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Effects of oestrous synchronization with altrenogest in gilts on endometrial and embryonic characteristics

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The use of altrenogest (ALT) supplementation for oestrous synchronization improves subsequent reproductive performance of gilts and sows. However, the causes of this improvement in reproductive performance after ALT treatment are not fully/clearly understood. The objective of this study was to evaluate the effects of ALT supplementation for oestrous synchronization in gilts on the endometrial glands and embryonic development characteristics at 28 days of pregnancy. Pregnant gilts were divided into two experimental treatments: Control (did not receive ALT; n = 9 gilts) and ALT (ALT feeding at 20 mg/day for 18 days; n = 9 gilts). At 28 days of pregnancy, six gilts from each treatment were slaughtered, and reproductive tracts were immediately evaluated. There was no statistical difference ($P > 0.05$) between treatments regarding ovulation rate, number of embryos, number of vital embryos and number of non-vital embryos. Embryo weight, length and embryonic vesicle weight were lower in ALT treatment compared with Control ($P < 0.01$), and it was lower in the cervical uterine region compared with apex uterine region, respectively ($P < 0.05$). Higher values of gland duct area, gland duct perimeter, percentage of the glandular area and total endometrial area were observed in ALT treatment compared with Control ($P < 0.05$). The use of ALT during 18 days for oestrous synchronization in gilts increased the gland duct area, perimeter and total endometrial area but did not increase the embryo number and embryo size at day 28 of pregnancy.

Keywords: altrenogest, embryonic development, endometrium, gilts, pregnancy

Implications

With advances in the genetic improvement of sows and selection for increased litter size, the numbers of low-weight piglets and variation in birth weight increased due to competition for nutrients and oxygen, typical of an overcrowded intrauterine environment. Embryo development is directly related to ovulation rate, embryonic survival rate, placental development and intrauterine environments. This paper shows that oestrous synchronization with altrenogest in gilts did not increase numbers and embryos' size but results in increased gland duct area, perimeter and total endometrial area at day 28 of pregnancy.

Introduction

Altrenogest (ALT) is a progestin that inhibits ovarian follicular development by negative feedback on gonadotropin-releasing

hormone. The ALT inhibits FSH and LH release (Dos *et al.*, 2004). Therefore, it blocks follicular growth and so prevents oestrus from occurring (De Rensis *et al.*, 2017). Altrenogest is mostly used for accurate oestrous synchronization at rates up to 93% for 4 to 7 days after administration in gilts and sows (Martinat-Botté *et al.*, 1995; Fernandez *et al.*, 2005; Kraeling and Webel, 2015; Lopes *et al.*, 2017).

Several studies have indicated that ALT supplementation improves subsequent reproductive performance of gilts and sows by enhancing the total number of piglets born, the number of piglets born alive and pregnancy rates (Wang *et al.*, 2018). However, the causes of this improved performance after treatment with ALT are not yet entirely understood. Although, these increases in litter size with ALT treatment have been associated with an increased ovulation rate regardless of the genotype (Kirkwood *et al.*, 1986; Martinat-Botté *et al.*, 1995; Wang *et al.*, 2018). Further, litter size may also be related to factors such as intrauterine

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environment and placental development (Quesnel *et al.*, 2008; Da Silva *et al.*, 2016).

The uterine environment may be influenced by histotrophic nutrition, which is defined as the provision of nutrients through secretions from endometrial glands to the uterine lumen for developing blastocysts. Thus, during the early stage of pregnancy, the histotroph represents the main form of nutrition of the conceptuses (Bazer, 1975; Gelsert *et al.*, 1982; Spencer *et al.*, 2019). Several trials have reported that progesterone stimulated the production of histotroph by uterine glands (Bazer, 1975) and the expression of various components of histotroph as uteroferrin protein (Knight *et al.*, 1974; Roberts and Bazer, 1988). However, there is no scientific evidence of the effects of oestrous synchronization with ALT on endometrial gland parameters in pigs during pregnancy.

