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Original Research Article

Metabolic profile and glycemic response in fully-grown sows born using assisted reproductive technologies

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ABSTRACT

The aim of the present work was to gain insight into the metabolism of pigs derived from assisted reproductive technologies during their adulthood. Approximately 4h after feeding, a blood sample was taken from 3.5 year old sows born by artificial insemination (AI group, n = 7) and transfer of in vitro produced embryos (IVP group, n = 11) to determine the physiological concentrations of the main biomarkers of carbohydrates (glucose and lactate), proteins (albumin, creatinine and urea) and lipids (cholesterol and triglycerides). Four weeks later, an oral glucose tolerance test (OGTT; 1.75g glucose/kg body weight) was performed after an overnight fast and 1h of water withdrawal. Blood samples were obtained prior (T = 0 min; fasting conditions) and 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min after glucose intake. At each time point, glycemia was measured immediately using glucometer test strips, and serum was collected to determine the above metabolites along with insulin and glucagon. After OGTT, the area under the curve (AUC) between sampling times and homeostasis model assessment of insulin resistance (HOMA) indices were calculated. Under physiological conditions, the concentration of metabolites studied was similar between AI and IVP sows. In both groups, fasting decreased cholesterol and increased triglycerides and urea ($P < 0.001$). However, creatinine and lactate were similar in both groups under physiological and fasting conditions. The expected increase in albuminemia and decrease in glycaemia after fasting was only observed in IVP sows. OGTT revealed a different glucose curve pattern (monophasic in AI and biphasic in IVP group), a lower mean concentration of cholesterol, glucose, lactate, triglycerides in IVP compared to AI pigs ($P < 0.01$), and a higher mean concentration of albumin, creatinine and insulin in IVP compared to AI group ($P < 0.05$). On the contrary, no differences were found between groups for mean serum glucagon and urea levels, nor for glucose homeostasis indices HOMA-IR and HOMA-%B. The AUC differed between groups at several time points with larger AUC for creatinine, and smaller AUC for glucose, glucagon, and triglycerides, in IVP pigs than in AI pigs at 180–210 min ($P < 0.05$). In conclusion, under physiological conditions the metabolic profile of fully-grown AI and IVP sows is similar and within normal ranges. Glucose challenge revealed differences in metabolic and insulin responses between groups but with normal glucose tolerance in both cases.

1. Introduction

The association of assisted reproductive technologies (ART) with long-term effects on fetal, postnatal and adult health has been known for decades, with most of the evidence coming from studies in mice and cattle (reviewed by Ref. [1]). There is clear evidence that ART involving in vitro fertilization predisposes individuals from the above species to long-term effects on molecular physiology and metabolic dysfunction [2–5]. Unfortunately, studies in the porcine model in this regard are very limited, although the human and porcine species share anatomical and physiological characteristics [6] and the porcine species has been proposed as the ideal non-rodent mammalian model in the clinical trials for human metabolic disorders [7,8].

In previous studies, our group reported small differences in haematological indices, biochemical profile and glucose tolerance between growing pigs (45 days) derived from ART and their in vivo-derived

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counterparts, with in vitro-derived piglets exhibiting higher plasma glucose concentrations [9,10]. However, it is unknown whether these differences persist or are corrected in late adulthood.

The 2-h oral glucose tolerance test (OGTT) is traditionally used to diagnose diabetes in humans [11]. The OGGT is also commonly used to diagnose conditions such as diabetes mellitus, impaired glucose tolerance, or insulin resistance in pigs [12], although there is controversy [10,13,14] about the effect of animal weight on the test results. However, it is not known whether the metabolic differences and likely effect of birth weight and embryonic origin (in vivo *vs*. in vitro) are maintained into adulthood and how pigs respond to metabolic stressors such as fasting and glucose challenge, as there are no studies of key metabolic biomarkers and OGTT response beyond 12 months of age. Accordingly, the response of 3.5 year old ART-derived pigs to fasting and oral glucose tolerance was investigated.

