


Supplemental progesterone during early pregnancy exerts divergent responses on embryonic characteristics in sows and gilts

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Progesterone (P4) plays a key role in pregnancy establishment and maintenance; during early pregnancy, P4 stimulates the production and release of uterine secretions necessary for conceptus growth prior to implantation; therefore, exogenous P4 supplementation may improve embryo development. This study evaluated the effects of supplementation during early pregnancy with long-acting injectable progesterone or altrenogest on embryonic characteristics of sows and gilts. Thus, a total of 32 sows and 16 gilts were used. On day 6 of pregnancy sows and gilts were allocated to one of the following groups: non-supplemented; supplemented with 20 mg of altrenogest, orally, from days 6 to 12 of pregnancy; supplemented with 2.15 mg/kg of long-acting injectable progesterone on day 6 of pregnancy. Animals were killed on day 28 of pregnancy, and ovulation rate, embryo survival, embryo weight, crown-to-rump length, uterine glandular epithelium and endometrial vascularization were assessed. Treatments had no effect on pregnancy rate, embryo survival or endometrial vascular density ($P > 0.05$). Non-supplemented gilts presented larger and heavier embryos compared to gilts from supplemented groups ($P < 0.05$). Sows in the altrenogest group presented larger and heavier embryos compared to non-supplemented sows and sows supplemented with long-acting injectable progesterone. In conclusion, supplementation of sows and gilts with progestagen from day 6 of pregnancy can be used as a means to improve embryo survival without deleterious effects.

Keywords: embryo development, uterine histoarchitecture, progestagen, altrenogest, swine

Implications

Progesterone supplementation during early pregnancy affects the uterine histoarchitecture and changes the composition of uterine secretions. However, progesterone supplementation prior to day 6 of pregnancy impairs embryonic survival or pregnancy rate in pigs, which hampers the progress of studies regarding the effects of progesterone supplementation on litter size and weight. Based on the results from this study, day 6 of pregnancy can be used as a basis to initiate exogenous progestagen treatment without deleterious effects on embryo survival. In addition, progestagen supplementation from day 6 of pregnancy improved embryonic development in sows; thus, it could be used to modulate early embryo growth in pigs.

Introduction

The vigorous genetic selection for increased litter size has been associated with lower birth weight (**BW**) and higher within-litter birth weight variation. Low birth weight piglets (<1.10 kg) are a serious economic and welfare problem in a pork production systems since there are permanent negative impacts on organ structure, neonatal adjustment, vitality, colostrum intake, postnatal growth, and consequently, account for 76% of pre-weaning deaths (Kirkden *et al.*, 2013; Muns *et al.*, 2016; Ji *et al.*, 2017).

The peri-implantation period, between days 10 and 30 of pregnancy, is essential for differences in piglets' birth weight; the preimplantation conceptuses undergo elongation to increase their physical contact area with the endometrium, and differences in the final size of elongated embryos will

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determine the amount of the uptake of nutrients of each embryo throughout the gestation (Patterson *et al.*, 2008; Wang *et al.*, 2017). During this period a fine-tune communication between conceptus, uterus and ovaries is required for an optimal embryo development and placentation (Bazer *et al.*, 2012). One of the key hormones in the control of early embryo development is progesterone (P4), which stimulates the production and release of uterine secretions necessary for conceptus growth prior to implantation (Bazer and Johnson 2014; Okrasa *et al.*, 2014; Chen *et al.*, 2016). Several studies with ruminants described the benefits of exogenous P4 supplementation on embryonic development. Cows supplemented prior to day 8 of pregnancy had significantly larger conceptuses on day 14 of pregnancy (Garrett *et al.*, 1988; Carter *et al.*, 2008). Similarly, when ewes were supplemented 36 h post mating, blastocyst diameter increased by 220% by day 9 (Satterfield *et al.*, 2006). Notwithstanding, in pigs the benefits of P4 supplementation during early pregnancy are inconsistent; Szymanska and Blitek (2016) noted that P4 supplementation increased the size and weight of the uterus, stimulated genes related to endometrial vascularization and increased the amount of total protein in uterine lumen. However, when supplemented with P4 or altrenogest prior to day 6 of pregnancy, pigs have shown impaired embryo survival and/or pregnancy rate, and no effect on embryo growth was observed (Mao and Foxcroft 1998; Soede *et al.*, 2012; Szymanska and Blitek, 2016). Based on the foregoing, the objective of the current study was to evaluate the effects of supplemental P4 from day 6 of pregnancy on (1) endometrial histoarchitecture, (2) embryo survival and (3) embryo development at 28 days of pregnancy in sows and gilts.

