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Effects of intravaginal devices containing different dosages of medroxyprogesterone acetate for the control of the estrous cycle in gilts

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ABSTRACT

This aim of this study was to investigate the effectiveness of intravaginal devices (IVDs), containing medroxyprogesterone acetate (MPA), for controlling the estrous cycle in pubertal gilts. Gilts were assigned to four treatments: Control (no IVD); IVD containing 100 (IVD100), 200 (IVD200) or 400 mg (IVD400) of MPA. The IVDs were inserted on day 12 of the estrous cycle and maintained intra-vaginally for 14 days. The percentage of gilts in estrus, interval between IVD removal and estrous onset, adjusted farrowing rate (AFR), total number of piglets born (TPB), follicle size and serum progesterone (P4) were recorded. None of the gilts expressed estrus during the IVD treatment period. All gilts of the control group expressed estrus (15/15; 100%) which was greater ($P = 0.03$) than all IVD-treated gilts (33/44; 75%); however, there were no treatment differences ($P = 0.09$). The interval between IVD removal and estrous onset was shorter for IVD100 (3.8 ± 0.6 d) compared to IVD400 (5.3 ± 0.6 d; $P = 0.05$). The IVD400-treated gilts had smaller follicles than the IVD100-treated gilts ($P = 0.05$). The P4 concentrations were similar among treated groups ($P = 0.99$). The AFR did not differ among treatment groups ($P = 0.37$); however, the control group had a greater TPB than the other treatment groups ($P = 0.04$). The gilts treated with IVDs had longer interval to estrous expression. The most effective dosage was 400 mg of MPA, considering both the minimal follicular growth during the IVD treatment period and the lesser numbers of persistent follicles.

1. Introduction

The current knowledge about reproductive endocrine regulation, follicular development, and accessibility to active substances has facilitated the use of reproductive biotechnologies in sow farms. The synchronization of stage of the estrous cycle among sows or gilts (Brüssow et al., 2009) is an example of a biotechnology that could be used for several swine management strategies. This approach is also used for batch farrowing (14–28 d interval) in swine production, to allow the synchronization of management

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endeavors such as weaning, estrous detection, breeding, pregnancy detection, and farrowing. Furthermore, the use of these techniques has important implications for managing pig health and production (Knox, 2014).

Adequate control of reproductive functions is necessary to utilize new technologies in swine production. In this context, progesterone analogs are used for: (a) placing replacement gilts in breeding groups, synchronizing gilts for fixed-time insemination (Driancourt, 2013); (b) delaying breeding in young sows because of lactational catabolism (Fernández et al., 2005; Van Leeuwen et al., 2011); and (c) delaying farrowing to avoid early parturitions (Gaggini et al., 2013) or parturitions on weekends when there is limited personnel expertise to assist with farrowing (Freling et al., 2013).

The use of progestagens for controlling the estrous cycle in swine females date from the 1950s to the 1980s. In that period the use of daily injectable progesterone was efficient to control the estrous cycle in gilts (Ulberg et al., 1951; Baker et al., 1954). In later studies, progestagens administered by the oral route were extensively investigated. The 6-Methyl-17-acetoxyprogesterone (medroxyprogesterone acetate – MPA) is a compound reported to have a greater progestin-efficacy than 17-alpha-acetoxyprogesterone (Nellor, 1960). Allyl-trenbolone (Altrenogest) administered orally was also investigated and was effective for synchronization of estrus in swine (Kraeling et al., 1981). The use of altrenogest for estrous suppression was subsequently used extensively in the swine industry.