Nutrient transport efficiency and placental development are also crucial factors in determining the foetal weight (Che *et al.*, 2016). In hyperprolific sows, vital embryos have reduced uterine space and placental development, which might cause growth retardation and increased mortality in later stages of pregnancy (Wilson *et al.*, 1998; Da Silva *et al.*, 2016). On the other hand, embryonic development is also influenced by the uterine position. Several studies investigating the relationship between intrauterine position and foetal weight demonstrated that foetal weight increases linearly from the cervix to the ovarian region during gestation (Wise *et al.*, 1997; Kim *et al.*, 2009; Che *et al.*, 2016; Palencia *et al.*, 2018). Therefore, uterine positions represent an essential factor influencing foetal weight variation (Kim *et al.*, 2013) and may be associated with differences in nutrient transport efficiency and endometrial characteristics throughout the uterus (Che *et al.*, 2016).

Our central hypothesis was that endometrial glands and embryonic development are altered according to the uterine region in gilts supplemented with ALT for oestrous synchronization. The present study aimed to evaluate the effects of ALT supplementation for oestrous synchronization in gilts on the endometrial glands and embryonic development characteristics at day 28 of pregnancy.

Material and methods

The current experiment was conducted from May 2017 to October 2017 at the Swine Research Center of University of São Paulo. The Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal of Science University of São Paulo (CEUA/FMVZ, protocol number 6292300517) approved all experimental procedures.

Animals and feeding

Eighteen hybrid gilts (Large White × Landrace × Pietrain) from their third to sixth oestrus which were housed in individual gestation stalls were used in the trial. The oestrous detection management was evaluated starting at 150 days of age twice daily (0800 and 1600 h) by exposure to a mature

boar for 15 min. Gilts were fed with a developmental diet until artificial insemination. After insemination, gilts were fed a gestational diet. All gilts received *ad libitum* access to water, and diets were formulated according to Rostagno *et al.* (2017).

Experimental procedures

The gilts were allocated randomly within two experimental treatments: (1) feeding of ALT at 20 mg/day for 18 days (ALT; $n = 9$ gilts) and (2) that did not receive ALT (Control; $n = 9$ gilts). The ALT was administered top-dressed individually with the morning feed and initiated irrespective of the stage of the oestrous cycle.

All gilts were artificially inseminated at 220 ± 20 days of age and 136 ± 8.3 kg of BW using refrigerated semen from hybrid boars (DB LM6200, Patos de Minas, Brazil) at 24-h intervals until the end of oestrus. The ALT gilts were inseminated at the detection of their first oestrus after the last ALT treatment. Control gilts were similarly inseminated but at their natural oestrus. The semen was collected using the gloved-hand technique, and only ejaculates with total motility above 80% were used. The total ejaculates were diluted with extender for refrigerated semen Beltsville Thawing Solution (IMV Technologies, L'Aigle, France) to a final concentration of 30×10^6 sperm/ml and the seminal dose was 100 ml. All seminal doses were stored at 17°C and used for up to 3 days.

Ovulation day was considered as day 0 of pregnancy. Ovulation was evaluated every 8 h by ultrasound scanning, from the first oestrous behaviour, and the ovulation moment was considered when no follicles were found or when the number of follicles was lower than that in the previous scan (Viana *et al.*, 1999). Pregnancy was confirmed at days 23 to 25 by ultrasound scanning 100® (Pie Medical, Philipsweg, Belgium) equipped with a 3.5-MHz convex array probe.

Measurements

At 28 days of pregnancy, six gilts for each treatment (according to pregnancy rate) were slaughtered at a local abattoir (University of São Paulo) through electrical stunning and exsanguination. After the slaughter, the entire reproductive tract of each gilt was taken and samples were collected. All reproductive tracts were immediately evaluated. The weight of each uterus was recorded, both uterine horns were separated from the broad ligament, and the uterus length and uterine horn length were recorded. Each uterine horn was divided into two equal segments according to the total length. The two segments were apex positioned towards the ovary and cervical positioned towards the cervix. Moreover, the uterine horns were opened towards the cervix to view each embryo in their respective uterine segments.