Material and methods

Animals, housing and diet

This experiment was performed at the Swine Research Center of School of Veterinary Medicine and Animal Science of University of São Paulo, Brazil. A total of 48 hybrid commercial females (Landrace × Large White), consisting of 32 sows (parity 2 to 8) and 16 gilts were used. Animals were checked for signs of estrus twice daily (0800 and 0400 h) by fence-line contact with a mature boar and a back-pressure test. All animals were artificially inseminated with fresh diluted semen (3×10^9 sperm cells; >80% motility; less than 72 h old) from the same boars whitening each batch. Gilts were inseminated at first signs of standing estrus and every 24 h while ever the gilts were showing a standing estrus. Sows (239 ± 4.79 kg live weight, 15.3 ± 0.4 mm of backfat thickness and average weaning-to-estrus intervals of 5 ± 1 days) were inseminated in the first estrus following a lactation period of 21 days at 12 h after first signs of standing estrus and every 24 h while ever the sows were in standing estrus. Gilts were inseminated in the second observed standing estrus with approximately 240 days of age and 145 kg live weight. The ovulation was considered as occurred 48 h after

estrus detection in order to standardize day 0 of pregnancy. All animals were allocated in individual pens and submitted to similar nutritional and sanitary management. Animals were fed 2.4 kg/day of a standard gestation diet (2930 kcal ME/kg, 16% CP and 0.6% Lys) from days 0 to 28 of pregnancy.

Experimental design

The experiment was completely randomly designed, and the treatments were arranged in factorials (2×3). The first factor was the category (sow or gilt), and the second factor was treatment (non-supplemented, long-acting injectable progesterone or altrenogest). On day 6 of pregnancy sows and gilts were allocated at random to one of the following groups: non-supplemented (NS, $n = 15$; 4 gilts and 11 sows), serving as controls; (ALT, $n = 17$; 6 gilts and 11 sows) supplemented daily with 20 mg of altrenogest (Regumate®—Merck Animal health, São Paulo, Brazil), orally, from days 6 to 12 of pregnancy; (PG, $n = 16$; 6 gilts and 10 sows) females supplemented with a single intramuscular injection of 2.15 mg/kg of long-acting progesterone (Sincrogest, Ourofino Saúde animal, Ribeirão Preto, Brazil) on day 6 of pregnancy. All animals were euthanized on day 28 of pregnancy.

Ovulation rate and embryonic measurements

All animals were slaughtered at a local abattoir by stunning and exsanguination, and their reproductive tracts were collected. The ovaries were removed, and ovulation rate (OR) was assessed by counting the number of corpora lutea on both ovaries.

The uterine horns were separated from the mesometrium and incised on the anti-mesometrial side. Pregnancies were confirmed by observing placentas containing embryos. All the embryonic vesicles were individually separated from the uterus and counted to determine the total number of embryos (TE). The vesicle was opened, and the amniotic fluid was weighed (vesicle weight). The embryo was separated from its respective vesicle, weighed, and crown-to-rump length was measured with a digital caliper (Absolute Digimatic; Mitutoyo Sul Americana Ltda. São Paulo, Brazil). Embryos were classified as vital or non-vital according to their visual appearance as described by Van der Waaij *et al.* (2010) and Da Silva *et al.* (2016). Embryos were considered non-vital when there was a presence of haemolysed amniotic fluid, resorbed embryonic membranes or both; and when there was evidence of implantation, combined with placental or embryonic remnants. Since the fertilization rate was considered 100% (Geisert and Schmitt 2002), embryo survival (ES) was defined as the number of vital embryos at slaughter, expressed as a percentage of the number of corpora lutea. The difference between OR and TE was considered as early embryonic mortality, and the number of non-vital embryos was considered as late embryonic mortality (Da Silva *et al.*, 2016).

