UNIVERSIDAD DE MURCIA

ESCUELA INTERNACIONAL DE DOCTORADO

Ovarian Follicular Development in Weaned Sows.
Determinants
and Potential Compensatory Treatments

Desarrollo Folicular Ovárico en Cerdas Destetadas.
Factores de Variación
y Posibles Tratamientos Correctores

Dª Tânia Marisa Piedade Lopes

2021

UNIVERSIDAD DE
MURCIA

Jordi Roca Aleu, Catedrático del Departamento de Medicina y Cirugía Animal de la Universidad de Murcia, y Alfonso Bolarín Guillén, Doctor en Veterinaria y director de Investigación de AIM Ibérica (Topigs Norsvin España),

AUTORIZAN

La presentación de la Tesis Doctoral titulada **"Ovarian follicular development in weaned sows. Determinants and potential compensatory treatments (Desarrollo folicular ovárico en cerdas destetadas. Factores de variación y posibles tratamientos correctores)"** realizada por Dña. **Tânia Marisa Piedade Lopes**, bajo nuestra inmediata dirección y supervisión, y que presenta para la obtención del grado de Doctor por la Universidad de Murcia.

En Murcia, a 29 de marzo de 2021

Fdo.: Jordi Roca Aleu

Fdo.: Alfonso Bolarín Guillén

Facultad de Veterinaria
Departamento de Medicina y Cirugía Animal (Reproducción y Obstetricia)
Campus Universitario de Espinardo. 30100 Murcia

PhD THESIS BY PUBLICATIONS

UNIVERSIDAD DE
MURCIA

D.ª TANIA MARISA PIEDADE LOPES

Vista la solicitud presentada el día 17 de febrero de 2021, por **D.ª Tania Marisa Piedade Lopes**, con NIE X6759646-S, sobre autorización para presentación de tesis doctoral como compendio de publicaciones con carácter previo a la tramitación de la misma en la Universidad de Murcia, le comunico que la Comisión de General de Doctorado, vistos:

- el informe previo de la Comisión Académica del Programa de Doctorado en CIENCIAS VETERINARIAS

- el visto bueno de la Comisión de Ramas de Conocimiento en CIENCIAS DE LA SALUD

resolvió, en su sesión de 19 de mayo de 2021, **ACCEDER** a lo solicitado por la interesada pudiendo, por tanto, presentar su tesis doctoral en la modalidad de compendio de publicaciones siempre que aporte, con antelación a la presentación de la tesis doctoral, originales de los documentos que contienen firmas manuscritas, con los siguientes artículos:

1. Relevance of ovarian follicular development to the seasonal impairment of fertility in weaned sows.
2. Altrenogest treatment before weaning improves litter size in sows.
3. Weaned sows with small ovarian follicles respond poorly to the GnRH agonist buserelin.
4. Ovarian follice growth during lactation determines the reproductive performance of weaned sows.

La presente resolución no pone fin a la vía administrativa. Frente a ella, de conformidad con lo previsto en el capítulo II del título V de la Ley 39/2015, de 1 de octubre, del Procedimiento Administrativo Común de las Administraciones Públicas y en el artículo 21 de los Estatutos de la Universidad de Murcia, aprobados por Decreto 85/2004, de 27 de agosto, los interesados pueden interponer recurso de alzada ante el Rector de la Universidad de Murcia, en el plazo de un mes, contado desde el día siguiente al de la notificación o publicación, sin perjuicio de que puedan intentar cualquier otro recurso que a su derecho convenga.

Lo que en cumplimiento del artículo 40.1 de la vigente Ley 39/2015, de 1 de octubre del Procedimiento Administrativo Común de las Administraciones Públicas, se notifica a **D.ª Tania Marisa Piedade Lopes**.

La Vicerrectora de Estudios, y
Presidenta de la Comisión General de Doctorado
Sonia Madrid Cánovas

Documento firmado electrónicamente

Edificio Rector Soler 3ª planta. Campus Universitario de Espinardo. 30100 Murcia
T. 968 364 294 – F. 968 363 304 – *email:* 3rciclo@um.es – **www.um.es/academic/sec-postgrado/**

PhD THESIS BY PUBLICATIONS

1. Lopes T.P., Sánchez-Osorio J., Bolarín A., Martínez E.A., Roca J. **Relevance of ovarian follicular development to the seasonal impairment of fertility in weaned sows**. The Veterinary Journal. **2014** Mar;199(3):382-6. doi: 10.1016/j.tvjl.2013.11.026. Epub 2013 Dec 2. PMID: 24461203.

2. Lopes T.P., Bolarín A., Martínez E.A., Roca J. **Altrenogest treatment before weaning improves litter size in sows**. Reproduction in Domestic Animals. **2017** Oct 52; Suppl 4:75-77. doi:10.1111/rda.13063. PMID: 29052320.

3. Lopes T.P., Padilla L., Bolarín A., Rodríguez-Martínez H., Roca J. **Ovarian follicle growth during lactation determines the reproductive performance of weaned sows**. Animals (Basel). **2020** Jun 10;10(6):1012. doi: 10.3390/ani10061012. PMID: 32532102; PMCID: PMC7341282.

4. Lopes T.P., Padilla L., Bolarín A., Rodríguez-Martínez H., Roca J. **Weaned sows with small ovarian follicles respond poorly to the GnRH agonist Buserelin**. Animals (Basel). **2020** Oct 28;10(11):1979. doi: 10.3390/ani10111979. PMID: 33126684; PMCID: PMC7692150.

Department of Medicine and Animal Surgery,
Faculty of Veterinary Science.
University of Murcia.

The research reported in this Doctoral Thesis was
supported by the Spanish Ministry of Economy, Industry
and Competitiveness (MINECO, Spain) and
FEDER funds (EU, AGL2015-69738-R); and
the Seneca Foundation, Murcia (Spain, GERM 04543/07 &
19892/GERM/15)

Dedicado a Alfonso, Daniela, Sofia y Tiago

ACKNOWLEDGMENTS

ACKNOWLEDMENTS

After almost a decade of a gestation with several complications, this work that you have in your hands finally sees the light of day. During such a long time, there have been many people to whom I want to thank for their help and understanding that have made possible the happy end of this work.

First of all, I would like to thank my director Dr. Jordi Roca for all his patience and perseverance even in the moments when I have not made it easy. I have in mind that all the hard field work would not have been visible if it were not for his insistence that I sit behind a table and put it on paper. Thank you very much.

I would also like to thank

To all the team of the Department of Reproduction and Obstetrics of the Veterinary Faculty of the University of Murcia, for their companionship and teachings in unknown areas to me. They made me look at research with more respect and admiration. Especially to Emilio, Toñi, Cristina, Xiomara, Lorena and Jonatan.

Especial thanks to Inma for her silent and constant help throughout the years. Not only for her encouragement, but also for solving my doubts and problems caused by my lack of understanding in the logistics of the PhD.

To Maria José and Lola for turning a companionship into a friendship that I know I can count on through the good and the bad times.

To Jeanne for her help in performing some of the ultrasound scans.

To the company Agropor SL for giving me the possibility of doing the field work in their farm and giving me all the freedom of schedules and work that made this study possible. To the veterinarians Juan Conesa and Paula for authorizing me to finish the tests even when there was a sanitary alert. To all the farm staff, especially Pepe Alguazas for his availability and help any time, but also to Ivan, Pascual and Chuca.

To MSD for providing me with the products with which I performed some of the tests published in this work.

But, mainly, to my family in Portugal. Even though they are far away I always feel them close to me. They have taught me that in life you don't have to give up on things simply because they are not easy. Thank you grandma, mum, dad, Marta, David, Francisco, Tomás, Madalena, Alice, Palhetas and Claudia.

Also, thanks to my Spanish family in-law for their welcome into their family and supporting me all the way. Thank you Mariano, Mariola, José Miguel, Maximilian and Birgit.

To all my friends, my chosen family, for those beers that we stopped drinking when I had to dedicate my time to my thesis. To Catarina, Joana, Ritas, Laura, Pablo, Fuen, Andrés, Fernando (soda in your case), Rafi, Miriam and Juanpe.

And finally, to my new family that in the time that this adventure has lasted, grew, and went from two to five. Alfonso, Daniela, Sofia and Tiago, you are the most important thing, the reason for my smile when circumstances lead me to discourage, my desire to continue improving as a person so that you can feel proud of me. You are all that matters.

Alfonso, you are the most hard-working and persistent person I know and I am proud to be able to call you briefly husband and I hope I can enjoy your company until you finally teach me how to play chess.

CONTENTS

INTRODUCTION

INTRODUCTION

Swine production is an economic activity that demands high production efficiency as it has low profit margins and high global competitiveness. This efficiency is mainly focused on the sow and is largely, but not exclusively, based on the reduction of non-productive days (NPD), i.e., those in which the sow is not pregnant or lactating. Non-productive days in sows would be associated to the absence or delay of oestrus sign after weaning and pregnancy loss after artificial insemination (AI).

Weaned sows account for more than 80% of total mating on a production farm. Therefore, weaned sows are the focus of major efforts to improve productivity. After weaning, sows exhibit a fertile oestrus in a short interval of 3-6 days (Knox and Zas, 2001). Nevertheless, a significant number of sows fail to exhibit oestrus within the 14 d after weaning, thus increasing the NPD. In addition, long wean-to-oestrus (WOI) intervals are closely related to poorer reproductive performance, i.e., pregnancy losses, decreasing farrowing rates and smaller litter sizes (Knox, 2019).

Research have been conducted aiming to minimize the incidence of sows failing to become pregnant after weaning and to identify the possible causes behind (Koketsu & Dial, 1997; Vargas et al., 2009; de Jong et al., 2013). Thus, for example, body condition at weaning, improved oestrus detection, quality of AI semen doses and on-farm AI strategy have been found to influence post-weaning pregnancy success. Nevertheless, the causes of sows not exhibiting oestrus properly after weaning remain poorly understood.

Seasonal infertility in weaned sows

The domestic pig is a species considered non-seasonal from the reproductive point of view. In fact, sows included in AI programs farrow litters throughout the year. However, farrowing rates and litter sizes show clear seasonal differences. A decrease in farrowing rate and a lower number of piglets per litter are noticed in late summer and autumn, especially in sows living in temperate geographic regions with well-defined seasons (Bertoldo et al., 2012; De Rensis et al., 2017). This fact is known as seasonal infertility in domestic pigs (*Sus scrofa domesticus*). The origin of this seasonal infertility can be probably found in the strong seasonal reproduction ability of its ancestor, the wild boar (*Sus scrofa*), in which females are in anoestrus (Mauget, 1985) and males show significant decreased libido (Weiler et al., 1996) in summer and autumn as well. Changes in day length and hight air temperature and humidity are determinant factors explaining seasonal infertility, and they are particularly severe in geographic areas with hot summers, as occurs in Mediterranean countries (Dominguez et al., 1996; Love et al., 1993).

In addition to decreased farrowing rates and reduced litter sizes, seasonal infertility in sows includes other reproductive disorders such as increased WOI and higher incidence of pregnancy losses (Peltoniemi and Virolainen, 2006; Bertoldo et al., 2012). Seasonality also affects ovarian functionality (Love et al., 1993) but specific studies remain limited. Little is known about how follicular growth occurs in sows affected by seasonal infertility and how such follicular growth influences subsequent reproductive performance. The clarification of these uncertainties will be one of the main objectives of this doctoral Thesis.

Other factors affecting the reproductive performance of weaned sows

In addition to seasonal environmental factors, other factors can also influence the reproductive performance of weaned sows. One of the most relevant is the nutritional level. Sows with poor body condition at weaning showed delayed WOI (Prunier et al., 1996), more non-productive days, lower farrowing rates, lower birthweight of piglets at farrowing and fewer piglets born in the following cycle (Koketsu et al., 1996). In addition, they account for a large proportion of sows culled for reproductive failures (Koketsu et al., 1996). Number of parities and lactation length is also considered to be one of the main factors influencing reproductive performance of weaned sows (Bracken et al., 2003). Sows with short lactation periods and/or low parity tend to have reduced reproductive performance (Knox et al., 2019). All these other factors would have a negative impact on the reproductive performance of sows through their direct influence on the development of ovarian follicles (Quesnel, 2009). The mean size of ovarian follicles at weaning varies significantly among sows (Knox, 2019). Whether the above-mentioned causal factors influence this heterogeneity remains to be clarified. The present Thesis aims to address this issue.

Ovarian follicle growth in weaned sows

The follicular ovarian phase of the estrous cycle is the short period of time following to luteal phase (*corpora lutea*) or lactation, and includes the final growth of follicles from preantral to large antral or mature. In sows, the follicular phase extends over 4-6 days, involving about 20% of the oestrus cycle. Accordingly, sows that not show oestrus during the first 8 d after weaning are considered to be in delayed oestrus or even anoestrus (Soede et al., 2011).

The follicular ovarian phase comprises two well defined subphases being the first one the so-called *recruitment* that starts with the release of FSH from adenohypophysis that stimulates growth of the pool of preantral follicles present in the ovary (Soede et al., 2011). At this stage, follicles grow from 0.2-0.4 cm to 0.5-0.6 cm in diameter. Some of these follicles may still experience atresia. Increased FSH secretion together with pulsatile LH secretion of low frequency leads to the subsequent so-called *selection* subphase, in which healthy follicles continue to grow reaching the preovulatory size, between 0.7 and 0.9 cm in diameter. These follicles secrete large amounts of 17β-oestradiol that stimulates hypothalamus-adenohypophysis axis to secrete high-frequency pulsatile LH (LH peak), ultimately responsible for ovulation. Ovulation occurs 27-33 h after the LH peak (Soede et al., 1995a) and the ovulation process, from the ovulation of the first follicle to that of the last follicle, lasts between 1 and 3 h. 17β-oestradiol secretion is also responsible for the psychosomatic signs that characterize oestrus. The duration of oestrus is variable among sows, ranging from 24 to 96 h. Ovulation will occur around 70% of the oestrus duration (Soede & Kemp, 1997). Unfortunately, the variable duration of oestrus makes it difficult to predict the time of ovulation.

