Ring finger protein 149	-1,92
CPA5	511416_at	Carboxypeptidase A5	-1,85	*TMEM74B*	539523_at	Transmembrane protein 74B	-1,92
KLHDC7A	530171_at	Kelch domain containing 7A	-1,85	*LOC100336892*	100336892_at	Uncharacterized LOC100336892	-1,92
ICOSLG	507857_at	Inducible T-cell co-stimulator ligand	-1,85	*IFI16*	506759_at	Interferon, gamma-inducible protein 16	-1,92
PLAU	281408_at	Plasminogen activator, urokinase	-1,85	*PLCE1*	519037_at	Phospholipase C, epsilon 1	-1,92
LOC785805	785805_at	Collagen alpha-5(VI) chain-like	-1,85	*VSTM4*	100336454_at	V-set and transmembrane domain containing 4	-1,93
LOC100847471	100847471_at	ADAM DEC1-like	-1,85	*CX3CL1*	517354_at	Chemokine (C-X3-C motif) ligand 1	-1,93
LOC508879	508879_at	Aldehyde dehydrogenase family 3 member B1-like	-1,87	*TMEM95*	614631_at	Transmembrane protein 95	-1,93
LOC527805	527805_at	Uncharacterized LOC527805	-1,87	*PDE1A*	281969_at	Phosphodiesterase 1A, calmodulin-dependent	-1,93
	100851409_at		-1,87	*RFK*	514697_at	Riboflavin kinase	-1,95
MIR2431	100313212_at	Microrna mir-2431	-1,87	*SERHL2*	531992_at	Serine hydrolase-like 2	-1,95
TRAF5	507234_at	TNF receptor-associated factor 5	-1,87	*PLAT*	281407_at	Plasminogen activator, tissue	-1,95
ITGA9	532127_at	Integrin, alpha 9	-1,87	*PGM2L1*	515366_at	Phosphoglucomutase 2-like 1	-1,95
JAG1	783681_at	Jagged 1	-1,87	*LOC617016*	617016_at	Olfactory receptor, family 8, subfamily A, member 1-like	-1,95
ENOX1	615969_at	Ecto-NOX disulfide-thiol exchanger 1	-1,88	*CCDC89*	538595_at	Coiled-coil domain containing 89	-1,96
CCDC122	525313_at	Coiled-coil domain containing 122	-1,88	*SUSD3*	512524_at	Sushi domain containing 3	-1,96
ATP8A2	617199_at	Atpase, aminophospholipid transporter, class I, type 8A, member 2	-1,88	*MIR708*	100313079_at	Microrna mir-708	-1,96
LACC1	537649_at	Laccase (multicopper oxidoreductase) domain containing 1	-1,89	*LOC784007*	784007_at	Uncharacterized LOC784007	-1,96
MGC151671	531076_at	Uncharacterized LOC531076	-1,89	*LOC100335155*	100335155_at	Uncharacterized LOC100335155	-1,96
ANKRD31	515838_at	Ankyrin repeat domain 31	-1,89	*TLR5*	444870_at	Toll-like receptor 5	-1,96
GPR75	539470_at	G protein-coupled receptor 75	-1,89	*KLHL3*	533364_at	Kelch-like 3 (Drosophila)	-1,97

ATAD2	522920_at	Atpase family, AAA domain containing 2	-1,97	*CPNE5*	508482_at	Copine V	-2,03
PRPS2	537688_at	Phosphoribosyl pyrophosphate synthetase 2	-1,97	*FUT1*	281174_at	Fucosyltransferase 1 (galactoside 2-alpha-L-fucosyltransferase, H blood group)	-2,03
LOC505183	505183_at	Histone H2B type 1-like	-1,97	*C3H1orf189*	504348_at	Chromosome 3 open reading frame, human c1orf189	-2,04
PLK2	539449_at	Polo-like kinase 2	-1,97	*ST3GAL5*	404164_at	ST3 beta-galactoside alpha-2,3-sialyltransferase 5	-2,04
TTC28	537659_at	Tetratricopeptide repeat domain 28	-1,97	*TPM1*	281544_at	Tropomyosin 1 (alpha)	-2,06
ATP1A2	515161_at	Atpase, Na+/K+ transporting, alpha 2 polypeptide	-1,99	*HS3ST5*	540355_at	Heparan sulfate (glucosamine) 3-O-sulfotransferase 5	-2,06
LOC100335299	100335299_at	Uncharacterized LOC100335299	-1,99	*OBFC2A*	613474_at	Oligonucleotide/oligosaccharide-binding fold containing 2A	-2,07
TREH	522889_at	Trehalase (brush-border membrane glycoprotein)	-1,99	*DIRAS3*	504559_at	DIRAS family, GTP-binding RAS-like 3	-2,07
CCDC103	509424_at	Coiled-coil domain containing 103	-1,99	*KRT5*	281268_at	Keratin 5	-2,07
H2AFY2	537167_at	H2A histone family, member Y2	-1,99	*NAGA*	533357_at	N-acetylgalactosaminidase, alpha-	-2,07
ZNF804B	100295505_at	Zinc finger protein 804B	-1,99	*FRAS1*	537989_at	Fraser syndrome 1	-2,07
THBS3	504323_at	Thrombospondin 3	-2,00	*PTPLAD2*	618814_at	Protein tyrosine phosphatase-like A domain containing 2	-2,07
SNCAIP	540156_at	Synuclein, alpha interacting protein	-2,00	*GBA3*	539625_at	Glucosidase, beta, acid 3 (cytosolic)	-2,07
LEPREL1	511799_at	Leprecan-like 1	-2,00	*PEAR1*	787112_at	Platelet endothelial aggregation receptor 1	-2,07
TCTEX1D4	516323_at	Tctex1 domain containing 4	-2,00	*LOC508589*	508589_at	Olfactory receptor, family 8, subfamily A, member 1-like	-2,07
CACNB2	327667_at	Calcium channel, voltage-dependent, beta 2 subunit	-2,00	*ASAP1*	327705_at	Arfgap with SH3 domain, ankyrin repeat and PH domain 1	-2,08
CHODL	613942_at	Chondrolectin	-2,00	*SEMA3E*	535644_at	Semaphorin-3E-like	-2,08
ZEB1	535183_at	Zinc finger E-box binding homeobox 1	-2,01	*LOC527645*	527645_at	Histone cluster 1, h4i-like	-2,08
MED12L	538979_at	Mediator complex subunit 12-like	-2,01	*VAT1L*	618809_at	Vesicle amine transport protein 1 homolog (T. Californica)-like	-2,08
LOC787649	787649_at	Histone H4-like	-2,01	*LOC784305*	784305_at	ATP-binding cassette, sub-family C, member 4-like	-2,08
ZPLD1	527953_at	Zona pellucida-like domain containing 1	-2,01	*SNX10*	508836_at	Sorting nexin 10	-2,10
SLC12A1	407161_at	Solute carrier family 12 (sodium/potassium/chloride transporters), member 1	-2,01	*MYC*	511077_at	V-myc myelocytomatosis viral oncogene homolog (avian)	-2,11
ABCG2	536203_at	ATP-binding cassette, sub-family G (WHITE), member 2	-2,03	*MIR29C*	791043_at	Microrna mir-29c	-2,11
LRP4	504317_at	Low density lipoprotein receptor-related protein 4	-2,03	*LOC527388*	527388_at	Histone cluster 1, h4i-like	-2,11

LOC613739	613739_at	Pregnancy-associated glycoprotein 2-like	-2,13	KIT	280832_at	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	-2,23
FRK	509227_at	Fyn-related kinase	-2,14	HTR2A	407230_at	5-hydroxytryptamine (serotonin) receptor 2A	-2,25
FAM117A	509931_at	Family with sequence similarity 117, member A	-2,14	FAM194A	100335773_at	Family with sequence similarity 194, member A	-2,25
CCND2	615414_at	Cyclin D2	-2,16	NPNT	513362_at	Nephronectin	-2,25
SLC16A7	614573_at	Solute carrier family 16, member 7 (monocarboxylic acid transporter 2)	-2,17	TTLL6	526482_at	Tubulin tyrosine ligase-like family, member 6	-2,25
PPP1R1A	767949_at	Protein phosphatase 1, regulatory (inhibitor) subunit 1A	-2,17	BEND4	614525_at	BEN domain containing 4	-2,25
EFNB1	534413_at	Ephrin-B1	-2,17	CRABP2	493998_at	Cellular retinoic acid binding protein 2	-2,25
MIR29B-2	791042_at	Microrna mir-29b-2	-2,17	SLC14A1	493988_at	Solute carrier family 14 (urea transporter), member 1 (Kidd blood group)	-2,27
LOC100335682	100335682_at	Ataxin-3-like	-2,17	C2H2orf72	787439_at	Chromosome 2 open reading frame, human c2orf72	-2,28
PAX6	286857_at	Paired box 6	-2,17	TEX11	515297_at	Testis expressed 11	-2,28
CEP170	529230_at	Centrosomal protein 170kda	-2,19	RBFOX1	521304_at	RNA binding protein, fox-1 homolog (C. Elegans) 1	-2,31
DENND5B	516544_at	DENN/MADD domain containing 5B	-2,19	PPP1R3C	539466_at	Protein phosphatase 1, regulatory subunit 3C	-2,33
HECW2	531691_at	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2	-2,19	GAS1	540336_at	Growth arrest-specific 1	-2,33
HIST1H2BD	519935_at	Histone cluster 1, h2bd	-2,20	GPR162	540410_at	G protein-coupled receptor 162	-2,33
TEX26	508945_at	Testis expressed 26	-2,20	JAM3	513412_at	Junctional adhesion molecule 3	-2,33
C9H6orf186	785203_at	Chromosome 9 open reading frame, human c6orf186	-2,20	PPP4R4	537521_at	Protein phosphatase 4, regulatory subunit 4	-2,33
MIR135A-2	100313470_at	Microrna mir-135a-2	-2,20	KIAA0319	520603_at	KIAA0319 ortholog	-2,35
COL15A1	100139730_at	Collagen, type XV, alpha 1	-2,20	HUNK	537640_at	Hormonally up-regulated Neu-associated kinase	-2,35
LOC616868	616868_at	Histone H2B type 1-like	-2,20	S100A13	404146_at	S100 calcium binding protein A13	-2,35
NRG1	281361_at	Neuregulin 1	-2,20	ANKS1B	615777_at	Ankyrin repeat and sterile alpha motif domain containing 1B	-2,36
LOC618824	618824_at	Histone cluster 1, h2ai-like	-2,22	PLA2G1B	282457_at	Phospholipase A2, group IB (pancreas)	-2,38
TRPC1	282100_at	Transient receptor potential cation channel, subfamily C, member 1	-2,23	KLHL23	100140861_at	Kelch-like 23 (Drosophila)	-2,39
ENPP6	537431_at	Ectonucleotide pyrophosphatase/phosphodiesterase 6	-2,23	BOC	512018_at	Boc homolog (mouse)	-2,39
PLA2G2C	504978_at	Phospholipase A2, group IIC	-2,23	MOG	280863_at	Myelin oligodendrocyte glycoprotein	-2,39
UBE2U	784986_at	Ubiquitin-conjugating enzyme E2U (putative)	-2,23	MME	536741_at	Membrane metallo-endopeptidase	-2,39

ELTD1	535066_at	EGF, latrophilin and seven transmembrane domain containing 1	-2,41	*PRKG1*	282004_at	Protein kinase, cgmp-dependent, type I	-2,58
PON1	523798_at	Paraoxonase 1	-2,41	*MAP1B*	514739_at	Microtubule-associated protein 1B	-2,58
LOC537655	537655_at	Dystrophin-like	-2,43	*HIST2H2AA4*	100297758_at	Histone cluster 2, h2aa4	-2,58
IGFBP5	404185_at	Insulin-like growth factor binding protein 5	-2,43	*KCND3*	539739_at	Potassium voltage-gated channel, Shal-related subfamily, member 3	-2,58
KCNS3	541460_at	Potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3	-2,45	*KCNRG*	404166_at	Potassium channel regulator	-2,60
GRIK4	526363_at	Glutamate receptor, ionotropic, kainate 4	-2,45	*LOC518961*	518961_at	Histone cluster 1, h4i-like	-2,60
C10H14orf37	508562_at	Chromosome 10 open reading frame, human c14orf37	-2,46	*CTNNA2*	527492_at	Catenin (cadherin-associated protein), alpha 2	-2,60
LOC100336854	100336854_at	Uncharacterized LOC100336854	-2,46	*SYCP2*	784000_at	Synaptonemal complex protein 2	-2,60
NTRK3	539126_at	Neurotrophic tyrosine kinase, receptor, type 3	-2,46	*HIST3H2A*	538911_at	Histone cluster 3, h2a	-2,60
UNC13D	506146_at	Unc-13 homolog D (C. Elegans)	-2,48	*ROR1*	783965_at	Receptor tyrosine kinase-like orphan receptor 1	-2,60
NETO2	520056_at	Neuropilin (NRP) and tolloid (TLL)-like 2	-2,48	*ADCY8*	535017_at	Adenylate cyclase 8 (brain)	-2,60
GRIP1	525592_at	Glutamate receptor interacting protein 1	-2,48	*HOXD8*	100295814_at	Homeobox D8	-2,60
SLC16A10	541240_at	Solute carrier family 16, member 10 (aromatic amino acid transporter)	-2,48	*CYP1B1*	511470_at	Cytochrome P450, family 1, subfamily B, polypeptide 1	-2,60
HOXD3	100295744_at	Homeobox D3	-2,50	*SLC26A5*	536341_at	Solute carrier family 26, member 5 (prestin)	-2,62
CAPN14	515741_at	Calpain 14	-2,50	*ADAMTS5*	286805_at	ADAM metallopeptidase with thrombospondin type 1 motif, 5	-2,62
ASGR2	531519_at	Asialoglycoprotein receptor 2	-2,50	*TRIM55*	616362_at	Tripartite motif containing 55	-2,64
CDC42EP1	511099_at	CDC42 effector protein (Rho gtpase binding) 1	-2,51	*TMEM74*	539995_at	Transmembrane protein 74	-2,66
C27H8orf48	100271834_at	Chromosome 27 open reading frame, human c8orf48	-2,51	*CACNA2D3*	519644_at	Calcium channel, voltage-dependent, alpha 2/delta subunit 3	-2,68
MIR15A	100170925_at	Microrna mir-15a	-2,51	*LOC516742*	516742_at	Histone cluster 1, h4i-like	-2,69
MACROD2	100125389_at	MACRO domain containing 2	-2,53	*BICD1*	533796_at	Bicaudal D homolog 1 (Drosophila)	-2,71
B4GALT4	511328_at	UDP-Gal:betaglcnac beta 1,4-galactosyltransferase, polypeptide 4	-2,53	*DENND2C*	522279_at	DENN/MADD domain containing 2C	-2,71
ZDHHC2	536310_at	Zinc finger, DHHC-type containing 2	-2,53	*GABBR2*	517040_at	Gamma-aminobutyric acid (GABA) B receptor, 2	-2,73
NPR1	533048_at	Natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	-2,53	*FAM65B*	539635_at	Family with sequence similarity 65, member B	-2,75
TPBG	540358_at	Trophoblast glycoprotein	-2,53	*MIR218-1*	100313258_at	Microrna mir-218-1	-2,77
ADRB2	281605_at	Adrenergic, beta-2-, receptor, surface	-2,55	*GABRA2*	282236_at	Gamma-aminobutyric acid (GABA) A receptor, alpha 2	-2,77

EMCN	616367_at	Endomucin	-2,77	*GAS7*	614517_at	Growth arrest-specific 7	-3,05
GPC5	522828_at	Glypican 5	-2,79	*ARHGAP29*	504657_at	Rho gtpase activating protein 29	-3,05
LOC513333	513333_at	Olfactory receptor, family 8, subfamily A, member 1-like	-2,79	*S100A14*	618250_at	S100 calcium binding protein A14	-3,05
CUEDC2	516091_at	CUE domain containing 2	-2,83	*LOC100337478*	100337478_at	Glutamate receptor interacting protein 1-like	-3,07
PRKCB	282325_at	Protein kinase C, beta	-2,83	*ARMCX2*	767841_at	Armadillo repeat containing, X-linked 2	-3,10
EFNA5	616742_at	Ephrin-A5	-2,85	*LOC616254*	616254_at	Intercellular adhesion molecule 2-like	-3,10
DTNA	541153_at	Dystrobrevin, alpha	-2,85	*LOC785870*	785870_at	PDYN protein-like	-3,10
LOC617833	617833_at	Mal, T-cell differentiation protein-like	-2,85	*BVES*	539988_at	Blood vessel epicardial substance	-3,10
GLYATL3	787783_at	Glycine-N-acyltransferase-like 3	-2,89	*ADAMTS12*	525276_at	ADAM metallopeptidase with thrombospondin type 1 motif, 12	-3,12
PALMD	509823_at	Palmdelphin	-2,89	*CTNND2*	523661_at	Catenin (cadherin-associated protein), delta 2 (neural plakophilin-related arm-repeat protein)	-3,12
SCG5	508224_at	Secretogranin V (7B2 protein)	-2,89	*MEIS2*	539573_at	Meis homeobox 2	-3,14
MAOA	281293_at	Monoamine oxidase A	-2,91	*AGPAT4*	507456_at	1-acylglycerol-3-phosphate O-acyltransferase 4 (lysophosphatidic acid acyltransferase, delta)	-3,14
POPDC3	100139174_at	Popeye domain containing 3	-2,91	*ENPEP*	504350_at	Glutamyl aminopeptidase (aminopeptidase A)	-3,16
TGFBI	539596_at	Transforming growth factor, beta-induced, 68kda	-2,91	*LOC530341*	530341_at	Cortactin-binding protein 2-like	-3,18
ALDH1A3	507093_at	Aldehyde dehydrogenase 1 family, member A3	-2,93	*EPHA6*	100336601_at	EPH receptor A6	-3,18
SLITRK6	781119_at	SLIT and NTRK-like family, member 6	-2,93	*MORC4*	539471_at	MORC family CW-type zinc finger 4	-3,18
MYOM3	532872_at	Myomesin family, member 3	-2,93	*GLP1R*	517420_at	Glucagon-like peptide 1 receptor	-3,18
NCKAP5	786958_at	NCK-associated protein 5	-2,95	*ATP8A2*	508723_at	Atpase, aminophospholipid transporter, class I, type 8A, member 2	-3,18
ARHGEF25	506075_at	Rho guanine nucleotide exchange factor (GEF) 25	-2,95	*SLIT2*	534164_at	Slit homolog 2 (Drosophila)	-3,23
ST3GAL1	282351_at	ST3 beta-galactoside alpha-2,3-sialyltransferase 1	-2,95	*HOXD4*	513306_at	Homeobox D4	-3,25
CDKN2C	505691_at	Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	-2,97	*SERPING1*	281035_at	Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1	-3,27
GRIN2B	537804_at	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	-2,99	*LOC534360*	534360_at	Poliovirus receptor-related 3-like	-3,29
LPL	280843_at	Lipoprotein lipase	-2,99	*TM4SF1*	533038_at	Transmembrane 4 L six family member 1	-3,32
LOC527083	527083_at	Cytochrome P450 2G1-like	-3,03	*HPSE*	281230_at	Heparanase	-3,39