Histology of uterine glandular epithelium

The analyses of uterine glands were performed for all the animals used in this study. On day 28 of pregnancy, three

samples per animal (approximately 4 cm²) were collected from different regions of the left uterine horn to assess the uterine glandular epithelium. The regions studied were the utero-tubal junction (A1), the central region (A2) and the utero-cervical junction (A3). After collection, all the samples were stored in 10% buffered formalin for 24 h and routinely processed for histology. Sections (4 µm) were obtained and stained with hematoxylin and eosin, as previously described (Dunlap *et al.*, 2008; Bailey *et al.*, 2010). Three high-power fields (HPFs, 40× magnification) per slide were captured as 'jpg' files, from the glandular epithelium area, using a microscope (Leica DM500) coupled with a high-definition camera (Leica ICCD50 HD). Glandular density (GD) was determined by the number of glands divided by the area of endometrium in the HPFs. For the mean glandular area (MGA), the average area of 50 glands per photomicrograph was measured using ImageJ® software. The total glandular area (TGA) per square millimetre was calculated by GD × MGA. The same observer in a blind evaluation performed all the histological analyses.

Endometrial vascularization

The immunohistochemistry technique was used to evaluate the vascular density of the endometrium of a total of 18 animals (3 gilts and 3 sows per group). Animals with similar ovulation rate were chosen for each group.

Histological sections were adhered to silanized slides, dewaxed in xylene, rehydrated in graded alcohol followed by distilled water. Endogenous peroxidase was blocked for 30 min in 3% hydrogen peroxide solution. Antigen retrieval (Von-Willebrand Factor antibody – ABCAM, Cod. Ab6994, 1 : 100) was performed with trypsin (Trypsin Enzymatic Antigen Retrieval Solution ab970, Abcam), for 15 min, at room temperature. After incubation with 5% skimmed milk diluted in distilled water for 30 min to block non-specific protein binding, the slides were washed with TBS buffer (Tris-buffered saline) with 1% Tween20 and incubated with a rabbit polyclonal primary antibody anti-Von Willebrand Factor antibody, for 16 h, in a humid chamber, at 4°C. After washing with buffer, the samples were incubated with secondary antibody (Easylink ONE – EasyPath – Erviegas, Brazil), following manufacturer instructions. The reaction was visualized with 3,3'-diaminobenzidine and counterstained with Harris' hematoxylin. For negative control, slides were incubated with rabbit IgG, at the same concentration, and processed simultaneously as those used for primary antibodies. Ten photomicrographs using the 100× magnification were captured as 'jpg' files along each histological section, using the aforementioned microscope. The vascular density (VD) was determined by the count of immunolabelled blood vessels divided by the area of endometrium in each image.

Progesterone and 17β-estradiol measurements

Blood samples from eight animals per group (four sows and four gilts) were collected by venipuncture on days 5, 6, 8, 12, 16, 22 and 28 of pregnancy for serum P4 concentrations measurements. Samples from day 12 of pregnancy were also used

for serum 17β-estradiol determinations. The samples were centrifuged for 10 min at 1500×g (centrifuge Excelsa II model 206; Fanem, São Paulo, Brazil) and stored into 2.0 ml microtubes at –20°C for subsequent analysis.

The P4 and 17β-estradiol levels were obtained by the solid phase radioimmunoassays using commercial kits (RIA PROGESTERONE, Beckman Coulter and RIA 17β-ESTRADIOL, Beckman Coulter, respectively). The hormonal assays were performed according to the protocol provided by the manufacturer and previously tested for swine serum (Mao and Foxcroft 1998; Novak *et al.*, 2002). The samples for progesterone measurements were analysed in duplicate assay, the sensitivity was 89%, intraassay CV ranged from 3.19% to 7.35% and interassay CV ranged from 3.99% to 7.25%. The samples for 17β-estradiol measurements were analysed in simple assay. The sensibility was 90%, and intraassay CV ranged from 6.01% to 7.00%.

Statistical analyses

Prior to the analysis, all the data were tested for normality and homogeneity of the variances, and when necessary, they were transformed. In this experiment, a completely randomized design with a factorial arrangement was used. The factor 1 was the category of females (gilt or sow), and the factor 2 was the treatment used (altrenogest, long-acting progesterone and non-supplemented). SAS software (Statistical Analysis System, Cary, NC, USA, 2002) was used. The pregnancy rate was analysed by the χ^2 test. The LSD was used to evaluate the treatment and/or category effect, and the effect of time was evaluated by the Tukey's test. Correlations were analysed by Pearson correlation coefficient. The data were presented as mean ± SEM, and the level of significance considered was 5%.