2. Material and methods

2.1. Animals

Oral glucose tolerance test (OGTT), weighing, and blood sampling were performed in collaboration with a pig breeding company (Cefu S. A., Murcia, Spain) in a group of crossbred sows (Landrace × Large White) 3.5 years old, born after routine commercial breeding by artificial insemination (AI group; n = 7; 1298.42 ± 9.48 days; 224.85 ± 17.01 kg) and transfer of in vitro produced embryos (IVP group, n = 11, 1312.72 ± 8.14 days; 244.54 ± 4.12 kg). The animals in this study were never inseminated (no farrowing) and shared the genetic background in the paternal line, as the same boar was used to produce the animals [9]. For the maternal line, AI animals were gestated by 2–3 parity sows from the collaborating pig farm, and IVP animals were produced from the ovaries of gilts from the same farm. At the 1 year of age, AI and IVP animals in this study were grouped together and housed in the same outdoor pen, with natural light regime, ad libitum access to water and a standard 2.5-3-kg barley-corn-soybean meal diet (13 % crude protein, 2.80 % crude fat, 35.88 % starch, 9.08 % crude fiber, 5.45 % crude ash, 0.75 % lysine, 0.27 % methionine, 0.90 % calcium, 0.19 % sodium and 0.55 % phosphorus) delivered daily in a controlled manner by an electronic feeding system (Compident ESF, Schauer, Austria). Body condition on the day of blood sampling and OGTT was scored out of 5, with all pigs used in this study scoring 3–4.

2.2. Habituation

From birth, both groups of pigs (AI and IVP) were fed the same diet, housed in the same conditions, and handled daily by several trained personnel, so that each animal experienced a high level of human contact, allowing effective, stress-free blood sampling. In addition, two weeks prior to the OGTT, special attention was given to the ear region to accustom the animals to specific contact in this area for blood sampling. Pigs were also trained to drink the glucose solution by using a calf feeding bottle with a metal nipple.

2.3. Weighing, blood sampling and physiological biochemical profile

Weight was recorded on the day of birth using a digital hanging scale and on the day of OGTT using the weighing machine installed in the electronic sow feeding system (Compident ESF, Schauer, Austria). Four weeks before the OGTT (1230–1340 days of age), a blood sample was obtained by puncturing the retro-orbital sinus with an 18G needle between 9.00 and 11.00 a.m., approximately 4 h after the pigs had consumed the daily feed ration, and collected in lithium heparin tubes. Blood plasma was obtained by centrifugation (1200 g, 20 min, 4 °C, Eppendorf 5810 R). Plasma concentrations of metabolites were determined using an automated clinical chemistry analyzer (Olympus AU400, Japan) with the following commercial kits (Beckman Coulter, California, USA): albumin (g/dL; cat. No. OSR6102), cholesterol (mg/dL; cat. No. OSR6116), creatinine (mg/dL; cat. No. OSR6178), glucose (mg/dL; cat. No. OSR6121), lactate (mmol/L; cat. No. OSR6193), triglycerides (mg/dL; cat. No. OSR6118) and urea (mg/dL; cat. No. OSR6134). After serial dilution, the inter- and intra-assay precision of the methods were linear and less than 15 %.

2.4. Oral glucose tolerance test (OGTT)

The OGTT was performed between 8.00 a.m. and 13.00 p.m. as previously described [10]. Briefly, after an overnight fast and 1h of water deprivation, pigs were given an oral glucose solution (100 % Glucose carbs, Myprotein; 1.75 g/kg body weight) dissolved in distilled water. Pigs drank the glucose solution within 3–5 min and with minimal loss due to the previous training. The pigs were restrained with a nose sling, and blood samples were collected before oral glucose administration to determine fasting glucose (T = 0 min) and at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after glucose ingestion. Blood samples were collected from the auricular vein using a 1 mL syringe with a 25G needle, immediately transferred to sterile tubes to allow blood clotting, and kept at 4 °C to prevent insulin degradation according to the manufacturer's instructions. Blood glucose concentration was measured immediately at each time point with a glucometer (GlucoMenLX Plus) using test strips to avoid degradation of glucose molecules and to compare results obtained in a previous study using the same method and animals [10]. Serum was obtained by centrifugation of blood samples (1200 g, 20 min, 4 °C, Eppendorf 5810 R) and stored in aliquots at −80 °C until determination of insulin, glucagon, and metabolites (albumin, cholesterol, creatinine, lactate, triglycerides and urea). Normal feeding regime and water access were restored immediately after the OGTT.

Samples were assayed in duplicate at all time points of the OGTT from 0 min (fasting conditions) to 240 min, except for insulin and glucagon, which were assayed in triplicate. Serum insulin concentrations (μIU/mL) were analysed by a porcine-specific insulin assay (Porcine Insulin ELISA 10–1200-01, Mercodia AB, Uppsala, Sweden), a two-site solid phase sandwich ELISA test using two mouse anti-insulin monoclonal antibodies to bind to with porcine insulin. The assay sensitivity was ≤1.15 mU/L, and coefficients of variation 7.1, 14.6 and 27.5 respectively for low, medium and high concentrations of insulin. Serum glucagon concentrations (pmol/L) were measured using a commercially available porcine ELISA assay kit according to the manufacturer's instructions (Mercodia Glucagon ELISA (10-1281-01, Mercodia AB, Uppsala, Sweden). The assay sensitivity was 1 pmol/L, and coefficients of variation 3.0, 5.2 and 21.9 for low, medium and high concentrations of glucagon.