Ovarian follicular activity during lactation is characterized by waves of follicles that initiate growth and eventually become atresia so that sows are in anoestrus throughout the lactation period. However, some factors can trigger follicle recruitment and selection during this period. For example, loss of body reserves during lactation, decreased suckling stimulation or prolonged lactation periods (Kemp & Soede, 2012). Once weaned, the WOI will depend on the phase of the predominant follicular population at weaning. The WOI will be short if the follicles are beginning a recruitment phase. However, the WOI will be longer if the follicles are still in an early growth phase or undergoing atresia. In the latter situation a new follicular wave should start to develop (Lucy et al., 2001). It is interesting to recall that the size of the ovarian follicle pool at weaning is highly variable among sows (Britt et al., 1985; Bracken et al., 2003) and factors responsible of this variability remain still unclear (Knox, 2019). Elucidating and controlling on the factors behind this variability would decrease the NPD of sows on the farm. This knowledge would help to design effective reproductive management strategies to achieve and unify optimal WOIs. Accordingly, achieving this will be one of the goals of the present Thesis.

Exogenous hormone treatments for control of ovary function in the sow

Hormonal treatments for the control of ovarian function are commonly used in swine (De Rensis & Kirkwood, 2016). Hormonal ovulation induction and synchronization treatments can significantly improve pig farm management. They facilitate sow flock management by (1) optimizing AI and (2) grouping future farrowings. Focusing on AI, ovulation induction and synchronization in addition to oestrus synchronization allows significantly to decrease the number of semen doses per cycle and sow, facilitating to perform a single fixed-time AI (Driancourt, 2013; Kirkwood & Kauffold, 2015; Roca et al, 2016; Knox, 2019). Fewer AIs per cycle save labour and reduce the number of boars used in AI programs, increasing the genetic value of the entire boar population and indirectly of the entire sow population. It also reduces the environmental pollutants generated by the consumables used for insemination (catheters, semen bags, ...). Another advantage of ovulation induction and synchronization treatments is to homogenize the litters because more farrowing will occur simultaneously and thus more piglets will be of the same exact age in each batch of sows (Bortolozzo et al., 2015; Kirkwood & Kauffold, 2015; De Rensis & Kirkwood, 2016). Consequently, hormonal treatments for oestrus synchronization in gilts, especially through the use of Altrenogest, and for ovulation induction and synchronization in any sow, are routinely used in pig farms

Altrenogest is a synthetic progestogen that, when administered orally, inhibits the secretion of GnRH and gonadotropins by the hypothalamic-pituitary axis in sows and thus the functional activity of the ovary. As mentioned above, the treatment is used to synchronize the oestrus of gilts that are incorporated into the production batches of weaned sows. At the end of the 18-day treatment, these gilts show oestrus at the same time as the weaned sows (Kraeling & Webel, 2015; van Leeuwen et al., 2015; Wang et al., 2018). This treatment also improves reproductive performance of sows (Boyer & Almond, 2014; van Leeuwen et al., 2015). However, treatment with Altrenogest has not demonstrated ability to homogenize follicular size in the follicular wave that begins after treatment (van Leeuwen et al, 2010; van Leeuwen et al, 2011b).

For induction and synchronization of ovulation there are several useful hormones such as human chorionic gonadotropin (hCG; Nissen et al., 1995; Cassar et al., 2004; Bolarín et al., 2009), porcine luteinizing hormone (pLH; Fontana et al., 2014; McBride et al., 2019) and GnRH analogues (Martinat-Botte et al., 2010). Nowadays, the GnRH analogues, such as buserelin (Martinat-Botte et al., 2010; Driancourt et al., 2013; Pearodwong et al., 2019), licerelin (Fries et al., 2010) and triptorelin (Knox et al., 2011; Dillard and Flowers, 2020) are the most widely recommended as they are able to induce a surge of endogenous LH similar to physiological LH peak. In addition, they can be administered by different routes, and several synthetic agonists with different bioactivities and half-lives are commercially available (Knox, 2019). Nevertheless, sow response to these treatments is quite variable, with less than 80% of sows ovulating within the expected window (Knox et al., 2011; Driancourt et al., 2013). The causes behind this suboptimal response variability remain still unclear. The timing and route of administration together with the number of previous farrowings are among the possible causes (Driancourt et al., 2013; Knox et al., 2011). However, other causes have been neglected or poorly evaluated to date, such as the season of weaning and the size of the ovarian follicles, either at weaning or at the time of treatment (Knox et al. (2019; Pearodwong et al., 2019). Clarifying the influence of these poorly studied possible causes is one of the objectives of the present Thesis.

Transrectal ultrasonography

In the sow, ovaries and follicle growth dynamics can be monitored throughout the estrous cycle by sequential ultrasonography every few hours, namely every 6-12 h (Bolarín et al., 2009). The procedure provides accurate information on the size and numbers of follicles present in the ovary and how these measurements change over time (Soede et al., 1998). Ultrasonography can be performed transabdominally (Kauffold et al., 2019) or transrectally (Soede et al., 1998; Bolarín et al., 2009). The transrectal approach allows for a better accuracy in counting and measuring follicles in the ovary (Bolarín et al., 2008) and causes little stress to the sow and do not alter her physiology and reproductive performance (Kauffold & Althouse, 2007). Transrectal ultrasonography also allows to screen the ovulation time, which is recorded when the follicle count is noticeably lower than the count of the previous scanning. In addition to monitoring the growth of ovarian follicles and subsequent ovulation, ultrasound can also identify pathologies of the reproductive system, including the ovaries. Thus, for example, follicular cysts (anechoic spherical structures larger than 1.1 cm) or inactive ovaries (no follicles bigger than 0.3 cm) can be identified (Kauffold et al., 2019).

OBJECTIVES

OBJECTIVES

The main objective of the present Thesis was to investigate and hormonally control the dynamics of follicular growth in sow ovaries during lactation and weaning in order to establish strategies to reduce non-productive days.

To achieve this goal, the Thesis contemplates four different specific objectives:

1. To evaluate seasonal differences in ovarian follicular development in weaned sows and to assess the implications of these differences on the clinical reproductive consequences of seasonal infertility.

2. To reveal whether the weaning season, parity, body condition and lactation length would be causal factors explaining the heterogeneity among sows in follicular size at weaning. And, to analyse whether this heterogeneity already existed during lactation.

3. To explore whether a short-term treatment of Altrenogest during the last days of lactation could regulate follicular waves at weaning and thereby improve the reproductive performance of weaned sows, regardless of the weaning season.

4. To evaluate whether the response of weaned sows to treatment with the GnRH agonist buserelin to induce and synchronize ovulation depends on the size of ovarian follicles at the time of treatment.

EXTENDED SUMMARY

EXTENDED SUMMARY

Introduction

Pig industry is defined by efficiency in meat production. An important indicator of efficiency on pig farms is the non-production days, i.e., the days that the sows are neither pregnant nor lactating. Basically, they are represented by sows that have estrus problems after weaning (no or delayed estrus) or that do not become pregnant after artificial insemination (AI). The present Thesis includes four experimental studies focused on ultrasound examination of ovarian follicle growth dynamics in lactating and weaned sows showing differences in reproductive performance after weaning caused by environmental (weaning and mating season), nutritional (body condition) or management (parity and lactation duration) factors. The Thesis also addresses the effectiveness of hormonal treatments (Altrenogest and the GnRH agonist buserelin) to control ovarian follicular growth dynamics and ovulation in weaned sows.

Material and Methods

The experiments performed in the present Thesis involved the handling of animals. The procedures used for such handling were carried out in accordance with the current 2010/63/EU ECC Directive on the protection of animals used for scientific purposes, and were reviewed and approved by the Ethical Committee for Experimentation with Animals of the University of Murcia (Murcia, Spain).

Farm location, Animals, Housing and Handling

Experiments were conducted under field conditions in a large intensive production pig farm of 1,800-2,500 sows. The farm was located in the south-eastern Spain (37°59'NL, 1°08'WL), with daylight ranging from 14 h 48 min on the summer solstice to 9 h 32 min on the winter solstice. The mean maximum air temperature ranged from 32.8 (summer) to 17.7°C (winter) during the experimental years. The farm fulfilled the guidelines of the European Union and local Spanish legislation in terms of production, health, biosecurity, and animal welfare. The farm had climate control only in the farrowing rooms, where evaporative cooling systems and exhaust fans kept the room temperature at around 24°C. All other farm facilities were open to environment temperature and natural light. The sows always had free access to water, and they were fed with commercial feed whose composition varied according to the physiological state of the sow. The farm had a consistent history of seasonal infertility that was manifested by an increased rate of delayed return to oestrus and reduced farrowing rates and litter sizes in summer and early autumn.

Sows were crossbred breeding sows (Landrace x Large White) of parities 1-6 and were randomly selected at farrowing or weaning. Previous reproductive data of the sows as well as their body condition at the beginning of the experiments were recorded.

On-farm breeding management

After a lactation period of 20-28 d, weaning sows were placed in individual cages for oestrus detection, insemination, and subsequent pregnancy diagnosis. Oestrus detection started the day after weaning, twice per day, at 07:00-08:00 h and 18:00-19:00 h. Detection was performed by experienced farm staff in the presence of a healthy adult boar. The start of the oestrus was defined as 6 h before the first time that the sows showed an immobile standing in response to back pressure tests during snout-to-snout contact with the mature boar located in the alley in front of the crate. Once in oestrus, sows were two or three times post-cervically inseminated by experienced farm staff, using liquid semen doses of 40 ml containing 1.5 x 10^9 total spermatozoa, aiming ≥75% fresh motility and morphologically altered sperm. Semen doses were from a mixture of ejaculates from several (1-5) undefined boars (heterospermic doses) and were purchased from an independent boar station (AIM Ibérica, Topigs Norsvin España, Spain). The semen doses remained stored at 15-17°C for 12-48 h before use. Twenty-eight days after AI, pregnancy diagnosis was performed by trans-abdominal ultrasonography. Pregnant sows were grouped in 25 m^2 pens (10 sows per pen), where they remained until seven days before the scheduled day of farrowing. Then, sows were moved to farrowing rooms where they were placed in individual farrowing crates, where they remained until weaning.

Transrectal ultrasound of the ovaries

Ovaries were examined with a portable ultrasound machine (LOGIQ Book XP; GE Healthcare) equipped with a cine loop and fitted with a transrectal 4-10 MHz multiple scan linear transducer (i739-RS, GE Healthcare). The methodology followed for ovarian scanning was the same in all the experiments and was that proposed by Bolarín et al. (2009), which is described in detail below. First, the rectum was manually cleaned of faeces prior to scanning. Then, the transducer is introduced facing down using a long exploration glove covered with vaseline. The contact between the transducer and the rectal mucosa should be close. The preferred frequency of the transducer was 8 MHz, which is the best option in a linear transducer for the rectal counting and measuring of the ovarian follicles. This approach allows the recording of follicles from a size of 0.2 cm. The ovaries are usually located next to the bladder, about 35-45 cm beyond the anal sphincter. They are characterized as anechoic structures with irregular, thin, and echogenic borders. These follicles are identified from the ovarian stroma as circular anechoic structures with smooth, well-defined thin borders. The follicles reach a size between 0.7 and 1.0 cm when they are close to ovulation. At this size, the shape of the follicles changes from circular to irregular due to the compression exerted between them. Follicles with diameters larger than 1.1 cm were recorded as follicular cysts, which normally do not ovulate. Ovulation is identified because the follicles collapse and they are replaced by irregular echogenic structures that correspond to haemorrhagic bodies, early stages of the corpora lutea. The corpora lutea are shown as 0.8-1.4 cm circular hyperechogenic structures with diffuse borders, which are difficult to count and measure by ultrasonography.

General guidelines followed in all experiments

All ovarian ultrasound scans were performed by the same researcher (T. Lopes). Ultrasonographic scans were started on the same day of weaning, except in some specific experiments where they were also performed during the lactation period, specifically at days 7, 14 and 21 from the start of lactation. The timing of the scans in weaned sows was every 24 h until the onset of estrus and, thereafter, every 12 h until ovulation. Transrectal ultrasounds focused on identifying the presence of ovarian follicles, which is expected in reproductively healthy weaned sows. However, sows with inactive ovaries (follicles smaller than 0.3 cm) or with other structures, either functional (haemorrhagic or corpora lutea) or non-functional (follicular cysts), were also recorded. In each sow, both ovaries were scanned separately in different cross sections. At least three video sequences per ovary were recorded using the calibrated digital cine software provided by the scanner manufacturer. The videos were subsequently analyzed and the diameter of two-three representative follicles in each ovary was measured and the mean measurement in cm was recorded. The sows were considered to be ovulating at the ultrasound scan when 50% fewer follicles were counted than at the previous scan. From ultrasound recordings and estrus detection, intervals from wean-to-oestrus (WOI), oestrus-to-ovulation (OOI) and wean-to-ovulation were recorded. Sows that showed no signs of estrus during the first eight days after weaning were recorded as in anoestrus. Pregnancy and farrowing rates and total number of piglets born per litter were also recorded.

Statistical Analysis

IBM SPSS software (IBM Spain, Madrid, Spain) was used for data analysis. The Wilk-Shapiro test was used to test the normality of the count data, and those that did not follow a normal distribution were log-transformed or analysed by nonparametric tests. Student's t-test and ANOVA approaches were used to evaluate normally distributed or log-transformed count data. The Mann-Whitney test was used for analyzing non-normally distributed count data. The Chi-square test or Fisher's exact test were used to analyse differences in percentage data and the frequency distribution of intervals (WOI, OOI, wean-to-ovulation). Pearson's and Spearman's correlation tests were used to calculate the relationships between variables. Hierarchical cluster analysis was used to objectively group sows according to mean ovarian follicle size. Differences were considered statistically significant at $p < 0.05$.