The embryonic vesicles were removed intact from the uterus and weighed individually. Embryos were separated from their vesicle to determine the length and weight of each embryo. Also, the length of each embryonic vesicle on the uterine wall was measured. Embryonic vesicle sites

were recognized by the reddening of the endometrium, compared to the whiter area (unoccupied area). To assess the apex and cervix regions, were used data from the three embryos closest to the ovary and three embryos closest to the cervix with their respectively embryonic vesicle measurements, corresponding to the apex and cervical regions, respectively.

The number of corpora lutea (CL) on each ovary was separated from ovaries and individually weighed. The ovulation rate was assessed by counting the number of CL on both ovaries. Vital or non-vital embryos were determined according to their visual appearance. The non-vital embryos were considered when there was a presence of haemolysed amniotic fluid, resorbed embryonic membranes or both (Van der Waaij *et al.*, 2010; Da Silva *et al.*, 2016). The fertility rate was calculated as the ratio of the total number of embryos to the number of CL. The number of non-vital embryos was considered as late embryonic mortality.

Histological analysis

Uterine samples (space 1 cm²) from the right uterine horn (dorsal position) were collected according to the apex and cervical regions. Three sections from a single vital-embryonic vesicle site closest to the ovary and three sections from a single vital-embryonic vesicle site closest to the cervix were collected, corresponding to the apex and cervical regions, respectively. Each uterine section was stored for 24 h in 10% neutral buffered formalin and then routinely processed for histology.

Three-micrometer sections (4 µm) were obtained and stained with haematoxylin–eosin (Prophet *et al.*, 1992). The uterine horn sections were examined using a Nikon Eclipse Ni-U 80i[®] microscope (Nikon Instruments Inc., Melville, NY, USA). Three high-power fields (4× objective) were evaluated for counting endometrial glands (area = 0.977 mm² per field). The endometrial gland number, per mm², was estimated between the area measured by field and the number of endometrial glands counted. For the perimeter (µm) and area (µm²), the average area and perimeter of all glands observed per field were measured using three high-power fields (10× objective). Total endometrial area (µm²) per field was also determined by the uterine endometrium, including stratum compactum and stratum spongiosum, but excluded the myometrium and uterine lumen. The histological analysis was measured in a software image analyzer system ImageJ IJ 1.46r (Rasband and Ferreira 2012).

Statistical analyses

Data were analysed using the SAS software (SAS, 2011). The experiment was conducted as a completely randomized design within a factorial arrangement of treatments with two treatments (ALT and Control) and two uterine regions (apex and cervical). To evaluate the uterine regions, within each uterine horn, we considered data from three embryos, their embryonic vesicle measurements from a vital-embryonic vesicle site nearest to the ovary and three from a vital-embryonic vesicle site nearest to cervix corresponding to the apex and cervical regions, respectively.

The statistical model included fixed effects of ALT, uterine regions, ALT × uterine region, gestational age, gilt, cycle number and gilt weight. The total number of embryos was used as a covariate. Further, the covariates gilt, cycle number, gilt weight and the total number of embryos were used only when significant effect ($P < 0.05$). The statistical model for variables that did not include the uterine region effect included fixed effects of ALT and covariates gestational age and the total number of embryos.

Analysis of variance of embryos and histological analysis were performed using the PROC MIXED of SAS, and the Tukey–Kramer test made comparisons of the mean. All data are presented as means ± SEM, and effects were considered significant when $P < 0.05$.

Results

From 18 inseminated gilts, only 6 of each treatment were slaughtered according to the pregnancy rate (ALT = 9; 100.0% and Control = 6; 66.66%; $P > 0.05$). In the Control group, all pregnancy gilts ($n = 6$) were selected, and six gilts from ALT group were randomly selected. Unselected gilts were removed from the experiment. Furthermore, from the 12 gilts slaughtered, data from 11 gilts were used for the statistical analysis. One gilt of Control treatment had only four total embryos and 71.4% of early embryonic mortality. We considered that it was not due to physiological causes; therefore, the number of females in Control treatment was reduced to five gilts.