Serum concentrations of albumin (g/dL), cholesterol (mg/dL), creatinine (mg/dL), lactate (mmol/L), triglycerides (mg/dL) and urea (mg/dL) were measured using an automated clinical chemistry analyser (Olympus AU400, Japan) as previously described. For the OGTT response, blood glucose, serum insulin, glucagon, and other metabolites were assessed separately by calculating the total area under the response curve (AUC) determined for the specified time period after oral glucose ingestion (e.g. AUC0-15 represents the integrated area between 0 and 15 min after ingestion, AUC15-30 between 15 and 30 min after ingestion, and so on up to AUC210-240). The AUC is an index of total glucose transport and subsequent metabolic response that provides more information about glucose tolerance and subsequent response than analysis of metabolite levels at a single time point [15].

Rates of decline in serum insulin and glucose concentrations were calculated from the slope of the linear portion of the response curve from 0 to 30 min post-OGTT [16]. The results were then expressed as a fractional rate constant determined from the slope of the natural logarithm of serum concentrations versus time [17]. Fractional turnover rates (k) or disappearance rates of plasma insulin and glucose in %/min, were calculated using the formula [18]:

$k = (Ln_1 - Ln_2)/(T_2 - T_1)$

where Ln1 and Ln2 are the natural logarithms of plasma insulin (μIU/mL) or glucose (mg/dL) concentrations at times T_1 (0 min) and T_2 (30 min), respectively.

From the k value, the half-life, $T_{1/2}$ (min), may be calculated as:

$T_{1/2} = 100 \times 0.693/k$

For insulin sensitivity, indices used in human medicine were used. Homeostasis model assessment of insulin resistance (HOMA) [19] was calculated to estimate insulin resistance (HOMA-IR) and β-cell function (HOMA-%B) under fasting conditions, as follows:

HOMA-IR = fasting plasma insulin (μIU/mL) x fasting plasma glucose (mM)/22.5

HOMA-%B = (20 x fasting plasma insulin (μIU/mL))/(fasting plasma glucose (mM) - 3.5)

It is assumed that individuals who are not insulin resistant have a β-cell function of 100 % and an insulin resistance of 1.

The quantitative insulin sensitivity check index (QUICKI [20]; was computed as:

$QUICKI = 1/[Ln(I_0) + Ln(G_0)]$

where I_0 is the fasting insulin (μIU/mL), and G_0 is the fasting glucose (mg/dL).

2.5. Statistical analysis

Metabolite levels were assessed using a GLM procedure (version 9.0; PROC GLM, SAS Institute Inc., Cary, NC, USA) with experimental group of animals (AI versus IVP) and sampling time at OGTT (0-15-30-45-60-90-120-150-180-210-240 min) as fixed effects. The interaction between the two independent variables was also examined. The normal distribution of the variables was assessed using the Kolmogorov-Smirnov test and when there was no normal distribution, a logarithmic transformation (Log10) of the data was performed. Birth weight was used as a covariate to account for unequal variances over time, unequal correlations and covariance between different pairs of measurements. The average concentration for each metabolite during the OGTT was calculated using the values obtained at all sampling times. The pig was considered as the experimental unit and as a random effect. When differences in metabolite concentration were found between groups and sampling times, a test of means was performed using the LS means option. Differences between feeding conditions within a group (physiological versus fasting conditions) were evaluated by one-way ANOVA. Outliers were identified and removed, and data for the different metabolites were presented including mean, minimum and maximum values, and SEM was generated as a residual error. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Physiological metabolic profile and fasting effect

Under physiological conditions (approximately 4h after ingestion of the ration and water ad libitum), plasma concentrations of albumin, cholesterol, creatinine, glucose, lactate, triglycerides and urea were similar between AI and IVP sows (Table 1). In both groups, fasting induced a decrease in cholesterol of 15–20 mg/dL ($P \leq 0.01$), an increase in triglycerides of 30–40 mg/dL ($P < 0.001$) and an increase in urea of 10–15 mg/dL ($P < 0.001$). Under fasting conditions, IVP sows significantly reduced their albuminemia and glycaemia compared to physiological conditions ($P < 0.05$). Fasting did not alter lactate and creatinine concentrations.

3.2. Metabolic response to OGTT and glycemic indices

The weight of the pigs included in the study was similar between the two experimental groups, both at birth and on the day of OGTT. Birth weight was 1.05 ± 0.10 kg (AI group) and 1.36 ± 0.11 kg (IVP group) ($P = 0.060$); and 224.85 ± 17.01 kg (AI group) and 244.54 ± 4.12 kg (IVP group) ($P = 0.173$) on the day of OGTT. Regarding the possible effect of birth weight on the metabolic profile, only serum lactate concentration was affected ($P < 0.001$).