H2B	787581_at	Histone H2B	-3,39	*DAPK2*	529131_at	Death-associated protein kinase 2	-4,03
THEM5	525765_at	Thioesterase superfamily member 5	-3,41	*PTN*	280904_at	Pleiotrophin	-4,14
MASP1	522347_at	Mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	-3,41	*NID1*	534319_at	Nidogen 1	-4,32
RND1	508869_at	Rho family gtpase 1	-3,43	*NRIP3*	538608_at	Nuclear receptor interacting protein 3	-4,38
	100849181_at		-3,46	*HIST2H2BF*	615091_at	Histone cluster 2, h2bf	-4,47
NRG3	539977_at	Neuregulin 3	-3,48	*SLAMF9*	613822_at	SLAM family member 9	-4,50
FOXRED2	532871_at	FAD-dependent oxidoreductase domain containing 2	-3,48	*CCL26*	508387_at	Chemokine (C-C motif) ligand 26	-4,59
SERPINA1	280699_at	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	-3,58	*SERPINE3*	513955_at	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 3	-4,66
SYNPR	613433_at	Synaptoporin	-3,61	*DCHS2*	539323_at	Dachsous 2 (Drosophila)	-4,69
SLC38A11	523418_at	Solute carrier family 38, member 11	-3,66	*MMP7*	286794_at	Matrix metallopeptidase 7 (matrilysin, uterine)	-4,82
LOC100297621	100297621_at	Dystrophin-like	-3,71	*CLDN11*	508268_at	Claudin 11	-4,86
NTRK2	505824_at	Neurotrophic tyrosine kinase, receptor, type 2	-3,76	*CDKL1*	523900_at	Cyclin-dependent kinase-like 1 (CDC2-related kinase)	-4,89
PCDH11Y	538674_at	Protocadherin 11 Y-linked	-3,78	*MILR1*	789682_at	Mast cell immunoglobulin-like receptor 1	-5,10
SLIT3	615883_at	Slit homolog 3 (Drosophila)	-3,81	*MEST*	404180_at	Mesoderm specific transcript homolog (mouse)	-5,28
SCUBE2	529947_at	Signal peptide, CUB domain, EGF-like 2	-3,84	*THY1*	614712_at	Thy-1 cell surface antigen	-5,31
KIF19	538109_at	Kinesin family member 19	-3,84	*C3*	280677_at	Complement component 3	-5,39
LOC617905	617905_at	Histone cluster 1, h4i-like	-3,84	*NT5E*	281363_at	5'-nucleotidase, ecto (CD73)	-5,43
H2B	615043_at	Histone H2B-like	-3,84	*LOC516661*	516661_at	Leucine-rich repeat LGI family member 3-like	-5,50
ANO3	100139986_at	Anoctamin 3	-3,86	*LOC100296742*	100296742_at	Beta-defensin 142	-5,54
PTGIS	282021_at	Prostaglandin I2 (prostacyclin) synthase	-3,89	*ATRNL1*	504617_at	Attractin-like 1	-5,54
CBLN4	539114_at	Cerebellin 4 precursor	-3,92	*PROS1*	282006_at	Protein S (alpha)	-5,86
DSEL	538958_at	Dermatan sulfate epimerase-like	-3,92	*MEF2C*	512254_at	Myocyte enhancer factor 2C	-6,02
TMEM45A	509461_at	Transmembrane protein 45A	-3,92	*KDR*	407170_at	Kinase insert domain receptor (a type III receptor tyrosine kinase)	-6,41
PIGR	281401_at	Polymeric immunoglobulin receptor	-3,94	*HPGD*	512259_at	Hydroxyprostaglandin dehydrogenase 15-(NAD)	-6,63
LOC521580	521580_at	Histone H2B type 1-like	-3,97	*PPP2R2B*	509290_at	Protein phosphatase 2, regulatory subunit B, beta	-6,63
LOC613926	613926_at	Histone cluster 1, h2ai-like	-4,03	*PGA5*	414350_at	Pepsinogen 5, group I (pepsinogen A)	-7,06

UPK1B	282113_at	Uroplakin 1B	-7,31
PTGDS	286858_at	Prostaglandin D2 synthase 21kda (brain)	-8,69
PLEKHG7	519417_at	Pleckstrin homology domain containing, family G (with rhogef domain) member 7	-8,88
FMO2	504401_at	Flavin containing monooxygenase 2 (non-functional)	-9,51
GATA6	654400_at	GATA binding protein 6	-14,62

Supplemental Table 3.1. Overrepresented gene ontologies (GO_FAT) associated with differential expressed genes up-regulated in the isthmus.

GOBPID	Term	Entrez Gene Symbol
GO:0006796	Phosphate metabolic process	*FGFR2, FGFR1, MAPKAPK5, SYNJ1, MKNK1, PINK1, BRSK1, MAPKAPK2, TRIB1, EPHB2, ACVR1C, MTMR2, ADCK1, BCL2, CAMK2B, GK5, THBS1, ATP6V0D1, PTPRB, ADAM10, SGK2, PTPRF, LIMK2, LYN, PIK3CB, NLK, MET, PTPRS, TRIO, CDK7, ALK, CDC25A, MARK2, PRKD1, ACVR2B, EYA2, KSR2, RPS6KA1, PSEN1, CSNK1E, ARAF, JAK1, RIPK2, PPM1L, MERTK, GADD45B, NEK6*
GO:0006793	Phosphorus metabolic process	*FGFR2, FGFR1, MAPKAPK5, SYNJ1, MKNK1, PINK1, BRSK1, MAPKAPK2, TRIB1, EPHB2, ACVR1C, MTMR2, ADCK1, BCL2, CAMK2B, GK5, THBS1, ATP6V0D1, PTPRB, ADAM10, SGK2, PTPRF, LIMK2, LYN, PIK3CB, NLK, MET, PTPRS, TRIO, CDK7, ALK, CDC25A, MARK2, PRKD1, ACVR2B, EYA2, KSR2, RPS6KA1, PSEN1, CSNK1E, ARAF, JAK1, RIPK2, PPM1L, MERTK, GADD45B, NEK6*
GO:0007242	Intracellular signaling cascade	*RHOJ, ADCY7, ADCY6, MKNK1, ABCA1, RGL2, AGTR1, RASAL1, GMIP, RAB11B, GUCY1A3, RAB25, AGAP1, THBS1, PLCB1, RASA4, RHOG, NET1, ARL2, VAV3, BCR, LYN, PIK3CB, RASEF, RAB4B, MET, ARHGEF11, PRKD1, GNAL, KSR2, PLCG1, NUPR1, RPS6KA1, PSEN1, ARAF, JAK1, PPM1L, GUCY1B3, SMC1A, GADD45B, RAPGEFL1, PTAFR*
GO:0006811	Ion transport	*ATP1B1, ATP1B2, SLC38A7, TPCN1, KCNIP3, SHKBP1, LTF, TRPV4, CCS, CAMK2B, ATP6V0D1, SLC31A2, SCNN1A, SLC1A1, SLC39A1, SLC12A2, GRIN2A, CFTR, ATP13A5, TMEM38B, ATP13A4, SLC34A2, SLC26A3, SLC4A11, PSEN1, ATP2A3, CLIC5, KCNN2, KCNH7, SLC5A6, KCNH8, GUCY1B3, KCTD15, PLLP, PDZK1, CHRNE, CACNA1A, CLCN4*
GO:0016310	Phosphorylation	*FGFR2, FGFR1, MAPKAPK5, MKNK1, PINK1, BRSK1, MAPKAPK2, TRIB1, ACVR1C, EPHB2, ADCK1, BCL2, CAMK2B, THBS1, ATP6V0D1, ADAM10, SGK2, LIMK2, LYN, PIK3CB, NLK, MET, TRIO, CDK7, ALK, MARK2, PRKD1, ACVR2B, KSR2, PSEN1, RPS6KA1, CSNK1E, ARAF, JAK1, RIPK2, MERTK, GADD45B, NEK6*
GO:0006468	Protein amino acid phosphorylation	*FGFR2, FGFR1, MAPKAPK5, MKNK1, PINK1, BRSK1, MAPKAPK2, TRIB1, ACVR1C, EPHB2, ADCK1, BCL2, CAMK2B, THBS1, ADAM10, SGK2, LIMK2, LYN, NLK, MET, TRIO, CDK7, ALK, MARK2, PRKD1, ACVR2B, KSR2, PSEN1, RPS6KA1, CSNK1E, ARAF, JAK1, RIPK2, MERTK, GADD45B, NEK6*
GO:0055114	Oxidation reduction	*CYB5R3, TM7SF2, HSD17B10, SEPX1, ME3, ALDH18A1, HMGCR, CYP51A1, GLUD1, OGDH, ALDH3A2, FDFT1, MTHFR, GPX3, FASN, ALOX12B, CCS, ACAD8, DUS1L, NSDHL, LOC537017, DECR2, FADS2, PPARGC1A, POR, DHRS1, ACADVL, RDH11, DHRS4, CYP27A1, SQLE, RRM1, AKR1B1, TMLHE, PHGDH, ALOX12*
GO:0016192	Vesicle-mediated transport	*ARFGAP3, SNAP91, LDLR, AP1G2, SYNJ1, CDC42SE1, NOSTRIN, ABCA1, AP1S3, PICALM, GSN, STX17, THBS1, GHR, ELMOD1, MICALL2, STX5, CLN3, STX3, VAV3, SCRN1, GARS, STXBP2, ELMO3, PSEN1, CDC42SE2, VAMP7, VAMP2, VAMP1, GGA2, CACNA1A*
GO:0006812	Cation transport	*ATP1B1, ATP1B2, SLC38A7, KCNIP3, SHKBP1, LTF, CAMK2B, CCS, ATP6V0D1, SLC31A2, SCNN1A, SLC39A1, SLC12A2, ATP13A5, TMEM38B, ATP13A4, SLC34A2, PSEN1, ATP2A3, KCNN2, KCNH7, KCTD15, GUCY1B3, KCNH8, SLC5A6, PDZK1, CACNA1A*

GO:0042127	Regulation of cell proliferation	*FGFR2, SAT1, PPARD, PRRX1, BCL2L1, TIMP2, CALR, FANCL, CD9, CASP3, BCL2, AGT, THBS1, APC, ADAM10, LOC783195, RNASE4, ANG2, ESR2, CDKN1C, MSX1, NUPR1, IL20RB, GRN, RIPK2, PTCH1, ALOX12, NFIB*
GO:0022610	Biological adhesion	*PPARD, CLDN4, CLDN3, ITGB4, CDH1, ITGB3, PXN, ALCAM, CD9, PCDH1, EZR, CD44, SORBS1, CTGF, BCL2, AGT, COL12A1, THBS1, PTPRF, PIK3CB, NLGN3, DSG2, PSEN1, DSG3, FBLN5, DSC3*
GO:0007155	Cell adhesion	*PPARD, CLDN4, CLDN3, ITGB4, CDH1, ITGB3, PXN, ALCAM, CD9, PCDH1, EZR, CD44, SORBS1, CTGF, BCL2, AGT, COL12A1, THBS1, PTPRF, PIK3CB, NLGN3, DSG2, PSEN1, DSG3, FBLN5, DSC3*
GO:0010605	Negative regulation of macromolecule metabolic process	*PPARA, SRP14, PPARD, SBNO2, NOSTRIN, ITGB3, CALR, PAX2, KCNIP3, HOXA2, AES, HEXIM1, THBS1, SATB1, CLN3, LOC783195, RNASE4, MTA2, ANG2, SNW1, SIRT7, CBY1, FURIN, CDKN1C, MSX1, PSEN1*
GO:0044271	Nitrogen compound biosynthetic process	*BCAT1, MOCOS, ATP1B1, ALDH18A1, ASS1, ADCY7, ATP1B2, ADCY6, ATP13A5, ATP13A4, GCH1, GOT2, ATP2A3, RRM1, TMLHE, LOC510369, NPPC, LOC534520, PHGDH, GUCY1A3, QPRT, GUCY1B3, ATP6V0D1, ATP8A1*
GO:0030001	Metal ion transport	*ATP1B1, SLC12A2, ATP1B2, SLC38A7, SLC34A2, KCNIP3, TMEM38B, SHKBP1, ATP2A3, KCNN2, KCNH7, LTF, SLC5A6, CAMK2B, GUCY1B3, KCNH8, CCS, KCTD15, SLC31A2, SCNN1A, CACNA1A, SLC39A1*
GO:0009890	Negative regulation of biosynthetic process	*SRP14, PPARA, SATB1, SBNO2, PPARD, LOC783195, RNASE4, MTA2, ANG2, NOSTRIN, SNW1, ITGB3, CBY1, SIRT7, CALR, PAX2, KCNIP3, CDKN1C, HOXA2, AES, MSX1, HEXIM1, CACNA1A*
GO:0016044	Membrane organization	*MICALL2, CLN3, SNAP91, VAV3, LDLR, SYNJ1, CDC42SE1, NOSTRIN, ABCA1, BCL2L1, ELMO3, CD9, PICALM, CDC42SE2, VAMP7, BCL2, VAMP2, THBS1, GHR, ELMOD1*
GO:0010558	Negative regulation of macromolecule biosynthetic process	*SRP14, PPARA, SATB1, SBNO2, PPARD, LOC783195, RNASE4, MTA2, ANG2, NOSTRIN, SNW1, ITGB3, CBY1, SIRT7, CALR, PAX2, KCNIP3, CDKN1C, HOXA2, AES, MSX1, HEXIM1*
GO:0031327	Negative regulation of cellular biosynthetic process	*SRP14, PPARA, SATB1, SBNO2, PPARD, LOC783195, RNASE4, MTA2, ANG2, NOSTRIN, SNW1, CBY1, SIRT7, CALR, PAX2, KCNIP3, CDKN1C, HOXA2, AES, MSX1, HEXIM1, CACNA1A*
GO:0016265	Death	*BCL2L15, SRA1, BCL2L1, ELMO3, KCNIP3, BAG5, TMEM173, CASP3, EYA2, PSEN1, LOC781146, BAG3, BCL2, SLC18A2, RIPK2, FAIM, TSTA3, GADD45B, C27H8ORF4, CACNA1A*
GO:0043009	Chordate embryonic development	*FGFR2, GINS1, ADAM10, SYVN1, TAF8, PRRX1, CDH1, BCL2L1, NMT1, TJP1, HOXA2, MSX1, PLCG1, PSEN1, SFRP2, GRN, PHGDH, PYGO2, PTCH1*
GO:0009792	Embryonic development ending in birth or egg hatching	*FGFR2, GINS1, ADAM10, SYVN1, TAF8, PRRX1, CDH1, BCL2L1, NMT1, TJP1, HOXA2, MSX1, PLCG1, PSEN1, SFRP2, GRN, PHGDH, PYGO2, PTCH1*
GO:0008219	Cell death	*BCL2L15, SRA1, BCL2L1, ELMO3, KCNIP3, BAG5, TMEM173, CASP3, EYA2, PSEN1, LOC781146, BAG3, BCL2, RIPK2, FAIM, TSTA3, GADD45B, C27H8ORF4, CACNA1A*
GO:0051056	Regulation of small gtpase mediated signal transduction	*ALS2, ARFGAP3, VAV3, BCR, SIPA1, ARHGEF16, TRIO, RGL2, ARHGEF11, RASAL1, SIPA1L1, PLEKHG5, TBC1D30, AGAP1, ARAP2, RASA4, ARHGEF10L, NET1*
GO:0008202	Steroid metabolic process	*CYB5R3, TM7SF2, LDLR, CYP51A1, HMGCR, RUSC1, OSBPL7, CFTR, ABCA1, PMVK, FDFT1, CEL, SULT1A1, INSIG1, OSBPL10, IDI1, NSDHL*
GO:0008283	Cell proliferation	*FGFR2, GINS1, SATB1, PPARD, PDXK, TAF8, SRA1, MET, FURIN, MIF, PROK2, PSEN1, AGT, BCL2, NUMB, RIPK2, PTCH1*
GO:0016481	Negative regulation of transcription	*PPARA, SATB1, SBNO2, PPARD, MTA2, NOSTRIN, SNW1, CBY1, SIRT7, CALR, PAX2, KCNIP3, CDKN1C, HOXA2, AES, MSX1, HEXIM1*

GO:0045934	Negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	*PPARA, SATB1, SBNO2, PPARD, MTA2, NOSTRIN, SNW1, CBY1, SIRT7, CALR, PAX2, KCNIP3, CDKN1C, HOXA2, AES, MSX1, HEXIM1*
GO:0007610	Behavior	*CLN3, HMGCR, ENPP2, MET, NLGN3, KCNIP3, PROK2, NPAS2, PSEN1, AGT, BCL2, LOC510369, GAA, SLC18A2, SEPP1, CACNA1A, PTAFR*
GO:0051172	Negative regulation of nitrogen compound metabolic process	*PPARA, SATB1, SBNO2, PPARD, MTA2, NOSTRIN, SNW1, CBY1, SIRT7, CALR, PAX2, KCNIP3, CDKN1C, HOXA2, AES, MSX1, HEXIM1*
GO:0008610	Lipid biosynthetic process	*CYB5R3, TM7SF2, LOC783195, HMGCR, CYP51A1, RNASE4, RUSC1, ANG2, FADS2, CFTR, PMVK, LPCAT3, MIF, FDFT1, PEX7, FASN, IDI1, NSDHL, AGPAT1*
GO:0010629	Negative regulation of gene expression	*PPARA, SATB1, SBNO2, PPARD, MTA2, NOSTRIN, SNW1, CBY1, SIRT7, CALR, PAX2, KCNIP3, CDKN1C, HOXA2, AES, MSX1, HEXIM1*
GO:0070271	Protein complex biogenesis	*TAF4, SNAP91, LOC783195, RNASE4, ANG2, CDH1, CALR, GCH1, MIF, MTMR2, PICALM, GSN, RRM1, GPX3, LOC510369, QPRT, CACNA1A, DCTPP1, APC*
GO:0006461	Protein complex assembly	*TAF4, SNAP91, LOC783195, RNASE4, ANG2, CDH1, CALR, GCH1, MIF, MTMR2, PICALM, GSN, RRM1, GPX3, LOC510369, QPRT, CACNA1A, DCTPP1, APC*
GO:0022402	Cell cycle process	*LOC512293, GAS2, RBM7, SIRT7, CALR, CDC25A, CCNB1, CDKN1C, MACF1, BCL2, PHGDH, GAS2L1, CAMK2B, AKAP8, SMC1A, THBS1, GADD45A, APC*
GO:0008285	Negative regulation of cell proliferation	*PPARD, LOC783195, RNASE4, ANG2, ESR2, TIMP2, CDKN1C, CD9, CASP3, MSX1, IL20RB, NUPR1, BCL2, AGT, PTCH1, THBS1, APC, NFIB*
GO:0006915	Apoptosis	*BCL2L15, SRA1, BCL2L1, ELMO3, KCNIP3, BAG5, CASP3, TMEM173, EYA2, PSEN1, BAG3, BCL2, RIPK2, FAIM, GADD45B, C27H8ORF4*
GO:0010033	Response to organic substance	*ADAM10, PFKL, BCL2L1, GCH1, GOT2, GNAL, ACVR2B, MSX1, SORBS1, BCL2, SLC18A2, RIPK2, PIK3R3, THBS1, PTAFR, GHR*
GO:0012501	Programmed cell death	*BCL2L15, SRA1, BCL2L1, ELMO3, KCNIP3, BAG5, CASP3, TMEM173, EYA2, PSEN1, BAG3, BCL2, RIPK2, FAIM, GADD45B, C27H8ORF4*
GO:0030030	Cell projection organization	*KLF5, MYO1A, VAV3, BAIAP2L2, KIF5C, EFHD1, HOXA2, BCL2, CLIC5, CAPG, NUMB, PHGDH, ROBO2, CACNA1A, APC*
GO:0045892	Negative regulation of transcription, DNA-dependent	*PPARA, SATB1, PPARD, SBNO2, MTA2, NOSTRIN, CBY1, SIRT7, CALR, PAX2, KCNIP3, CDKN1C, HOXA2, MSX1, HEXIM1*
GO:0051253	Negative regulation of RNA metabolic process	*PPARA, SATB1, PPARD, SBNO2, MTA2, NOSTRIN, CBY1, SIRT7, CALR, PAX2, KCNIP3, CDKN1C, HOXA2, MSX1, HEXIM1*
GO:0007167	Enzyme linked receptor protein signaling pathway	*FGFR2, MET, ALK, EPHB2, GRB10, ACVR2B, MSX1, SORBS1, CTGF, JAK1, PPM1L, PIK3R3, CACNA1A, GHR, PLEKHA1*
GO:0060548	Negative regulation of cell death	*CLN3, SYVN1, BCL2L1, PAX2, MIF, PROK2, CASP3, MSX1, AGT, BAG3, BCL2, FAIM, CACNA1A, ALOX12, APC*
GO:0043069	Negative regulation of programmed cell death	*CLN3, SYVN1, BCL2L1, PAX2, MIF, PROK2, CASP3, MSX1, AGT, BAG3, BCL2, FAIM, CACNA1A, ALOX12, APC*