Altrenogest is a synthetic progestagen, registered as a hormonal feed additive for synchronization of estrus in mature swine (Knox, 2014). This analog could be orally administrated daily (15–20 mg/day) for 14–18 days if the stage of the estrous cycle is not known. Estrous behavior in gilts is then observed for 4–7 days after progestagen withdrawal (Knox, 2016). Although the effects of feeding altrenogest are satisfactory, the daily administration protocol that extends for several days is laborious. Furthermore, individual oral administration is not practical in group-housed gilts because of partial dosing and to maintain the efficacy of the product (Knox, 2014). To overcome these challenges, a potential alternative is the use of intravaginal devices (IVDs), which are used extensively in cattle and sheep for synchronization of estrus.

Most of the studies performed in the past were conducted by daily administration of altrenogest or MPA via the oral route (Estill, 2000). Compared to altrenogest, the use of IVD requires less labor for its administration and provides for more precise control as compared with progestagen dosage because there is not dependence on the daily intake of the product (Haas et al., 2017). The IVDs, however, are not yet available for commercial swine production and few studies have been conducted to assess the use of these devices compared to progestagens administered by the oral route. The effects of IVDs containing MPA were analyzed by Gasperin et al. (2011) and Freling et al. (2013), and there were promising results on parturition-delaying effects in sows with use of these IVDs. The use of IVDs containing MPA resulted in an effective maintenance of pregnancy after prostaglandin-induced luteolysis. There is an efficacy of IVDs for estrous suppression in sows after weaning (Gasperin et al., 2011). These results encouraged the investigation of synchronization of estrus in gilts using IVDs. The present study was designed to evaluate the use of IVDs containing different dosages of MPA for the synchronization of time of estrus among gilts. The hypothesis for this study is that an adequate dose of MPA in the IVD can be used to more precisely suppress the release of gonadotropins, preventing the development of ovulatory follicles and, therefore, allowing for the synchronization of time of estrus among gilts after IVD removal from the vagina.

2. Materials and methods

The study protocol was approved by the Ethics Committee of Animal Utilization (CEUA) of the Federal University of Rio Grande do Sul (UFRGS), under Process No. 35497.

2.1. Location

The study was performed in a commercial gilt development unit, with an inventory of 1200 gilts, located in the Midwest of Santa Catarina State (27° 00′30″ S, 51° 09′ 06″ W), southern Brazil. The study was conducted between September and November with an average, minimum, and maximum temperature of 20.8, 15.5, and 25.7 °C, respectively, and relative humidity of 85.3%, during the spring season in the southern hemisphere.

2.2. Animals, housing and management

A total of 59 gilts (PIC Camborough®, Hendersonville, TN, Landrace × Large White crossbred) were used. The gilts were housed in collective pens of 25–30 animals with automatic feeders (floor feeding). At 1 week after the first estrous detection, the gilts were stall-housed (2.20 × 0.60 m). Starting 2 weeks before insemination, the gilts were fed 3 kg/day of a standard corn-soybean diet (3.2 Mcal ME/kg, 14% crude protein and 0.7% SID Lys). From insemination until 86 days of gestation, gilts were fed 2.4 kg/day. From 87 days of gestation until the transfer to the farrowing rooms, gilts were fed 2.6–2.8 kg/day. There was *ad libitum* access to water throughout the experimental period. Puberty induction started at 160 days of age, being induced by daily boar exposure for 20 min in each pen, with the estrous detection observations were confirmed using the back-pressure test. The gilts that did not express estrus until 15 days after boar exposure were not included in the study. At the first estrus, the gilts were an average of 168.1 ± 4.6 days of age and weighed 130.6 ± 6.6 kg with no differences among treatments ($P \geq 0.58$).

2.3. Experimental design

Polyurethane IVDs, with dimensions of 4 × 4 × 5 cm, were infused with different dosages of MPA using procedures previous described by Freling et al. (2013). At day 12 of the estrous cycle (Day 0 = estrus onset), the gilts were assigned according to age and body weight into homogeneous groups of gilts for distribution into the following treatments: Control with no IVD; IVD100; IVD200; and IVD400, with the IVDs containing 100, 200, and 400 mg of MPA, respectively. There were insertions of the IVDs into the vagina and the IVDs remained in the vagina for 14 days before being removed. Estrous detection using fence-line boar contact and the back-pressure test was performed once a day from day 12 of the estrous cycle (beginning of treatments) to the time standing estrus was detected or day 10 after IVD removal.