Experimental Designs

Four different experimental studies are included, each addressing a specific objective, namely.

Objective 1. To evaluate seasonal differences in ovarian follicular development and to assess the implication of these putative differences in the clinical reproductive disorders characterizing seasonal infertility.

Experimental Design

A total of 58 and 52 healthy crossbred sows were randomly selected at weaning (8-10 sows every 2 weeks) from February to March (winter-spring period, WS) and from July to September (summer-autumn period, SA), respectively. The WOI and OOI were recorded. Sows that did not show oestrus within 14 d of weaning were considered to be in *anoestrus.* Sows showing oestrus were AI at 12 and 36 h after the beginning of oestrus. The inseminated sows were exposed to a healthy mature boar at 18-28 d after the onset of oestrus to identify potential returns to oestrus. Pregnancy was confirmed 28 d after AI. Transrectal ultrasounds of the ovaries were performed, as described above, once daily from weaning until the start of oestrus and twice daily (every 12 h) thereafter until ovulation.

Results

Six sows, all belonging to the WS period, were removed from the experiment due to the absence of follicles in the ovaries at the first scan after weaning. Therefore, follicular growth and follicle numbers were analysed in 52 weaned sows in each of the two seasonal periods. The ovaries of 10 sows had growing follicles between 0.2 and 0.5 cm in diameter at weaning, but they did not trespass 0.5 cm in diameter during the subsequent 14 d. None of these sows showed signs of oestrus. Of the 10 sows, only one was recorded in the WS groups, while the other nine were recorded in the SA group ($p < 0.05$). WS sows had the largest follicles at weaning, at the start of oestrus and at the ovulation time ($p < 0.01$). There were no differences in follicular counts between WS and SA sows.

The distribution of WOIs differed between the two seasons ($p < 0.001$). WOIs of 3-6 d were recorded in 50/51 WS sows (98.0%) and in only 31/43 SA sows (72.1%). Five of the 43 SA sows (11.6%) experienced a WOI higher than 10 d. The distribution of OOIs did not differ between seasons. Nevertheless, 2/43 (4.6%) SA sows in oestrus had a longer OOI than usual. The average diameter of the ovarian follicles at weaning was negatively correlated with the WOI ($p < 0.001$).

Fifty-one of 52 (98.1%) WS sows and 43/52 (82.7%) SA sows came into oestrus and were inseminated. The percentage of pregnant sows that failed to farrow was lower in WS (1/47, 2.1%) than in SA (5/43, 11.6%; $p < 0.05$). The percentage of sows that farrowed was higher in WS (46/51, 90.2%) than in SA (32/43, 74.4%; $p < 0.05$). Similarly, the number of piglets born per litter was larger in WS than in SA ($p < 0.01$). The WS sows had on average 1.5 piglets more than SA sows. Finally, sows with a WOI of 3 to 6 d had lower gestation loss rates ($p < 0.05$) and higher farrowing percentages ($p < 0.001$) than those with a WOI > 6 d, regardless season.

Conclusions

The development of ovarian follicles is affected in sows weaned during the summer-autumn period when sows manifest signs of seasonal infertility. This impairment in follicular development could be the origin of the clinical reproductive disorders that characterize seasonal infertility in weaned sows.

Objective 2. To reveal whether the weaning season, parity, body condition and lactation length would be causal factors explaining the heterogeneity among sows in follicular size at weaning. And also to analyse whether this heterogeneity already existed during lactation.

Experimental Design

This objective was approached through 2 experiments, namely 2.1. and 2.2. Experiment 2.1. aimed to elucidate the factors influencing the size of ovarian follicles at weaning and their influence on the reproductive performance of weaned sows. Experiment 2.2. explored the ovarian follicle growth during lactation period and monitored its influence on reproductive performance of sows after weaning.

In the experiment 2.1., serial ultrasound studies of the ovaries were performed on 191 weaned sows, 95 during SA period and 96 during WS period. The factors evaluated were season of weaning, parity, body condition and lactation length. WOI, OOI, weaning to ovulation interval, farrowing rates and total numbers of piglets born were recorded, following the procedures described above.

In experiment 2.2., the ovaries of 40 lactating sows were scanned, 20 during the SA period and 20 during the WS period. Specifically, the ovaries were scanned on days 7, 14 and 21 of the lactation period, and follicle sizes were recorded.

Results

In experiment 1.1, weaning season and parity influenced ($p < 0.01$) follicular size at weaning, but neither body condition (ranging from 2.5 to 3.5) nor lactation length (length between 20 and 28 d) did. There were more sows with small follicles (0.2 to 0.3 cm) at weaning in SA than in WS, while there were more sows with large follicles (0.4 to 1.0 cm) in WS than in

SA. The percentage of sows with small follicles was higher among those of lower parities (1-3) than among those of higher parity (4-6), whereas the percentage of sows with large follicles was higher among those of parity 4-6. A total of 26 sows showed anoestrus (no post-weaning oestrus, or detected later than 8 d after weaning), 24 during SA and 2 during WS ($p < 0.001$). The incidence of anoestrus was higher ($p < 0.01$) in sows with small follicles (29.7%) than in those with medium (0.31 to 0.39 cm; 13.3%) or large follicles (7.25%). Follicular size at weaning influenced ($p < 0.01$) WOI, OOI and the wean-to-ovulation interval. Sows with small follicles had the longest intervals. Follicle size at weaning did not influence farrowing rates but it did influence litter size ($p < 0.05$).

In experiment 2.2, ovarian follicle sizes varied among lactating sows at each of the three measurement times. Despite this, there was a consistent relationship between the three measurements ($R^2 = 0.85$). The mean follicle size at the three measurements was larger ($p < 0.05$) in WS sows than in SA sows.

Conclusions

Sows showed clear differences in the diameter of ovarian follicles at weaning, differences that were sustained from the beginning of lactation. Sows weaned during the summer-autumn period and those with fewer farrowing showed a higher incidence of small follicles (less than 0.3 cm in diameter). Overall, the sows with small follicles at weaning had lower reproductive performance after weaning.

Objective 3. To explore whether a short-term treatment of Altrenogest during the last days of lactation could regulate follicular waves at weaning and thereby improve the reproductive performance of weaned sows, regardless of weaning season.

Experimental Design

A total of 90 sows, 50 in WS period and 40 in SA period, were randomly selected 8 d before weaning and randomly distributed in two experimental groups. The sows of one group (23 sows in WS and 20 sows in SA) were orally treated during 6 d, finishing 2 d before weaning, with altrenogest (20 mg of Regumate®, MSD Animal Health, Kenilworth, NJ, USA). The sows of the second group, control group, were untreated (27 sows in WS and 20 sows in SA). Ovarian functional status, oestrus control, inseminations and reproductive records were evaluated and recorded as mentioned above.

Results

Thirteen of the 90 sows showed no signs of oestrus within 8 d after weaning. They were distributed similarly between the experimental groups, six in the control (12.76%) and seven in the altrenogest group (16.28%) but differently ($p < 0.01$) between seasons, one in WS (2.00%) and 12 in SA (30.00%). The number of ovarian follicles at the beginning of oestrus was similar between groups, regardless of season. However, follicle diameter differed between groups and season ($p < 0.01$). Diameter was greater in sows of the altrenogest group (mean 0.76 ± 0.01 cm) than in those of the control group (mean 0.73 ± 0.01 cm).

The percentage of sows farrowed was similar between altrenogest and control (94.4% and 90.2%, respectively) and between WS and SA (93.9% and 89.3%, respectively). However, the number of piglets born per litter was higher ($p < 0.01$) in sows of altrenogest group (14.00 ± 0.46) than in sows of control group (12.27 ± 0.44), regardless of season.

Conclusions

Short-term treatment with altrenogest during some days prior to weaning improves reproductive performance of weaned sows. In particular, the treatment improves litter size, probably by ensuring a large and homogeneous number of follicles able to ovulate at the onset of oestrus.

Objective 4. To evaluate whether the response of weaned sows to treatment with the GnRH agonist buserelin to induce and synchronize ovulation depends on the size of ovarian follicles at the time of treatment.

Experimental Design

A total of 352 sows were randomly selected at weaning, 174 in WS period and 178 SA period. The sows were randomly distributed in two groups, one with 172 sows (84 in WS and 88 in SA) was treated at 86 h after weaning with GnRH agonist buserelin (10 µg of buserelin acetate, Porceptal®, MSD, Animal Health, Kenilworth, NJ, USA). The other 180 sows (90 in WS and 90 in SA) were untreated (control group). Ovulation after GnRH administration was expected between 120 and 132 h after weaning (32-44 h after treatment). Sows were clustered into three groups according to whether they had a small (< 0.5 cm in diameter), medium (0.5-0.64 cm) or large (0.65 - 1.19 cm) mean ovarian follicular diameter at the time of treatment. Ultrasound monitoring of ovarian follicular development and ovulation, estrus control, WOI and OOI intervals, inseminations and reproductive records of pregnant sows were evaluated and recorded following the above procedures.

Results

The GnRH agonist treatment did not modify the incidence of sows showing anoestrus post-weaning. None of the anoestrus sows ovulated. The percentage of sows that ovulated was similar in both groups, 87.2% in the treatment and 88.3% in the control. Intervals (WOI and OOI) were shorter ($p < 0.001$) for GnRH agonist-treated sows than for control sows. More GnRH treated than control sows ovulated within the expected time window, 71.5% vs. 26.11%, respectively ($p < 0.001$). There were no differences between the two groups of sows in farrowing rate and litter size.

The proportion of sows that ovulated within the expected time window (WOI of 120-132 h) increased in both sow groups as follicular size increased. Among sows with large and medium follicles, 84.6% of treated sows ovulated within the expected time window while only 36.2% of control sows did ($p < 0.001$). In contrast, there was no difference between the two groups in the percentage of sows with small follicles that ovulated, being 28.6% in those treated with GnRH and 0% in the control. Most of the sows with small ovarian follicles were found during the SA period (20,5% in SA and 4.8% in WS; $p < 0.01$). There were no differences between the two groups of sows in farrowing rate and litter size.

Conclusions

As expected, the GnRH agonist buserelin is effective in inducing and synchronizing ovulation in most weaned sows. The great majority of sows that do not respond to GnRH-treatment have small ovarian follicles at the time of treatment, and they are more frequent among those weaned during the summer-autumn than during the winter-spring.

CONCLUSIONS

CONCLUSIONS

1. Disorders in ovarian follicular development would be the primary cause of the poor reproductive performance shown by weaned sows during so-called seasonal infertility.

2. Sows show clear differences in the diameter of ovarian follicles at weaning, differences that have been present since the beginning of lactation and are more noticeable in sows weaned during summer and autumn.

3. Sows with small ovarian follicles at weaning are prone to poor post-weaning reproductive performance and their incidence is higher during the summer and autumn and in sows with fewer parities.

4. Short-term treatment with Altrenogest during the last days of lactation ensures a large and homogeneous number of follicles capable of ovulating at the onset of estrus, thereby increasing the total number of piglets born in inseminated sows.

5. Treatment with the GnRH agonist buserelin, commonly used to induce and synchronize ovulation in weaned sows, is of low effectivity in sows with small ovarian follicles at the time of the treatment.

ABBREVIATIONS

ABBREVIATIONS

NPD	Non-Productive Days
AI	Artificial Insemination
WS	Winter-Spring period
SA	Summer-Autumn period
FR	Farrowing Rate
LS	Litter Size
WOI	Weaning-to-Oestrus Interval
OOI	Oestrus-to-Ovulation Interval
BCS	Body Condition Score
LH/pLH	Luteinising Hormone
GnRH	Gonadotropin-Releasing Hormone
hCG	Human Chorionic Gonadotropin
FSH	Follicle Stimulating Hormone
eCG	Equine Chorionic Gonadotropin
IGF-1:	Insuline-like Growth Factor I

RESUMEN GENERAL

RESUMEN GENERAL

Introducción

La industria cárnica porcina se caracteriza por la necesidad de alta eficiencia en la producción cárnica. De hecho, a menudo la eficiencia se mide en kg de carne producida por unidad productiva, que es la cerda. Un indicador importante respecto de la eficiencia en las granjas de porcino es el de *días no productivos*, que son los días que una cerda no está gestante ni en lactación. El indicador lo componen básicamente cerdas que presentaron problemas de expresión de celo tras el destete (celo retrasado o ausente) o cerdas que no quedan gestantes después de la inseminación artificial (IA).

La cerda destetada supone más del 80% de las cubriciones en la granja de producción cárnica. Por consiguiente, es un foco de atención donde centrar los esfuerzos de mejora en productividad. Tras el destete, las cerdas muestran un celo fértil en 3-6 d (Knox & Zas, 2001). Sin embargo, las cerdas con largos intervalos destete-estro (IDE) o que no muestran signos de estro tras el destete aún son comunes en las granjas. Las razones que hay tras esta pérdida de eficiencia reproductiva que provocan un incremento en los días no productivos no están del todo dilucidadas aún.

En la cerda doméstica, la fertilidad está disminuida durante el verano y el otoño, especialmente en las regiones cálidas con estaciones bien definidas (de Rensis et al., 2017). Esto se conoce como *infertilidad estacional* y conlleva descensos moderados de tasas de parto y prolificidad (Bertoldo et al., 2012), incrementos en el IDE, mayor incidencia de abortos (Peltoniemi & Virolainen, 2006) y mayor depresión de la actividad ovárica (Love et al., 1993). Los efectos de la estación del año sobre la función ovárica durante la gestación, lactación y destete no están bien elucidados. Un objetivo de la presente tesis es el estudio de las diferencias estacionales en el desarrollo folicular ovárico en cerdas destetadas, y la evaluación las implicaciones de estas diferencias, si son halladas, en las manifestaciones clínicas de la infertilidad estacional.