GO:0009165	Nucleotide biosynthetic process	*ATP1B1, ADCY7, ATP1B2, ADCY6, ATP13A5, ATP13A4, ATP2A3, LOC510369, RRM1, NPPC, GUCY1A3, GUCY1B3, QPRT, ATP6V0D1, ATP8A1*
GO:0034404	Nucleobase, nucleoside and nucleotide biosynthetic process	*ATP1B1, ADCY7, ATP1B2, ADCY6, ATP13A5, ATP13A4, ATP2A3, LOC510369, RRM1, NPPC, GUCY1A3, GUCY1B3, QPRT, ATP6V0D1, ATP8A1*
GO:0034654	Nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic process	*ATP1B1, ADCY7, ATP1B2, ADCY6, ATP13A5, ATP13A4, ATP2A3, LOC510369, RRM1, NPPC, GUCY1A3, GUCY1B3, QPRT, ATP6V0D1, ATP8A1*
GO:0042325	Regulation of phosphorylation	*VAV3, HMGCR, MET, CDKN1C, ACVR2B, SPRY1, CASP3, PSEN1, HEXIM1, BCL2, GADD45B, THBS1, GADD45A, APC, GHR*
GO:0008203	Cholesterol metabolic process	*TM7SF2, CYB5R3, LDLR, CYP51A1, HMGCR, RUSC1, CFTR, ABCA1, PMVK, FDFT1, CEL, INSIG1, IDI1, NSDHL*
GO:0016125	Sterol metabolic process	*TM7SF2, CYB5R3, LDLR, CYP51A1, HMGCR, RUSC1, CFTR, ABCA1, PMVK, FDFT1, CEL, INSIG1, IDI1, NSDHL*
GO:0001501	Skeletal system development	*FGFR2, SOX5, PRRX1, ACP2, PEX7, ACVR2B, HOXA2, AES, PSEN1, CLEC3B, CTGF, BCL2, IGFBP3, PLEKHA1*
GO:0006163	Purine nucleotide metabolic process	*ATP1B1, ADCY7, ATP1B2, AK5, ATP13A5, ATP13A4, GCH1, ATP2A3, LOC510369, NPPC, GUCY1A3, GUCY1B3, ATP6V0D1, ATP8A1*
GO:0043066	Negative regulation of apoptosis	*CLN3, SYVN1, BCL2L1, MIF, PROK2, CASP3, MSX1, AGT, BAG3, BCL2, FAIM, CACNA1A, ALOX12, APC*
GO:0055082	Cellular chemical homeostasis	*CLN3, PPARD, PIK3CB, FOXA3, NLGN3, BCL2L1, AGTR1, CD9, PSEN1, BCL2, LTF, CHRNE, EIF2B4, CACNA1A*
GO:0040007	Growth	*GINS1, PPARD, HPN, TAF8, BCL2L1, ACVR2B, PSEN1, BCL2, PYGO2, SEPP1, CACNA1A, BMP5, PLEKHA1*
GO:0006897	Endocytosis	*MICALL2, CLN3, LDLR, SYNJ1, CDC42SE1, NOSTRIN, ABCA1, ELMO3, CDC42SE2, VAMP7, THBS1, ELMOD1, GHR*
GO:0010324	Membrane invagination	*MICALL2, CLN3, LDLR, SYNJ1, CDC42SE1, NOSTRIN, ABCA1, ELMO3, CDC42SE2, VAMP7, THBS1, ELMOD1, GHR*
GO:0001701	In utero embryonic development	*GINS1, FGFR2, SYVN1, ADAM10, TAF8, CDH1, BCL2L1, NMT1, TJP1, MSX1, PLCG1, GRN, PYGO2*
GO:0006631	Fatty acid metabolic process	*PPARA, PPARD, ECHDC2, FADS2, ACSF2, PEX7, MIF, ACOX3, ACADVL, FASN, ALOX12B, ALOX12, GHR*
GO:0043549	Regulation of kinase activity	*VAV3, HMGCR, MET, CDKN1C, ACVR2B, SPRY1, CASP3, PSEN1, HEXIM1, THBS1, GADD45B, GADD45A, APC*
GO:0046903	Secretion	*ARFGAP3, GARS, SCRN1, STXBP2, ABCA1, FURIN, CEL, ACVR2B, PSEN1, VAMP7, AGT, VAMP2, CACNA1A*
GO:0051338	Regulation of transferase activity	*VAV3, HMGCR, MET, CDKN1C, ACVR2B, SPRY1, CASP3, PSEN1, HEXIM1, THBS1, GADD45B, GADD45A, APC*
GO:0030182	Neuron differentiation	*KIF5C, SOX5, CDKN1C, EFHD1, HOXA2, PSEN1, BCL2, CLIC5, NUMB, PHGDH, ROBO2, CACNA1A, APC*
GO:0001775	Cell activation	*SATB1, SBNO2, ADAM10, SWAP70, PIK3CB, STXBP2, PSEN1, VAMP7, AGT, BCL2, RIPK2, NDRG1, APC*
GO:0046578	Regulation of Ras protein signal transduction	*ALS2, ARFGAP3, VAV3, BCR, ARHGEF16, TRIO, ARHGEF11, PLEKHG5, AGAP1, TBC1D30, ARAP2, ARHGEF10L, NET1*
GO:0040008	Regulation of growth	*FGFR2, ADAM10, AES, CTGF, AGT, BCL2, NPPC, PTCH1, IGFBP3, CRIM1, IGFBP4, ALOX12, GHR*

GO:0046942	Carboxylic acid transport	*SLC1A4, GOT2, SLC1A5, CLN3, PPARD, PSEN1, SLC38A7, SLC6A6, PDZK1, SLC7A5, SLC1A1, CACNA1A*
GO:0015849	Organic acid transport	*SLC1A4, GOT2, SLC1A5, CLN3, PPARD, PSEN1, SLC38A7, SLC6A6, PDZK1, SLC7A5, SLC1A1, CACNA1A*
GO:0048666	Neuron development	*CDKN1C, EFHD1, HOXA2, PSEN1, BCL2, CLIC5, KIF5C, NUMB, PHGDH, ROBO2, CACNA1A, APC*
GO:0006164	Purine nucleotide biosynthetic process	*ATP1B1, ADCY7, ATP1B2, ATP2A3, LOC510369, NPPC, GUCY1A3, GUCY1B3, ATP6V0D1, ATP13A5, ATP8A1, ATP13A4*
GO:0005996	Monosaccharide metabolic process	*ALDOA, AMDHD2, PFKL, GCK, GAA, CHST4, GALE, OGDH, PCK2, FUCA1, CACNA1A, PYGB*
GO:0032989	Cellular component morphogenesis	*ACTG2, CD9, HOXA2, EZR, BCL2, CLIC5, KIF5C, NUMB, ROBO2, PAX2, CACNA1A, APC*
GO:0030036	Actin cytoskeleton organization	*RHOJ, LOC783195, DIAPH1, RNASE4, ANG2, CALR, ACTG2, EZR, SORBS1, GSN, BCL2, DBN1, LCP1*
GO:0016042	Lipid catabolic process	*PLD2, CEL, PPARD, SMPDL3B, PLCG1, SMPDL3A, ENPP2, PLCB1, PEX7, ACOX3, PLBD2*
GO:0030029	Actin filament-based process	*RHOJ, LOC783195, DIAPH1, RNASE4, ANG2, CALR, ACTG2, EZR, SORBS1, GSN, BCL2, DBN1, LCP1*
GO:0000122	Negative regulation of transcription from RNA polymerase II promoter	*CDKN1C, SATB1, PPARA, HOXA2, PPARD, MSX1, HEXIM1, MTA2, CALR, PAX2, KCNIP3*
GO:0007169	Transmembrane receptor protein tyrosine kinase signaling pathway	*FGFR2, GRB10, SORBS1, CTGF, MET, PIK3R3, ALK, CACNA1A, EPHB2, PLEKHA1, GHR*
GO:0032940	Secretion by cell	*ARFGAP3, ACVR2B, PSEN1, VAMP7, SCRN1, GARS, STXBP2, ABCA1, VAMP2, FURIN, CACNA1A*
GO:0009100	Glycoprotein metabolic process	*CLN3, GALNT1, MGAT3, B3GALT6, PSEN1, FUT10, FUT5, ST8SIA5, B3GNT3, DOLPP1, PORCN*
GO:0051240	Positive regulation of multicellular organismal process	*FGFR2, PROK2, ACVR2B, IL20RB, PSEN1, BCL2, AGT, RIPK2, THBS1, AGPAT1, GHR*
GO:0045321	Leukocyte activation	*SATB1, SBNO2, ADAM10, PSEN1, SWAP70, BCL2, VAMP7, RIPK2, STXBP2, NDRG1, APC*
GO:0045859	Regulation of protein kinase activity	*SPRY1, CASP3, ACVR2B, PSEN1, HMGCR, HEXIM1, MET, GADD45B, THBS1, GADD45A, APC*
GO:0006695	Cholesterol biosynthetic process	*CYB5R3, TM7SF2, HMGCR, CYP51A1, RUSC1, CFTR, IDI1, PMVK, NSDHL, FDFT1*
GO:0016126	Sterol biosynthetic process	*CYB5R3, TM7SF2, HMGCR, CYP51A1, RUSC1, CFTR, IDI1, PMVK, NSDHL, FDFT1*
GO:0006694	Steroid biosynthetic process	*CYB5R3, TM7SF2, HMGCR, CYP51A1, RUSC1, CFTR, IDI1, PMVK, NSDHL, FDFT1*
GO:0001822	Kidney development	*FGFR2, AGTR1, SPRY1, ACVR2B, BCL2, AGT, PYGO2, ROBO2, PAX2, APC*
GO:0001655	Urogenital system development	*FGFR2, AGTR1, SPRY1, ACVR2B, BCL2, AGT, PYGO2, ROBO2, PAX2, APC*
GO:0042391	Regulation of membrane potential	*CLN3, CD9, PPARD, PSEN1, BCL2, NLGN3, BCL2L1, CHRNE, EIF2B4, CACNA1A*
GO:0060284	Regulation of cell development	*HOXA2, SERPINF1, PSEN1, BCL2, SOX5, SEMA4D, CALR, TIMP2, IGFBP3, CACNA1A*
GO:0043086	Negative regulation of catalytic activity	*CDKN1C, CLN3, SPRY1, CASP3, PSEN1, HMGCR, HEXIM1, GADD45B, GADD45A, APC*
GO:0006260	DNA replication	*GINS1, TOP1, POLE2, CTGF, POLD2, RRM1, TOM1, NFIX, NFIB, REPIN1*
GO:0044092	Negative regulation of molecular function	*CDKN1C, CLN3, SPRY1, CASP3, PSEN1, HMGCR, HEXIM1, GADD45B, GADD45A, APC*
GO:0035295	Tube development	*FGFR2, SPRY1, ACVR2B, PSEN1, CTGF, BCL2, AGT, PTCH1, PAX2, NFIB*
GO:0033673	Negative regulation of kinase activity	*CDKN1C, SPRY1, CASP3, PSEN1, HMGCR, HEXIM1, GADD45B, GADD45A, APC*
GO:0051348	Negative regulation of transferase activity	*CDKN1C, SPRY1, CASP3, PSEN1, HMGCR, HEXIM1, GADD45B, GADD45A, APC*
GO:0006865	Amino acid transport	*SLC1A4, CLN3, PSEN1, SLC38A7, SLC6A6, PDZK1, SLC7A5, SLC1A1, CACNA1A*

GO:0050767	Regulation of neurogenesis	*HOXA2, SERPINF1, PSEN1, BCL2, SOX5, SEMA4D, CALR, TIMP2, CACNA1A*
GO:0015837	Amine transport	*SLC1A4, CLN3, PSEN1, SLC38A7, SLC6A6, PDZK1, SLC7A5, SLC1A1, CACNA1A*
GO:0007398	Ectoderm development	*PPARA, CASP3, PPARD, PSEN1, BCL2, ELF5, PTCH1, APC, NSDHL*
GO:0051259	Protein oligomerization	*MTMR2, LOC510369, RRM1, GPX3, CDH1, QPRT, DCTPP1, GCH1, MIF*
GO:0051960	Regulation of nervous system development	*HOXA2, SERPINF1, PSEN1, BCL2, SOX5, SEMA4D, CALR, TIMP2, CACNA1A*
GO:0035023	Regulation of Rho protein signal transduction	*ALS2, BCR, VAV3, PLEKHG5, ARHGEF16, TRIO, ARHGEF10L, ARHGEF11, NET1*
GO:0070085	Glycosylation	*GALNT1, MGAT3, B3GALT6, PSEN1, FUT10, FUT5, ST8SIA5, B3GNT3, DOLPP1*
GO:0006486	Protein amino acid glycosylation	*GALNT1, MGAT3, B3GALT6, PSEN1, FUT10, FUT5, ST8SIA5, B3GNT3, DOLPP1*
GO:0043413	Biopolymer glycosylation	*GALNT1, MGAT3, B3GALT6, PSEN1, FUT10, FUT5, ST8SIA5, B3GNT3, DOLPP1*
GO:0031175	Neuron projection development	*EFHD1, HOXA2, BCL2, KIF5C, NUMB, PHGDH, ROBO2, CACNA1A, APC*
GO:0000904	Cell morphogenesis involved in differentiation	*HOXA2, BCL2, CLIC5, KIF5C, NUMB, ROBO2, PAX2, CACNA1A, APC*
GO:0009101	Glycoprotein biosynthetic process	*GALNT1, MGAT3, B3GALT6, PSEN1, FUT10, FUT5, ST8SIA5, B3GNT3, DOLPP1*
GO:0007015	Actin filament organization	*ACTG2, EZR, SORBS1, LOC783195, RNASE4, GSN, BCL2, ANG2, DBN1, LCP1*
GO:0006469	Negative regulation of protein kinase activity	*SPRY1, CASP3, PSEN1, HMGCR, HEXIM1, GADD45B, GADD45A, APC*
GO:0045664	Regulation of neuron differentiation	*HOXA2, PSEN1, BCL2, SOX5, SEMA4D, CALR, TIMP2, CACNA1A*
GO:0009791	Post-embryonic development	*ACVR2B, PSEN1, BCL2, NPPC, SLC18A2, PYGO2, SEPP1, PLEKHA1*
GO:0007050	Cell cycle arrest	*CDKN1C, MACF1, GAS2L1, GAS2, CALR, THBS1, GADD45A, APC*
GO:0031589	Cell-substrate adhesion	*PPARD, SORBS1, CTGF, BCL2, AGT, ITGB4, ITGB3, PXN*
GO:0008544	Epidermis development	*PPARA, CASP3, PPARD, PSEN1, BCL2, PTCH1, APC, NSDHL*
GO:0006790	Sulfur metabolic process	*MTHFR, GGT7, PHGDH, CHST4, GSTT3, SEPP1, CACNA1A, GHR*
GO:0009309	Amine biosynthetic process	*BCAT1, GOT2, ALDH18A1, ASS1, TMLHE, PHGDH, LOC534520, GCH1*
GO:0045165	Cell fate commitment	*FGFR2, SPRY1, HOXA2, CASP3, PSEN1, BCL2, SOX5, PAX2*
GO:0048667	Cell morphogenesis involved in neuron differentiation	*HOXA2, BCL2, CLIC5, KIF5C, NUMB, ROBO2, CACNA1A, APC*
GO:0030155	Regulation of cell adhesion	*ADAM10, VAV3, PIK3CB, BCL2, SMOC1, THBS1, APC, ALOX12*
GO:0006820	Anion transport	*SLC26A3, SLC4A11, SLC12A2, PSEN1, CLIC5, SLC1A1, CLCN4, SLC34A2*
GO:0006909	Phagocytosis	*CDC42SE2, VAMP7, CDC42SE1, ABCA1, THBS1, ELMO3, ELMOD1*
GO:0048511	Rhythmic process	*PROK2, NPAS2, LOC783195, RNASE4, BCL2, AGT, ANG2, BCL2L1, EIF2B4*
GO:0044242	Cellular lipid catabolic process	*CEL, PPARD, SMPDL3B, PLCG1, SMPDL3A, PEX7, ACOX3*
GO:0007160	Cell-matrix adhesion	*SORBS1, CTGF, BCL2, AGT, ITGB4, ITGB3, PXN*
GO:0022604	Regulation of cell morphogenesis	*RHOJ, EZR, PSEN1, CDC42SE2, CDC42SE1, SEMA4D, CACNA1A*
GO:0032269	Negative regulation of cellular protein metabolic process	*CLN3, SRP14, LOC783195, PSEN1, RNASE4, ANG2, ITGB3, THBS1, FURIN*