2.4. IVD insertion and removal

The IVD was inserted in the vagina using a speculum. Before IVD insertion, the vulva was cleaned with a paper towel, and a spray containing 6.8 g terramycin and 2.0 g hydrocortisone (Terra-Cortril® Spray, Zoetis, Brazil) was used on the surface of the IVD. The retention of or loss of the IVD from the vagina was verified twice a day in all gilts (0800 and 1500 h) to ascertain the capacity of maintaining the device in the vagina during the treatment period. At the time of removal, the IVD was examined for traces of blood and secretions. Presence or absence of vulvar discharge was also recorded.

2.5. Artificial insemination

The protocol involved artificial inseminations at 0, 24 and 48 h after the onset of estrus, for as long as the gilts were in standing estrus. Heterospermic semen doses, with 2.2 billion sperm cells in 90 mL diluent, were deposited into the cervix while there was a presence of a mature boar.

2.6. Ultrasonic evaluations

Transabdominal ultrasonography (Sonoscape AV6, Sonoscape Brazil, Rio de Janeiro) using a 5 MHz transducer was performed for ovarian evaluation of all gilts. From the day of IVD insertion until its removal, the gilts were evaluated at 48 h intervals. After IVD removal until ovulation, the evaluations were performed at 24 h intervals. The presence or absence of follicles was recorded and the diameters of the three largest follicles were determined at each assessment. Follicles larger than 12 mm in diameter were considered to be persistent follicles. Pregnancy was detected 25 days after insemination with trans-abdominal ultrasonic assessments.

2.7. Blood collection and hormone assays

On days 0, 7, and 14 during the period of IVD treatment, blood samples (10 mL each) were collected from the jugular vein of 12 gilts (IVD100 – 4; IVD200 – 3; IVD400 – 5). There was collection of additional blood samples from eight gilts that did not express estrus within 10 days after IVD removal. The blood samples for progesterone analysis were collected in polypropylene tubes containing EDTA solution and centrifuged at 3000*g* for 10 min. Plasma was collected and stored at -20 °C for further analysis. Plasma progesterone concentrations were determined using a competitive immunoassay utilizing direct chemiluminescence technology (ADVIA Centaur® Siemens Healthcare Ltd, São Paulo, SP, Brazil). The assay had a sensitivity of 0.15 ng/mL and intra- and inter-assay coefficients of variation of less than 10%.

2.8. Statistical analysis

Data were evaluated for normality and analyzed using the Statistical Analysis System SAS 9.1. The results are expressed as least squares (LS) means ± standard error of the mean (SEM) or percentages, depending on the variable. Continuous variables were evaluated using the MIXED procedure with the comparison of means by the Tukey–Kramer test. The group of gilts and the week were included in the model as random variables. The follicle size and progesterone concentration during the period of IVD treatment were analyzed using repeated measures (MIXED procedure), considering the effect of treatment, time point of evaluation and the interaction as fixed effects, and also including group of gilts and week as random variables. After IVD removal, analysis of follicle size was performed until day 5 after IVD removal and values at each time point were evaluated using the MIXED procedure. The percentage of follicles larger than 4 mm was analyzed as binary response using logistic regression models utilizing the GLIMMIX procedure. The other frequency variables were analyzed using the Fisher Exact test. The percentages of stillborn piglets and mummified fetuses were analyzed using the NPAR1WAY procedure and the treatment effects were compared utilizing the Kruskal–Wallis test. The adjusted farrowing rate (AFR) was calculated considering the exclusion of dead gilts and those culled for non-reproductive reasons.