Altrenogest effects on ovarian, uterine and embryo characteristics

The results of ALT effects on ovarian, uterine and embryo characteristics are presented in Table 1. There was no

Table 1 Effects of altrenogest supplementation during 18 days to oestrous synchronization in gilts on ovarian, uterine and embryo characteristics at day 28 of pregnancy

Parameters	Treatments ¹		SEM	P-values
	ALT	Control		
<i>n</i>	6	5		
Ovarian characteristics				
Ovulation rate	16.50	16.60	0.58	0.937
CL diameter (cm)	1.02	0.97	0.02	0.206
CL weight (g)	0.37	0.35	0.02	0.612
Ovarian weight (g)	7.98	8.08	0.43	0.916
Uterine characteristics				
Uterine weight (kg)	4.16	5.52	0.31	0.029
Uterine length (cm)	328.30	271.80	29.05	0.367
Embryo characteristics				
Number of embryos	14.83	12.00	1.02	0.179
Number of vital embryos	13.83	12.00	0.96	0.370
Number of non-vital embryos	1.00	0	0.28	0.074
Fertility rate (%)	89.48	72.46	5.24	0.108

CL = Corpora lutea.

¹Control = unsupplemented; ALT = feeding of altrenogest.

Table 2 Effects of altrenogest supplementation during 18 days to oestrous synchronization in gilts according to uterine region on embryonic characteristics at day 28 of pregnancy

Parameters	Treatments		Region		SEM	P-values ¹		
	Control	ALT	Apex	Cervical		ALT	R	ALT × R
<i>n</i> ²	54	72	71	55				
Embryo weight (g)	1.60	1.39	1.50	1.44	0.03	<0.001	0.413	0.741
Embryo length (cm)	2.22	2.15	2.20	2.15	0.01	0.001	0.033	0.951
Embryonic vesicle weight (g)	268.74	200.43	239.21	213.37	6.06	<0.001	0.042	0.746
Embryonic vesicle length (cm)	19.20	19.79	20.08	18.81	0.63	0.185	0.473	0.580
Embryonic vesicle weight : embryo weight ratio	178.12	144.09	164.18	149.95	4.45	0.004	0.050	0.890

Control = unsupplemented; ALT = Feeding of altrenogest.

¹ALT = altrenogest effect; R = uterine region effect.

²Number of embryos evaluated from 5 and 6 gilts from Control and ALT treatments, respectively.

statistical difference ($P > 0.05$) between treatments regarding ovulation rate, CL diameter, CL weight and ovarian weight. Higher uterine weight was observed in Control gilts compared with the ALT group ($P < 0.05$). However, the uterine length was not affected by ALT treatment ($P > 0.05$). Furthermore, with respect to embryo characteristics, number of embryos, number of vital embryos, number of non-vital embryos and fertility rate did not differ ($P > 0.05$) among treatments.

Altrenogest and uterine regions effects on embryonic characteristics

The results of ALT and uterine region effects on embryonic characteristics are presented in Table 2. There was no interaction of treatment with uterine regions for embryo characteristics evaluated. Embryo weight was lower in ALT treatment compared with Control ($P < 0.01$); however, it was not affected by the uterine region. Embryo length and embryonic vesicle weight were lower in the ALT group, and it was lower in the cervical region compared with Control and apex groups, respectively ($P < 0.05$). No differences ($P > 0.05$) were found between groups regarding embryonic vesicle length. Embryonic vesicle weight : embryo weight ratio was lower in ALT treatment compared with Control and lower in the cervical region when compared to apex ($P < 0.05$).

The CV of embryo characteristics according to treatments is presented in Table 3. The CV of embryo weight, embryo length, embryonic vesicle weight and embryonic vesicle length did not differ ($P > 0.05$) among treatments.