Results

Ovulation rate and embryonic measurements

The treatments did not affect the pregnancy rate (PR), and it was similar between sows and gilts. The variables OR, TE, vital embryos, early embryonic loss and late embryonic loss were higher ($P < 0.05$) for sows compared to gilts, but they were not affected by treatments, as shown in Table 1. The embryo survival was similar among treatments, but a difference was observed related to categories; gilts had higher embryo survival than sows (Table 1). For all embryo measurements evaluated (vesicle weight, embryo weight and crown-to-rump length) there was a significant effect ($P < 0.05$) on the interaction between treatment and category. Both crown-to-rump and embryo weight were higher ($P < 0.05$) for G-NS compared to gilts from treated groups; PG and ALT were similar. For sows, the ALT group had higher crown-to-rump compared to NS and PG groups, which were similar to each other. Embryos were heavier for ALT compared to NS, which in turn was heavier than PG (Table 1). The G-NS had vesicle weight higher than gilts from treated groups, which were similar to each other (261.7 ± 5.4 ,

Table 1 Pregnancy rate, embryonic losses and embryo development at 28 days of pregnancy in sows and gilts supplemented with altrenogest or long-term progesterone

Item	Category	Groups			Mean	T ¹	C ²	T × C ³
		NS	PG	ALT				
Pregnancy rate	Gilt	4/4 (100%)	5/6 (83%)	5/6 (83%)	14/16 (88%)	0.99	0.99	0.94
	Sow	11/11 (100%)	9/10 (90%)	10/11 (91%)	30/32 (94%)			
	Mean	15/15 (100%)	14/16 (88%)	15/17 (88%)				
Ovulation rate	Gilt	14.0	15.0	14.2	14.5 ± 0.5 ^B	0.76	<0.01	0.69
	Sow	29.8	27.3	26.8	28.0 ± 1.0 ^A			
	Mean	25.2 ± 2.3	21.9 ± 1.9	22.6 ± 2.1				
Total embryos	Gilt	12.0	12.8	13.2	12.7 ± 0.6 ^B	0.68	<0.01	0.38
	Sow	24.6	20.5	19.5	21.3 ± 1.2 ^A			
	Mean	21.0 ± 2.2	17.1 ± 1.5	17.4 ± 1.7				
Vital embryos	Gilt	11.7	11.7	10.8	11.4 ± 0.5 ^B	0.47	<0.01	0.65
	Sow	20.9	16.7	17.0	18.2 ± 1.1 ^A			
	Mean	18.2 ± 1.7	14.5 ± 1.2	14.9 ± 1.5				
Embryo survival	Gilt	83.2	78.3	75.9	78.8 ± 2.7 ^A	0.45	0.01	0.87
	Sow	69.9	68.1	63.4	67.1 ± 2.7 ^B			
	Mean	73.7 ± 4.1	72.9 ± 3.4	67.6 ± 3.8				
Late embryo loss	Gilt	0.25	1.14	2.40	1.31 ± 0.40 ^B	0.49	0.01	0.21
	Sow	2.90	3.77	2.50	3.03 ± 0.44 ^A			
	Mean	2.14 ± 0.60	2.62 ± 0.63	2.46 ± 0.55				
Early embryo loss	Gilt	2.0	2.1	1.0	1.7 ± 0.3 ^B	0.76	<0.01	0.20
	Sow	4.6	5.4	7.3	5.8 ± 0.6 ^A			
	Mean	3.8 ± 0.8	3.7 ± 0.8	5.2 ± 1.0				
Crown-to-rump length	Gilt	22.84 ^a	22.02 ^b	21.74 ^b	22.12 ± 0.11	0.01	<0.01	<0.01
	Sow	20.88 ^b	20.62 ^b	21.69 ^a	21.06 ± 0.07			
	Mean	21.23 ± 0.10	21.11 ± 0.12	21.70 ± 0.09				
Embryo weight	Gilt	1.71 ^a	1.56 ^b	1.49 ^b	1.57 ± 0.01	<0.01	<0.01	<0.01
	Sow	1.38 ^b	1.22 ^c	1.46 ^a	1.36 ± 0.01			
	Mean	1.44 ± 0.01	1.34 ± 0.01	1.47 ± 0.01				

NS = non-supplemented; PG = supplemented with long-term progesterone on day 6 of pregnancy; ALT = supplemented with altrenogest from days 6 to 12 of pregnancy. Different superscript letters in the same row or in the same column indicate significant differences ($P < 0.05$).

¹Effect of treatment.