La nutrición, la temperatura ambiental y el fotoperiodo son los factores más influyentes en la infertilidad estacional. Consecuentemente, estas variables fueron controladas durante los experimentos de esta tesis.

La dinámica folicular puede ser monitorizada mediante ecografía. La aplicación transrectal permite, adicionalmente, un contaje y medición precisos de los folículos (Soede et al., 1998; Bolarín et al., 2008; 2009). La dinámica folicular es un proceso de crecimiento y regresión de los folículos en el ovario durante la fase folicular, y está influenciada por la estación del año, la condición corporal, la edad de parto y la duración de la lactación (Knox, 2019). Los folículos en el ovario son reclutados y seleccionados para crecer y posteriormente ovular. El reclutamiento, sin embargo, sucede seguido de atresia folicular en oleadas durante toda la lactación, con la cerda en anoestro. Sin embargo, los folículos aún pueden pasar a la fase de reclutamiento, dependiendo de factores externos tales como un descenso en la intensidad del estímulo de succión de los lechones, o largos períodos de lactación (Kemp & Soede, 2012). Al destete, los folículos se hallan en diferentes momentos de reclutamiento y atresia, en una dinámica que definirá un más o menos largo IDE (Lucy et al., 2001). Consecuentemente, el tamaño de los folículos al destete es marcadamente diferentes entre las cerdas al destete (Bracken et al., 2003). Los factores detrás de esta variabilidad permanecen poco claros

(Knox, 2019), y su definición y control es uno de los objetivos de la presente tesis.

El control exógeno de la función ovárica es una práctica común en la industria porcina. La sincronización del celo se consigue mediante el uso de Altrenogest, un progestágeno sintético que inhibe el desarrollo folicular (Wang et al., 2018). Sin embargo, no consiguió homogeneizar el tamaño folicular durante la dinámica folicular alrededor del destete (van Leeuwen et al., 2010; 2011). Existen otras hormonas para la sincronización del celo que son análogas de la GnRH, tales como la buserelina (Pearodwong et al., 2019). Sin embargo, la respuesta de las cerdas a este tratamiento es variable, con menos del 80% de las cerdas ovuladas en el momento esperado (Knox et al., 2011; Driacourt et al., 2013). Las causas que provocan esta variabilidad y respuesta subóptimas no están claras. Se han estudiado algunas de ellas, tales como el momento y la ruta de administración (Driancourt et al., 2013; Knox et al., 2011), pero otras no han sido estudiadas, como es el caso del estado de los folículos ováricos en el momento del destete, y la estación del destete. Este tema constituye uno de los objetivos de la presente tesis doctoral, enfocada al uso de Altrenogest y el análogo de GnRH acetato de buserelina. Además, la presente tesis incluye cuatro estudios experimentales enfocados al examen ecográfico de la dinámica de crecimiento folicular ovárico en las cerdas en lactación y tras el destete que muestran diferencias en la capacidad reproductiva tras el destete por factores ambientales (estación del año del destete y la IA), nutricionales (condición corporal) o de manejo (edad de parto y duración de la lactación).

Objetivos

Por lo anteriormente expuesto, el principal objetivo de esta tesis es el de investigar y controlar hormonalmente la dinámica de crecimiento folicular ovárico en la cerda durante la lactación y el destete, para establecer estrategias que conlleven una reducción de los días no productivos de las cerdas en las granjas. Para alcanzar este objetivo general, la tesis contempla cuatro objetivos específicos:

1. Evaluar las diferencias estacionales en el desarrollo folicular ovárico en cerdas destetadas, y establecer las implicaciones de estas diferencias en las consecuencias reproductivas clínicas relacionada con la infertilidad estacional.

2. Revelar si la época de destete, la edad de parto, la condición corporal y la duración de la lactación podrían ser factores causales que explicaran la heterogeneidad entre cerdas en el tamaño folicular al destete. Asimismo, analizar si esta heterogeneidad ya existe al inicio de la lactación.

3. Explorar si un tratamiento de corta duración de Altrenogest durante los últimos días de lactación podría regular las oleadas foliculares en el destete y por consiguiente mejorar la eficiencia reproductiva de las cerdas destetadas, independientemente de la estación del año del destete.

4. Evaluar si la respuesta de las cerdas destetadas al tratamiento con el agonista de la GnRH buserelina para inducir y sincronizar la ovulación depende del tamaño de los folículos ováricos en el momento del tratamiento.

Material y Métodos

Los experimentos llevados a cabo en esta tesis involucraron animales. Los procedimientos de manejo empleados se llevaron a cabo de acuerdo con la Directiva vigente 2010/63/EU ECC de 22 de septiembre de 2010 relativa a la protección de los animales utilizados para fines científicos, y fueron revisados y aprobados por el Comité Ético para la Experimentación con Animales de la Universidad de Murcia (Murcia, España).

Localización de la granja, animales, alojamiento y manejo

Los experimentos fueron llevados a cabo bajo condiciones de campo en una granja comercial porcina de explotación intensiva de 1,800-2,500 cerdas, localizada en el sudeste de España (37°59'NL, 1°08'WL). La insolación en la granja oscila entre las 14 h y 48 min en el solsticio de verano y las 9 h y 32 min en el solsticio de invierno. La temperatura de aire máxima media varió entre los 32.8 (verano) y los 17.7°C (invierno) durante los años que duraron los experimentos. La granja cumplía con los mandatos legislativos de la Unión Europea española en términos de producción, salud, bioseguridad y bienestar animal. Por otro lado, la granja contaba con control ambiental sólo en las salas de parto, donde sistemas evaporativos y ventiladores de pared mantenían la temperatura alrededor de 24°C. El resto de las dependencias no contaban con control de temperatura y tenían acceso libre a la luz natural. Las cerdas siempre disfrutaban de acceso libre a agua de bebida, y eran alimentadas con ración comercial cuya composición variaba según su requerimiento fisiológico. La granja, además, tenía un historial consistente de infertilidad estacional, que se manifestaba recurrentemente con tasas incrementadas de cerdas con largos IDE y reducidas tasas de parto y tamaños de camada en verano y a principio de otoño.

Las cerdas que participaron en los estudios fueron híbridas Landrace x Large White de edad de parto 1-6. Fueron seleccionadas aleatoriamente en el momento del parto o del destete, de entre las cerdas propias de la granja. Los datos reproductivos previos de las cerdas, así como su condición corporal al principio del experimento siempre fueron registrados y analizados.

Manejo de la reproducción en la granja

Tras un período de lactación de 20-28 d, las cerdas destetadas eran ubicadas en jaulas individuales para la detección del celo, inseminación y posterior diagnóstico de gestación. La detección del celo comenzaba el día después del destete, y se realizaba dos veces por día, a las 07:00-08:00 h y a las 18:00-19:00 h. La detección de celo se llevaba a cabo por personal experimentado bajo la supervisión de los investigadores, en presencia de un verraco adulto maduro. El inicio del celo se definía como 6 h antes de la primera vez que la cerda mostraba signos de inmovilidad a la presión dorso-lumbar ejercida por el operario durante el contacto nasal entre la hembra y el verraco, situado en un pasillo central en frente de las jaulas. Una vez en celo, las cerdas eran inseminadas mediante inseminación post-cervical 2 o 3 veces, con una dosis con 40 ml que contenía 1.5×10^9 espermatozoides totales, con motilidad en fresco ≥75% y formas anormales espermáticas ≤30%. Las dosis seminales provenían de una mezcla de 1-5 eyaculados de varios verracos no pre-seleccionados (heterospermias) y eran adquiridas a un centro de inseminación independiente (AIM Ibérica, Topigs Norsvin España, España). Las dosis seminales permanecían a 15-17°C durante 12-48 h antes de su uso. Veintiocho días después de la IA, se realizaba un diagnóstico de gestación mediante ecografía transabdominal. Las cerdas gestantes eran agrupadas en grupos de 10 en cuadras de 25 m^2 donde permanecían hasta 7 días previos al parto, cuando eran trasladadas a las salas de parto donde permanecían en jaulas de parto individuales hasta el destete.

Ecografía transrectal ovárica

Para el examen ecográfico ovárico se utilizó un equipo ecográfico portátil (LOGIQ Book XP; GE Healthcare) equipado con software de registro de imágenes y con una sonda transrectal lineal de frecuencia múltiple de 4-10 MHz (i739-RS, GE Healthcare). La metodología que se siguió es la propuesta por Bolarín et al., (2009); primero, retiramos el contenido fecal de la ampolla rectal de forma manual. Seguidamente, introducimos el transductor en posición ventral utilizando un guante de exploración largo recubierto con vaselina. El contacto entre la mucosa rectal y el transductor debe ser estrecho. La frecuencia preferida en el transductor lineal es de 8 MHz, que es la mejor opción para el contaje y medición de folículos ováricos. Mediante esta técnica, podemos medir y contar folículos a partir de 0.2 cm. Los ovarios se localizan usualmente cerca de la vejiga de la orina, a unos 35-45 cm del esfínter anal. Los folículos se caracterizan por ser estructuras anecoicas con bordes irregulares, finos y ecogénicos, bien diferenciados del estroma ovárico. Estos folículos alcanzan un tamaño de 0.7-1.0 cm antes de su ovulación. Con este tamaño, la forma de los folículos cambia y se vuelve más irregular debido a la compresión que ejercen unos sobre otros en un ovario con espacio limitado. Aquellos folículos que superan un diámetro de 1.1 cm se consideran quistes foliculares, y normalmente no ovularán. La ovulación se identifica ecográficamente porque los folículos aparecen colapsados y en su lugar se pueden identificar estructuras irregulares hiperecogénicas que corresponden con cuerpos hemorrágicos o estados tempranos de cuerpos lúteos. Los cuerpos lúteos se evidencian como estructuras circulares hiperecogénicas de 0.8-1.4 cm de bordes difusos, que son difíciles de contar y medir mediante ecografía.

Pautas seguidas en los experimentos

Todas las ecografías transrectales fueron realizadas por la misma investigadora (T. Lopes), y comenzaban el mismo día del destete, excepto en algunos experimentos específicos en los que las ecografías comenzaban durante el período de lactación, en los días 7, 14 y 21 tras el inicio de éste. Cada cerda fue ecografiada cada 24 h hasta la detección del celo, y después cada 12 h hasta el diagnóstico de ovulación. Las ecografías transrectales se centraban en identificar la presencia de folículos en los ovarios, lo cual indica cerdas destetadas reproductivamente sanas. No obstante, las cerdas que presentaron ovarios inactivos (con folículos menores de 0.3 cm) o con otras estructuras funcionales (cuerpos lúteos o cuerpos hemorrágicos) o no funcionales (quistes foliculares) también fueron registradas. Para cada cerda, ambos ovarios fueron escaneados cada vez, por separado y en diferentes secciones, y fueron registrados al menos 3 secuencias de video en cada uno usando el software digital calibrado del propio ecógrafo. Los vídeos fueron después analizados y sobre ellos se contó los folículos ováricos y se midió el diámetro promedio en dos o tres de sus folículos más representativos. Las cerdas se consideraban ovulando cuando en la ecografía se hallaba un 50% menos de folículos con respecto a la ecografía previa. Los siguientes intervalos fueron registrados: intervalo destete-estro (IDE), intervalo estro-ovulación (IEO) e intervalo destete-ovulación (IDO). Las cerdas que no mostraron signos de celo durante los primeros 8 días después del destete fueron registradas como en anoestro. Finalmente, se registró la tasa de parto y el número total de lechones nacidos de cada cerda implicada en el estudio.

Análisis estadístico

Para el análisis de datos se utilizó el software IBM SPSS (IBM Spain, Madrid, España). El test Wilk-Saphiro se aplicó como test de normalidad de los datos, y aquellos que no presentaron una distribución normal fueron transformados logarítmicamente o analizados mediante técnicas no paramétricas. Para analizar los datos con distribución normal o transformados logarítmicamente se aplicaron las pruebas t de Student y ANOVA. Para analizar los datos con distribución no normal se aplicó la prueba Mann-Whitney. Las diferencias en los datos expresados como porcentaje y las frecuencias de distribución de intervalos (WOI, OOI, destete-ovulación) fueron analizados mediante el test Chi-cuadrado o la prueba exacta de Fisher. Para calcular la relación entre variables se aplicaron las pruebas de correlación de Pearson y Spearman. Con el objetivo de agrupar a las cerdas según el promedio de tamaño folicular se implementaron análisis jerárquicos grupales. Se consideraron diferencias estadísticamente significativas con valor $p < 0.05$.

EXPERIMENTOS Y RESULTADOS

Para la consecución del objetivo principal de esta tesis se planificaron cuatro estudios experimentales, cada uno con sus propios objetivos.

Objetivo 1. Evaluación de diferencias estacionales en el desarrollo folicular ovárico, y valoración de la implicación de estas diferencias putativas en los desórdenes clínicos reproductivos que caracterizan a la infertilidad estacional.

Diseño experimental

Un total de 58 y 52 cerdas híbridas fueron seleccionadas en el momento del destete (8-10 cerdas cada 2 semanas) desde febrero a marzo (periodo invierno-primavera, IP) y desde julio a septiembre (periodo verano-otoño, VO), respectivamente. Los intervalos IDE y IEO fueron registrados para cada cerda. Aquellas cerdas que no mostraron signos de celo en los 14 d post-destete, fueron consideradas en anoestro. Las cerdas que mostraron celo fueron inseminadas tras la su detección, a las 12 y 36 h. Las cerdas inseminadas fueron expuestas a un verraco sano maduro tras la detección del celo en los días 18-28 para identificar posibles retornos a celo y ausencia de gestación. La gestación fue confirmada a los 28 d tras la IA. Las ecografías transrectales ováricas se realizaron tal y como se describió anteriormente, una vez al día desde el destete hasta el inicio del celo, y luego dos veces al día (cada 12 h) hasta el diagnóstico de ovulación.