GO:0051248	Negative regulation of protein metabolic process	*CLN3, SRP14, LOC783195, PSEN1, RNASE4, ANG2, ITGB3, THBS1, FURIN*
GO:0048705	Skeletal system morphogenesis	*HOXA2, ACVR2B, PSEN1, CTGF, PRRX1, PEX7, PLEKHA1*
GO:0001558	Regulation of cell growth	*ADAM10, CTGF, BCL2, IGFBP3, IGFBP4, CRIM1, ALOX12*
GO:0006887	Exocytosis	*PSEN1, VAMP7, SCRN1, GARS, STXBP2, VAMP2, CACNA1A*
GO:0048812	Neuron projection morphogenesis	*HOXA2, BCL2, KIF5C, NUMB, ROBO2, CACNA1A, APC*
GO:0001656	Metanephros development	*FGFR2, SPRY1, BCL2, AGT, ROBO2, PAX2*
GO:0016050	Vesicle organization	*SNAP91, VAV3, PICALM, BCL2, VAMP7, ABCA1*
GO:0043583	Ear development	*FGFR2, HOXA2, BCL2, CLIC5, PRRX1, PAX2*
GO:0048589	Developmental growth	*GINS1, PPARD, PSEN1, BCL2, TAF8, PYGO2*
GO:0009064	Glutamine family amino acid metabolic process	*GOT2, ALDH18A1, ASS1, GLUD1, GFPT2, PHGDH*
GO:0051260	Protein homooligomerization	*LOC510369, GPX3, CDH1, DCTPP1, GCH1, MIF*
GO:0043523	Regulation of neuron apoptosis	*CLN3, BCL2, AGT, BCL2L1, CACNA1A, KCNIP3*
GO:0001657	Ureteric bud development	*FGFR2, SPRY1, BCL2, AGT, PAX2*
GO:0034330	Cell junction organization	*CD9, SORBS1, BCL2, NUMB, ITGB3*
GO:0006944	Membrane fusion	*CLN3, CD9, VAV3, VAMP7, VAMP2*
GO:0022405	Hair cycle process	*PPARD, PSEN1, BCL2, APC, NSDHL*
GO:0002274	Myeloid leukocyte activation	*SBNO2, ADAM10, VAMP7, STXBP2, NDRG1*
GO:0022404	Molting cycle process	*PPARD, PSEN1, BCL2, APC, NSDHL*
GO:0001942	Hair follicle development	*PPARD, PSEN1, BCL2, APC, NSDHL*
GO:0007033	Vacuole organization	*CLN3, PSEN1, GAA, ACP2, ABCA1*
GO:0009190	Cyclic nucleotide biosynthetic process	*ADCY7, ADCY6, NPPC, GUCY1A3, GUCY1B3*
GO:0042303	Molting cycle	*PPARD, PSEN1, BCL2, APC, NSDHL*
GO:0042633	Hair cycle	*PPARD, PSEN1, BCL2, APC, NSDHL*
GO:0042471	Ear morphogenesis	*FGFR2, HOXA2, CLIC5, PRRX1, PAX2*
GO:0022602	Ovulation cycle process	*LOC783195, RNASE4, BCL2, AGT, ANG2, BCL2L1, EIF2B4*
GO:0008585	Female gonad development	*LOC783195, RNASE4, BCL2, AGT, ANG2, BCL2L1, EIF2B4*
GO:0042698	Ovulation cycle	*LOC783195, RNASE4, BCL2, AGT, ANG2, BCL2L1, EIF2B4*
GO:0046660	Female sex differentiation	*LOC783195, RNASE4, BCL2, AGT, ANG2, BCL2L1, EIF2B4*
GO:0046545	Development of primary female sexual characteristics	*LOC783195, RNASE4, BCL2, AGT, ANG2, BCL2L1, EIF2B4*
GO:0034097	Response to cytokine stimulus	*ADAM10, BCL2, RIPK2, BCL2L1, GCH1*
GO:0009187	Cyclic nucleotide metabolic process	*ADCY7, ADCY6, NPPC, GUCY1A3, GUCY1B3*
GO:0050678	Regulation of epithelial cell proliferation	*CDKN1C, PPARD, GRN, PTCH1, APC*
GO:0050905	Neuromuscular process	*CLN3, CLIC5, GAA, CACNA1A, GCH1*

GO:0031668	Cellular response to extracellular stimulus	*CLN3, AES, PSEN1, SFRP2, FOXA3*
GO:0043524	Negative regulation of neuron apoptosis	*CLN3, BCL2, AGT, BCL2L1, CACNA1A*
GO:0006720	Isoprenoid metabolic process	*DHRS4, HMGCR, RUSC1, IDI1, FDFT1*
GO:0045785	Positive regulation of cell adhesion	*VAV3, SMOC1, THBS1, APC, ALOX12*
GO:0030031	Cell projection assembly	*KLF5, MYO1A, VAV3, BAIAP2L2, CAPG*
GO:0015807	L-amino acid transport	*SLC1A4, SLC7A5, SLC1A1, CACNA1A*
GO:0050680	Negative regulation of epithelial cell proliferation	*CDKN1C, PPARD, PTCH1, APC*
GO:0007040	Lysosome organization	*CLN3, GAA, ACP2, ABCA1*
GO:0034329	Cell junction assembly	*CD9, SORBS1, BCL2, ITGB3*
GO:0001541	Ovarian follicle development	*LOC783195, RNASE4, BCL2, ANG2, BCL2L1, EIF2B4*
GO:0043648	Dicarboxylic acid metabolic process	*GOT2, ME3, QPRT, GHR*
GO:0015718	Monocarboxylic acid transport	*SLC1A4, GOT2, PPARD, SLC6A6*
GO:0060021	Palate development	*ACVR2B, PRRX1, PYGO2, PLEKHA1*
GO:0050885	Neuromuscular process controlling balance	*CLN3, CLIC5, GAA, CACNA1A*
GO:0042472	Inner ear morphogenesis	*FGFR2, CLIC5, PRRX1, PAX2*
GO:0030902	Hindbrain development	*HOXA2, BCL2, CACNA1A, NFIB*
GO:0002366	Leukocyte activation during immune response	*SBNO2, PSEN1, VAMP7, STXBP2*
GO:0002263	Cell activation during immune response	*SBNO2, PSEN1, VAMP7, STXBP2*
GO:0008299	Isoprenoid biosynthetic process	*HMGCR, RUSC1, IDI1, FDFT1*
GO:0048634	Regulation of muscle development	*FGFR2, BCL2, LUC7L, IGFBP3*
GO:0016202	Regulation of striated muscle tissue development	*FGFR2, BCL2, LUC7L, IGFBP3*
GO:0051262	Protein tetramerization	*MTMR2, LOC510369, GPX3, DCTPP1*
GO:0034440	Lipid oxidation	*PPARD, PEX7, ALOX12, ACOX3*
GO:0045834	Positive regulation of lipid metabolic process	*AGTR1, VAV3, SORBS1, AGT*
GO:0019395	Fatty acid oxidation	*PPARD, PEX7, ALOX12, ACOX3*
GO:0051402	Neuron apoptosis	*CASP3, PSEN1, BCL2*
GO:0006684	Sphingomyelin metabolic process	*CLN3, SMPDL3B, SMPDL3A*
GO:0015804	Neutral amino acid transport	*SLC1A4, SLC6A6, SLC7A5*
GO:0030149	Sphingolipid catabolic process	*CEL, SMPDL3B, SMPDL3A*
GO:0046466	Membrane lipid catabolic process	*CEL, SMPDL3B, SMPDL3A*

GO:0002275	Myeloid cell activation during immune response	*SBNO2, VAMP7, STXBP2*
GO:0007044	Cell-substrate junction assembly	*SORBS1, BCL2, ITGB3*
GO:0015695	Organic cation transport	*SLC12A2, PSEN1, PDZK1*
GO:0006911	Phagocytosis, engulfment	*VAMP7, ABCA1, THBS1*
GO:0006182	Cgmp biosynthetic process	*NPPC, GUCY1A3, GUCY1B3*
GO:0045736	Negative regulation of cyclin-dependent protein kinase activity	*CASP3, HEXIM1, APC*
GO:0045055	Regulated secretory pathway	*VAMP7, GARS, STXBP2*
GO:0051017	Actin filament bundle formation	*EZR, SORBS1, LCP1*
GO:0051289	Protein homotetramerization	*LOC510369, GPX3, DCTPP1*
GO:0046068	Cgmp metabolic process	*NPPC, GUCY1A3, GUCY1B3*
GO:0001836	Release of cytochrome c from mitochondria	*CASP3, BCL2, BCL2L1*
GO:0009081	Branched chain family amino acid metabolic process	*BCAT1, ACAD8, GHR*

Supplemental Table 3.2. Overrepresented gene ontologies (GO_FAT) associated with differential expressed genes up-regulated in the ampulla.

GOBPID	Term	Genes
GO:0006796	Phosphate metabolic process	*STYXL1, FGFR4, STK31, CDC14B, CAMK2G, KIT, PRKG1, CAMKK2, DUPD1, GALK2, EPHB6, VRK1, DUSP14, TEK, MAP3K8, IPMK, GPD2, TYRO3, ALPK1, SGK3, MAK, NPR1, MAPK10, DAPK2, CDC25C, PTPDC1, NEK11, TBCK, PRKCB, KDR, DAPK1, RPS6KA5, HUNK, DDR1, ACVR2A, MAP4K5, CDKL1, PPM1E, EPHA6, NTRK2, ROR1, ERN1, NEK4, F2R*
GO:0006793	Phosphorus metabolic process	*STYXL1, FGFR4, STK31, CDC14B, CAMK2G, KIT, PRKG1, CAMKK2, DUPD1, GALK2, EPHB6, VRK1, DUSP14, TEK, MAP3K8, IPMK, GPD2, TYRO3, ALPK1, SGK3, MAK, NPR1, MAPK10, DAPK2, CDC25C, PTPDC1, NEK11, TBCK, PRKCB, KDR, DAPK1, RPS6KA5, HUNK, DDR1, ACVR2A, MAP4K5, CDKL1, PPM1E, EPHA6, NTRK2, ROR1, ERN1, NEK4, F2R*
GO:0006508	Proteolysis	*CUEDC2, MASP1, C3, MMP7, LOC613739, ASB14, ENPEP, FBXW9, USP27X, ECE1, TPP2, ADAM32, PREPL, SCG5, ZMPSTE24, PGA5, ADAMTS12, PCSK5, FBXO7, CPA5, PLAT, NRIP3, FBXL21, FBXO2, AGBL5, UBE2L6, CELA1, BRCA1, FEM1C, LAP3, F2, CAPN14, DDB2, LTA4H, CTSC, FBXO15, ADAMTS5, PLAU, FBXL2, UBE2U*
GO:0016310	Phosphorylation	*FGFR4, STK31, CAMK2G, KIT, PRKG1, CAMKK2, GALK2, VRK1, EPHB6, TEK, MAP3K8, IPMK, TYRO3, ALPK1, SGK3, MAK, NPR1, MAPK10, DAPK2, TBCK, NEK11, DAPK1, KDR, PRKCB, RPS6KA5, HUNK, ACVR2A, DDR1, MAP4K5, EPHA6, CDKL1, NTRK2, ERN1, ROR1, NEK4, F2R*
GO:0006468	Protein amino acid phosphorylation	*FGFR4, STK31, CAMK2G, KIT, PRKG1, CAMKK2, VRK1, EPHB6, TEK, MAP3K8, TYRO3, ALPK1, SGK3, MAK, NPR1, MAPK10, DAPK2, TBCK, NEK11, DAPK1, KDR, PRKCB, RPS6KA5, HUNK, ACVR2A, DDR1, MAP4K5, EPHA6, CDKL1, NTRK2, ERN1, ROR1, NEK4, F2R*

GO:0006811	Ion transport	*PLCZ1, SLC39A10, CAMK2G, GRIK4, CACNB2, SLC38A11, KCNA5, KCNJ2, KCNRG, KCNIP4, KCNS3, GRIN2B, CHRNA5, PKD2, SLC4A9, MYB, SLC39A3, TRPC1, KCND3, GLRB, GABRA2, SLC22A7, ATP1A2, SLC9A11, ITPR1, CNGA1, PRKCB, SLC26A5, SLC16A7, LASP1, PLN, CACNA1H, F2R*
GO:0007242	Intracellular signaling cascade	*PLCZ1, RP1, THRA, ADCY8, DCDC2B, ASB14, NR3C1, KIT, RHOV, CDC42EP1, DIRAS3, RAC2, PLEKHG7, NSMCE1, PKD2, RHOBTB1, RAB26, RASA2, NPR1, ARHGAP29, MAPK10, ARL6, DCDC1, PRKCB, ADRB2, PLCE1, RND1, RASSF1, CNIH4, PECAM1, IRAK1BP1, F2R*
GO:0022610	Biological adhesion	*DCHS2, TYRO3, ICAM1, PCDHGA10, THRA, COL15A1, ITGA1, NID1, CELSR1, CLDN11, CDH3, BTBD9, THY1, CTNNA2, CD97, ITGA9, DDR1, CD36, ITGAV, PECAM1, TEK, LAMC2, THBS3, SPON1*
GO:0007155	Cell adhesion	*DCHS2, TYRO3, ICAM1, PCDHGA10, THRA, COL15A1, ITGA1, NID1, CELSR1, CLDN11, CDH3, BTBD9, THY1, CTNNA2, CD97, ITGA9, DDR1, CD36, ITGAV, PECAM1, TEK, LAMC2, THBS3, SPON1*
GO:0042127	Regulation of cell proliferation	*FGFR4, AIMP1, EFNB1, ST8SIA1, PAX6, CD276, MMP7, IL15, ZEB1, KIT, KDR, DDR1, HDAC2, KRT5, RAC2, CDKN2C, TEK, TRAF5, SMARCA2, MYC, PLAU, IGFBP5, F2R*
GO:0043933	Macromolecular complex subunit organization	*LOC617905, LOC523214, ALDOB, RBM5, LOC527388, TSPYL4, LOC614881, NAP1L4, LOC527645, LOC613926, GTF2E1, EIF3D, LOC614376, LOC518961, C1QTNF1, SRR, HIST3H2A, PTBP2, LOC505183, TUBG2, LPL, LOC516742, LOC618824, LOC618012, PFKM, LOC616868, LOC521580, H2AFY2, LRP4*
GO:0030001	Metal ion transport	*PLCZ1, TRPC1, KCND3, SLC39A10, CAMK2G, CACNB2, SLC38A11, KCNA5, ATP1A2, KCNJ2, KCNRG, ITPR1, KCNIP4, PRKCB, KCNS3, PLN, PKD2, MYB, SLC39A3, F2R*
GO:0065003	Macromolecular complex assembly	*LOC617905, LOC523214, ALDOB, RBM5, LOC527388, TSPYL4, LOC614881, NAP1L4, LOC527645, LOC613926, GTF2E1, EIF3D, LOC614376, LOC518961, C1QTNF1, SRR, HIST3H2A, PTBP2, LOC505183, TUBG2, LOC516742, LOC618824, LOC618012, PFKM, LOC616868, LOC521580, H2AFY2, LRP4*
GO:0006928	Cell motion	*DNAH11, PLAT, ICAM1, AIMP1, FUT8, EFNB1, PAX6, ABI2, ENPEP, KIT, PRKG1, KDR, CXCR4, TEKT2, EFNA5, AKAP3, PLAU, GFRA3, MYH10*
GO:0048878	Chemical homeostasis	*LPL, TRPC1, GLRB, CLDN11, PFKM, KCNA5, ATP1A2, SLC9A3R1, LDLRAP1, ITPR1, PRKCB, KDR, ABCG8, ASGR2, PLN, NAB1, F2, PKD2, F2R*
GO:0051674	Localization of cell	*DNAH11, PLAT, ICAM1, FUT8, AIMP1, EFNB1, PAX6, ABI2, ENPEP, KIT, PRKG1, KDR, CXCR4, TEKT2, PLAU, GFRA3, MYH10*
GO:0048870	Cell motility	*DNAH11, PLAT, ICAM1, FUT8, AIMP1, EFNB1, PAX6, ABI2, ENPEP, KIT, PRKG1, KDR, CXCR4, TEKT2, PLAU, GFRA3, MYH10*
GO:0034622	Cellular macromolecular complex assembly	*LOC617905, LOC618824, LOC516742, LOC523214, LOC618012, ALDOB, RBM5, LOC527388, TSPYL4, LOC614881, LOC613926, LOC616868, NAP1L4, LOC527645, LOC521580, EIF3D, LOC614376, LOC518961, H2AFY2, HIST3H2A, PTBP2, LOC505183, TUBG2, LRP4*
GO:0034621	Cellular macromolecular complex subunit organization	*LOC617905, LOC618824, LOC516742, LOC523214, LOC618012, ALDOB, RBM5, LOC527388, TSPYL4, LOC614881, LOC613926, LOC616868, NAP1L4, LOC527645, LOC521580, EIF3D, LOC614376, LOC518961, H2AFY2, HIST3H2A, PTBP2, LOC505183, TUBG2, LRP4*
GO:0016477	Cell migration	*PLAT, ICAM1, FUT8, AIMP1, EFNB1, PAX6, ABI2, KIT, ENPEP, PRKG1, KDR, CXCR4, PLAU, GFRA3, MYH10*
GO:0007017	Microtubule-based process	*DNAH11, BBS4, MAP1B, KIF9, KIF3C, BRCA1, DNAH6, BBS2, KIF2C, TEKT1, LOC528767, TEKT2, KIF19, TEKT4, TUBG2*

GO:0007167	Enzyme linked receptor protein signaling pathway	*PLAT, FGFR4, FUT8, KIT, KDR, DDR1, ACVR2A, EPHB6, EPHA6, TEK, NTRK2, ROR1, HPGD, AKAP3, LRP4*
GO:0043009	Chordate embryonic development	*BBS4, PAX6, ZEB1, TPM1, BRCA1, FZD6, ACVR2A, ECE1, HOXD4, PKD2, IFT52, MYB, IPMK, MYH10*
GO:0009792	Embryonic development ending in birth or egg hatching	*BBS4, PAX6, ZEB1, TPM1, BRCA1, FZD6, ACVR2A, ECE1, HOXD4, PKD2, IFT52, MYB, IPMK, MYH10*
GO:0007010	Cytoskeleton organization	*BBS4, MAP1B, BRCA1, THY1, BBS2, RND1, RAC2, TEKT1, SVIL, KRT14, TEKT2, TEKT4, EHD2, MYH10*
GO:0050801	Ion homeostasis	*TRPC1, GLRB, CLDN11, KCNA5, ATP1A2, SLC9A3R1, ITPR1, PRKCB, KDR, PLN, NAB1, F2, PKD2, F2R*
GO:0030030	Cell projection organization	*BBS4, EFNB1, MAP1B, PAX6, ABI2, PRKG1, BBS2, RAC2, CXCR4, EFNA5, TEKT4, MYH10, GFRA3*
GO:0048598	Embryonic morphogenesis	*BBS4, LMBR1, CRABP2, ZEB1, FZD6, ACVR2A, ECE1, HOXD4, IFT52, EXT1, MYC, IPMK, LRP4*
GO:0006873	Cellular ion homeostasis	*TRPC1, GLRB, CLDN11, KCNA5, ATP1A2, SLC9A3R1, ITPR1, PRKCB, PLN, NAB1, F2, PKD2, F2R*
GO:0055082	Cellular chemical homeostasis	*TRPC1, GLRB, CLDN11, KCNA5, ATP1A2, SLC9A3R1, ITPR1, PRKCB, PLN, NAB1, F2, PKD2, F2R*
GO:0040012	Regulation of locomotion	*ICAM1, BBS2, BBS4, HS3ST5, CXCR4, PECAM1, TEK, PAX6, IGFBP5, KDR, THY1, F2R*
GO:0007389	Pattern specification process	*DNAH11, ACVR2A, CXCR4, EFNB1, SOSTDC1, HOXD4, PKD2, PAX6, IFT52, ZEB1, LFNG, LRP4*
GO:0048666	Neuron development	*BBS4, RND1, CXCR4, EFNB1, MAP1B, PAX6, ABI2, EFNA5, PRKG1, GFRA3, THY1, MYH10*
GO:0006631	Fatty acid metabolic process	*LPL, PTGIS, PTGDS, PLA2G1B, ACSBG2, LTA4H, HSD17B4, ALOX5, HPGD, BRCA1, MGST2, DEGS1*
GO:0030182	Neuron differentiation	*BBS4, RND1, CXCR4, EFNB1, MAP1B, PAX6, ABI2, EFNA5, PRKG1, GFRA3, THY1, MYH10*
GO:0051270	Regulation of cell motion	*ICAM1, BBS2, BBS4, CXCR4, PECAM1, TEK, PAX6, IGFBP5, KDR, THY1, F2R*
GO:0007169	Transmembrane receptor protein tyrosine kinase signaling pathway	*PLAT, DDR1, FGFR4, EPHB6, EPHA6, TEK, NTRK2, ROR1, KIT, LRP4, KDR*
GO:0007423	Sensory organ development	*DDR1, BBS4, MEIS2, ECE1, PAX6, ABI2, ZEB1, MYC, FZD6, THY1, MYH10*
GO:0009100	Glycoprotein metabolic process	*ST3GAL1, ASGR2, ST3GAL3, HS3ST5, ST3GAL2, B3GNT5, ST3GAL5, FUT8, FBXO2, ST8SIA1, EXT1*
GO:0006333	Chromatin assembly or disassembly	*LOC617905, LOC618824, LOC516742, LOC523214, LOC618012, LOC527388, TSPYL4, LOC614881, LOC613926, LOC527645, LOC616868, NAP1L4, LOC521580, LOC614376, LOC518961, H2AFY2, SMARCA5, HIST3H2A, LOC505183*
GO:0006281	DNA repair	*XRCC5, POLI, MSH5, NSMCE1, TDP1, MGMT, DDB2, XRCC1, NTHL1, BRCA1, EEPD1*
GO:0031175	Neuron projection development	*BBS4, CXCR4, EFNB1, MAP1B, PAX6, ABI2, EFNA5, PRKG1, GFRA3, MYH10*
GO:0006816	Calcium ion transport	*PLCZ1, TRPC1, PLN, CAMK2G, PKD2, CACNB2, MYB, ITPR1, F2R, PRKCB*
GO:0006334	Nucleosome assembly	*LOC617905, LOC618824, LOC516742, LOC523214, LOC618012, LOC527388, TSPYL4, LOC614881, LOC613926, LOC527645, LOC616868, NAP1L4, LOC521580, LOC614376, LOC518961, H2AFY2, HIST3H2A, LOC505183*
GO:0031497	Chromatin assembly	*LOC617905, LOC618824, LOC516742, LOC523214, LOC618012, LOC527388, TSPYL4, LOC614881, LOC613926, LOC527645, LOC616868, NAP1L4, LOC521580, LOC614376, LOC518961, H2AFY2, HIST3H2A, LOC505183*
GO:0065004	Protein-DNA complex assembly	*LOC617905, LOC618824, LOC516742, LOC523214, LOC618012, LOC527388, TSPYL4, LOC614881, LOC613926, LOC527645, LOC616868, NAP1L4, LOC521580, LOC614376, LOC518961, H2AFY2, HIST3H2A, LOC505183*