²Effect of category (gilt × sow).

³Interaction between treatment and category.

211.5 ± 7.1, 202.8 ± 8.6 g; for NS, PG and ALT, respectively). Otherwise, sows from NS and ALT had vesicle weight similar to each other and higher than vesicle weight of sows from PG (201.3 ± 6.1, 175.9 ± 6.1, 191.8 ± 5.9 g; for NS, PG and ALT, respectively). Finally, there was a positive correlation between vesicle weight and crown-to-rump length ($P < .05$; $r = 0.35$) and also between vesicle weight and embryo weight ($P < 0.05$; $r = 0.46$).

Endometrial glands and vascularization

There were no differences in glandular epithelium among the three uterine regions analysed (A1, A2 and A3). The treatment did not influence the GD in both sows and gilts ($P > 0.05$) and, in addition, sows and gilts had similar GD independent of treatment. There was a significant interaction between treatment and category for MGA; treated gilts (PG and ALT) had similar MGA; however, they had higher ($P < 0.05$) MGA than G-NS. There was no difference between treatments in sows' MGA (Table 2). The treatment affected the TGA for both sows and gilts differently; gilts from PG

and ALT had higher TGA than G-NS (Table 2). Otherwise, S-NS had higher TGA when compared with S-ALT; sows from PG group had similar TGA compared with sows from NS and ALT (Figure S1 in Supplementary material). The VD was similar among groups in both sows and gilts. However, there was an effect of category for VD; gilts had higher VD than sows (Table 2; Figure S2 in Supplementary material).

Progesterone and 17β-estradiol

There was an effect of treatment for total P4 concentration in blood serum; PG-treated animals (sows and gilts) presented higher serum P4 concentrations when compared to the NS and ALT groups, which were similar (20.34 ± 1.05; 26.22 ± 1.10; 20.30 ± 0.85; NS, PG and ALT, respectively). There was an interaction of treatment × time for P4 levels on days 6, 8 and 12, as shown in Figure 1. Sows and gilts had similar P4 concentrations on all days analysed.

There was no effect of treatments on 17β-estradiol concentrations on day 12 of pregnancy (6.50 ± 0.70, 6.92 ± 1.54, 8.16 ± 0.82, for NS, PG and ALT, respectively). Sows and gilts

Table 2 Effects of altrenogest or long-term progesterone supplementation on endometrial glandular epithelium and vascularization on day 28 of pregnancy in sows and gilts

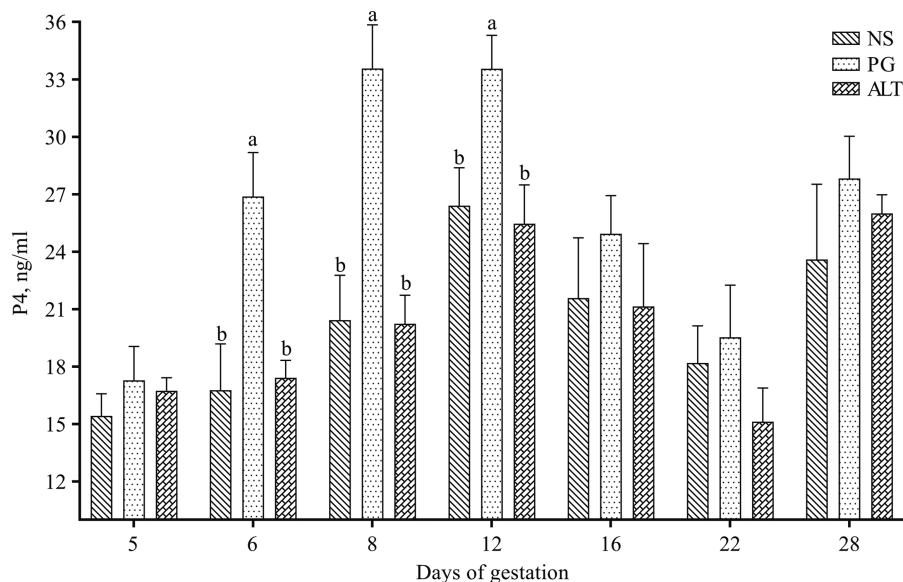
Item	Category	Groups			Mean	T ¹	C ²	T × C ³
		NS	PG	ALT				
GD	Gilt	46.5	48.3	44.9	46.8 ± 1.4	0.53	0.48	0.73
	Sow	48.3	46.8	44.8	46.7 ± 1.3			
	Mean	47.8 ± 1.8	47.5 ± 1.7	44.8 ± 1.7				
MGA	Gilt	1424.2 ^b	1841.9 ^a	1936.5 ^a	1761.8 ± 65.5	0.01	0.58	<0.01
	Sow	1743.3 ^a	1567.5 ^a	1647.9 ^a	1659.6 ± 41.3			
	Mean	1652.8 ± 59.9	1689.9 ± 59.6	1750.2 ± 65.3				
TGA	Gilt	0.061 ^b	0.084 ^a	0.083 ^a	0.078 ± 0.003	0.18	0.16	<0.01
	Sow	0.078 ^a	0.070 ^{ab}	0.066 ^b	0.072 ± 0.002			
	Mean	0.073 ± 0.003	0.076 ± 0.003	0.072 ± 0.002				
VD	Gilt	0.79	0.69	0.71	0.73 ± 0.06 ^A	0.82	0.02	0.50
	Sow	0.44	0.50	0.60	0.51 ± 0.05 ^B			
	Mean	0.61 ± 0.10	0.59 ± 0.09	0.65 ± 0.03				