3. Results

During the 14 days of treatment, two IVDs were not retained in the vagina of gilts from the IVD100 group. One of these was lost from the vagina on day 1 post-insertion and was replaced with a new IVD and the other was lost from the vagina on the day when there was withdrawal of IVDs from the vagina and was not replaced. Adherence of IVD to the vaginal wall was not detected, thereby facilitating ease of IVD removal from all gilts. Vaginal discharge was observed in 69.2%, 57.1% and 42.9% of the gilts in IVD100, IVD200 and IVD400, respectively ($P = 0.41$). The presence of blood on the IVD at the time of withdrawal from the vagina was observed in only 6.8% of the gilts (3/44; IVD100 – 2, IVD200 – 0, IVD400 – 1; $P = 0.31$).

Table 1
Reproductive variables of gilts submitted to the treatment with an intravaginal device (IVD) containing different dosages of medroxyprogesterone acetate (MPA) for 14 days (LS means ± SEM).

Variables	Treatments[1]			
	Control	IVD100	IVD200	IVD400
Number of gilts	15	14	15	15
Interval IVD removal–estrus (d)	–	3.8 ± 0.6 [a]	4.6 ± 0.5[ab]	5.3 ± 0.6[b]
Inter-estrus interval (d)[2]	19.4 ± 1.0 [a]	28.5 ± 1.1[b]	29.3 ± 0.8[b]	30.1 ± 1.0[b]
Estrus length (h)	60.8 ± 3.0 [a]	54.0 ± 3.4[ab]	45.8 ± 3.5[b]	52.8 ± 3.7[ab]
Females in estrus (%)[3]	15/15 (100)	12/14 (85.7)	11/15 (73.3)	10/15 (66.7)
Persistent follicles (%)	0/15 (0)[a]	6/14 (42.9)[b]	4/15 (26.7)[a]	1/15 (6.7)[a]
Adjusted farrowing rate (%)[4]	14/15 (93.3)	8/10 (80.0)	8/10 (80.0)	10/10 (100)
No. of total piglets born	13.4 ± 1.0 [a]	9.8 ± 1.3[b]	10.6 ± 1.2[ab]	10.9 ± 1.2[ab]
No. of total piglets born alive	12.2 ± 1.1 [a]	8.1 ± 1.4[b]	10.1 ± 1.2[ab]	9.4 ± 1.2[ab]
Stillborn piglets (%)[5]	3.2 ± 1.0 (0; 8.3)	1.8 ± 1.2 (0; 7.1)	1.1 ± 1.1 (0; 9.1)	3.7 ± 2.1 (0; 20.0)
Mummified fetuses (%)[5]	2.1 ± 1.2 (0; 15.4)	6.2 ± 5.3 (0; 42.8)	0 (0; 0)	3.6 ± 2.5 (0; 20.0)

[a–b]Represent differences between the treatment groups ($P \leq 0.05$).
[1] Control – no IVD; IVD100 – 100 mg of MPA; IVD200 – 200 mg of MPA; IVD400 – 400 mg of MPA.
[2] Inter-estrous interval is the interval between the previous and the subsequent estrus.
[3] Estrous expression was recorded during the normal estrous cycle in the control group and for 10 days after IVD removal in the IVD100, IVD200, and IVD400 groups.
[4] Three gilts were removed for non-reproductive reasons (culled or dead).
[5] Median and maximum values in parenthesis.