GO:0034728	Nucleosome organization	*LOC617905, LOC618824, LOC516742, LOC523214, LOC618012, LOC527388, TSPYL4, LOC614881, LOC613926, LOC527645, LOC616868, NAP1L4, LOC521580, LOC614376, LOC518961, H2AFY2, HIST3H2A, LOC505183*
GO:0016053	Organic acid biosynthetic process	*LPL, PTGIS, PTGDS, PLA2G1B, SRR, ASNS, LTA4H, BRCA1, MGST2, DEGS1*
GO:0046394	Carboxylic acid biosynthetic process	*LPL, PTGIS, PTGDS, PLA2G1B, SRR, ASNS, LTA4H, BRCA1, MGST2, DEGS1*
GO:0006323	DNA packaging	*LOC617905, LOC618824, LOC516742, LOC523214, LOC618012, LOC527388, TSPYL4, LOC614881, LOC613926, LOC527645, LOC616868, NAP1L4, LOC521580, LOC614376, LOC518961, H2AFY2, HIST3H2A, LOC505183*
GO:0015674	Di-, tri-valent inorganic cation transport	*PLCZ1, TRPC1, PLN, CAMK2G, PKD2, CACNB2, MYB, ITPR1, F2R, PRKCB*
GO:0019637	Organophosphate metabolic process	*GPD2, LPL, ISYNA1, PLA2G2D4, PLA2G1B, PON1, GPLD1, PLA2G2C, AGPAT4, PIP4K2B*
GO:0008285	Negative regulation of cell proliferation	*DDR1, KRT5, AIMP1, CDKN2C, CD276, PAX6, ZEB1, SMARCA2, IGFBP5, F2R*
GO:0055074	Calcium ion homeostasis	*TRPC1, PLN, F2, PKD2, ATP1A2, ITPR1, KDR, F2R, PRKCB*
GO:0030334	Regulation of cell migration	*ICAM1, CXCR4, PECAM1, TEK, PAX6, IGFBP5, KDR, THY1, F2R*
GO:0055065	Metal ion homeostasis	*TRPC1, PLN, F2, PKD2, ATP1A2, ITPR1, KDR, F2R, PRKCB*
GO:0009101	Glycoprotein biosynthetic process	*ST3GAL1, ST3GAL3, HS3ST5, ST3GAL2, B3GNT5, ST3GAL5, FUT8, ST8SIA1, EXT1*
GO:0016042	Lipid catabolic process	*HSD17B11, PLCZ1, ENPP6, LPL, PLA2G2D4, PLA2G1B, PLA1A, PLA2G2C, HSD17B4*
GO:0055066	Di-, tri-valent inorganic cation homeostasis	*TRPC1, PLN, F2, PKD2, ATP1A2, ITPR1, KDR, F2R, PRKCB*
GO:0006644	Phospholipid metabolic process	*LPL, ISYNA1, PLA2G2D4, PLA2G1B, PON1, GPLD1, PLA2G2C, AGPAT4, PIP4K2B*
GO:0003006	Reproductive developmental process	*ACVR2A, BBS2, BBS4, FOXJ1, CXCR4, SRD5A1, HSD17B4, KIT, KDR*
GO:0006633	Fatty acid biosynthetic process	*LPL, PTGIS, PTGDS, PLA2G1B, LTA4H, BRCA1, MGST2, DEGS1*
GO:0048610	Reproductive cellular process	*PLCZ1, ACVR2A, BBS2, BBS4, GLRB, CXCR4, HSD17B4, KIT*
GO:0006874	Cellular calcium ion homeostasis	*TRPC1, PLN, F2, PKD2, ATP1A2, ITPR1, F2R, PRKCB*
GO:0048858	Cell projection morphogenesis	*BBS2, BBS4, CXCR4, EFNB1, PAX6, EFNA5, GFRA3, MYH10*
GO:0006875	Cellular metal ion homeostasis	*TRPC1, PLN, F2, PKD2, ATP1A2, ITPR1, F2R, PRKCB*
GO:0032101	Regulation of response to external stimulus	*TNFRSF1B, C3, AOAH, F2, CD276, NT5E, KDR, F2R*
GO:0032990	Cell part morphogenesis	*BBS2, BBS4, CXCR4, EFNB1, PAX6, EFNA5, GFRA3, MYH10*
GO:0060429	Epithelium development	*BBS4, KRT5, UPK1B, PAX6, IFT52, IPMK, KDR, FZD6*
GO:0032844	Regulation of homeostatic process	*ACVR2A, TRPC1, F2, PKD2, MYC, THY1, F2R*
GO:0016051	Carbohydrate biosynthetic process	*GPD2, ISYNA1, PPP1R3C, ATF3, CHST9, EXT1, AGL*
GO:0000226	Microtubule cytoskeleton organization	*BBS2, BBS4, TEKT1, MAP1B, TEKT2, TEKT4, BRCA1*
GO:0001654	Eye development	*BBS4, MEIS2, PAX6, ABI2, ZEB1, THY1, MYH10*
GO:0010959	Regulation of metal ion transport	*TRPC1, ADRB2, F2, PKD2, KCNA5, THY1*
GO:0043269	Regulation of ion transport	*TRPC1, ADRB2, F2, PKD2, KCNA5, THY1*
GO:0007411	Axon guidance	*CXCR4, EFNB1, PAX6, EFNA5, GFRA3, MYH10*

GO:0033559	Unsaturated fatty acid metabolic process	*PTGIS, PTGDS, LTA4H, ALOX5, HPGD, MGST2*
GO:0006690	Icosanoid metabolic process	*PTGIS, PTGDS, LTA4H, ALOX5, HPGD, MGST2*
GO:0034637	Cellular carbohydrate biosynthetic process	*GPD2, ISYNA1, PPP1R3C, ATF3, EXT1, AGL*
GO:0042445	Hormone metabolic process	*HSD17B11, ECE1, CRABP2, SCG5, SRD5A1, NR3C1*
GO:0007218	Neuropeptide signaling pathway	*CD97, GLRB, GPR110, ELTD1, SCG5, CELSR1*
GO:0034754	Cellular hormone metabolic process	*HSD17B11, ECE1, CRABP2, SRD5A1, NR3C1*
GO:0021915	Neural tube development	*BBS4, PAX6, IFT52, IPMK, FZD6*
GO:0044264	Cellular polysaccharide metabolic process	*PPP1R3C, PHKB, AOAH, EXT1, AGL*
GO:0050727	Regulation of inflammatory response	*TNFRSF1B, C3, AOAH, CD276, NT5E*
GO:0001764	Neuron migration	*CXCR4, PAX6, PRKG1, GFRA3, MYH10*
GO:0051480	Cytosolic calcium ion homeostasis	*TRPC1, F2, PKD2, ATP1A2, F2R*
GO:0030326	Embryonic limb morphogenesis	*LMBR1, ECE1, CRABP2, IFT52, LRP4*
GO:0035113	Embryonic appendage morphogenesis	*LMBR1, ECE1, CRABP2, IFT52, LRP4*
GO:0042733	Embryonic digit morphogenesis	*LMBR1, ECE1, IFT52, LRP4*
GO:0050728	Negative regulation of inflammatory response	*TNFRSF1B, AOAH, CD276, NT5E*
GO:0016358	Dendrite development	*BBS4, MAP1B, ABI2, PRKG1*
GO:0031348	Negative regulation of defense response	*TNFRSF1B, AOAH, CD276, NT5E*
GO:0009855	Determination of bilateral symmetry	*DNAH11, ACVR2A, PKD2, IFT52*
GO:0009799	Determination of symmetry	*DNAH11, ACVR2A, PKD2, IFT52*
GO:0007368	Determination of left/right symmetry	*DNAH11, ACVR2A, PKD2, IFT52*
GO:0001841	Neural tube formation	*BBS4, IFT52, IPMK, FZD6*
GO:0001838	Embryonic epithelial tube formation	*BBS4, IFT52, IPMK, FZD6*
GO:0035148	Tube lumen formation	*BBS4, IFT52, IPMK, FZD6*
GO:0032102	Negative regulation of response to external stimulus	*TNFRSF1B, AOAH, CD276, NT5E*
GO:0051924	Regulation of calcium ion transport	*TRPC1, F2, PKD2, THY1*
GO:0046456	Icosanoid biosynthetic process	*PTGIS, PTGDS, LTA4H, MGST2*
GO:0006636	Unsaturated fatty acid biosynthetic process	*PTGIS, PTGDS, LTA4H, MGST2*
GO:0015914	Phospholipid transport	*ABCG8, LOC516849, ATP11B, ATP11C*
GO:0021537	Telencephalon development	*BBS2, BBS4, PAX6, MYH10*

GO:0051281	Positive regulation of release of sequestered calcium ion into cytosol	*TRPC1, F2, THY1*
GO:0044253	Positive regulation of multicellular organismal metabolic process	*ADRB2, F2, F2R*
GO:0051279	Regulation of release of sequestered calcium ion into cytosol	*TRPC1, F2, THY1*
GO:0010524	Positive regulation of calcium ion transport into cytosol	*TRPC1, F2, THY1*
GO:0044246	Regulation of multicellular organismal metabolic process	*ADRB2, F2, F2R*
GO:0043954	Cellular component maintenance	*PARD6A, BBS2, BBS4*
GO:0006020	Inositol metabolic process	*ISYNA1, PPIP5K1, IPMK*
GO:0032846	Positive regulation of homeostatic process	*TRPC1, F2, THY1*
GO:0070169	Positive regulation of biomineral formation	*ACVR2A, ADRB2, CD276*
GO:0030501	Positive regulation of bone mineralization	*ACVR2A, ADRB2, CD276*
GO:0010522	Regulation of calcium ion transport into cytosol	*TRPC1, F2, THY1*
GO:0045778	Positive regulation of ossification	*ACVR2A, ADRB2, CD276*
GO:0051928	Positive regulation of calcium ion transport	*TRPC1, F2, THY1*
GO:0048854	Brain morphogenesis	*BBS2, BBS4, CTNNA2*
GO:0042462	Eye photoreceptor cell development	*BBS4, PAX6, THY1*
GO:0021591	Ventricular system development	*MNAT1, MYH10*
GO:0002318	Myeloid progenitor cell differentiation	*KIT, MLF1*
GO:0060295	Regulation of cilium movement involved in ciliary motility	*BBS2, BBS4*
GO:0060632	Regulation of microtubule-based movement	*BBS2, BBS4*
GO:0060296	Regulation of cilium beat frequency involved in ciliary motility	*BBS2, BBS4*
GO:0021756	Striatum development	*BBS2, BBS4*

Supplemental Table 3.3. Overrepresented KEGG pathways associated with differential expressed genes up-regulated in the isthmus.

Category	Term	Genes
bta04310	Wnt signaling pathway	*WNT5A, TBL1XR1, TCF7, PPARD, NLK, FZD5, DAAM2, TCF7L1, PORCN, PSEN1, SFRP2, CSNK1E, NFAT5, LRP6, CAMK2B, WIF1, SOX17, PLCB1, APC*

bta04510	Focal adhesion	*VAV3, PIK3CB, DIAPH1, BCAR1, MET, ITGB4, ITGB3, FLNB, SRC, PXN, CHAD, COL6A6, BCL2, MAPK3, COL6A3, PIK3R3, THBS1, PARVA*
bta04360	Axon guidance	*PLXNC1, LIMK2, MET, NTN1, EPHB4, EPHB2, SEMA5A, SEMA6A, SEMA4G, UNC5B, ROBO1, MAPK3, SEMA3D, NFAT5, ROBO2, SEMA4D, EFNA4*
bta04520	Adherens junction	*PTPRB, FGFR1, TCF7, PTPRM, PTPRF, NLK, MET, CDH1, TCF7L1, SRC, ACVR1C, TJP1, SORBS1, MAPK3, MLLT4*
bta04670	Leukocyte transendothelial migration	*CLDN8, OCLN, VAV3, CLDN4, CLDN3, PIK3CB, BCAR1, SIPA1, CLDN10, CLDN23, PXN, EZR, PLCG1, PIK3R3, MLLT4*
bta04910	Insulin signaling pathway	*PTPRF, PIK3CB, PRKAG2, MKNK1, PCK2, PPARGC1A, CBLB, SORBS1, GCK, MAPK3, ARAF, FASN, PIK3R3, PYGB*
bta05210	Colorectal cancer	*TCF7, CASP3, PIK3CB, BCL2, ARAF, MET, MAPK3, PIK3R3, FZD5, TCF7L1, APC, ACVR1C*
bta04666	Fc gamma R-mediated phagocytosis	*PLD2, MYO10, PLD1, VAV3, LYN, LIMK2, PLCG1, GSN, PPAP2C, PIK3CB, MAPK3, PIK3R3*
bta05215	Prostate cancer	*FGFR2, FGFR1, TCF7, PIK3CB, BCL2, ARAF, MAPK3, CREB3L1, CREB3L4, PIK3R3, TCF7L1*
bta04916	Melanogenesis	*WNT5A, TCF7, ADCY7, ADCY6, MAPK3, CREB3L1, CAMK2B, CREB3L4, FZD5, PLCB1, TCF7L1*
bta03320	PPAR signaling pathway	*PPARA, PPARD, SORBS1, CYP27A1, FADS2, PCK2, SCP2, SLC27A2, ACSL3, ACOX3*
bta04370	VEGF signaling pathway	*PLA2G10, PLCG1, PIK3CB, MAPK3, NFAT5, HSPB1, MAPKAPK2, PIK3R3, SRC, PXN*
bta04914	Progesterone-mediated oocyte maturation	*CCNB1, RPS6KA1, ADCY7, PIK3CB, ARAF, ADCY6, MAPK3, ANAPC4, LOC512293, PIK3R3, CDC25A*
bta00520	Amino sugar and nucleotide sugar metabolism	*PGM2, CYB5R3, AMDHD2, LOC537017, GCK, GFPT2, NPL, GALE, TSTA3*
bta04730	Long-term depression	*NOS1, LYN, PLA2G10, ARAF, MAPK3, GUCY1A3, GUCY1B3, PLCB1, CACNA1A*
bta04070	Phosphatidylinositol signaling system	*INPP1, PLCG1, PIK3CB, INPPL1, SYNJ1, PIP4K2A, PIK3R3, PLCB1, INPP5A*
bta05213	Endometrial cancer	*TCF7, PIK3CB, ARAF, MAPK3, CDH1, PIK3R3, TCF7L1, APC*
bta00330	Arginine and proline metabolism	*SAT1, GOT2, NOS1, ALDH18A1, ASS1, GLUD1, LOC534520, ALDH3A2*
bta00562	Inositol phosphate metabolism	*INPP1, PLCG1, PIK3CB, INPPL1, SYNJ1, PIP4K2A, PLCB1, INPP5A*
bta00100	Steroid biosynthesis	*TM7SF2, CEL, CYP51A1, SQLE, NSDHL, FDFT1*
bta00052	Galactose metabolism	*PGM2, PFKL, GCK, AKR1B1, GAA, GALE*
bta00500	Starch and sucrose metabolism	*PGM2, ENPP1, GCK, GAA, UGT1A1, PYGB*
bta00565	Ether lipid metabolism	*PLD2, PLD1, PLA2G10, PPAP2C, ENPP2, AGPAT1*
bta00900	Terpenoid backbone biosynthesis	*HMGCR, RUSC1, IDI1, PMVK*

Supplemental Table 3.4. Overrepresented KEGG pathways associated with differential expressed genes up-regulated in the ampulla.

Category	Term	Genes
bta04010	MAPK signaling pathway	*MEF2C, FGFR4, TGFBR2, CACNB2, FGF10, HSPA1A, MAPK10, GNG12, CACNA2D3, TAB2, PRKCB, RPS6KA5, RAC2, DUSP14, PLA2G2D4, MAP3K8, NTRK2, PLA2G1B, CACNA1H, PLA2G2C, MYC, RASA2*
bta04144	Endocytosis	*PARD6A, FGFR4, CBL, TGFBR2, ASAP1, EEA1, HSPA1A, KIT, LDLRAP1, KDR, ADRB2, AP2B1, CXCR4, EHD2, EHD4, PIP4K2B, F2R*
bta04020	Calcium signaling pathway	*PLCZ1, TRPC1, PHKB, ADCY8, CAMK2G, TRHR, ITPR1, PRKCB, ADRB2, PLCE1, P2RX6, PLN, CACNA1H, F2R, HTR2A*
bta04360	Axon guidance	*EFNB1, DPYSL5, SLIT2, EPHA2, EPHB6, RND1, EPHA6, RAC2, CXCR4, SRGAP3, EFNA5, UNC5C, SRGAP1*
bta04610	Complement and coagulation cascades	*PLAT, MASP1, C3, F2, SERPINA1, SERPING1, PROS1, PLAU, F2R*
bta00590	Arachidonic acid metabolism	*PTGIS, PTGDS, PLA2G2D4, PLA2G1B, PLA2G2C, CYP4F3, LTA4H, ALOX5*
bta00603	Glycosphingolipid biosynthesis	*ST3GAL1, ST3GAL2, NAGA, ST8SIA1, FUT1*
bta00533	Keratan sulfate biosynthesis	*ST3GAL1, ST3GAL3, ST3GAL2, FUT8, B4GALT4*
bta00601	Glycosphingolipid biosynthesis	*ST3GAL3, B3GNT5, ST8SIA1, FUT1, B4GALT4*
bta00512	O-Glycan biosynthesis	*ST3GAL1, GALNT3, GCNT4, ST3GAL2, GALNT6*
bta00604	Glycosphingolipid biosynthesis	*ST3GAL1, ST3GAL2, ST3GAL5, ST8SIA1*

Chapter 3

Effect of hCG administration during *corpus luteum* establishment on subsequent *corpus luteum* development and circulating progesterone concentrations in beef heifers

ABSTRACT

This study examined the effect of a single administration of human chorionic gonadotrophin (hCG) on Day 1 to Day 4 after oestrus on *corpus luteum* (CL) development and circulating progesterone (P4). Oestrus-synchronized heifers (n=43) were administered a single intramuscular injection of saline on Day 1 (control) or 3000 IU hCG on Day 1, 2, 3, or 4 after oestrus. Administration of hCG on Day 1 had no effect on CL area, on Day 2 increased CL area from Day 6 to 12 ($P<0.05$), on Day 3 increased CL area from Day 9 to 11, while on Day 4 increased CL size on Days 9 and 10 ($P<0.05$). Administration of hCG on Day 4 induced the formation of accessory CL in 89% of heifers, resulting in a significant increase in total luteal tissue area on the ovaries compared with all other groups. Consistent with the effects on the CL, hCG on Day 1 did not affect P4 concentrations, on Day 2 significantly increased P4 compared with the control from Day 6 to 11 ($P<0.05$), on Day 3 resulted in a non-significant increase in P4 while hCG on Day 4 increased P4 from Day 8 to 13 compared with the control ($P<0.05$). In conclusion administration of hCG as early as Day 2 after oestrus results in increased P4 in circulation from Day 6, which should have beneficial downstream effects in terms of uterine receptivity and conceptus elongation.