Histological analysis. The results of ALT and uterine regions' effects on morphometric characteristics of endometrial glands are presented in Table 4 and Figure 1. There was no interaction of treatment with the uterine region for all morphometric characteristics evaluated ($P > 0.05$). Gland duct number was not affected by ALT treatment, and no differences were found among uterine regions ($P > 0.05$). Higher values of gland duct area, gland duct perimeter and total endometrial area were observed in ALT treatment compared with Control

Table 3 Effects of altrenogest supplementation during 18 days to oestrous synchronization in gilts on CV of embryo characteristics at day 28 of pregnancy

Parameters	Treatments ¹		SEM	P-values
	Control	ALT		
<i>n</i> ²	54	72		
CV (%)				
Embryo weight	10.30	14.26	1.44	0.183
Embryo length	3.00	3.29	0.39	0.730
Embryonic vesicle weight	18.51	23.44	2.19	0.286
Embryonic vesicle length	23.58	29.05	1.68	0.107

¹Control = unsupplemented; ALT = Feeding of altrenogest.

²Number of embryos evaluated from 5 and 6 gilts from Control and ALT treatments, respectively.

($P < 0.05$); besides, they were not affected by uterine region ($P > 0.05$).

Correlation coefficients between embryonic and morphometric characteristics of endometrial glands. Pearson correlation values between embryonic and morphometric characteristics are presented in Table 5. Embryo number was negatively correlated with embryonic vesicle weight and also negatively correlated with embryonic vesicle weight : embryo weight ratio ($r = -0.56$; $P < 0.001$). Embryo weight was positively correlated with embryo length, embryonic vesicle weight, embryonic vesicle length and gland duct number. Embryo length was also positively correlated with embryonic vesicle weight, embryonic vesicle length and gland duct number.

Discussion

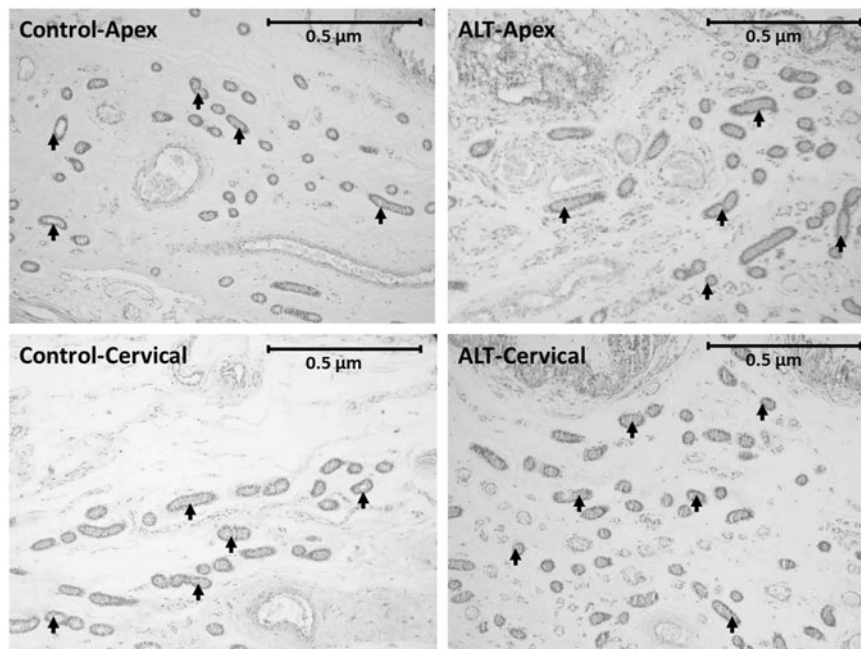
The oral ALT supplementation has been widely used for oestrous synchronization in gilts and sows (Kraeling and Webel, 2015; Lopes *et al.*, 2017). Moreover, ALT treatment also allows for improving the reproductive performance of gilts and primiparous sows (Wang *et al.*, 2018). The increased litter size with ALT treatment has been associated with an increased ovulation rate (Kirkwood *et al.*, 1986; Martinat-Botté *et al.*, 1995; Wang *et al.*, 2018) and higher embryonic survival (He *et al.*, 2017; Wang *et al.*, 2018).