Resultados

Se eliminaron del estudio 6 cerdas, todas ellas pertenecientes al periodo IP, debido a la ausencia de folículos ováricos en la primera ecografía realizada tras el destete. Consecuentemente, el crecimiento y el contaje folicular fue realizado en 52 cerdas destetadas en cada uno de los dos períodos estacionales definidos. Los ovarios de 10 cerdas presentaron folículos en crecimiento entre 0.2 y 0.5 cm al destete, pero no traspasaron los 0.5 cm de diámetro durante los siguientes 14 d. Ninguna de estas cerdas presentó signos de celo. De las 10 cerdas, sólo una fue registrada en el grupo IP, mientras que las otras 9 pertenecieron al grupo VO ($p < 0.05$). Las cerdas del grupo IP presentaron folículos mayores al destete, al inicio del celo y en el momento de la ovulación ($p < 0.01$). No se encontraron diferencias en el número de folículos entre las cerdas IP y VO.

La distribución del IDE fue diferente entre los dos períodos ($p < 0.001$). Se encontraron IDE de 3-6 d en 50/51 de las cerdas del grupo IP (98.0%) pero sólo en 31/43 de las cerdas del grupo VO (72.1%). Cinco de las 43 cerdas del grupo VO (11.6%) experimentaron un IDE mayor de 10 d. La distribución del IEO no difirió entre los períodos VO e IP. Sin embargo, 2/43 cerdas de las cerdas del grupo VO (4.6%) que mostraron celo presentaron un IEO mayor de lo normal. El diámetro promedio de los folículos ováricos al destete se correlacionó negativamente con el IDE ($p < 0.001$).

Cincuenta y una de 52 (98.1%) cerdas del grupo IP y 43/52 (82.7%) cerdas del grupo VO mostraron signos de celo y fueron inseminadas. El porcentaje de cerdas gestantes que no llegó a parir fue menor en IP (1/47, 2.1%) que en VO (5/43, 11.6%: $p < 0.05$). Paralelamente, el porcentaje de cerdas que parió fue mayor en IP (46/51, 90.2%) que en VO (32/43, 74.4%; $p < 0.05$). Similarmente, el número de lechones nacidos por camada fue mayor en el período IP que en VO ($p < 0.01$). Las cerdas del período IP tuvieron de media 1.5 lechones más que las cerdas del período VO. Finalmente, las cerdas con un IDE de 3 a 6 d presentaron menores pérdidas de gestación ($p < 0.05$) y mayores porcentajes de parto ($p < 0.001$) que aquellas con IDE > 6 d, independientemente del período seleccionado.

Conclusiones

El desarrollo de los folículos ováricos se vio afectado en las cerdas durante el período verano-otoño cuando las cerdas mostraron signos de infertilidad estacional. Esta discapacidad en el desarrollo folicular ovárico podría ser el origen de los desórdenes que caracterizan a la infertilidad estacional en las cerdas destetadas.

Objetivo 2. Confirmación de si la época del año del destete, la edad de parto, la condición corporal y la duración de la lactación podrían ser factores causales que expliquen la heterogeneidad entre las cerdas en cuanto al tamaño folicular ovárico al destete. También, el análisis de si esta heterogeneidad ya está establecida durante la lactación.

Diseño experimental

El presente objetivo se abordó mediante 2 experimentos. El experimento 2.1. trató de dilucidar los factores que influyen en el tamaño folicular ovárico al destete y su influencia en la capacidad reproductiva de la cerda destetada. El experimento 2.2. exploró el crecimiento folicular ovárico durante el período de lactación y monitorizó su influencia en la capacidad reproductiva de la cerda tras el destete.

En el experimento 2.1. se realizaron estudios ecográficos seriados en 191 cerdas: 95 durante el período VO y 96 durante el período IP. Los ovarios fueron escaneados a los 7, 14 y 21 d del período de lactación tras el parto, registrando el tamaño y número de folículos ováricos en cada caso.

Resultados

En el experimento 1.1. la época de destete y la edad del parto influyó ($p < 0.01$) en el tamaño folicular ovárico al destete, pero no influyó la condición corporal (que se situó entre 2.5 y 3.5) ni la duración de la lactación (entre 20 y 28 d). Hubo más cerdas con folículos pequeños (0.2-0.3 cm) al destete en VO que en IP, mientras que hubo más cerdas con folículos grandes (0.4-1.0 cm) en IP que en VO. El porcentaje de cerdas con folículos pequeños fue mayor entre aquellas cerdas de menos partos (1-3) que entre aquellas de más partos (4-6), mientras que el porcentaje de cerdas con folículos grandes fue mayor entre las cerdas con más partos (4-6). Un total de 26 cerdas mostraron anoestro (ausencia de signos de estro en los 8 d posteriores al destete), 24 durante el período VO y 2 durante el período IP ($p < 0.001$). La incidencia de anoestro fue mayor ($p < 0.01$) en cerdas con folículos pequeños (29.7%) que en aquellas con folículos medianos (0.31-0.39 cm; 13.3%) o grandes (7.25%). El tamaño folicular al destete influyó ($p < 0.01$) los IDE, IEO e IDO. Las cerdas con folículos pequeños mostraron los intervalos mayores. El tamaño folicular al destete no influyó la tasa de partos, pero si el tamaño de camada ($p < 0.05$).

En el experimento 2.2. el tamaño folicular ovárico varió entre las cerdas lactantes en cada una de las tres sesiones de medida. Sin embargo, se encontró una correlación consistente entre las tres mediciones ($R^2 = 0.85$). El tamaño folicular promedio en las tres medidas fue mayor ($p < 0.05$) en las cerdas del período IP que en las del período VO.

Conclusiones

Se demostró una claras diferencia entre los diámetros foliculares ováricos en las cerdas al destete, las cuales se mantuvieron desde el inicio de la lactación. Las cerdas destetadas durante el período verano-otoño y aquellas con menos partos mostraron mayor incidencia de folículos pequeños (menos de 0.3 cm de diámetro). Resumidamente, las cerdas con folículos pequeños al destete presentaron menor capacidad reproductiva tras el destete.

Objetivo 3. Explorar si un tratamiento limitado de Altrenogest durante los últimos días de lactación puede regular las oleadas foliculares al destete y así mejorar la capacidad reproductiva de las cerdas destetadas, independientemente de la estación de destete.

Diseño experimental

Se seleccionó un total de 90 cerdas, 50 en el período IP y 40 en el período VO, 8 d antes del destete, y fueron distribuidas aleatoriamente en dos grupos experimentales. Las cerdas del primer grupo (23 cerdas en IP y 20 cerdas en VO) fueron tratadas oralmente durante 6 d, terminando 2 d antes del destete, con Altrenogest (20 mg de Regumate®, MSD Animal

Health, Kenilworth, NJ, EEUU). Las cerdas del segundo grupo, el grupo control, no fueron tratadas (27 cerdas en IP y 20 cerdas en VO). El estado funcional ovárico, el control del celo, las inseminaciones y los registros reproductivos subsecuentes fueron evaluados y registrados.

Resultados

Trece de las 90 cerdas no llegaron a mostrar signos de celo en los 8 d tras el destete, y se distribuyeron de forma similar entre los grupos experimentales, seis en el grupo control (12.76%) y siete en el grupo administrado con Altrenogest (16.28%), pero se distribuyeron de forma diferente ($p < 0.01$) entre los períodos del año, una en IP (2.00%) y doce en VO (30.00%). El número de folículos ováricos al principio del estro fue similar entre los dos grupos, independientemente del período estacional. Sin embargo, el diámetro folicular ovárico fue diferente entre grupos y período estacional ($p < 0.01$). El diámetro fue mayor en las cerdas administradas con Altrenogest (media 0.76 ± 0.01 cm) que en las cerdas control (media 0.73 ± 0.01 cm).

El porcentaje de cerdas que parió fue similar entre los grupos Altrenogest y control (94.4% y 90.2%, respectivamente) y entre los períodos IP y VO (93.9% y 89.3%, respectivamente). Sin embargo, el número total de lechones nacidos por camada fue mayor ($p < 0.01$) en las cerdas del grupo administrado con Altrenogest (14.00 ± 0.46) que en las cerdas del grupo control (12.27 ± 0.44), independientemente de la época del año.

Conclusiones

Un tratamiento convencional de corta duración con Altrenogest durante algunos días previos al destete mejora la capacidad reproductiva de las cerdas destetadas. En particular, el tratamiento mejora el tamaño de camada, probablemente al garantizar más grandes y homogéneos folículos ováricos capaces de ovular al inicio del estro.

Objetivo 4. Evaluación de si la respuesta de las cerdas destetadas al tratamiento con el agonista de la GnRH buserelina para inducir y sincronizar la ovulación depende del tamaño de los folículos ováricos en el momento del tratamiento.

Diseño experimental

Un total de 352 cerdas fueron seleccionadas aleatoriamente al destete, 174 en el período IP y 178 en el período VO. Las cerdas fueron distribuidas aleatoriamente en dos grupos, uno con 172 cerdas (84 en IP y 88 en VO) que fue tratado a las 86 h post-destete con el agonista de la GnRH buserelina (10 µg de acetato de buserelina, Porceptal®, MSD, Animal Health, Kenilworth, NJ, EEUU). Las otras 180 cerdas (90 en IP y 90 en VO) formaron parte del grupo control y no fueron tratadas. La ovulación tras la administración del agonista de la GnRH se espera entre las 120 y las 132 h tras el destete (32-44 h tras el tratamiento). Las cerdas fueron agrupadas en tres lotes dependiendo de si mostraron un tamaño folicular pequeño (< 0.5 cm de diámetro), medio (0.5 – 0.64 cm) o grande (0.65 – 1.19 cm) en el momento del tratamiento. Se evaluaron y registraron las siguientes variables: monitorización ecográfica del desarrollo folicular y ovulación, control del celo, IDE y IEO, las IA y los registros reproductivos de las cerdas gestantes.

Resultados

El tratamiento con el agonista de la GnRH no modificó la incidencia de cerdas en anoestro tras el destete. Ninguna de las cerdas en anoestro llegó a ovular. El porcentaje de cerdas que ovuló fue similar en los grupos tratados y control (87.2% y 88.3% respectivamente). Los intervalos IDE y IEO fueron significativamente menores ($p < 0.001$) en las cerdas tratadas con el agonista de la GnRH que en las cerdas control. Las cerdas tratadas con el agonista de la GnRH ovularon dentro de la ventana esperada de ovulación más frecuentemente ($p < 0.001$) que el grupo control (71.5% vs 26.11%, respectivamente). No se encontraron diferencias entre los dos grupos en la tasa de partos ni en el tamaño de camada.

La proporción de cerdas que ovularon dentro de la ventana esperada (IDE de 120-132 h) se incrementó en ambos grupos según el tamaño folicular ovárico en el momento del tratamiento era mayor. Entre las cerdas con folículos grandes y medianos, el 84.6% de las cerdas tratadas ovuló en el momento esperado, mientras que sólo el 36.2% de las cerdas control lo hizo ($p < 0.001$). En contraste, no se encontró diferencias entre los dos grupos en el porcentaje de cerdas con folículos pequeños que ovularon en el momento previsto (28.6% en las cerdas tratadas con GnRH y 0% en las cerdas control). La mayoría de las cerdas con folículos ováricos pequeños se encontraron en el período VO (20.5% en SA frente al 4.8% en IP; $p < 0.01$). Finalmente, tampoco se encontró diferencias entre los grupos en tasa de partos ni en el número de lechones nacidos totales.

Conclusiones

Como suponíamos, el agonista de la GnRH acetato de buserelina es efectivo en la inducción y sincronización de la ovulación en la mayoría de las cerdas destetadas. La gran mayoría de cerdas que no respondieron a los tratamientos de GnRH presentaron tamaños foliculares ováricos pequeños en el momento del tratamiento, lo cual fue más frecuente entre aquellas cerdas destetadas en el período verano-otoño que en el período invierno-primavera.

CONCLUSIONES

1. Los desórdenes en el desarrollo folicular ovárico podrían ser la causa primaria de la pobre capacidad reproductiva de la cerda destetada durante el período de infertilidad estacional.

2. Las cerdas presentan claras diferencias en el diámetro folicular ovárico al destete, las cuales están presentes desde el inicio de la lactación y son más marcadas en las cerdas destetadas durante verano y otoño.

3. Las cerdas con folículos ováricos pequeños en el momento del destete son más propensas a una peor capacidad reproductiva post-destete, y su incidencia es mayor durante el verano y el otoño, y en cerdas con menos partos.

4. Un tratamiento corto con Altrenogest durante los últimos días de lactación asegura un mayor y más homogéneo número de folículos capaces de ovular al inicio del estro, incrementando así el número total de lechones nacidos por cerda inseminada.

5. El tratamiento con el agonista de la GnRH buserelina, comúnmente usado para sincronizar la ovulación en las cerdas destetadas, es de baja efectividad en cerdas con folículos ováricos pequeños en el momento del tratamiento.

ABREVIATURAS

IA	Inseminación Artificial.
IDE	Intervalo Destete-Estro.
IEO	Intervalo Estro-Ovulación.
IDO	Intervalo Destete-Ovulación.
IP	Período estacional Invierno-Primavera.
VO	Período estacional Verano-Otoño.
GnRH	Hormona Liberadora de Gonadotropinas (*Gonadotropin Releasing Hormone)*

REFERENCES

REFERENCES

Bertoldo M.J.; Holyoake P.K.; Evans G.; Grupen C.G. Seasonal variation in the ovarian function of sows. *Reprod. Fertil. Dev.* **2012**, 24(6), 822-34.