INTRODUCTION

Increased concentrations of circulating progesterone (P4) in the first week after conception are associated with altered gene expression in the uterine endometrium (Forde *et al.,* 2009a), larger conceptuses (Garrett *et al.,* 1988b; Satterfield *et al.,* 2006; Carter *et al.,* 2008), increased interferon-tau (IFNT) production (Mann and Lamming 1999; 2001), and greater pregnancy rate in cattle and sheep (Ashworth *et al.,* 1989; Stronge *et al.,* 2005; McNeill *et al.,* 2006).

The administration of human chorionic gonadotrophin (hCG) during the early luteal phase has been used to induce the ovulation of the first wave dominant follicle and subsequent formation of a functional accessory CL, in turn leading to increased circulating concentrations of P4 [for review see De Rensis *et al.,* (2010); Lonergan (2011)]. Increased concentrations of P4 have been reported in lactating dairy cows (Santos *et al.,* 2001; Hanlon *et al.,* 2005; Stevenson *et al.,* 2007; Vasconcelos *et al.,* 2011; Nascimento *et al.,* 2013b), dairy heifers (Diaz *et al.,* 1998; Chagas e Silva and Lopes da Costa 2005), beef cows (Nishigai *et al.,* 2002) and beef heifers (Funston *et al.,* 2005). Furthermore, an association with larger conceptuses and increased IFNT secretion has been demonstrated (Kerbler *et al.,* 1997; Rizos *et al.,* 2012), suggesting positive benefits for the developing embryo, presumably mediated through changes in endometrial gene expression (Forde *et al.,* 2009a).

Reported effects of hCG on pregnancy rate have been variable (Lonergan 2011; Wiltbank *et al.,* 2011), although those studies that used large numbers of animals have generally reported a modest improvement, irrespective of whether artificial insemination (AI) (Santos *et al.,* 2001; Stevenson *et al.,* 2007) or embryo transfer [ET; Nishigai *et al.,* (2002); Wallace *et al.,* (2011)] was used. However, such positive effects on pregnancy rate are not equivocal, with others failing to demonstrate an effect of hCG administration on pregnancy rate (Galvão *et al.,* 2006). Indeed, in several studies, the improvement in pregnancy rate was more apparent after ET compared to AI (Chagas e Silva and Lopes da Costa 2005; Vasconcelos *et al.,* 2011). Recently, using a model of compromised embryos, Torres *et al.,* (2013) reported increased pregnancy rate after hCG administration in lactating dairy cow recipients following transfer of demi-embryos.

hCG has activity similar to LH, binds to LH receptors and mimics the effects of LH by stimulating small luteal cells to increase production of P4 (Niswender *et al.,* 1985a; 2000). Administration of hCG on Day 5-7 does not impact on circulating P4 for several days, partly due to the time required for ovulation and accessory CL formation (Rizos *et al.,* 2012). However, given its luteotrophic nature, hCG also appears to stimulate development of the original CL, giving rise to a double effect on P4 concentrations. Given that an early rise in P4 has been associated with improved conceptus development (Carter *et al.,* 2008; Forde *et al.,* 2011c), the aim of this study was to examine the effect of early administration of hCG (on Day 1, 2, 3 or 4 after oestrus) on CL development and function in terms of P4 secretion in beef heifers.

MATERIALS AND METHODS

Animals and treatments

All experimental procedures involving animals were licensed by the Department of Health and Children, Ireland. Protocols were in accordance with the Cruelty to Animals Act (Ireland 1876) and the European Community Directive 86/609/EC and were sanctioned by the Institutional Animal Research Ethics Committee.

For the duration of the experiment, all animals were housed indoors on a slatted floor and were fed a diet consisting of grass and maize silage supplemented with a standard beef ration. The oestrous cycles of cross-bred beef heifers [n = 50, predominantly Charolais and Limousin cross; 22.6 ± 0.32 months old (mean ± SEM); weight at start of experiment 583 ± 6 kg (mean ± SEM)] were synchronized using a 7-day controlled internal drug release (CIDR 1.38 g; Pfizer, Sandwich, UK) insert with administration of 15 mg of a prostaglandin $F_{2\alpha}$ analogue (Prosolvin; Intervet, Dublin, Ireland) given on the day before CIDR removal. Heifers were observed for signs of oestrus four times per day commencing 30 h after CIDR withdrawal and only those recorded in standing oestrus (Day 0; n=43) were used. Heifers were assigned randomly to one of five treatments and administered a single intramuscular injection of 3000 IU hCG (5 mL Chorulon; Intervet) either (1) 24 h after oestrus onset (n= 8), (2) 48 h after oestrus onset (n=9), (3) 72 h after oestrus onset (n=9), (4) 96 h after oestrus onset (n=9) or (5) 5 mL of the saline diluent provided with the product at 24 h after oestrus onset (n=8, control group). Daily blood samples were collected from each heifer from Day 0 to Day 14 by coccygeal venipuncture to measure serum concentration of P4. The dimensions of the original CL and induced CL, when present, were measured by daily ultrasound scanning beginning on Day 3 after oestrus.

Ultrasonography of the CL

Daily transrectal ultrasonography commenced on Day 3 (where Day 0 = oestrus). Ultrasound examination of the ovaries was performed using an ALOKA SSD-900V (Aloka Co., LTD, Tokyo, Japan) equipped with a 60 mm linear reproductive transducer emitting a frequency of 7.5 MHz. All ultrasound examinations were performed by the same operator. Different images were taken to measure the maximum diameter of the CL and the CL cavity, if present. The horizontal and vertical diameters were recorded, respectively, and the average was used in the analysis. All the measurements were made by the same operator.

Progesterone measurement

Following collection, blood samples were refrigerated (4 ºC) for 12-24 h before being centrifuged at 1500 g for 20 min at 4 ºC. Serum was separated and stored at -20 ºC until it was assayed to determine P4 concentrations by solid phase radioimmunoassay using a Coat-A-Count Progesterone kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA), as described previously (Forde *et al.,* 2011c). The sensitivity of the assay was 0.03 ng/ml. The inter- and intraassay CV were 10.14 and 10.56%

respectively, for the low P4 standards, 5.85 and 5.93% respectively, for the medium P4 standards and 4.67 and 4.70%, respectively, for the high P4 standards.

Statistical Analysis

Data were checked for normality and homogeneity of variance using histograms, qplots, and formal statistical tests in the UNIVARIATE procedure (SAS Version 9.1.3, 2006; SAS Institute, Cary, NC, USA). Data that were not normally distributed were transformed by raising the variable to the power of lambda. The appropriate lambda value was obtained by conducting a Box-Cox transformation analysis using the TRANSREG procedure of SAS. The transformed data were used to calculate *P*-values. The corresponding least squares means and SEM of the non-transformed data are presented in the results for clarity. Variables having more than one observation such as the P4 metabolic concentrations and ovarian ultrasonic measures were analyzed in groups using a repeated measures analysis with the MIXED procedure of SAS. Fixed effects included experimental treatments, day, and their interaction. The interaction term if not statistically significant ($P>0.10$) was subsequently excluded from the final model. Animal within treatment was included as a random effect in the model with the most appropriate covariance structure between records within animal determined by minimizing the Akaike Information Criterion (AIC). Models were run under compound symmetry, unstructured, autoregressive, or Toeplitz variance-covariance structures. Differences between treatments were determined by *F*-tests using Type III sums of squares. The PDIFF command incorporating the Tukey test was applied to evaluate pair wise comparisons between treatment means. Change in progesterone concentrations during the test period (between Days 2 and 7) for each animal was computed as the coefficient of the linear regression of measurements upon time (day) using the REG procedure of SAS. The linear trapezoidal equation was used to estimate the area under the P4 time curve (AUC) of the function $f(x)$ by calculating the total area of adjacent trapezoid shapes.

RESULTS

Four animals were removed from the study, two because they failed to ovulate (control group) and two because they had a double ovulation (not induced by hCG treatment; one from hCG Day 2 and one from hCG Day 3 groups).

CL development

The effect of hCG administration on Day 1, 2, 3 or 4 on CL development from Day 0 to Day 14 is shown in Figure 1. Compared to the control treatment, administration of hCG on Day 1 had no effect on CL area, administration on Day 2 increased CL area from Day 6 to 12 ($P<0.03$), hCG on Day 3 increased CL area from Day 9 to 11 ($P<0.05$), whilst administration on Day 4 increased CL from Day 9 and 10 ($P<0.03$). Additionally, hCG on Day 4 induced the formation of an accessory CL in 89% of heifers, resulting in a significant increase in total luteal tissue area on the ovaries compared to all other groups (different from the control from Day 9 to 14, $P<0.001$; Figure 1).

P4 Concentration

Consistent with the effects on the CL, administration of hCG on Day 2 significantly increased circulating P4 concentration compared to the control from Day 6 to 11 ($P<0.05$), while hCG on Day 4 increased P4 from Day 8 to 13 ($P<0.05$). hCG administration on Day 1 or 3 resulted in a non-significant ($P>0.10$) increase in P4 relative to the control treatment (Figure 2).

The daily change in P4 from Day 2 to Day 7 was assessed using regression analysis. Compared to the control, there was no difference ($P>1.0$) in the rate of change of P4 concentrations between Days 2 to 7 following hCG administration on Day 1, Day 3 or Day 4. However, when administered on Day 2 the rate of change of P4 between Days 2 to 7 was significantly increased ($P=0.02$) following hCG administration compared to the control (0.185±0.007 vs. 0.153±0.009 ng ml^{-1} day^{-1}). Furthermore, mean serum AUC for P4 over the entire period (Day 0 to Day 14) was greatest ($P<0.05$) following hCG administration on Day 2 and no differences were observed between the remaining experimental treatments (Figure 3).

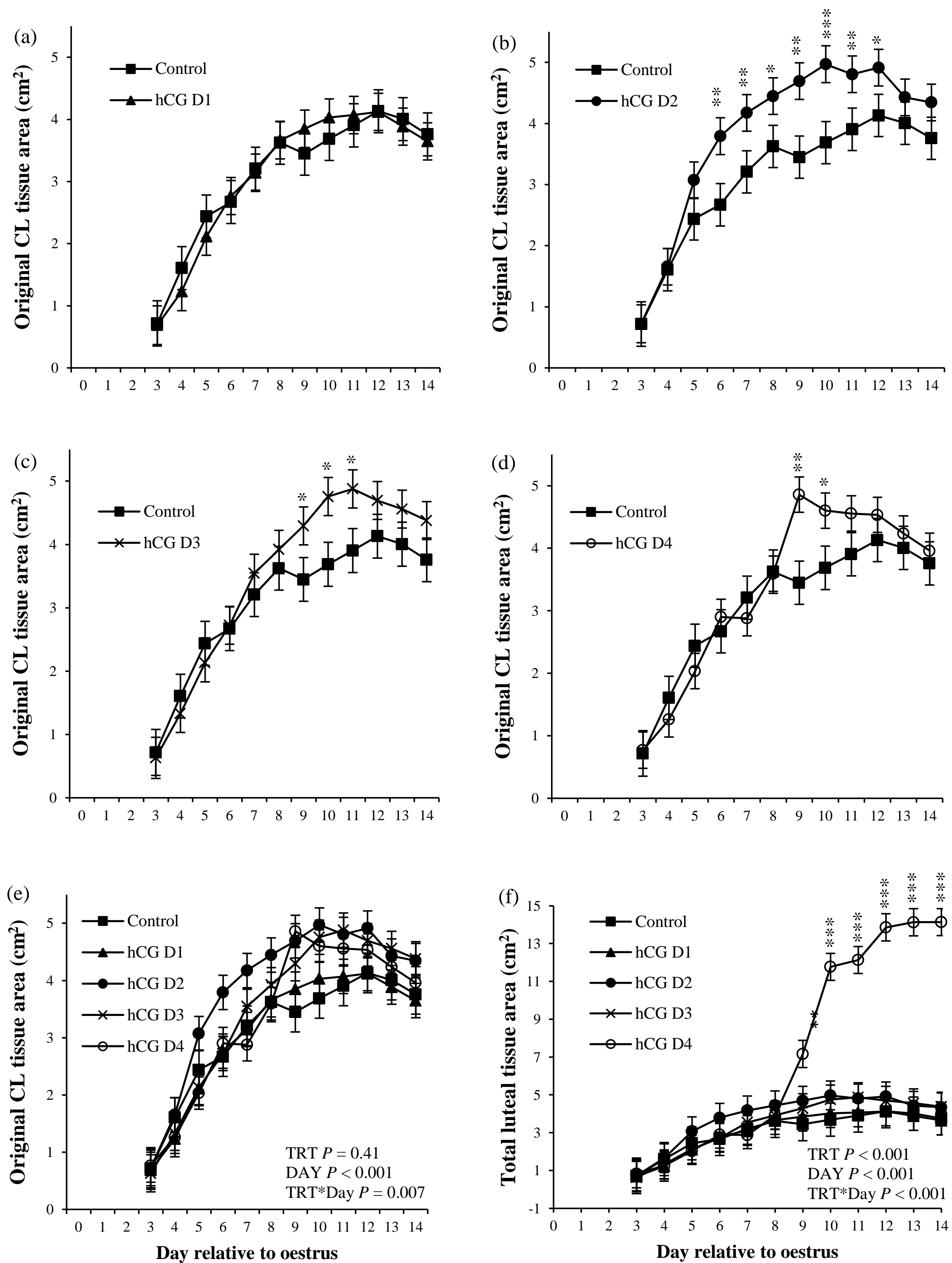

Figure 1. Luteal tissue area of the original corpus luteum (CL, cm^2) from heifers treated with saline (control group n=6) or with 3000 IU of human chorionic gonadotrophin (hCG) on (*a*) Day 1 (n=8), (*b*) Day 2 (n=8), (*c*) Day 3 (n=8) or (*d*) Day 4 (n=9). (*e*) All groups together. (*f*) Total luteal tissue area (cm^2), including accessory CLs, when present, when hCG was injected on Day 4. No accessory CLs were formed following administration of hCG on Days 1, 2 or 3.

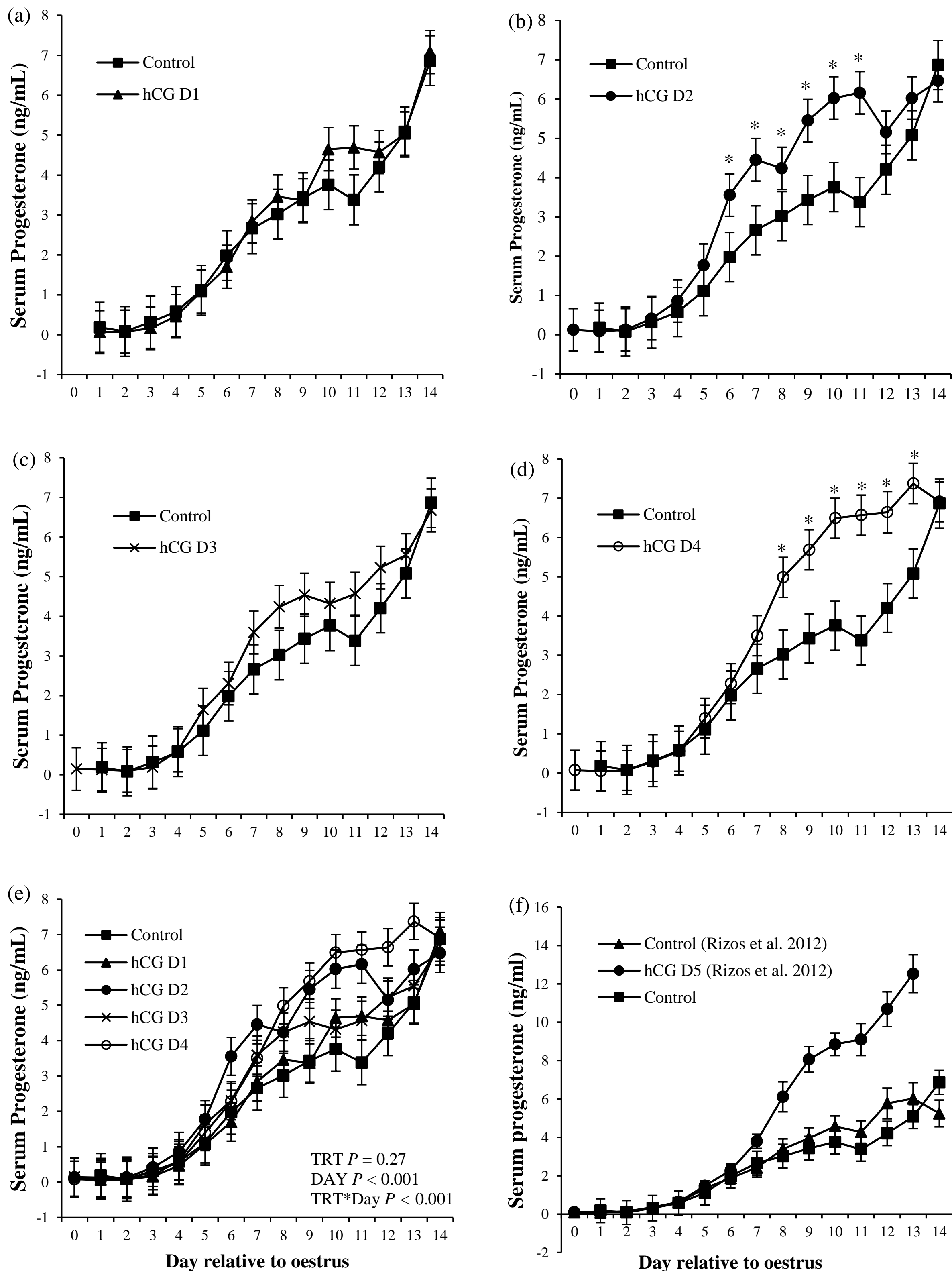

Figure 2. Serum progesterone profiles for heifers treated with saline (control group; n=6) or with 3000 IU human chorionic gonadotrophin (hCG) on (*a*) Day 1 (n=8), (*b*) Day 2 (n=8), (*c*) Day 3 (n=8) or (*d*) Day 4 (n=9). (*e*) All groups together. (*f*) Comparison with data from (Rizos *et al.* 2012) when hCG was injected on Day 5.