Table 4 Effects of altrenogest supplementation during 18 days to oestrous synchronization in gilts according to uterine region on morphometric characteristics of endometrial glands at day 28 of pregnancy

Parameters	Treatments ¹		Region		SEM	P-values ²		
	Control	ALT	Apex	Cervical		ALT	R	ALT × R
<i>n</i>	5	6	11	11				
Gland duct number (n ^o /mm ²)	43.50	38.77	41.67	39.88	1.87	0.349	0.634	0.710
Gland duct area (μm ²)	2142.18	2378.80	2326.97	2217.68	57.00	0.041	0.366	0.793
Gland duct perimeter (μm)	210.10	230.39	226.47	216.64	4.60	0.028	0.326	0.918
Total endometrial area (mm ²)	1.89	2.05	2.01	1.95	0.04	0.033	0.511	0.688

¹Control = unsupplemented; ALT = feeding of altrenogest.

²ALT = altrenogest effect; R = uterine region effect.

**Figure 1** Photomicrography of the endometrial glands at day 28 of pregnancy of altrenogest-supplemented gilts during 18 days to oestrous synchronization. Control = unsupplemented; ALT = feeding of altrenogest. Objective 10×; endometrial glands (arrows); scale bar = 0.5 μm.**Table 5** Pearson correlation (*r*) and probability (*P*) values between embryonic and morphometric characteristics of endometrial glands of altrenogest-supplemented gilts during 18 days to oestrous synchronization

Parameters	Embryo weight		Embryo length		Embryonic vesicle weight		Embryonic vesicle length	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Number of embryos					-0.24	0.005		
Embryo weight					0.36	<0.001	0.32	<0.001
Embryo length	0.65	<0.001			0.55	<0.001	0.29	<0.001
Gland duct number	0.25	0.004	0.22	0.015				
Glandular area	0.32	<0.001	0.21	0.018				

In the present study, ALT treatment did not influence ovulation rate, CL diameter, CL weight, ovarian weight, uterine length, number of embryos, vital embryos and non-vital embryos. In contrast to previous studies (Kirkwood *et al.*, 1986; Martinat-Botté *et al.*, 1995; Wang *et al.*, 2018),

we suggest that ALT treatment does not increase ovulation rate in hyperprolific gilts (higher ovulation rates). However, additional studies should be conducted to investigate the effects of ALT on the ovulation rate in hyperprolific gilts using more number of animals. Uterine weight was 32% higher in

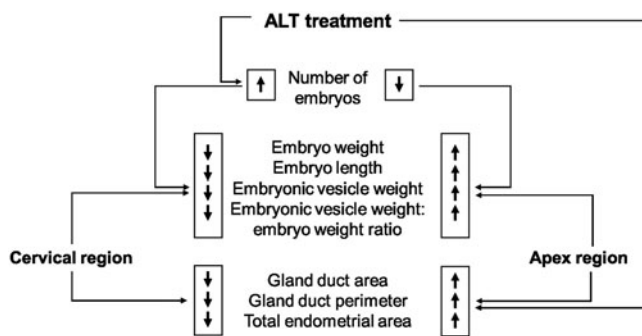


Figure 2 Summary of the effects of altrenogest supplementation during 18 days for oestrous synchronization in gilts on the endometrial glands and embryonic development characteristics at 28 days of pregnancy according to uterine region. ALT = feeding of altrenogest.

Control treatment, which may be related to the higher embryo weight and embryonic vesicle weight presented in this treatment. Embryo weight, embryo length and embryonic vesicle weight were also higher in Control treatment compared to ALT treatment. Embryo weight and embryo length are composite variables that can be influenced by ovulation rate, embryonic survival rate and placental development (Quesnel *et al.*, 2008; Da Silva *et al.*, 2016). We found +2.83 more embryos in ALT group, which would explain the higher embryonic vesicle weight : embryo weight ratio in Control treatment. Therefore, we suggest that the more significant placental development observed in Control treatment is the most likely reason for the higher embryo weight and embryo length observed (Figure 2).