NS = non-supplemented; PG = supplemented with long-term progesterone on day 6 of pregnancy; ALT = supplemented with altrenogest from day 6 to day 12 of pregnancy; GD = glandular density; MGA = median glandular area; TGA = total glandular area; VD = vascular density. Different superscript letters in the same row or in the same column indicate significant differences ($P < 0.05$).

¹Effect of treatment.

²Effect of category (gilt × sow).

³Interaction between treatment and category.

**Figure 1** Progesterone (P4) concentrations on days 5, 6, 8, 12, 16 and 28 in female pigs supplemented with long-term progesterone on day 6 of pregnancy (PG) and altrenogest, from days 6 to 12 of pregnancy (ALT) and non-supplemented females (NS).

also had similar 17β -estradiol concentrations on day 12 of pregnancy (7.01 ± 0.60 , 7.32 ± 1.14 , for gilts and sows, respectively).

Discussion

Exogenous P4 supplementation during early pregnancy is regarded as a potential tool to increase embryo size in ruminants. However, in pigs, the deleterious effects of supplemental P4 on embryo survival still prohibit its practical application.

Ovulation rate and embryonic measurements

In the current study, supplementing sows and gilts with long-acting injectable P4 on day 6 of pregnancy or orally with altrenogest from days 6 to 12 of pregnancy resulted in no detrimental effect on PR or embryo survival in both sows and gilts, contradicting the results of previous experiments in which female pigs were supplemented with exogenous P4. Mao and Foxcroft (1998) found an impaired embryonic survival in gilts supplemented with injectable P4 from 36 to 96 h after the onset of standing estrus. Correspondingly, Soede *et al.* (2012), supplementing gilts from days 1 to 4 or 4 to 6 after onset of standing estrus,

observed reduced embryo survival and reduced litter size, respectively. It is worth mentioning that in the current study day 0 of pregnancy – from which the start of supplementation was based – was designated as 48 h after onset of standing estrous since this corresponds to the period that most female pigs tend to ovulate (Soede *et al.*, 2011) in opposition to the aforementioned studies in which first day of standing estrous was considered day 0; this may represent a difference of 2 days on the starting of supplementation. It is important to emphasize that the increase in P4 concentration down-regulates its own receptors (PGR) in the uterine epithelium in several mammalian species (Spencer *et al.*, 2004; Mathew *et al.*, 2011). In pigs, the down-regulation of PGR occurs between days 8 and 12 after onset of estrus and enables conceptus attachment to the uterine surface (Sukjumlong *et al.*, 2005; Mathew *et al.*, 2011). Mechanisms that interfere with the spatiotemporal transcriptome profile could impair embryo survival (Clemente *et al.*, 2009; Forde *et al.*, 2009; Spencer *et al.*, 2015). Therefore, the disagreement between our results in relation to aforementioned studies probably is due to the difference in the starting date of the supplementation. Indeed, an overload on PGR before the day 6 of pregnancy, through exogenous P4 treatment, was associated with early down-regulation of PGR, which represents an asynchrony between embryonic development and uterine function, leading to embryonic losses by the inability of less-developed embryos to maintain their rates of development in the face of an increasingly hostile uterine environment (Geisert and Schmitt 2002; Kridli *et al.*, 2016). Contrastingly, the lower embryonic survival presented by sows compared to gilts might be a consequence of the differences in OR, since there is a positive correlation between OR and embryonic mortality (Vallet *et al.*, 1998; Van der Waaij *et al.*, 2010; Da Silva *et al.*, 2016). Using multiparous sows with ORs varying from 17 and 38, Da Silva *et al.* (2016) showed that each extra ovulation represented an increase of 0.49 and 0.24 in the incidence of early and late embryonic mortality, respectively. Besides the lower embryonic survival, the current study indeed showed that sows, which had higher OR than gilts, had higher early and late embryonic mortality than gilts.