The average concentrations of metabolites during the OGTT are shown in Table 2, with cholesterol, glucose, lactate and triglyceride concentrations lower in IVP sows compared to AI sows ($P < 0.01$). However, the mean concentrations of albumin, creatinine and insulin were higher in IVP sows than in the AI group ($P < 0.05$). Mean glucagon and urea concentrations were similar between groups. Concentrations of glucose ($P < 0.05$), insulin ($P < 0.01$), and triglycerides ($P < 0.001$) changed throughout the sampling time (Figs. 1 and 2). No significant interaction between time and group was found for the concentration of any metabolite.

Regarding the glycaemic indices, no significant differences were observed for fasting glucose (AI 57.85 mg/dL and IVP 51.30 mg/dL) and fasting insulin (AI 60.73 μIU/mL and IVP 66.22 μIU/mL) (Table 3). In addition, disappearance rates and half-life for blood glucose and serum insulin were similar between groups, as were indices of quantitative insulin sensitivity (QUICKI), insulin resistance (HOMA-IR), and β-cell function (HOMA-%B) (Table 3).

Table 1
Metabolic parameters in 3.5-year-old sows born after artificial insemination (AI group) and in vitro produced embryo transfer (IVP group) under physiological (approximately 4 h after feed intake and water ad libitum) and fasting conditions (overnight fasting and 1 h water deprivation before glucose intake). The study under physiological conditions was performed four weeks before the fasting conditions. Minimum and maximum values for each metabolite are given in brackets.

	AI (n = 7)				IVP (n = 11)			
	Physiological conditions (min-max)	Fasting conditions (min-max)	SEM	P value	Physiological conditions (min-max)	Fasting conditions (min-max)	SEM	P value
Albumin (g/dL)	3.42 (3.10–3.74)	3.58 (3.34–3.86)	0.06	0.163	3.44 (3.10–3.80)	3.68 (3.22–3.99)	0.05	0.021
Cholesterol (mg/dL)	72.88 (53.22–86.34)	52.73 (37.23–63.59)	3.83	<0.001	68.24 (57.10–98.06)	52.93 (34.89–73.66)	3.02	0.010
Creatinine (mg/dL)	2.13 (1.82–2.44)	2.08 (1.75–2.27)	0.05	0.666	2.31 (1.84–3.20)	2.36 (1.96–2.71)	0.06	0.743
Glucose (mg/dL)	65.20 (55.90–75.30)	57.86 (40.00–72.00)	2.49	0.152	61.46 (42.40–77.90)	46.99 (39.00–60.00)	3.23	0.020
Lactate (mmol/L)	2.45 (1.92–2.88)	2.92 (1.30–4.20)	0.21	0.287	2.26 (1.38–3.53)	2.79 (1.42–3.89)	0.18	0.134
Triglycerides (mg/dL)	27.14 (20.46–38.16)	56.06 (24.64–74.31)	5.05	<0.001	27.01 (21.12–38.16)	67.08 (29.49–100.95)	5.60	<0.001
Urea (mg/dL)	20.93 (17.00–28.20)	31.86 (26.30–41.90)	1.98	<0.001	20.44 (15.60–24.10)	34.64 (24.50–46.80)	1.93	<0.001

No differences between groups (AI vs. IVP) were observed under physiological and fasting conditions.

Table 2
Average concentration of metabolites recorded during the oral glucose tolerance test (OGTT, 1.75 g/kg body weight) in 3.5-year-old sows born by artificial insemination (AI group) and in vitro produced embryo transfer (IVP group). The minimum and maximum values obtained during the OGTT for each metabolite are given in brackets.

	AI (n = 7)	IVP (n = 11)	SEM	*P*-value Group	Time
Albumin (g/dL)	3.27 (3.25–4.40)	3.62 (3.36–3.49)	0.02	<0.001	0.999
Cholesterol (mg/dL)	71.28 (65.63–74.68)	63.62 (61.53–68.23)	1.07	<0.001	0.751
Creatinine (mg/dL)	2.14 (2.11–2.23)	2.40 (2.31–2.49)	0.02	<0.001	0.999
Glucagon (pmol/L)	2.73 (1.80–7.12)	2.88 (1.69–3.59)	0.32	0.750	0.640
Glucose (mg/dL)	66.18 (57.86–74.57)	60.09 (46.99–69.73)	1.23	0.006	0.018
Insulin (μIU/mL)	58.77 (34.86–74.50)	74.57 (61.11–88.16)	5.15	0.041	0.009
Lactate (mmol/L)	3.61 (3.08–3.73)	3.19 (2.79–3.79)	0.10	0.006	0.670
Triglycerides (mg/dL)	29.69 (22.93–40.89)	25.54 (20.74–35.32)	0.74	<0.001	<0.001
Urea (mg/dL)	21.64 (21.14–22.73)	21.40 (20.43–22.06)	0.40	0.695	0.999

No significant interaction (Group x Time) was detected.