Bolarín A.; Vázquez, J.M.; Parrilla, I.; Vázquez, J.L.; Martínez, E.A.; Roca, J. Validation of trans-rectal ultrasonography for counting preovulatory follicles in weaned sows. *Anim. Reprod. Sci.* **2009**, 13(1-4): 137-42.

Bolarín, A.; Hernández, M.; Vázquez, J.M.; Rodríguez-Martínez, H.; Martínez, E.A.; Roca, J. Use of frozen-thawed semen aggravates the summer-autumn infertility of artificially inseminated weaned sows in the Mediterranean region. *J. Anim. Sci.* **2009**, 87, 3967-3975.

Boyer, P.E., Almond, G.W. Use of altrenogest at weaning in primiparous sows. *J. Swine Health Prod.*, **2014**, 22(3), 134-137.

Bortolozzo, F.P.; Menegat, M.B.; Mellagi, A.P.G.; Bernardi, M.L.; Wentz, I. New artificial insemination technologies for swine. *Reprod. Domest. Anim.* **2015,** 50 Suppl 2:80-4.

Bracken, C.J.; Lamberson, W.R.; Safranski, T.J.; Lucy, M.C. Factors affecting follicular populations on Day 3 postweaning and interval to ovulation in a commercial sow herd. *Theriogenology,* **2003**, 60, 11-20.

Britt, J.H.; Armstrong, J.D.; Cox, N.M.; Ebenshade, K.L. Control of follicular development during and after lactation in sows. *J. Reprod. Fertil. Suppl.* **1985**, 33, 37-54.

Cassar, G.; Kirkwood, R.N.; Poljak, Z.; Friendship, R. Effect of estrogen formulation and site of deposition on fertility of artificially inseminated sows treated with human chorionic gonadotrophin to induce ovulation. *J. Swine Health Prod.* **2004**, 12, 285-287.

Coffey, R.D.; Parker, G.R.; Laurent, K.M. Assessing sow body condition. Publication ASC 158. Cooperative Extension Service, College of Agriculture, University of Kentucky, Lexington, Kentucky, USA, 2 pp. http://www2.ca.uky.edu/agc/pubs/asc/as158/asc158.pdf (accessed 27th November 2013).

Dominguez, J.C.; Peña, F.J.; Anel, L.; Carbajo, M. Swine summer infertility syndrome in northwest Spain. *Vet. Rec.* **1996**, 139, 93-94.

Driancourt, M.A.; Cox, P.; Rubion, S.; Harnois-Milon, G.; Kemp, B.; Soede, N.M. Induction of an LH surge and ovulation by buserelin (as Receptal) allows breeding of weaned sows with a single fixed-time insemination, *Theriogenology*, **2013**, 80(4):391-399.

Fontana, D.L.; Ulguim, R.R.; Sbardella, P.E.; Bernardi, M.L.; Wentz, I.; Bortolozzo, F.P. Fixed-time post-cervical artificial insemination in sows receiving porcine luteinising hormone at oestrus onset. *Anim. Reprod. Sci.* **2014**, 144, 109-114.

Fries, H.C.C.; Souza, L.P.; Faccin, J.E.G.; Reckziegel, M.V.; Hernig, L.; Marimon, B.T.; Bernardi, M.L. Induction and synchronization of ovulation in sows using a Gonadotropin-releasing Hormone Analog (Lecirelin). *Anim. Reprod.* **2010**, 55, 362-366.

de Jong, E.; Laanen, M.; Dewulf, J.; Jourquin, J.; de Kruif, A.; Maes, D. Management factors associated with sow reproductive performance after weaning. *Reprod. Domest. Anim.* **2013**, Jun;48(3):435-40.

Kauffold, J.; Althouse, G.C. An update on the use of B-mode ultrasonography in female pig reproduction. *Theriogenology*. **2007**, 67(5), 901-11.

Kauffold, J.; Peltoniemi, O.; Wehrend, A.; Althouse, G.C. Principles and clinical uses of real-time ultrasonography in female swine reproduction. *Animals* **2019**, 9, 950.

Kemp, B.; Soede, N.M. Should weaning be the start of the reproductive cycle in hyper prolific sows? A physiological view. *Reprod. Domest. Anim.* **2012**, 47 (Suppl.), 320-326.

Kirkwood, R.N.; Kauffold, J. Advances in breeding management and use of ovulation induction for fixed-time AI. *Reprod. Domest. Anim.*, **2015**, 50 Suppl 2:85-9.

Knox, R.V. Factors influencing follicle development in gilts and sows and management strategies used to regulate growth for control of estrus and ovulation, *J. Anim. Sci.* **2019**, 97, 1433–1445.

Knox, R.V.; Willenburg, K.L.; Rodríguez-Zas, S.L.; Greger, D.L.; Hafs, H.D.; Swanson, M.E. Synchronization of ovulation and fertility in weaned sows treated with intravaginal triptorelin is influenced by timing of administration and follicle size. *Theriogenology*, **2011**, 75, 308-319.

Knox, R.V.; Zas, S.L. Factors influencing estrus and ovulation in weaned sows as determined by transrectal ultrasound. *J. Anim. Sci.* **2001**, 79, 2957-2963.

Koketsu, Y.; Dial, G.D. Factors influencing the post-weaning reproductive performance of sows on commercial farms. *Theriogenology* **1997**, 47, 1445-1461.

Koketsu, Y.; Dial, G.D.; Pettigrew, J.E.; King, V.L. Feed intake pattern during lactation and subsequent reproductive performance of sows. *J. Anim. Sci.* **1996**, 74(12), 2875-84.

Kraeling, R., Webel, S.K. Current strategies for reproductive management of gilts and sows in North America, *J. Anim. Biotechnol.* **2015**, 6(1):3.

van Leeuwen, J.J.; Martens, M.R.T.M; Jourquin, J.; Driancourt, M.A.; Wagner, A.; Kemp, B.; Soede, N.M. Follicle size and reproductive hormone profiles during a post-weaning altrenogest treatment in primiparous sows. *Reprod. Fertil. Develop.*, **2015**, 27(2), 304-12.

van Leeuwen, J.J.; Williams, S.I.; Kemp, B.; Soede, N.M. Post-weaning altrenogest treatment in primiparous sows; the effect of duration and dosage on follicular development and consequences for early pregnancy, *Anim. Reprod. Sci.*, **2010**, 119(3-4):258-64.

van Leeuwen, J.J.; Williams, S.I.; Martens, M.R.; Jourquin, J.; Driancourt, M.A.; Kemp, B.; Soede, N.M. The effect of different postweaning altrenogest treatments of primiparous sows on follicular development, pregnancy rates, and litter sizes, *J. Anim. Sci.*, **2011b**, 89(2):397-403.

Love, R.J.; Evans, G.; Klupiec, C. Seasonal effects on fertility in gilts and sows. *Reprod. Fertil. suppl.* **1993**, 48, 191-206.

Lucy, M.C.; Liu, J.; Boyd, C.K.; Bracken, C.J. Ovarian follicular growth in sows. *Reprod.* **2001**, 58 (Suppl.), 31-45. Review.

Martinat-Botté, F.; Venturi, E.; Guillouet, P.; Driancourt, M.A.; Terqui, M. Induction and synchronization of ovulations of nulliparous and multiparous sows with an injection of gonadotropin-releasing hormone agonist (Receptal), *Theriogenology*, **2010**, 73:332-342.

Mauget, R. Seasonal Anoestrus in Wild Sows. *In* Endocrine Causes of Seasonal and Lactational Anestrus in Farm Animals **1985**, 108–118. *Springer Netherlands*.

McBride, M.; Armezcua, R.; Cassar, G.; O'Sullivan, T.; Friendship, R. Combining Fixed-Time Insemination and Improved Catheter Design in an Effort to Improve Swine Reproduction Efficiency. *Animals* **2019**, 9, 748.

Nissen, A.K.; Lehn-Jensen, H.; Hyttel, P.; Greve, T. Follicular development and ovulation in sows: Effect of hCG and GnRH treatment. *Acta Veter. Scand.* **1995**, 36, 123-133.

Pearodwong, P.; Tretipskul, C.; Soede, N.M.; Tummaruk, P. Factors affecting estrus and ovulation time in weaned sows with induced ovulation by GnRH administration in different seasons. *J. Veter. Med. Sci.* **2019**, 81, 1567-1574.

Peltoniemi, O.A.; Virolainen, J.V. Seasonality of reproduction in gilts and sows. *Soc. Reprod. Fertil. Suppl.* **2006**, 62, 205-18.

Prunier, A.; Quesnel, H.; de Bragança, M.M.; Kermabon, A.Y. Environmental and seasonal influences on the return-to-oestrus after weaning in primiparous sows: a review. *Livest. Prod. Sci.* **1996**, 45, 103-110.

Quesnel H. Nutritional and lactational effects on follicular development in the pig. *Soc. Reprod. Fertil. Suppl.* **2009**, 66, 121-34.

de Rensis, F.; Kirkwood, R.N. Control of estrus and ovulation: Fertility to timed insemination of gilts and sows. *Theriogenology* **2016**, 86(6), 1440-1466.

de Rensis, F.; Ziecik, A.J.; Kirkwood, R.N. Seasonal infertility in gilts and sows: Aetiology, clinical implications and treatments. *Theriogenology* **2017**, 96, 111-117.

Roca. J.; Parrilla. I.; Bolarín, A.; Martínez, E.A.; Rodríguez-Martínez, H. Will AI in Pigs become more efficient. *Theriogenology* **2016**, Jul 1;86(1):187-93.

Soede, N.M.; Hazeleger, W.; Kemp, B. Follicle size and the process of ovulation in sows as studied with ultrasound. *Reprod. Dom. An.* **1998**, 33, 239-244.

Soede, N.M., Kemp, B. Oestrus expression and timing of ovulation in pigs. *J. Reprod. Fert.* **1997**, 52 (Suppl.), 91-103.

Soede, N.M.; Langendijk, P.; Kemp, B. Reproductive cycles in pigs. *Anim. Reprod. Sci.* **2011**, 124, 251-258.

Soede, N.M.; Wetzels, C.C.H.; Zondag, W.; de Koning, M.A.I.; Kemp, B. Effects of time of insemination relative to ovulation, as determined by ultrasonography, on fertilization rate and accessory sperm count in sows. *J. Reprod. Fertil.* **1995a**, 104, 99-106.

Vargas, A.J.; Bernardi, M.L.; Bortolozzo, F.P.; Mellagi, A.P.; Wentz, I. Factors associated with return to estrus in first service swine females, *Prev. Vet. Med.* **2009**, 89, 75-80.

Wang, Z.; Liu, B.S.; Wang, X.Y.; Wei, Q.H.; Tian, H.; Wang, L.Q. Effects of altrenogest on reproductive performance of gilts and sows: a meta-analysis, *Anim. Reprod. Sci.*, **2018**, 197:10-21.

Weiler, U.; Claus, R.; Denhard, M.; Hofäcker, S. Seasonal infertility, stress and adrenocritical responsiveness in pigs. *Anim. Reprod. Sci.* **1996**, 34, 265-279.

ARTICLE 1

The Veterinary Journal, 2014

Relevance of ovarian follicular development to the seasonal impairment of fertility in weaned sows

A field study was conducted to estimate seasonal differences in follicular development in weaned sows and to evaluate the implication of these differences on seasonal infertility. A total of 110 sows were selected at weaning during winter–spring (WS, n = 58) and summer–autumn (SA, n = 52). Ovaries were scanned once daily from weaning to the onset of oestrus and twice daily from then until ovulation. Six sows during WS were removed from study for not showing growing follicles at weaning. Oestrus was evaluated twice daily from day 1 after weaning to day 14 post-weaning. One of 52 (1.9%) sows in WS and 9/52 (17.3%) in SA showed no signs of oestrus within 14 days of weaning ($P < 0.05$). The diameters of the follicles at weaning, at the onset of oestrus and just before ovulation were smaller ($P < 0.01$) in SA sows than in WS sows. There were fewer follicles in SA sows than in WS sows just before ovulation ($P < 0.05$). Fifty of 51 (98.0%) sows in WS and 31/43 (72.1%) sows in SA experienced a weaning-to-oestrus interval (WOI) of 3–6 days ($P < 0.05$). Fifty-one of 52 (98.1%) sows in WS and 43/52 (82.7%) sows in SA were inseminated; the percentage of pregnant sows that failed to farrow was lower in WS (1/51, 2.0%)

than in SA (5/43, 11.6%; $P < 0.05$). The percentage of farrowed sows was greater in WS (46/51, 90.2%) than in SA (32/43, 74.4%; $P < 0.05$). Sows in WS had on average 1.5 more piglets than sows in SA ($P < 0.05$). Sows with a WOI of 3–6 days had lower rates of pregnancy losses ($P < 0.05$) and higher farrowing percentages ($P < 0.01$) than those with a WOI > 6 days, irrespective of season.