Gilts treated with all doses of MPA did not have a standing estrous response during the period of IVD treatment. The results with regard to the interval between the previous estrus and the estrus following IVD removal clearly indicated the capacity of the different dosages of MPA to regulate timing of estrous behavior in gilts (Table 1). The interval between IVD removal and symptoms of estrus was shorter ($P = 0.05$) in gilts of the IVD100 as compared with the IVD400 treatment group; however, there were no differences between gilts of the IVD200 and other treatment groups ($P > 0.05$; Table 1). A larger proportion of gilts in the IVD100 treatment group expressed estrus within 3 days after IVD removal when compared to the IVD200 and IVD400 groups ($P = 0.04$; Fig. 1). At 5 days post-IVD removal, the cumulative percentage of gilts in estrus in the IVD100 and IVD200 treatments was similar, whereas there was a lesser percentage of gilts in the IVD400 group that expressed estrus compared to those of the IVD100 group ($P = 0.03$; Fig. 1). All gilts in the control group expressed standing estrus (15/15; 100%), with the percentage being greater ($P = 0.03$) than that for all IVD treatment groups together (33/44; 75%). There, however, were no differences in the percentage of gilts in estrus among treatment groups (P = 0.09; Table 1). When considering all groups, there was a 10-days period after IVD removal when 25.0% (11/

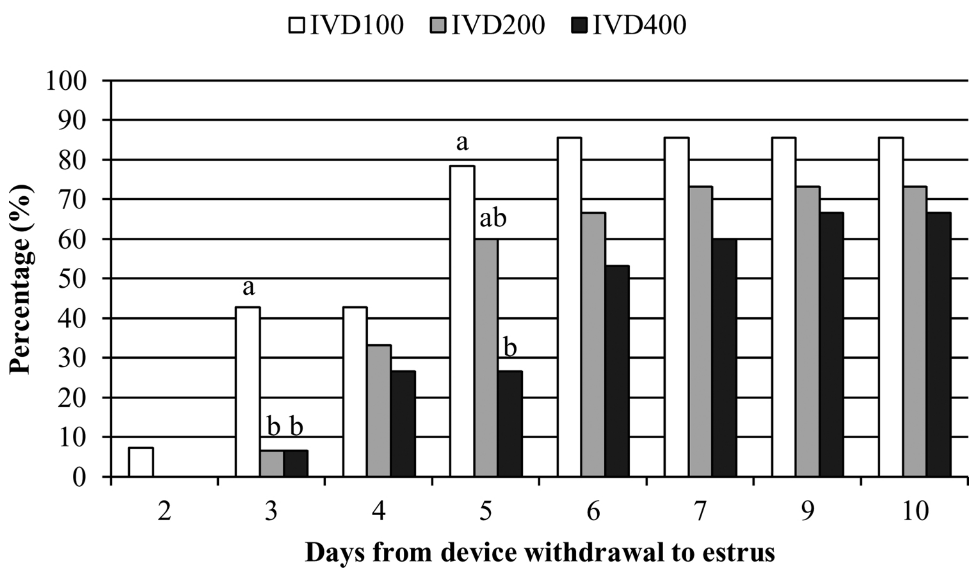

Fig. 1. Cumulative frequency of standing estrus in the gilts that expressed estrus during the 10 days after the removal of the intravaginal device (IVD) containing different dosages of medroxyprogesterone acetate (MPA).
IVD100 – 100 mg of MPA; IVD200 – 200 mg of MPA; IVD400 – 400 mg of MPA.
[a–b]Represent differences among the treatments within each day of evaluation ($P \leq 0.04$).