3.3. Metabolites curves and AUC results

Plasma glucose concentration increased steadily after glucose ingestion, with the maximum peak being reached earlier in the IVP group (30 min) than in the AI group (60 min) (Fig. 1). In addition, in the IVP group, the glucose concentration decreased after the maximum peak between 30 and 60 min, and a second peak was observed at 90 min. As a result, a monophasic glycaemic curve was observed in the AI group, whereas IVP described a biphasic curve. Once the decrease in plasma glucose was initiated, the lowest concentration (glucose nadir) was found at 120 min after glucose ingestion (AI group), and at 60 and 210 min (IVP group). With regard to insulin, a biphasic response was observed in both groups of pigs following the OGTT, with the first decrease in glycaemia in IVP pigs following the maximum discharge of insulin at 30 min post-glucose intake. In AI pigs, however, the first insulin peak was observed earlier, at 15 min post-glucose intake, and then declined rapidly until 45 min to enter the second phase, peaking at 120 min and declining sharply to end the OGTT (180–240 min) with insulin concentrations below initial fasting levels. This marked decrease in insulin at the end of the OGTT in AI pigs coincides with the higher glucose AUC (180–240 min) and glucagon AUC (150–210 min) (Table 4). As for glucagon the response, a clear peak was observed at 15 min post-glucose in the AI group, whereas the glucagon increase in IVP animals was more stable over time (Fig. 1).

In both experimental groups, lactate increased after the glucose ingestion, describing a serrated pattern, whereas triglycerides and cholesterol decreased after glucose ingestion. Later, triglycerides in AI pigs showed an ascending curve ending with significantly higher values at 150–210 min (Fig. 2) and a higher AUC at 120–210 min than in IVP sows (Table 4). However, serum creatinine and albumin concentrations did not vary with time, with the exception of a significant increase in creatinine in IVP pigs at 210 and 240 min (Fig. 2) and higher AUC values (180–240 min) than in the AI group (Table 4). There were no differences between groups in AUC values for albumin, cholesterol, lactate and urea, but time significantly affected the results (Table 4).

4. Discussion

The literature shows different metabolic profiles and subtle but significant changes in glucose metabolism in animals and human offspring

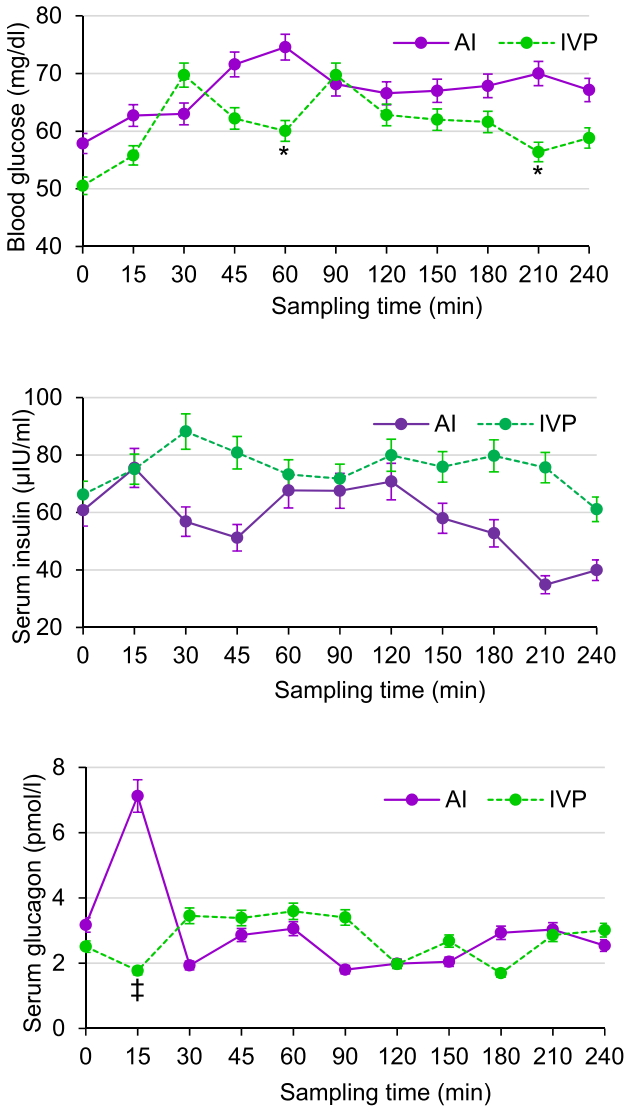

Fig. 1. Glucose, insulin, and glucagon concentrations during the oral glucose tolerance test (OGTT, 1.75 g/kg body weight) in sows conceived after artificial insemination (AI) and in vitro produced embryo transfer (IVP). Data are expressed as mean ± SEM. Differences between groups at a given time point are indicated as * ($P < 0.05$) and ‡ ($P = 0.06$).

derived from ART [2,5,9,10,21]. Here, for the first time, we report baseline concentrations of key biomarkers of carbohydrate, protein and lipid metabolism in ART-derived sows at 3.5 years of age.