http://dx.doi.org/10.1016/j.tvjl.2013.11.026

ARTICLE 2

Reproduction in Domestic Animals, 2017

Altrenogest treatment before weaning improves litter size in sows

The study aimed to assess whether altrenogest treatment, fed before weaning (from −8 to −2 days), could improve fertility of sows showing reproductive seasonality. Ninety sows (50 in winter–spring [WS] and 40 in summer–autumn [SA]) were randomly selected and assigned to control (C; 27 in WS and 20 in SA) or altrenogest treatment (A; 23 in WS and 20 in SA) groups. The diameter and number of ovarian follicles were transrectally scanned at the onset of oestrus. Oestrus was evaluated twice daily from weaning to day 8 post-weaning. Sows in oestrus were post-cervically inseminated at 0 and 24 hr after the onset of oestrus with liquid stored semen (1.5 × 109 sperm/doses), and farrowing rates (FR) and total piglets born (LS) were recorded. More ($p < .01$) sows showed no signs of oestrus within 8 days after weaning in SA (30%) than in WS (2%), without differences between A and C groups. The diameter (cm) of the follicles at the onset of oestrus was larger in A than in C sows (0.76 ± 0.01 vs 0.73 ± 0.01; $p < .01$), irrespective of the season. No differences in the number of follicles were found. FR did not differ between seasons and groups, being always above 85%. LS was larger ($p < .01$) in A (14.00 ± 0.46) than C (12.27 ± 0.44) sows, irrespective of the season. In conclusion, a short-term altrenogest treatment at the end of lactation improves the total number of piglets born from weaned sows, probably by promoting a better and more homogeneous follicular development at the start of oestrus.

https://doi.org/10.1111/rda.13063

ARTICLE 3

Animals, 2020

Ovarian Follicle Growth during Lactation Determines the Reproductive Performance of Weaned Sows

Factors causing variability in ovarian follicle size among weaned sows are not well known. This field study aimed to disclose influencing factors and evaluate if the differences at weaning were established during lactation. Ovaries were scanned using transrectal ultrasound. The first experiment was conducted over a year with 191 randomly chosen sows that were hierarchically grouped ($p < 0.001$) according to ovarian follicle diameter reached at weaning: Small (0.20–0.30 cm; n = 37), medium (0.31–0.39 cm; n = 75), and large (0.40-1.00 cm; n = 69). Sows with small follicles showed a higher incidence of post-weaning anestrus ($p < 0.01$), longer wean-to-estrus/ovulation intervals ($p < 0.01$) and farrowing smaller litters ($p < 0.05$). Ovaries with small follicles were more common among sows weaned in summer–autumn than in winter–spring ($p < 0.01$) and among sows of lower parity (1–3) ($p < 0.05$). In the second experiment, with 40 sows randomly chosen at farrowing, the ovaries were scanned at 7, 14, and 21 d post-partum. Sows showed great variability in ovarian follicular size during lactation with a consistent relationship between the three measurement times ($r = 0.84$, $p < 0.01$). Follicle size was smaller in sows nursing in summer–autumn than in winter–spring ($p < 0.05$). In conclusion, early lactation dictates the great variability in ovarian follicular diameter at weaning shown by sows. Sows with smaller follicles at weaning had longer intervals for estrus and ovulation and smaller litters at farrowing and they were in greater numbers among sows weaned during the summer and fall and among those with fewer previous farrowing.

https://doi.org/10.3390/ani10061012

ARTICLE 4

Animals, 2020

Weaned Sows with Small Ovarian Follicles Respond Poorly to the GnRH Agonist Buserelin

The GnRH agonist buserelin (GnRH), used to synchronize ovulation in weaned sows, attains only 70–80% effectivity, owing to several reasons of ovarian origin. This study evaluated in particular whether mean ovarian follicle size at treatment and the season of weaning are among those influencing GnRH responsiveness. The experiment was carried out in a temperate-region farm with 352 sows of 1–6 parities weaned either in winter–spring (WS, 174 sows) or in summer–autumn (SA, 178 sows). The sows were randomized into two groups: GnRH (10 g of buserelin acetate at 86 h after weaning, 172 sows) and control (180 sows). The ovaries were transrectally scanned from weaning to ovulation and the sows clustered according to their mean follicular size at treatment time: small (<0.5 cm in diameter), medium (0.5 to 0.64 cm) and large (0.65 to 1.09 cm). In total, 88.33% of the GnRH-treated sows ovulated, with 82% of them within the expected time window (120–132 h after weaning). In contrast, 95.45% of the unresponsive sows had small follicles at the time of treatment and were mostly weaned in SA (20.45%) than in WS (4.76%). In conclusion, the conspicuous presence of sows having small ovarian follicles at treatment time compromises the efficiency of the GnRH agonist buserelin to synchronize ovulation in weaned sows, which occurs more frequently in summer–autumn weaning.

https://doi.org/10.3390/ani10111979

GRAPHIC APPENDIX

GRAPHIC APPENDIX

Figure 1

Overhead image of the farm where the experiments were performed, in the Southeast of Spain (37°59'NL, 1º08'WL)

Figure 2

Photography of a sow farrowing room of the farm where the experiments were performed, with controlled environment.

Figure 3

Subjective Body Condition Score (BCS) implemented in the sows included in the experiments. The most emaciated sows correspond to the value 1 and the sows with over fat to value 5 (adapted from Coffey et al., 1999).

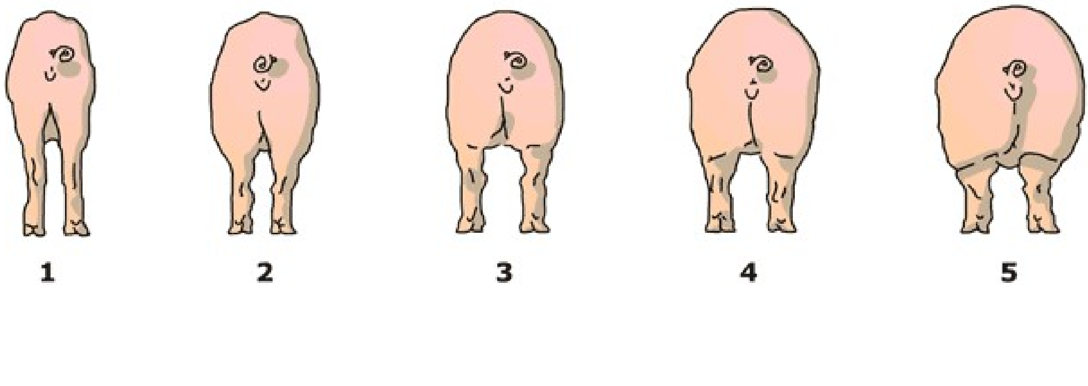

1
2
3
4
5

Figure 4

Weaned sows allocated in individual crates for oestrus detection using one or several teaser boars placed in an alley with snout-to-snout contact with the sows.

Figure 5

[Up] Oestrus detection in the presence of one or several boars. Standing heat response is assessed by experienced staff of the farm.

[Down] Artificial insemination after oestrus detection using a plastic disposable insemination spirette.

Figure 6

[Up] Oral administration of Regumate® (MSD)

[Down] Picture of the hormones (Altrenogest, Regumate®, MSD; and busereline, Porceptal®, MSD) used in the experiments.


Regumate
Regumate
MSD


Porceptal
Solución Inyectable
USO VETERINARIO
1 X 10 ml
Porceptal
MSD

Figure 7

[Up] Picture of the scanner Logiq Book XP (GE Healthcare) and the 4-10 MHz multiple scan linear transducer (i739-RS, GE Healthcare) and the expendables used during the experiments.

[Down] Transrectal scanning sequence, including (1) the use of a long silicone exploration glove, (2) gentle application of vaseline to the glove, (3) cleaning of the rectum ampulla of faeces, (4) rectal introduction of the transducer and (5) scan, (6) removal and (7) subsequent cleaning of the materials.


vet
miniLube
VETEUR
100 PCS


1
2
3
4
5
6
7

Figure 8

Sketch of the transrectal introduction of the transducer during the scanning, and organs and structures involved (own source).

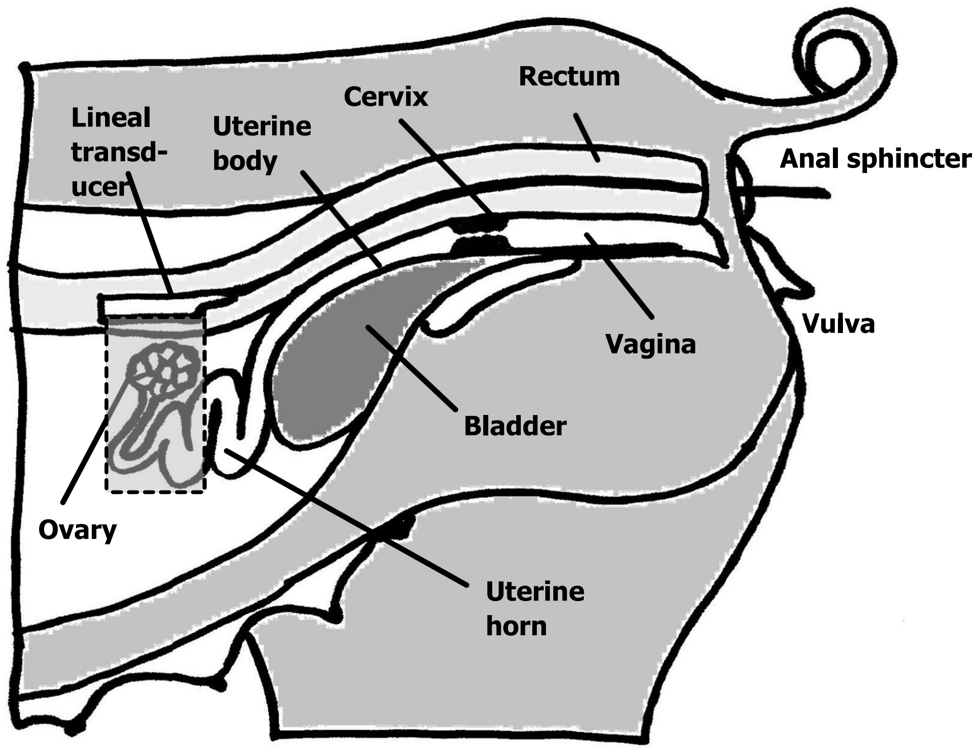
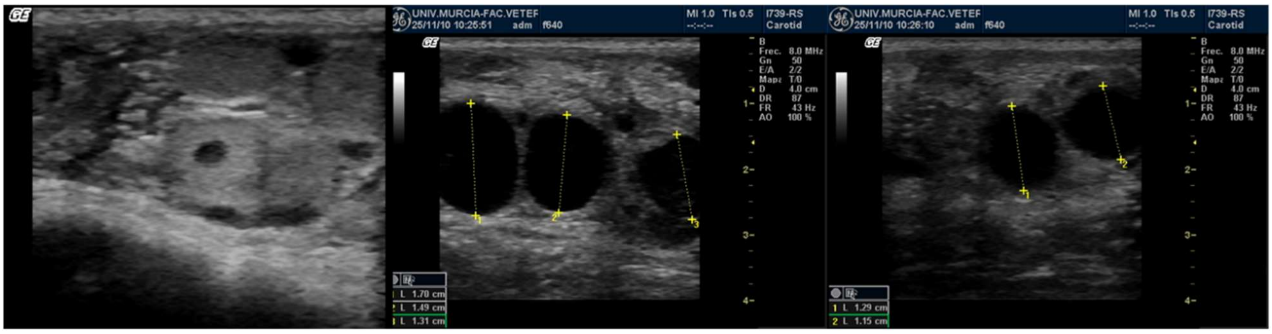

Rectum
Cervix
Lineal transd-ucer
Uterine body
Anal sphincter
Vulva
Vagina
Bladder
Ovary
Uterine horn

Figure 9

[Up] *Corpora lutea* with follicles (left) and cysts (center and right) with a diameter greater than 1.1 cm in sows at the weaning day.

[Down] Evolution of the follicular diameter in the ovary of one sow during 6 days from weaning (day 0) every 24h; from oestrus detection (day 4) every 12h; up to ovulation assessment (in day 7).