Da Silva *et al.* (2016) demonstrated that sows with higher early embryonic mortality had a longer placental length around each embryonic-placental unit. The surviving embryos of early embryonic mortality may be provided of more space and, therefore, a better opportunity to grow. In our experiment, although the number of embryos (+2.83) was not significantly influenced by ALT, we found a positive correlation between embryo weight and length with embryonic vesicle weight. Further, the number of embryos was negatively correlated with embryonic vesicle weight : embryo weight ratio ($r = -0.56$; $P < 0.001$).

On the other hand, late mortality may be related to less faetal spacing and smaller empty spaces around each embryonic-placental unit (Da Silva *et al.*, 2016). Thus, increased late embryonic mortality is already a consequence of uterine crowding. So, the smallest space also compromises placental development (Town *et al.*, 2004), and these recently non-viable embryos still occupy space that cannot be used by the surviving embryos (Vallet *et al.*, 2011).

The uterine region may also be associated with faetal development during pregnancy. Several studies investigated the relationship between intrauterine position and faetal weight and showed an increase in faetal weight according to uterine regions from cervix to the uterine apex region during late gestation (Wise *et al.*, 1997; Kim *et al.*, 2009; Che *et al.*, 2016; Palencia *et al.*, 2018). Similar to these trials, in our experiment, higher values of embryo length, embryonic vesicle weight and embryonic vesicle weight : embryo weight

ratio were observed at the apex region of the uterus. However, embryo weight was similar between the apex and cervical regions of the uterus.

Che *et al.* (2016) suggested the highest faetal weights in the uterine apex region are related to higher placental efficiency at the uterine apex region, and these placentas showed more significant transport and metabolism of amino acids. Moreover, differences in the vascularization of the different uterus region were also described. Guimarães *et al.* (2014) observed larger vessels in the apex, which likely related to the proximity of this region with the first branches emitted by the uterine artery. Therefore, similar to these studies, we show that the uterine region of apex and cervix has different embryonic development.

In the present study, ALT treatment improved the endometrial characteristics, gland duct area, gland duct perimeter and total endometrial area compared to Control treatment. Progesterone has an essential role in the activity of uterine glands. Several studies indicate that progesterone and other additional factors derived from the mother and placenta are associated with uterine gland hypertrophy and hyperplasia (Bailey *et al.*, 2010). Furthermore, progesterone can increase the expression of various uterine secretory proteins that are components of histotroph in pregnant pigs as well as stimulation of acid phosphatase 5, tartrate resistant (also known as uteroferrin) (Roberts and Bazer, 1988; Bailey *et al.*, 2010; Spencer *et al.*, 2019). Therefore, we suggest that ALT supplementation may be related to improvements in the uterine gland development during early pregnancy.

In this study, there were no effects in embryo size according to the uterine gland improvements observed in ALT treatment. The glandular epithelium on 17 days of pregnancy remains low cuboidal, with a greater rough endoplasmic reticulum and Golgi but low secretory activity. The glandular epithelium remains simple, coiled and tubular until day 30 of pregnancy, developing characteristics of increased secretory activity around day 35 (Spencer *et al.*, 2019). Therefore, the largest uterine gland hypertrophy occurs primarily after day 30 of pregnancy and relates with the increased production of histotroph that fills and increases the lumina of uterine glands with secretory products (Basha *et al.*, 1980; Perry and Crombie, 1982; Sinowatz and Friess, 1983; Spencer *et al.*, 2019). Effects of uterine gland improvements on embryo development or/and embryo survival are not expressed until a later stage of pregnancy, thus making it impossible to confirm this information with our experiment design.


The current experiment showed that maternal ALT supplementation might improve the endometrial histological characteristics on day 28 of pregnancy. Moreover, ovarian characteristics and embryo size were positively influenced by maternal ALT supplementation.

In conclusion, the use of ALT during 18 days to oestrous synchronization in gilts could improve the uterine gland's characteristics but did not increase the embryo number and did not improve embryo weight and length at day 28 of pregnancy. However, more studies should be performed

to better understand the effect of maternal ALT supplementation on embryo and endometrial characteristics on later stages of pregnancy.

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Declaration of interest

None.

Ethics statement

None.

Software and data repository resources

None of the data were deposited in an official repository.

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