As changes in diet or nutrient requirements affect metabolism, animals in the present study were not subjected to breeding programmes and were fed the same diet and ration throughout their lives and housed outdoors from 1 year of age. Data in the literature mainly refer to sows with multiple gestation-lactation cycles, on different diets and rations, and housed in farrowing crates. Even considering the differences in management conditions, the basal plasma concentrations of albumin, cholesterol, creatinine, glucose, lactate, triglycerides and urea obtained in our study are within the range of values reported in pregnant/lactating sows of age and weight close to those of our study [22–26]. Under basal physiological conditions, AI and IVP sows had similar metabolite concentrations, but their response to metabolic stressors was different.

In our study, fasted sows in both groups had decreased cholesterol and increased triglycerides and urea, as the expected response due to the use of stored fat and protein breakdown for producing energy [27].

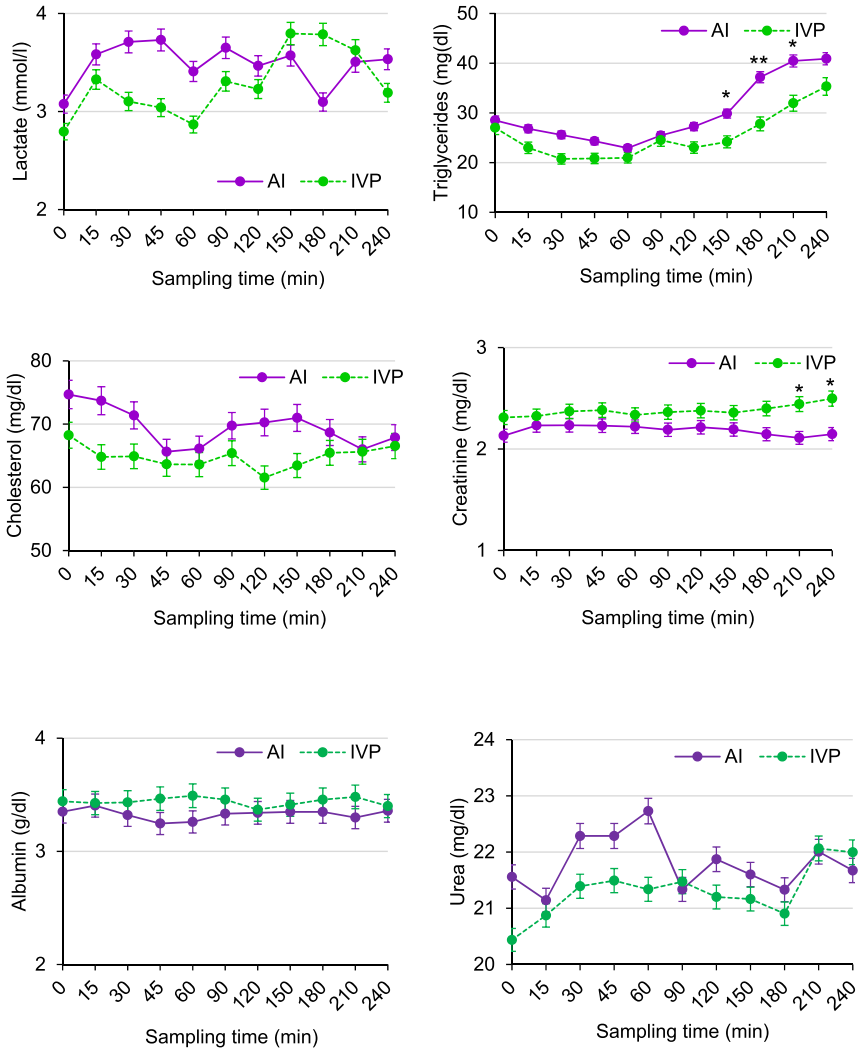

Fig. 2. Serum metabolites concentration during the oral glucose tolerance test (OGTT, 1.75 g/kg body weight) in sows conceived after artificial insemination (AI) and in vitro produced embryo transfer (IVP). Data are expressed as mean ± SEM. Differences between groups at a given time point are indicated as * ($P < 0.05$) and ** ($P < 0.01$).