Progesterone concentration and embryo survival

The final size of each embryo after the elongation process determines their physical contact area and, consequently, the amount of nutrient uptake throughout the gestation (Patterson *et al.*, 2008; Wang *et al.*, 2017). The positive correlation found in this study between vesicle weight and embryo development reinforces the importance of the elongation process for embryo development, since the vesicle weight was measured to estimate the size of each elongated conceptus. Sows and gilts responded divergently to treatment with long-acting injectable progesterone or altrenogest with respect to embryonic development; sows in the altrenogest group produced larger and heavier embryos compared to NS and PG. Interestingly, for gilts, a negative effect on embryo development occurred in both treatments (ALT and PG). These contradictory results might be caused

by excessive stimulation of P4 on embryos. Indeed, Starbuck *et al.* (2001) reported impaired embryo survival at progesterone concentrations greater than 9 ng/ml on day 5 of pregnancy in dairy cows, indicating that there is an optimal concentration range of P4 above which P4 levels can be harmful to embryo development. Since P4 levels were similar in gilts and sows during the entire experimental period and the OR was twice higher for sows, the amount of P4 per embryo was remarkably higher for gilts, and the combination with exogenous P4 treatment might have led to a harmful overdose of P4 for the embryos. To estimate the concentration of P4 per embryo during the early embryo development, the concentration of P4 per CL (P4/CL) was calculated in sows and gilts; indeed, the P4/CL was remarkably higher in gilts all days in which P4 was collected ($P < 0.05$), and also the average was twice as high (1.56 and 0.77, in gilts and sows, respectively; $P < 0.05$). These results showed that increased progesterone activity during early pregnancy in pigs can affect early embryonic development, therefore, contradicting the observations of Szymanska and Blitek (2016) which suggested that a mechanism to prevent changes in early embryonic development may exist.

Uterine histoarchitecture

The effects of treatments on glandular epithelium were different comparing sows and gilts, and it is not congruent to embryonic development; G-NS, which had larger and heavier embryos compared to treated gilts, had lower TGA. Similarly, ALT-treated sows had larger and heavier embryos although the lowest TGA among the groups. It is well known the importance of the endometrial histoarchitecture for early embryo development and embryo-maternal changes (Gray *et al.*, 2001; Bailey *et al.*, 2010). However, the lack of effects of long-acting injectable P4 or altrenogest supplementation on endometrial vascularization and the negative relation between uterine glandular epithelium and embryo development at day 28 of pregnancy suggest that there are other mechanisms determining embryonic growth prior to day 30 of pregnancy. More studies are needed to evaluate the effects of supplemental P4 supplementation on endometrial histoarchitecture and its consequences on uterine environment as well as embryo-maternal communication during the supplementation period and/or immediately after it in order to gain a better understanding of the mechanisms involved in modulation of pig embryonic development prior to implantation.

Conclusions

Day 6 of pregnancy can be used as a basis to initiate the supplementation of long-acting injectable progesterone or altrenogest without deleterious effects on embryo survival in sows and gilts. In addition, altrenogest supplementation from days 6 to 12 of pregnancy increased the embryo development at day 28 of pregnancy in sows; however, this topic warrants further studies to evaluate the effects of supplemental P4 on late pregnancy and on litter size.

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Declaration of interest

The authors declare no competing interest.

Ethics statement

All experimental procedures were performed according to the legal and ethical standards of the Ethics Committee for the use of Animals of the School of Veterinary Medicine and Animal Science of the University of São Paulo (CEUA / FMVZ-USP) under protocol 1160030817.

Software and data repository resources

None of the data were deposited in an official repository.

Supplementary material

To view supplementary material for this article, please visit <https://doi.org/10.1017/S1751731119002982>

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