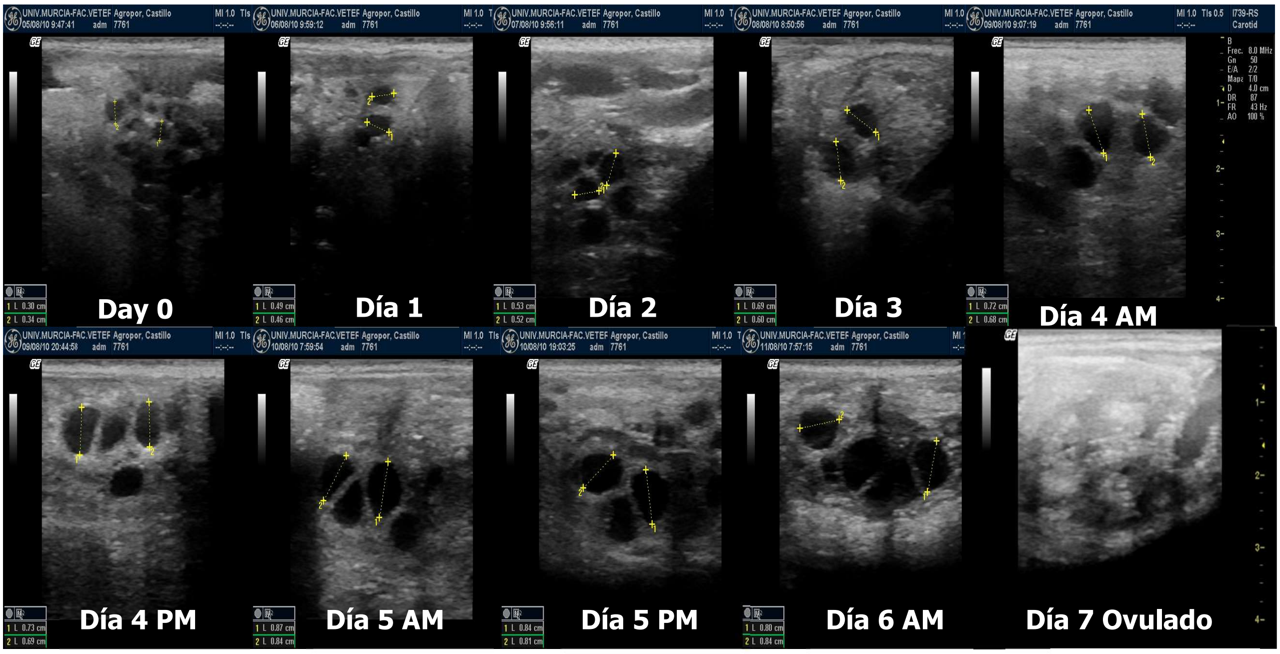
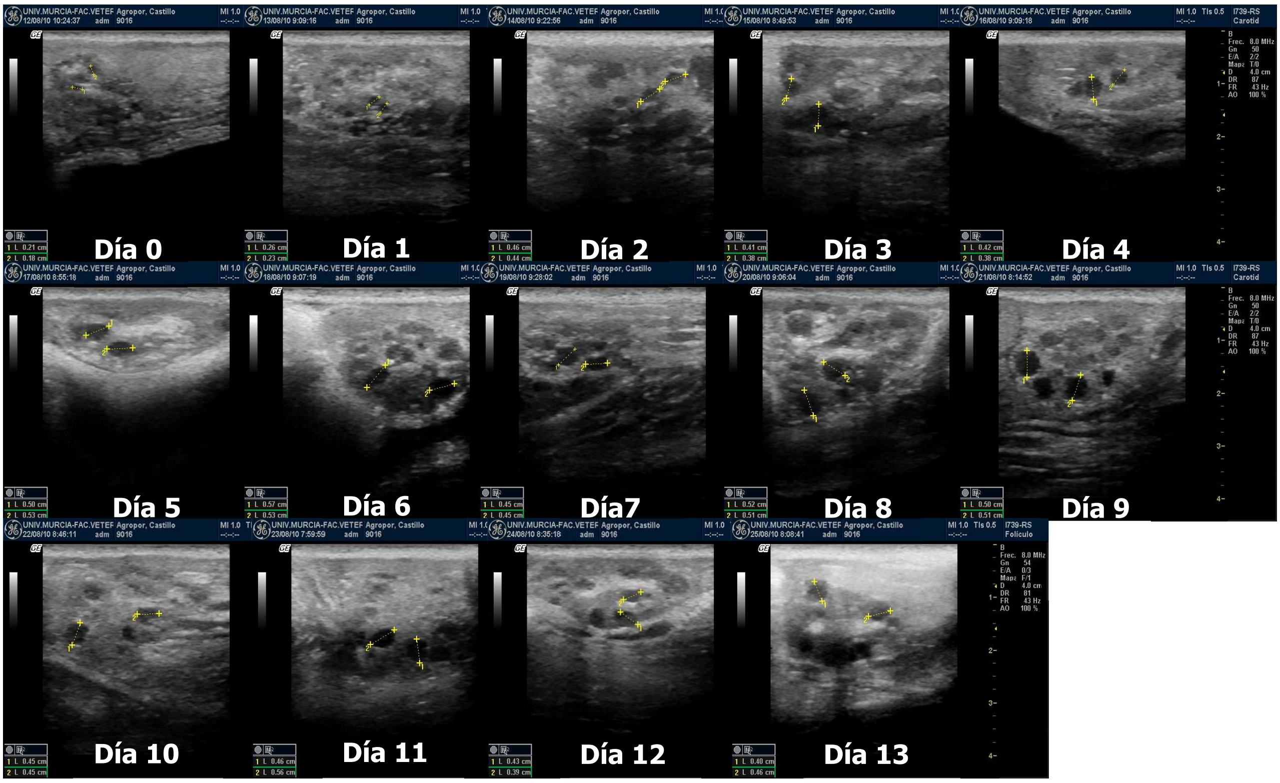

Day 0
Día 1
Día 2
Día 3
Día 4 AM
Día 4 PM
Día 5 AM
Día 5 PM
Día 6 AM
Día 7 Ovulado

Figure 10

Evolution of the follicular diameter in an inactive ovary of one sow during 13 days, from the weaning day (day 0) every 24h until its diagnostic as anoestrus.


Día 0
Día 1
Día 2
Día 3
Día 4
Día 5
Día 6
Día7
Día 8
Día 9
Día 10
Día 11
Día 12
Día 13

APPENDIX

THE VETERINARY JOURNAL

JCR YEAR

2014

VETERINARY JOURNAL

View title change

ISSN

1090-0233

EISSN

1532-2971

JCR ABBREVIATION

VET J

ISO ABBREVIATION

Vet. J.

Journal information

EDITION

Science Citation Index Expanded (SCIE)

CATEGORY

VETERINARY SCIENCES - SCIE

LANGUAGES	REGION	1ST ELECTRONIC JCR YEAR
ENGLISH	ENGLAND	1997

Publisher information

PUBLISHER	ADDRESS	PUBLICATION FREQUENCY
ELSEVIER SCI LTD	THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND	12 issues/year

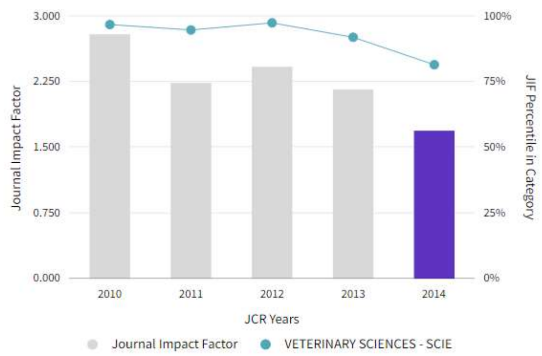
Journal Impact Factor

The Journal Impact Factor (JIF) is a journal-level metric calculated from data indexed in the Web of Science Core Collection. It should be used with careful attention to the many factors that influence citation rates, such as the volume of publication and citations characteristics of the subject area and type of journal. The Journal Impact Factor can complement expert opinion and informed peer review. In the case of academic evaluation for tenure, it is inappropriate to use a journal-level metric as a proxy measure for individual researchers, institutions, or articles. Learn more

2014 JOURNAL IMPACT FACTOR	JOURNAL IMPACT FACTOR WITHOUT SELF CITATIONS
1.693	1.525
View calculation	View calculation

Journal Impact Factor Trend 2014

Export



Rank by Journal Impact Factor

Journals within a category are sorted in descending order by Journal Impact Factor (JIF) resulting in the Category Ranking below. A separate rank is shown for each category in which the journal is listed in JCR. Data for the most recent year is presented at the top of the list, with other years shown in reverse chronological order. Learn more

EDITION

Science Citation Index Expanded (SCIE)

CATEGORY

VETERINARY SCIENCES

25/133

JCR YEAR	JIF RANK	JIF QUARTILE	JIF PERCENTILE
2020	26/146	Q1	82.53
2019	25/141	Q1	82.62
2018	25/141	Q1	82.62
2017	26/140	Q1	81.79
2014	25/133	Q1	81.58

REPRODUCTION IN DOMESTIC ANIMALS

Rank by Journal Impact Factor

Journals within a category are sorted in descending order by Journal Impact Factor (JIF) resulting in the Category Ranking below. A separate rank is shown for each category in which the journal is listed in JCR. Data for the most recent year is presented at the top of the list, with other years shown in reverse chronological order. Learn more

EDITION
Science Citation Index Expanded (SCIE)

CATEGORY
AGRICULTURE, DAIRY & ANIMAL SCIENCE
19/60

JCR YEAR	JIF RANK	JIF QUARTILE	JIF PERCENTILE
2020	27/63	Q2	57.94
2019	23/63	Q2	64.29
2018	17/61	Q2	72.95
2017	19/60	Q2	69.17
2017	19/60	Q2	69.17

JCR YEAR
2017

REPRODUCTION IN DOMESTIC ANIMALS

ISSN
0936-6768

EISSN
1439-0531

JCR ABBREVIATION
REPROD DOMEST ANIM

ISO ABBREVIATION
Reprod. Domest. Anim.

Journal information

EDITION
Science Citation Index Expanded (SCIE)

CATEGORY
REPRODUCTIVE BIOLOGY - SCIE
AGRICULTURE, DAIRY & ANIMAL SCIENCE - SCIE
VETERINARY SCIENCES - SCIE

LANGUAGES	REGION	1ST ELECTRONIC JCR YEAR
Multi-Language	GERMANY (FED REP GER)	1997

Publisher information

PUBLISHER	ADDRESS	PUBLICATION FREQUENCY
WILEY	111 RIVER ST, HOBOKEN 07030-5774, NJ	6 issues/year

Journal Impact Factor

The Journal Impact Factor (JIF) is a journal-level metric calculated from data indexed in the Web of Science Core Collection. It should be used with careful attention to the many factors that influence citation rates, such as the volume of publication and citations characteristics of the subject area and type of journal. The Journal Impact Factor can complement expert opinion and informed peer review. In the case of academic evaluation for tenure, it is inappropriate to use a journal-level metric as a proxy measure for individual researchers, institutions, or articles. Learn more

2017 JOURNAL IMPACT FACTOR	JOURNAL IMPACT FACTOR WITHOUT SELF CITATIONS
1.422	1.262
View calculation	View calculation

Journal Impact Factor Trend 2017

Export

Journal Impact Factor contributing items

Export

Citable items (325) | Citing Sources (164)

TITLE	CITATION COUNT
Recent Advances in Boar Sperm Cryopreservation: State of the Art and Current Perspectives	13
l-carnitine Mediated Reduction in Oxidative Stress and Alteration in Transcript Level of Antioxidant Enzymes in Sheep Embryos Produced In Vitro	9
The Impact of Reproductive Technologies on Stallion Mitochondrial Function	9
Effect of Sildenafil on Pre-Eclampsia-Like Mouse Model Induced By L-Name	8
Alpha-Linolenic Acid Supplementation in Tris Extender Can Improve Frozen-Thawed Bull Semen Quality	7
Effects of Bovine Serum Albumin on Boar Sperm Quality During Liquid Storage at 17 degrees C	7

ANIMALS

JCR YEAR

2020

Animals

Open Access since 2011

ISSN

2076-2615

EISSN

2076-2615

JCR ABBREVIATION

ANIMALS-BASEL

ISO ABBREVIATION

Animals

Journal information

EDITION

Science Citation Index Expanded (SCIE)

CATEGORY

AGRICULTURE, DAIRY & ANIMAL SCIENCE - SCIE

VETERINARY SCIENCES - SCIE

LANGUAGES	REGION	1ST ELECTRONIC JCR YEAR
English	SWITZERLAND	2018

Publisher information

PUBLISHER	ADDRESS	PUBLICATION FREQUENCY
MDPI	ST ALBAN-ANLAGE 66, CH-4052 BASEL, SWITZERLAND	12 issues/year

Rank by Journal Citation Indicator (JCI)

Journals within a category are sorted in descending order by Journal Citation Indicator (JCI) resulting in the Category Ranking below. A separate rank is shown for each category in which the journal is listed in JCR. Data for the most recent year is presented at the top of the list, with other years shown in reverse chronological order. Learn more

CATEGORY

AGRICULTURE, DAIRY & ANIMAL SCIENCE

9/79

JCR YEAR	JCI RANK	JCI QUARTILE	JCI PERCENTILE
2020	9/79	Q1	89.24
2019	11/62	Q1	83.06
2018	9/60	Q1	85.83
2017	n/a	n/a	n/a

CATEGORY

VETERINARY SCIENCES

17/166

JCR YEAR	JCI RANK	JCI QUARTILE	JCI PERCENTILE
2020	17/166	Q1	90.06
2019	22/136	Q1	84.19
2018	17/135	Q1	87.78
2017	n/a	n/a	n/a

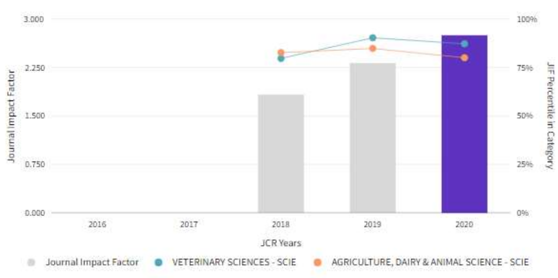
Journal Impact Factor

The Journal Impact Factor (JIF) is a journal-level metric calculated from data indexed in the Web of Science Core Collection. It should be used with careful attention to the many factors that influence citation rates, such as the volume of publication and citations characteristics of the subject area and type of journal. The Journal Impact Factor can complement expert opinion and informed peer review. In the case of academic evaluation for tenure, it is inappropriate to use a journal-level metric as a proxy measure for individual researchers, institutions, or articles. Learn more

2020 JOURNAL IMPACT FACTOR	JOURNAL IMPACT FACTOR WITHOUT SELF CITATIONS
2.752	1.967
View calculation	View calculation

Journal Impact Factor Trend 2020

Export



View all years

Journal Impact Factor contributing items

Export

Citable items (1,379) | Citing Sources (0)

TITLE	CITATION COUNT
The Potential Role of Insects as Feed: A Multi-Perspective Review	32
Animals Fed Insect-Based Diets: State-of-the-Art on Digestibility, Performance and Product Quality	27
Use of Licorice (Glycyrrhiza glabra) Herb as a Feed Additive in Poultry: Current Knowledge and Prospects	24
Omega-3 and Omega-6 Fatty Acids in Poultry Nutrition: Effect on Production Performance and Health	22
Assessment of Welfare in Zoo Animals: Towards Optimum Quality of Life	19
Effects of Dietary Betaine on Growth Performance, Digestive Function, Carcass Traits, and Meat Quality in Indigenous Yellow-Feathered Broilers under Long-Term Heat Stress	17
The Visitor Effect on Zoo Animals: Implications and Opportunities for Zoo Animal Welfare	17
Evaluation of Black Soldier Fly (Hermetia illucens) Larvae and Pre-Pupae Raised on Household Organic Waste, as Potential Ingredients for Poultry Feed	16