Table 3
Indices of glucose tolerance and insulin sensitivity after oral glucose tolerance test (OGTT, 1.75 g/kg body weight) in 3.5-year-old sows born by artificial insemination (AI group) and in vitro-produced embryo transfer (IVP group). QUICKI: quantitative insulin sensitivity check index. HOMA-IR: homeostasis model assessment for the estimation of insulin resistance. HOMA-%B: homeostasis model assessment for estimation of β-cell function.

	AI (n = 7)	IVP (n = 11)	SEM	*P*-value
Fasting glucose (mg/dL)	57.85	51.30	11.38	0.963
Fasting insulin (μIU/mL)	60.73	66.22	11.38	0.963
Glucose disappearance rate (%/min)	1.69	1.64	0.06	0.574
Insulin disappearance rate (%/min)	1.53	1.67	0.07	0.534
Glucose half-live (min)	41.29	42.39	0.65	0.422
Insulin half-live (min)	47.15	42.32	3.30	0.432
QUICKI	0.48	0.46	0.02	0.978
HOMA-IR	9.28	8.56	2.35	0.559
HOMA-%B	359.19	529.74	99.70	0.644

However, only IVP sows had a reduced glycaemia and a slight increase in albuminemia, which could be explained by the high fasting insulin levels in IVP sows. Reduced glycaemia and increased albuminemia have been observed in fasted rats [27] and 1-year-old miniature pigs [28] compared to fed animals, so the response of fasted IVP sows is within the expected normal response.

In pigs, oral glucose intake is preferred to other routes of administration (i.e. intravenous, peritoneal) because it induces a more physiological and greater insulin release [12], but no differences were observed between groups for insulin sensitivity indices, fasting glucose and fasting insulin. As for the glycaemic indices, differences have been found between obese (Iberian) and lean (Landrace) pig breeds [29], rather than between pigs of the same line [30]. In our study, the sows were from the same commercial cross, with similar weight at the time of OGTT and the same paternal genetic origin, reducing the possibility of finding differences in the glycaemic indices. In IVP-derived piglets at 45 days of age (Landrace x Large White), there is evidence of a higher glucose AUC with no effect of body weight in the response to OGTT [10]. Similar observations have been described in 3-month-old pigs (purebred Large White) [15] and 9.5-month-old miniature pigs [16]. However, when purebred animals reached adulthood (12 months), glucose AUC was negatively correlated with body weight and body mass index at

Table 4
Area under the curve (AUC) of blood glucose (mg x min/dl), glucagon (pmol x min/ml), insulin (μIU x min/ml), creatinine (mg x min/dL), triglycerides (mg x min/dl), albumin (g x min/dl), cholesterol (mg x min/dl), lactate (mmol x min/l) and urea (mg x min/dl) during oral glucose tolerance test (OGTT, 1.75 g/kg body weight) in 3.5-year-old sows conceived by artificial insemination (AI group; n = 7) and in vitro produced embryo transfer (IVP group; n = 11).

	AUC Time interval (min)													
Metabolite	Group	0–15	15–30	30–45	45–60	60–90	90–120	120–150	150–180	180–210	210–240	SEM	*P*-value group	*P*-value time interval
Glucose	**AI**	904.29	942.86	1009.29	1096.07a	2140.71	2020.71	2003.57	2022.86	2067.86a	2057.14a	27.88	0.006	0.018
	IVP	794.86	941.45	990.00	912.00b	1946.45	1988.18	1872.27	1855.50	1754.83b	1739.85b	22.59		
Glucagon	**AI**	85.52a	66.93a	42.85	45.35	87.45	61.58	64.22	87.68a	90.04a	76.85	7.63	0.032	0.016
	IVP	31.91b	38.44b	49.81	52.32	109.80	81.17	66.46	61.76b	57.32b	92.46	5.68		
Insulin	**AI**	1057.51	967.52	736.08	915.84	2081.50	2074.31	1930.93	1493.20	1130.85	1121.61	153.91	0.148	0.002
	IVP	1059.77	1190.81	1267.34	1107.71	2184.27	2271.17	2399.13	2274.22	2274.22	2278.62	121.91		
Creatinine	**AI**	18.88	18.99	18.96	18.88	35.07	35.40	35.11	34.38	33.80a	34.32a	1.00	<0.001	<0.001
	IVP	19.75	20.15	20.32	19.67	37.57	38.02	37.86	38.42	38.96b	39.79b	0.94		
Triglycerides	**AI**	229.56	218.65	207.96	196.26	404.85	434.17	475.17a	587.58a	643.95a	653.87	0.83	0.016	<0.001
	IVP	199.31	178.58	176.98	178.06	388.81	369.92	385.71b	441.08b	506.98b	561.80	0.65		
Albumin	**AI**	28.88	28.30	27.66	27.70	53.25	53.43	53.57	53.58	52.83	53.68	1.60	0.513	<0.001
	IVP	29.14	29.17	29.42	29.65	55.34	53.98	54.55	55.26	55.67	54.48	1.24		
Cholesterol	**AI**	627.39	608.99	563.59	561.49	1112.21	1123.60	1134.96	1101.01	1058.71	1084.07	34.41	0.174	<0.001
	IVP	554.25	551.57	542.27	540.66	1044.56	988.47	1013.27	1045.17	1049.90	1063.62	26.12		
Lactate	**AI**	49.94	54.70	55.80	53.54	105.88	106.74	105.56	100.03	99.04	105.58	31.90	0.584	<0.001
	IVP	45.91	48.22	46.07	44.30	92.63	98.06	105.37	113.73	111.16	102.23	36.42		
Urea	**AI**	180.13	188.29	189.43	192.75	342.74	349.41	345.87	341.53	351.41	347.08	11.44	0.742	<0.001
	IVP	176.98	181.30	182.57	181.51	343.43	339.47	338.65	334.72	351.86	352.00	8.47		