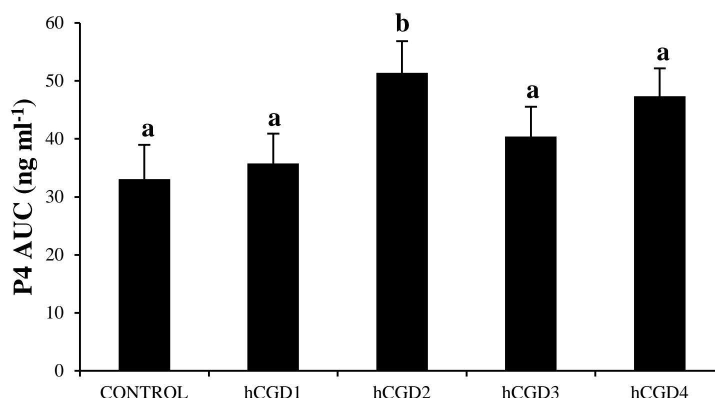

Figure 3. Mean serum progesterone area under the curve (AUC) for the entire period (Day 0 to 14) ($P<0.05$).

DISCUSSION

The main findings of the present study are that a single intramuscular administration of hCG (3000 IU/animal) to beef heifers in the early metoestrus period (Day 2-4) results in an increase in CL size and an associated increase in P4. Of particular interest, administration on Day 2 resulted in a significant deviation in circulating P4 from Day 6 onward, which, based on previous studies from our group (Carter *et al.,* 2008; Forde *et al.,* 2009a), would be beneficial for conceptus elongation.

It has been well demonstrated that administration of hCG on Day 5 after oestrus induces ovulation of the dominant follicle present resulting in the formation of an accessory CL and an eventual increase in circulating P4 concentrations [(for review see De Rensis *et al.,* (2010); Lonergan (2011)]. It has been suggested that the increase in P4 after hCG administration is due to the P4 produced by the induced CL. In the study of Schmitt *et al.,* (1996a), for example, plasma P4 did not differ between control and hCG groups after removal of the accessory CL on Day 13. However, other evidence indicates that the increased P4 cannot be entirely attributed the induced CL. For example, administration of hCG leads to an increase in the area of luteal tissue in the original CL in addition to the increase in total luteal tissue associated with the presence of the accessory CL (Rizos *et al.,* 2012). Such a hypertrophic effect has been reported previously (Farin *et al.,* 1988; Stevenson *et al.,* 2007). In support of this, despite the fact that the accessory CL was not apparent by ultrasonography until Day 9, a significant increase in serum P4 concentrations occurred from Day 7, suggesting increased output from the original CL (Rizos *et al.,* 2012), in agreement with the observations of Kerbler *et al.,* (1997).

The results of the current study provide evidence for a positive effect of hCG on the original CL as, in the absence of accessory CL formation (due to the lack of a dominant follicle capable of ovulating on Day 1 to 3), hCG administration on Day 2 or Day 3 increased original CL size and circulating P4. Consistent with this, administration of hCG on Day 5 resulted in a 46% increase in the weight of the original CL on Day 17 (5.63 vs. 8.22 g) (Schmitt *et al.,* 1996a). Furthermore, Beindorff *et al.,* (2009)

demonstrated increased P4 production by the native CL by aspirating all follicles >5 mm on Day 6, before hCG administration on Day 7. However, whether any luteal tissue formed after follicle aspiration was not recorded in that study and may have been a contributory factor to elevated P4, as aspirated dominant follicles do form P4-producing luteal structures (O'Hara *et al.,* 2012).

Pharmacokinetic studies of hCG indicate that it persists in circulation for a relatively long time and would be capable of stimulating CL proliferation over several days. Schmitt *et al.,* (1996b) reported that plasma concentrations of hCG were increased markedly for 30 h after administration of hCG and had not returned to baseline concentrations 66 h after treatment (Schmitt *et al.,* 1996b). In goats, plasma hCG profile after injection was characterized by rapid absorption (peak concentration reached at 11.6 h after administration) and slow elimination [70.0 h; Saleh *et al.,* (2012)]. This slow clearance of hCG is in broad agreement with studies in humans (Chan *et al.,* 2003). The prolonged activity and low clearance rate of hCG, in comparison to endogenous LH, are due to the high glycosylation rate (Cole 2010).

As reviewed by Niswender *et al.,* (2000), circulating concentrations of P4 are dependent on the amount of steroidogenic tissue, blood flow, and capacity of the steroidogenic tissue to synthesize progesterone. The amount of steroidogenic tissue is, in turn, dependent on number and size, of steroidogenic luteal cells, both of which increase during luteal development. Blood flow to the CL also increases as concentrations of progesterone in serum increase (Niswender *et al.,* 2000). The association between luteal blood flow and P4 production is interesting. Beindorff *et al.,* (2009) investigated the nature of the direct hCG effect on the original CL and it P4 synthesis. Intravenous administration of 3000 IU hCG on Day 7 after oestrus to nonlactating cows resulted in a transient 51% increase in luteal blood flow 1 h after administration and an increase in plasma P4 concentrations by 30% at 1 h, 15% at 12 h, 34% at 24 h and 81% at 48 h after administration (Beindorff *et al.,* 2009). hCG provoked an immediate and long-term increase in P4 but only a temporary elevation in luteal blood flow.

In the present study, administration of hCG on Day 1 had no effect on CL area or circulating P4 concentrations. This is perhaps not surprising given the fact that at this stage the CL is still forming. Administration on Day 2 increased CL area from Day 6 to 12 and was associated with increased P4 concentration compared to the control from Day 6 to 11. Surprisingly, while hCG on Day 3 increased CL area from Day 9 to 11, the increase in P4, although consistent, was not significantly greater than the control. Administration on Day 4 increased CL size on Days 9 and 10 and induced the formation of an accessory CL in ~90% of heifers, resulting in a significant increase in total luteal tissue area on the ovaries compared to all other groups; this was associated with increased P4 from Day 8 to 13 compared to the control.

We have previously demonstrated that P4 supplementation from Day 3 using an intravaginal P4-releasing device results in advanced conceptus elongation on Day 14-16 after oestrus (Clemente *et al.,* 2009; Forde *et al.,* 2011c). This is consistent with older publications that used P4 injections from Day 1-4 [e.g., Garrett *et al.,* (1988b)]. More recently, we have shown that short-term P4 supplementation, for as little as two days, in the early metoestrus period is sufficient to increase peripheral P4, increase conceptus

size and increase IFNT secretion, irrespective of whether ET or AI was used to establish pregnancy (O'Hara *et al.,* 2014). While such P4 supplementation treatments undoubtedly advance conceptus elongation, there is convincing evidence in the literature that administration of P4 early in the cycle may compromise CL function, ultimately leading to luteolysis and embryo loss (Ginther 1970; Garrett *et al.,* 1988a; Macmillan and Peterson 1993; Burke *et al.,* 1994; Pope *et al.,* 1995; Van Cleeff *et al.,* 1996). Strategies aimed at augmenting the endogenous supply of P4 through stimulation of the endogenous CL such as manipulation of preovulatory follicle development (Wiltbank *et al.,* 2011) or administration of luteotrophic agents such as hCG (De Rensis *et al.,* 2010; Lonergan 2011; Rizos *et al.,* 2012) rather than supplementation with exogenous P4 may be most effective in improving pregnancy rate.

In conclusion, hCG administration on Day 2 after oestrus leads to an increase in the luteal tissue area of the CL and an associated increase in circulating P4 concentration from Day 6 onwards which may be beneficial for early embryo development.

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General Discussion

Metabolic profile in lactating and nonlactating dairy cows from Day -14 to Day 95 postpartum

It is well known that after calving the metabolic profile of lactating dairy cows is altered. It is also clear the importance to characterize the metabolic profile between lactating and nonlactating cows in the early postpartum period; data not available in the literature when our study was in progress. In the first chapter, it was found that, in lactating cows, plasma levels of glucose, IGF-I and insulin were lower while NEFA and BHBA were higher compared to nonlactating cows. The changes in concentrations of these metabolites in lactating cows, from prepartum through the early postpartum period was in accordance with the results obtained in other studies where postpartum lactating dairy cows and/or dairy heifers were used (Paterson and Linzell 1974; Grummer *et al.,* 1995; McGuire *et al.,* 1995; Kobayashi *et al.,* 1999; Leroy *et al.,* 2004; Butler *et al.,* 2006; Bender *et al.,* 2010; Matoba *et al.,* 2012). Nevertheless, to study the specific effect of lactation on metabolic profile, heifers are not the most appropriate group to compare with because assuming that they are properly fed, they should not be under NEB. Thus, it is important to mention that our results comparing lactating with nonlactating cows after calving were one of the first available in the literature and they were in agreement with the other two studies, which came available at the same time (Green *et al.,* 2012; Thompson *et al.,* 2012). The differences observed in all metabolites between lactating and nonlactating cows, above all after calving, reinforce the fact that lactation alters metabolism.

Ability of the reproductive tract of postpartum dairy cows to support embryo development

Bearing in mind that after calving the metabolic profile between lactating and nonlactating cows varied, through two different experiments described in the first chapter, it was tested if this metabolic situation may be affecting: (1) the ability of the oviduct and the uterus to support early embryo development and (2) the capacity of the uterus to sustain embryo elongation. In these studies, to isolate the effect of the reproductive tract on embryo development, embryos produced *in vitro* were transferred into the oviduct or the uterus. Thus, fresh *in vitro* produced embryos were used due to higher pregnancy rate obtained compared to frozen-thawed IVP embryos (Putney *et al.,* 1989; Ambrose *et al.,* 1999; Al-Katanani *et al.,* 2002; Vasconcelos *et al.,* 2006; Demetrio *et al.,* 2007). In addition, a previously validated multiple embryo transfer model (Clemente *et al.,* 2009; Carter *et al.,* 2010; Forde *et al.,* 2011a), was used to avoid the potentially confounding factors associated with the endogenous oocyte.

The optimal productivity on a farm is to have one calf every 365 days. To achieve this objective, farmers should start to breed the animals around Day 60 to have the animal pregnant by Day 90. The antagonistic relationship between milk yield and fertility has made meeting this objective challenging. For this reason, the first experiment was carried out around Day 60 postpartum. By this time the uterus should be completely involuted following the previous pregnancy (Gier and Marion 1968; Royal *et al.,* 2000a; Scully *et al.,* 2013) and the estrous cyclicity reestablished (Opsomer *et al.,* 2000). To examine the ability of the postpartum reproductive tract of the dairy cow to support embryo development in the period encompassing the events between fertilization and Day 7, a multiple embryo transfer of *in vitro* produced

2- to 4-cell embryos was performed on Day 2 in the ipsilateral oviduct of lactating and nonlactating cows. The embryo transfer was done by endoscopy (Besenfelder and Brem 1998), a technique that has been used previously in bovine (Carter *et al.,* 2010; Havlicek *et al.,* 2010; Rizos *et al.,* 2010a; Kuzmany *et al.,* 2011a; Kuzmany *et al.,* 2011b) and other species including rabbits (Besenfelder and Brem 1993), pigs (Besenfelder *et al.,* 1997) and goats (Besenfelder *et al.,* 1994) with successful results in terms of embryo recovery after flushing and live offspring. Most of the studies carried out in bovine have been done in heifers with recovery rates at Day 7 that range from 65 to 81% (Carter *et al.,* 2010; Havlicek *et al.,* 2010; Kuzmany *et al.,* 2011a; Kuzmany *et al.,* 2011b), similar to those found in the present study for both groups (65.6% and 63.9% for lactating and nonlactating, respectively).

The ability of the reproductive tract to support embryo development was measured by the ability of the 2- to 4-cell transferred embryos to reach the blastocysts stage. Thus, the percentage of blastocysts recovered from lactating cows (26.3%) was significantly lower than in nonlactating cows (39.6%), which is in agreement with the results of a previous study from our group in lactating cows (Rizos *et al.,* 2010a). On the other hand, the number of blastocysts in the nonlactating group (39.6%) was higher compared to the majority of studies done with heifers (from 16.1 to 29.5%) (Havlicek *et al.,* 2010; Rizos *et al.,* 2010a; Kuzmany *et al.,* 2011a; Kuzmany *et al.,* 2011b) and lower than those found by Tesfaye *et al.,* (2007)(51.7%) and Carter *et al.,* (2010)(51.3%).

Using this model, where the only difference between both groups is lactating or not, embryo development is independent of the confounding factors potentially associated with the endogenous oocyte and must be a consequence of the ability of the reproductive tract to support development, considering that all transferred embryos were produced *in vitro* under the same conditions. It has been demonstrated that the effects of NEB, as seen in alterations of systemic concentrations of metabolites, is reflected in the FF (Cohick *et al.,* 1996; Landau *et al.,* 2000; Leroy *et al.,* 2004); thus, it is likely that such alterations are also reflected in the environments of the oviduct and the uterus. Apart from the negative effects of NEFA and BHBA on the oocyte, embryo development and quality *in vitro* (Leroy *et al.,* 2005c; Leroy *et al.,* 2006; Van Hoeck *et al.,* 2011), insulin, glucose or IGF-I concentrations at this time may also be crucial for the embryo. Amongst all the metabolites whose concentrations were different during the postpartum period, IGF-I was the only one with a big difference for the entire duration of the study between lactating and nonlactating cows. The genes for IGF-I, IGF-II and the receptor of IGF-I have been detected in the oviduct (Pushpakumara *et al.,* 2002) suggesting a direct or indirect effect on embryo development or transport. Besides, systemic low levels of IGF-I have been correlated with failure to reach ovulatory size and to produce sufficient estradiol to trigger ovulation (Taylor *et al.,* 2004). In the endometrium, the bioavailability of IGF-I and insulin was altered in severe NEB and could be related to a delay in the endometrial repair process (Wathes *et al.,* 2011). In relation to glucose, the requirements of this monosaccharide do not increase until morula to expanded blastocyst stage (Tiffin *et al.,* 1991; Rieger *et al.,* 1992a) but at the moment of EGA there is a slight but significant rise in glucose requirements (Rieger *et al.,* 1992a). Therefore, it can be concluded that when the synchronization is started on Day 60 postpartum, NEB is associated with impairment in the ability of the reproductive tract of lactating cows to support early embryo development to the blastocyst stage.

In the second experiment (Chapter 1) the aim was to examine the ability of the reproductive tract of the dairy cow to support conceptus elongation in the period encompassing the events between Day 7 and Day 14. For that purpose, a multiple embryo transfer of *in vitro* produced blastocysts was performed on Day 7 in the ipsilateral uterine horn of lactating and nonlactating cows on approximately Day 90 postpartum. No differences in either recovery rate or size of recovered Day 14 conceptuses were found between groups. In this experiment, higher plasma levels of P4 were observed in lactating animals associated with a bigger *corpus luteum* (CL); however, somewhat surprisingly conceptus elongation was not affected as has been reported in other studies were high levels of P4 have been related with longer embryos (Carter *et al.,* 2008; Clemente *et al.,* 2009; Forde *et al.,* 2009a; Forde *et al.,* 2009b) as well as with bigger CL (Farin *et al.,* 1988; Galvão *et al.,* 2006; Stevenson *et al.,* 2007; Rizos *et al.,* 2012). To summarise, by Day 90 the postpartum dairy cows have overcome the adverse effects of NEB represented by the same capacity of lactating and nonlactating cows to sustain conceptus elongation.

Transcriptome response of the oviduct to the presence of an early embryo

The second chapter of this thesis was developed based on: (1) the results of the first chapter where it was found that in postpartum dairy cows the capacity of the oviduct and the uterus to support early embryo development was reduced and (2) the existing amount of evidence for the interaction between the endometrium and the conceptus and the need to examine the communication between the early embryo and the oviduct. Moreover, understanding the molecular signals between the embryo and the reproductive tract may help on the one hand strategies to improve pregnancy rate in dairy cattle and on the other hand to enhance *in vitro* embryo production.

In this chapter it was found that at Day 3 after oestrus the embryo or unfertilized oocyte was present in the isthmus of the ipsilateral oviduct, in pregnant and cyclic animals, respectively. Under our experimental conditions, the presence of an embryo did not affect the transcriptome of the oviduct, i.e. no differences were found when the isthmus of the ipsilateral oviduct of pregnant animals was compared with its counterpart in cyclic animals. This is in contrast to other studies where the presence of embryos altered the expression of some genes in mice, rats and pigs (Lee *et al.,* 2002; Arganaraz *et al.,* 2007; Almiñana *et al.,* 2012; Arganaraz *et al.,* 2012). However, it is important to highlight that in these multiple-ovulating species several embryos are present compared to only one in the cow. In addition, in mares it has been suggested that the embryo produces prostaglandin E2 that favours its oviductal transport to the uterus (Weber *et al.,* 1991a; Weber *et al.,* 1991b) while non-fertilized oocytes are retained in the oviduct (Van Niekerk and Gerneke 1966). The bovine embryo (~120 µm) possibly exerts an important local paracrine interaction with the maternal epithelium that in our experimental conditions, where we used about 8 cm of epithelial tissue (oviductal isthmus) to get the samples, could not be captured. Therefore, any communication at the precise point where the embryo is located should not be dismissed.

Based on the results found in mice, pigs and horses it appears that the embryo should have an effect in the oviduct. However, there is also a possibility that the embryo does not alter the oviduct transcriptome. It is important to consider that in the presence of an embryo, gene expression changes in the endometrium are not detectable until Day 15 or Day 16 (Forde *et al.,* 2011c; Bauersachs *et al.,* 2012),

the time of maternal recognition of pregnancy. It may be that the oviduct and the uterus undergo the same temporal changes in pregnant and cyclic animals until the time of maternal recognition when it becomes necessary for the embryo to signal its presence in order to block the mechansims that bring about luteolysis around Day 15-16. In addition, it is worth noting that the presence or not of certain mRNA does not mean that the protein is being synthetized at that moment. Therefore, it is appropriate to evaluate the proteome of the oviductal fluid in this model to better characterize the secretory profile, taking into consideration that the gametes may specifically alter the proteome of the oviduct (Georgiou *et al.,* 2005; Georgiou *et al.,* 2007).

Effect of the CL on the oviduct transcriptome

At Day 3 after oestrus the proximity of the oviduct to the CL did not affect the gene expression in the isthmus in either pregnant or cyclic animals, as evidenced by a lack of DEGs between ipsilateral versus contralateral oviduct. This contrasts to Bauersachs *et al.,* (2003) who found a small number of DEGs (35) between the ipsilateral and contralateral oviduct in cyclic animals. However in that study, the technique used for microarray was different than ours and also the cells were taken from the entire oviduct epithelium. Thus, the differences in the technique and the origin of the samples may explain the discrepancy between the two studies.

Different gene expression between ampulla and isthmus of the ipsilateral oviduct in pregnant animals

The comparison between the ampulla and isthmus in pregnant animals revealed 2287 DEGs ($P<0.01$), of which 1132 and 1155 were up- and down-regulated in the isthmus, respectively. This is not surprising due to the fact that distinct functional differences exist between the different regions. Thus, the ampulla is responsible for recovering the ovulated oocyte and transporting it to the ampullary-isthmic junction where fertilization will take place (Halbert *et al.,* 1989; Croxatto 2002). The isthmus is involved, first of all in the formation of the sperm reservoir necessary for the sperm to become capacitated, and after fertilization to support early embryo development through important events like EGA which is crucial for the subsequent development of the embryo (Memili and First 1999; Schultz *et al.,* 1999). Therefore, it was expected that the gene expression should be different between both parts. These differences may be due to the diverse distributions of cells in the ampulla and isthmus. Thus, in the ampulla there are more ciliated cells during oestrus and more secretory cells during dioestrus, while in the isthmus the proportion of cells are more or less the same during the cycle (Abe 1996; Areekijseree 2003). In addition, oviduct fluid from different regions of the bovine oviduct, differentially facilitate sperm binding to the oocyte and fertilization *in vitro* (Way et al. 1997). Gene ontology analysis of our results revealed that in the ampulla some of the genes from the overrepresented categories were related with cell motion, motility and migration, ciliary motility and beat frequency, consistent with the greater population of ciliated cells there facilitating the transport of the oocyte to the site of fertilization (Halbert et al. 1989). In the isthmus, genes detected were related with vesicle-mediated transport, endocytosis, exocytosis, cell cycle and apoptosis, likely involved in the provision of an optimal environment to support early embryo development.