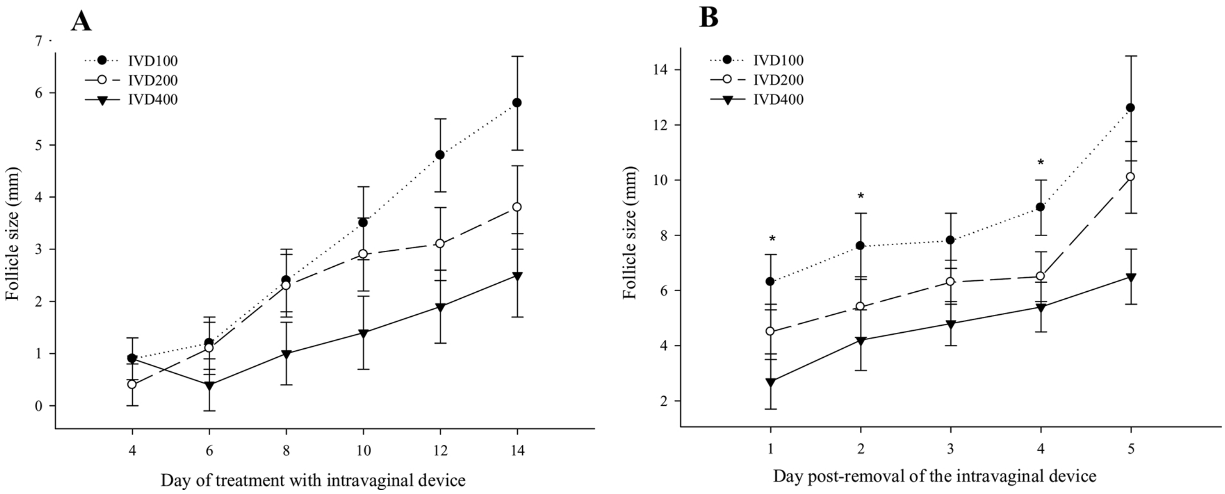

Fig. 2. Size of the three largest follicles at different days during the period of treatment (Fig.A) and until day 5 post-removal (Fig. B) of the intravaginal device (IVD) containing different dosages of medroxyprogesterone acetate (MPA).
Follicle size was considered to be zero in those gilts in which measurement was not possible or absent; Analyses included the follicle size until the day immediately before the standing estrus.
The analyses were performed as repeated measures (Fig. A) from day 4 to 14; (Treatment: $P = 0.05$; Day: $P < 0.01$; Treatment × Day: $P = 0.32$); After IVD removal, the comparison of means was performed at each day of evaluation (Fig. B).

44) of the gilts did not express symptoms of estrus. In 10 of these gilts (IVD100 – 1; IVD200 – 4; IVD400 – 5), estrus was detected approximately 21.0 ± 0.0, 23.0 ± 2.5, and 27.6 ± 2.2 days after IVD removal, respectively. It is noteworthy that these gilts had follicles larger than 4 mm nearly 5 days after IVD removal. The number of gilts with persistent follicles was greater in gilts of the IVD100 than other treatment groups ($P = 0.01$; Table 1). The AFR ($P = 0.37$), number of stillborn piglets ($P = 0.60$) and number of mummified fetuses did not differ ($P = 0.54$) among the treatment groups. The TPB ($P = 0.04$) and piglets born alive ($P = 0.02$), however, were greater in the control than IVD100 treatment group but similar for the other treatment groups.

During the period of IVD treatment, follicular growth was observed in gilts of all the treatment groups, being affected by the day of the IVD treatment period ($P < 0.01$) without there being an interaction treatment by day of the treatment period (Fig. 2A; $P = 0.32$). Overall, the follicle size was smaller during the period of IVD treatment in the gilts of IVD400 (1.4 ± 0.5 mm) than IVD100 (3.1 ± 0.5 mm; $P = 0.05$) group. Although the frequency of gilts with follicles larger than 4 mm was affected by the day of IVD treatment ($P = 0.01$), there was no interaction between the day of the IVD treatment period and treatment ($P = 0.86$; Fig. 3). The IVD400 group has a lesser frequency of gilts with follicles larger than 4 mm compared with the gilts of the IVD100 and IVD200 group, in which there was no difference between these two groups ($P < 0.01$; Fig. 3). The gilts of the control group had larger follicles ($P < 0.01$; Table 2) on day 18 of the estrous cycle (analogous to day 6 of the IVD treatment period) than gilts of all the groups treated with the IVDs and there was no difference in follicle sizes among gilts of the groups treated with the IVDs.