birth [15].

The values recorded for all metabolites during the glucose challenge were within the range of data reported for this test in pigs [15,29,31–33] and similar results for glucose and insulin have been reported in Landrace pigs [29]. The response of pigs to the OGTT can vary depending on a number of factors, but typically the insulin is secreted in a biphasic form and glycaemia rises rapidly after glucose ingestion, peaking at 30–60 min, followed by a decline without a second rise [34]. Indeed, in the current study, the biphasic insulin response was observed in both groups of pigs following the OGTT, but a secondary glucose peak at 90 min was observed in the IVP sows. Both groups of pigs used in this study had a monophasic glycaemia curve at 45 days of age [10]. Age-dependent changes in glucose tolerance have been reported in pigs and other species [13,35], and the biphasic pattern of the glycaemic curve has been observed both in pigs and humans [11,36]. IVP sows in our study had higher mean insulin levels during the glucose challenge, which would explain the lower glucose AUC observed in this group compared to AI sows. In addition, the glucose nadir in the IVP group was found at 210 min, which is not consistent with the reported expected time of 45 min [29] and may also be explained by the higher insulin secretion observed in this group or by a prolonged duration of insulin action. In humans, the biphasic curve together with a high insulin concentration during the OGTT has been associated with both a higher risk of diabetes and insulin resistance [37,38] and a lower risk of progression to diabetes with greater insulin sensitivity/secretion [11,39]. Therefore, the results observed in our study in IVP sows should be interpreted as a different response to glucose compared to AI animals, but not necessarily as an impaired tolerance to glucose or a higher risk of diabetes, as has been described in other species for individuals derived from in vitro produced embryos (reviewed by Ref. [2]). Further research with larger numbers of animals and at older ages, using more accurate methods of glucose estimation than the glucometer test strips, would be required to clarify whether there is a real risk of diabetes or metabolic problems in IVP-derived pigs. Finally, the higher average albumin and creatinine, and lower lactate, triglycerides, and cholesterol in IVP pigs than in AI pigs, as well as the different curve patterns, support the different metabolic response between pigs of different embryonic origin (in vivo vs. in vitro).

In conclusion, the present study is the first to provide metabolic reference values for IVP sows in late adulthood that do not differ from animals conceived by AI. The metabolic response pattern was different when sows were subjected to fasting and glucose challenge. Further studies would explain the clinical significance of these metabolic differences and whether they are relevant to pig management practices and the use of ART-derived pigs in metabolic studies.

Ethics approval

The study was carried out in accordance with applicable regulations (Royal Decree No. 53/2013, Spain). The study was approved by the Ethics Committee for Animal Experimentation (CEEA) of the University of Murcia (Spain) and authorised by the "Dirección General de Agricultura, Ganadería, Pesca y Acuicultura" of the Región de Murcia (project No. A13170705).

Data and model availability statement

None of the data was deposited in an official repository. The data supporting the findings reported in this study are available from the corresponding author upon request.

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CRediT authorship contribution statement

S. Cánovas: Writing – review & editing, Methodology, Investigation, Conceptualization. **S. Heras:** Writing – review & editing, Methodology, Investigation. **J. Romero-Aguirregomezcorta:** Writing – review & editing, Methodology, Investigation. **A.A. Quintero-Moreno:** Methodology, Investigation, Formal analysis. **J. Gadea:** Writing – review & editing, Methodology, Investigation. **P. Coy:** Writing – review & editing, Methodology, Investigation, Funding acquisition. **R. Romar:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

Declaration of interest

None of the authors have any conflict of interest to declare.

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