Effect of hCG on Days 1, 2, 3 or 4 after oestrus on CL size and circulating P4

The third chapter of this thesis was developed based on previous studies from our group and others, in which it was shown that hCG has a hypertrophic effect on the original CL (Farin *et al.,* 1988; Galvão *et al.,* 2006; Stevenson *et al.,* 2007; Rizos *et al.,* 2012) that in turn was associated with an increase in conceptus size (Rizos *et al.,* 2012). In our study, treatment with hCG on Day 2 after oestrus, was accompanied by an increase in the luteal tissue area and P4 concentration, confirming its hypertrophic effect on the original CL. When hCG was used on Day 4 there was a dramatic increase in the luteal tissue area and P4, mainly because most heifers had an accessory CL as seen in other studies (Breuel *et al.,* 1989; Stevenson *et al.,* 2007). Administration of hCG on Day 1 did not have any effect on the CL, likely because the CL is not fully formed by this time.

High concentrations of P4 have been associated with conceptus elongation (Carter *et al.,* 2008; Clemente *et al.,* 2009; Forde *et al.,* 2009a; Forde *et al.,* 2009b) and sometimes with better pregnancy rate (Breuel *et al.,* 1989; Sianangama and Rajamahendran 1992; Santos *et al.,* 2001; Nishigai *et al.,* 2002; Chagas e Silva and Lopes da Costa 2005; Stevenson *et al.,* 2007; Shabankareh *et al.,* 2010; Dahlen *et al.,* 2011; Rossetti *et al.,* 2011; Vasconcelos *et al.,* 2011; Wallace *et al.,* 2011; Torres *et al.,* 2013). In particular, a high correlation between P4 concentration on Day 5 and 6 and conceptus size on Day 16 has been demonstrated (Beltman *et al.,* 2009). The use of an intravaginal P4-releasing device from Day 3 after oestrus (which achieves an immediate increase in P4) has been shown to advance conceptus elongation on Days 14-16 (Clemente *et al.,* 2009; Forde *et al.,* 2011c). However, this early increase in P4 has been associated with a shortening of the oestrous cycle (Ginther 1970; Garrett *et al.,* 1988a; Macmillan and Peterson 1993; Burke *et al.,* 1994; Pope *et al.,* 1995; Van Cleeff *et al.,* 1996). The advantage of using hCG is that it increases P4 gradually, and its long half-life makes its effect last longer compared with other hormones like GnRH (Schmitt *et al.,* 1996b; Chan *et al.,* 2003; Saleh *et al.,* 2012). In our study, when hCG was used on Day 2, P4 was incremented from Day 6 onwards; therefore based on the previous studies mentioned above hCG treatment on Day 2 would be beneficial for conceptus elongation and most likely pregnancy rate.

Perspectives for future research

Based on the results obtained in this thesis, much work is needed to fully understand maternal-embryonic interactions. Nowadays, our group is working to complete these results and some studies are in progress:

- Because no evidence was observed for an effect of the embryo on the oviduct transcriptome, a bovine multiple embryo transfer model has been developed to enhance the possibility to capture any signal triggered by the embryo.
- In the second chapter, oviductal fluid from each part of the oviduct was collected. Therefore, the next step is to analyse these samples by proteomic and metabolomic techniques to see if there is any specific signal secreted by the embryo.

- Knowing that hCG treatment on Day 2 or 5 is related with higher concentration of progesterone, a field study with dairy cows has been designed to evaluate its possible effect on pregnancy rate.

Understanding maternal-embryo interactions will provide us with new knowledge that will be essential to design new treatments to improve pregnancy rate in cattle and also to enhance *in vitro* embryo production, not only in cattle but also with a potential use in other species like humans.

Conclusions

1. Lactation induces a significant alteration in the pattern of many key metabolites associated with fertility in postpartum cows.
2. Lactation is associated with an impairment in the ability of the reproductive tract of the postpartum lactating dairy cow to support early embryo development to the blastocyst stage.
3. By Day 90 postpartum lactation did not affect the ability of the uterus to support conceptus elongation.
4. Under our experimental conditions, the presence of an 8-cell embryo in the isthmus did not affect the transcriptome of the oviduct, although a local effect at the precise position of the embryo cannot be ruled out.
5. Gene expression of the oviduct in pregnant or cyclic heifers is not modified by proximity to the CL
6. In pregnant heifers, major differences exist between the ampulla and isthmus regions of the oviduct ipsilateral to the CL.
7. Human chorionic gonadotrophin administration on Day 2 after oestrus leads to an increase in the luteal tissue area of the CL and an associated increase in circulating P4 concentration from Day 6 onwards, which may be beneficial for early embryo development.

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Xiao, Q., Challis, J.R., Fraser, M., Wlodek, M.E., Thorburn, G.D., Cuttita, F., Hill, D.J., St-Pierre, S., Spindel, E.R., and McDonald, T.J. (1996a) Locations and molecular forms of gastrin-releasing peptide-like immunoreactive entities in ovine pregnancy. *Peptides* **17**(3), 489-95

Xiao, Q., Han, X., Challis, J.R., Hill, D.J., Spindel, E.R., Prasad, C.J., Akagi, K., and McDonald, T.J. (1996b) Gastrin-releasing peptide-like immunoreactivity is present in human maternal and fetal placental membranes. *J Clin Endocrinol Metab* **81**(10), 3766-73

Yamashita, S., Abe, H., Itoh, T., Satoh, T., and Hoshi, H. (1999) A serum-free culture system for efficient in vitro production of bovine blastocysts with improved viability after freezing and thawing. *Cytotechnology* **31**(1-2), 123-31

Yániz, J.L., Lopez-Gatius, F., Santolaria, P., and Mullins, K.J. (2000) Study of the functional anatomy of bovine oviductal mucosa. *Anat Rec* **260**(3), 268-78

Zeder, M.A., Bradley, D.G., and Emshwiller, E. (2006) 'Documenting Domestication: New Genetic and Archæological Paradigms.' (University of California Press)

Zhang, L., Jiang, S., Wozniak, P.J., Yang, X., and Godke, R.A. (1995) Cumulus cell function during bovine oocyte maturation, fertilization, and embryo development in vitro. *Mol Reprod Dev* **40**(3), 338-44

Appendix A
Curriculum Vitae

Education

- PhD, Department of Animal Reproduction, INIA, Madrid, Spain. Since 2010.
- Master Degree in "Biology and Biotechnology of Reproduction in Mammals", University of Murcia, Spain, 2010.
- DVM, Complutense University, Madrid, Spain, 2008.

Recent Postgraduate Courses Attended

- **Reproductive problems in beef cattle.** Veterinary School, University Complutense of Madrid. 12 February 2014.
- **Applied Proteomics.** Conway Institute, University College Dublin (UCD), Ireland. April-May 2012
- **Data Analysis for Biological Scientists**. Conway Institute, UCD, Ireland. May 2012
- **Genomics: Principles and Practical Applications**. Conway Institute, UCD, Ireland. May-June 2012.
- **Flow Cytometry**. Conway Institute, UCD, Ireland. June 2012.
- **XXXIV International Course of Animal Reproduction**. INIA, Madrid, Spain. November 2011.
- **II Theoretical-Practical Course "Reproductive Efficiency and Ultrasound in Dairy Cows".** Clinic "Monge Veterinarios", Madrid, November 2011.

Research Experience

- October 2013- present. **Department of Animal Reproduction, INIA**, Madrid (Spain).
  Research assistant (contract).
- November 2010 - present. **Department of Animal Reproduction, INIA**, Madrid (Spain).
  PhD student investigating "Maternal-Embryo Interactions and Strategies to Improve Embryo Survival in Cattle" (under the supervision of Dr D. Rizos, INIA, and Prof. Patrick Lonergan, University College Dublin).
- November 2013. **Lyons Research Farm, University College of Dublin** (UCD, Ireland).
  Scientific visit to continue the studies in relation with oviduct-embryo interactions in cattle (under the supervision of Prof. Pat Lonergan).
- March-April 2013. **Lyons Research Farm, University College of Dublin** (UCD, Ireland).
  Scientific visit to study oviduct-embryo interactions in cattle (under the supervision of Prof. Patrick Lonergan). Supported by a *Short Term Scientific Mission Grant (STSM, COST-Epigenomics).*
- February-July 2012. **Lyons Research Farm, University College of Dublin** (UCD, Ireland).
  Scientific visit study strategies to reduce early embryonic loss in cattle (under the supervision of Prof. Patrick Lonergan).
- July- December 2011**. Department of Animal Reproduction, INIA,** Madrid (Spain).
  Research assistant (contract).
- November 2010-March 2011. **Lyons Research Farm, University College of Dublin** (UCD, Ireland).
  Scientific visit to study maternal-embryonic interaction in postpartum dairy cows (under the supervision of Prof. Pat Lonergan). Supported by a *Short Term Scientific Mission Grant (STSM, COST-Gemini).*
- May - June 2010. **Department of Animal Reproduction, INIA**, Madrid (Spain).
  Training period. *Mobility Grant.*

Publications in Indexed Journals

Ramos-Ibeas P, Pericuesta E, Calle A, Laguna-Barraza R, Moros-Mora R, Lopera-Vásquez R, **Maillo V**, Yáñez-Mó M, Gutiérrez-Adán A, Rizos D, Ramírez M.A (2014). A system to establish a biopsy-derived trophoblastic cell lines for bovine embryo genotyping and implantation studies. *Submitted Biology of Reproduction.*

Maillo V, Duffy P, O'Hara L, de Frutos C, Kelly AK, Lonergan P, Rizos D. (2014). Effect of hCG administration during corpus luteum establishment on subsequent corpus luteum development and circulating progesterone concentrations in beef heifers. *Reproduction, Fertility and Development* 26(3): 367-374.

O'Hara L, Forde N, Carter F, Rizos D, **Maillo V**, Ealy AD, Kelly AK, Rodriguez P, Isaka N, Evans ACO, Lonergan P. (2014). Paradoxical effect of supplementary progesterone between Day 3 and 7 on corpus luteum function and conceptus development in cattle. *Reproduction, Fertility and Development* 26 (2): 328-336.

Scully S, **Maillo V**, Duffy P, Kelly A, Crowe M, Rizos D, Lonergan P. (2013). The effect of lactation on postpartum uterine involution in Holstein dairy cows. *Reproduction in Domestic Animals* 48 (6): 888-92.

Maillo V, Rizos D, Besenfelder U, Havlicek V, Kelly AK, Garrett M, Lonergan P. (2012). Influence of lactation on metabolic characteristics and embryo development in postpartum Holstein dairy cows. *Journal of Dairy Science* 95(7): 3865-76.

O'Hara L, Scully S, **Maillo V**, Kelly AK, Duffy P, Carter F, Forde N, Rizos D, Lonergan P. (2012). Effect of follicular aspiration just before ovulation on corpus luteum characteristics, circulating progesterone concentrations and uterine receptivity in single-ovulating and superstimulated heifers. *Reproduction* 143(5): 673-82.

Maillo V, De Frutos C, O'Gaora P, Forde N, Spencer TE, Gutierrez-Adan A, Lonergan P, Rizos D. (2014). Oviduct-embryo interactions: two-way traffic or a one-way street? Transcriptomic response of the bovine oviduct to the presence of an embryo. *In preparation.*

Other Publications

Maillo V, Besenfelder U, Havlicek V, Garret M, Kelly AG, Rizos D, Lonergan P. (2011). Effect of lactation on circulating metabolic hormones and early embryo development in postpartum dairy cows. *European Embryo Transfer Society* (*AETE).* Newsletter 36: 5-6.

Posters and Oral Communications Presented at International Scientific Meetings as First Author

Maillo V, O'Gaora P, Mehta JP, De Frutos C, Forde N, Spencer TE, Lonergan P, Rizos D. (2014). Oviduct-embryo interactions: two-way traffic or a one-way street? Transcriptomic response of the bovine oviduct to the presence of an embryo. 40th *Annual Conference of the International Embryo Transfer Society (IETS). Reno, Nevada. 11-14 January 2014.* Reproduction, Fertility and Development 26(1): 152-3. Poster and Oral communication.

Maillo V, Besenfelder U, Havlicek V, Gutierrez-Adan A, Lonergan P, Rizos D. (2013). The effect of uterine environment on the transcriptional response of the embryo in postpartum dairy cows. *1st Cost-Action FA1201 EPICONCEPT, General Conference "Epigenetics and Periconception environment". Antalya, Turkey. 24-25 April 2013.* Poster.

Maillo V, Duffy P, O'Hara L, de Frutos C, Kelly AK, Lonergan P, Rizos D. (2013). Effect of Human Chorionic Hormone (hCG) administration on Days 1, 2, 3 or 4 post estrus on corpus luteum development and circulating progesterone concentrations in beef heifers. *39th Annual Conference of the International Embryo Transfer Society (IETS). Hannover, Germany. 19-23 January 2013.* Reproduction, Fertility and Development 25(1): 202-3. Poster.

Maillo V, Besenfelder U, Havlicek V, Garrett M, Kelly AK, Rizos D, Lonergan P. (2012). Effect of lactation on embryo development during the postpartum period in dairy cows. *38th Annual Conference of the International Embryo Transfer Society (IETS). Phoenix, Arizona. 7-10 January 2012.* Reproduction, Fertility and development 24(1)155-156. Poster.

Maillo V, Kelly AG, Lonergan P, Rizos D. (2011). Conceptus elongation in lactating and non-lactating postpartum dairy cows. *4th Cost Action – Gemini "Maternal Interactions with Gametes and Embryos". Gijon, Asturias, Spain. 29 September-2 October 2011.* Poster.

Maillo V, Besenfelder U, Havlicek V, Garrett M, Kelly AG, Rizos D, Lonergan P. (2011). Effect of lactation on circulating metabolic hormones postpartum and early embryo development in dairy cows. *27th Scientific Meeting of the European Embryo Transfer Society (AETE). Chester, England, 9-10 September 2011.* Poster and oral communication in the Student Competition.

Other contributions at Internacional and National Meetings

Lopera R, Hamdi M, Fuertes B, **Maillo V**, Beltrán P, Redruello A, Yañez Mó M, Ramírez MA, Rizos D. (2014). Depletion of extracellular vesicles from fetal calf serum improves the quality of bovine embryos produced in vitro. *12º Congreso de la Asociación Española de Reproducción Animal (AERA). Alicante, Valencia, Spain. 16-18 October 2014.*

Lopera R, Hamdi M, Fuertes B, **Maillo V**, Beltran P, Redruello A, Gutierrez-Adan A, Yáñez-Mó M, Ramírez MA, Rizos D. (2014). Effect of extracellular vesicles secreted by bovine oviductal epithelial cells in in vitro bovine embryo production**.** *International Society for Extracellular Vesicles (ISEV). Rotterdam, the Netherlands. 30th April – 3rd May.*

Lopera R, Hamdi M, Fuertes B, **Maillo V**, Beltrán P, Redruello A, Gutierrez-Adán A, Yañez-Mó M, Ramírez MA, Rizos D. (2013). Extracellular vesicles secreted by bovine oviductal epithelial cells increase the quality of in vitro produced bovine embryos. *29th Scientific Meeting of the European Embryo Transfer Society (AETE). Istambul, Turkey, 6-7 September 2013.*

O'Hara L, Forde N, Rizos D, **Maillo V**, Ealy AD, Kelly AK, Rodriguez P, Evans ACO, Lonergan P. (2013). Effect of short term progesterone supplementation on circulating progesterone concentration, corpus luteum size, and early embryo development in cattle. *39th Annual Conference of the International Embryo Transfer Society (IETS). Hannover, Germany, 19-23 January 2013.* Reproduction, Fertility and Development 25(1): 202.

Ramos-Ibeas P, Moros-Mora R, **Maillo V**, Lopera-Vasquez R, Laguna-Barraza R, Gutierrez-Adan A, Rizos D, Ramirez MA. (2012). A Biopsy-Derived Trophectoderm Cell Line for Bovine Embryo Genotyping. *45th Annual meeting of Society for the Study of Reproduction (SSR). The Pennsylvania State University State College, Pennsylvania, USA, 12-15 August 2012.*

Scully S, **Maillo V**, Duffy P, Rizos D, Kelly AK, Crowe MA, Lonergan P. (2012). The effect of lactation on postpartum uterine involution in Holstein dairy cows. *17th International Congress of Animal Reproduction (ICAR). Vancouver, Canada, 29 july-2 August 2012.* Reproduction of Domestic Animals 47 (suppl. 4): 538.

Laguna-Barraza R, De Frutos C, **Maillo V**, Gutierrez-Adan A, Rizos D. (2012). Histone deacetylation inhibition decreases transcription of imprinted genes during early embryo development in cattle. *17th International Congress of Animal Reproduction (ICAR). Vancouver, Canada, 29 july-2 August 2012.* Reproduction of Domestic Animals 47 (suppl. 4): 503.

O'Hara L, Scully S, **Maillo-Sevilla V**, Kelly AK, Duffy P, Carter F, Forde N, Rizos D, Lonergan P. (2012). Effect of follicular aspiration just prior to ovulation on corpus luteum characteristics, circulating progesterone concentrations and uterine receptivity in single-ovulating beef heifers. *38th Annual Conference of the International Embryo Transfer Society (IETS). Phoenix, Arizona. 7-10 January 2012.* Reproduction, Fertility and development 24(1)155.

Laguna-Barraza R, De Frutos C, **Maillo V**, Moros-Mora R, Beltran-Brena P, Guitierrez-Adan A, Rizos D. (2011). Effect of treatment with the histone deacetylase inhibitor, scriptaid, on development and quality of in vitro produced bovine embryos. *27th Scientific Meeting of the European Embryo Transfer Society (AETE). Chester, England. 9-10 September 2011.*

Attendance at International Meetings

- 40th Annual Conference of the International Embryo Transfer Society (IETS). Reno, Nevada. 11-14 January 2014.
- IETS 2014 Preconference Symposium: New Developments in Embryo Technologies and Embryo Transfer Techniques. Reno, Nevada. 11th January 2014. **Assistant** in the Module 1 "*In vitro* embryo production in cattle" led by Prof. Peter Hansen and Dr. Jeremy Block, University of Florida, USA.
- 1st Cost-Action FA1201 EPICONCEPT, General Conference "Epigenetics and Periconception Environment". Antalya, Turkey. 24-25 April 2013.
- 39th Annual Conference of the International Embryo Transfer Society (IETS). Hannover, Germany. 19-23 January 2013.
- 4th Cost Action – Gemini "Maternal Interactions with Gametes and Embryos". Gijon, Spain. 29 September-2 October 2011.
- 27th Scientific Meeting of the European Embryo Transfer Society (AETE). Chester, England. 9-10 September 2011.

Awards

- First prize in Student Competition in 27th Scientific Meeting of the European Embryo Transfer Society (AETE). Chester, England. 9-10 September 2011.