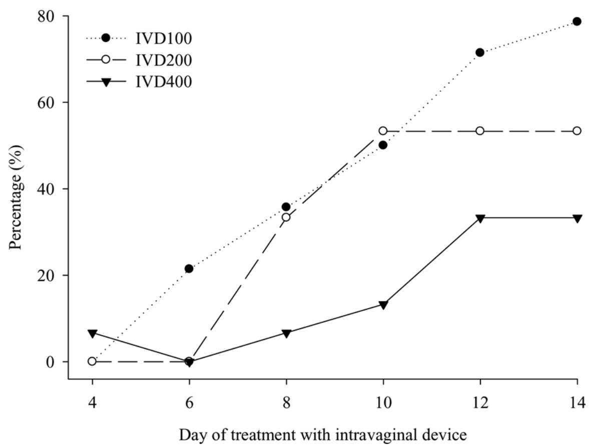

Fig. 3. Frequency of follicles greater than 4 mm on different days during the period of treatment (14 d) with the intravaginal device (IVD) containing different dosages of medroxyprogesterone acetate (MPA).
IVD100 – 100 mg of MPA; IVD200 – 200 mg of MPA; IVD400 – 400 mg of MPA.
Treatment: $P < 0.01$; Time of treatment period: $P = 0.01$; Treatment × time of treatment period: $P = 0.86$.

Table 2
Size of the three largest follicles (mm; LS means ± SEM) on days 4 and 6 of the treatment period with the intravaginal device (IVD) containing different dosages of medroxyprogesterone acetate (MPA), and at the day of estrus onset after the IVD removal.

Variable	Treatment[1]			
	Control	IVD100	IVD200	IVD400
Number of gilts	15	14	15	15
Day of IVD treatment period:[2]				
Day 4	1.7 ± 0.4[x]	0.9 ± 0.4	0.4 ± 0.4	0.9 ± 0.4
Day 6	3.9 ± 0.4[ay]	1.2 ± 0.4[b]	1.1 ± 0.4[b]	0.4 ± 0.4[b]
Number of gilts[3]	15	12	11	10
Estrus onset	5.5 ± 0.7[b]	8.5 ± 0.8 [a]	8.7 ± 0.8 [a]	6.5 ± 0.9[ab]

[a–b]Represents difference in the row ($P < 0.01$).
[x–y]Represents difference in the column ($P < 0.1$).
[1] Control – no IVD; IVD100 – 100 mg of MPA; IVD200 – 200 mg of MPA; IVD400 – 400 mg of MPA.
[2] Days 4 and 6 correspond to days 16 and 18 of the estrous cycle for all the treatments, respectively.
[3] For this analysis, only the gilts in estrus were included; In the gilts with no follicular growth at days 4 and 6 of the IVD treatment period, the follicle size was considered as zero; At estrous onset all the gilts had follicles.

On days 1, 2, and 4 after the IVD removal, follicle size was greater ($P \leq 0.05$) in gilts of the IVD100 than in IVD400 group (Fig. 2B). At estrous onset, the follicle sizes in gilts of the IVD100 and IVD200 groups were larger than in the control group ($P = 0.02$; Table 2). Considering the gilts that expressed estrus, ovulations were observed to have occurred in 100%, 75.0%, 90.9% and 100% in the gilts of the control, IVD100, IVD200, and IVD400 groups, respectively. The percentage of gilts having ovulations tended to be less in the IVD100 than in the control group ($P = 0.08$).

In the IVD-treated gilts, there was a lesser plasma progesterone concentration from the day of IVD insertion until removal ($P < 0.01$; Fig. 4). There was no effect of the treatment ($P = 0.99$) or for the interaction between the day of the IVD treatment period and the treatment group ($P = 0.59$; Fig. 4). Overall, the progesterone concentration in the subsample of anestrous gilts was 37.8 ± 7.4 ng/mL. In 77.8% of these gilts, the average plasma progesterone concentration was 47.7 ± 4.4 ng/mL, whereas for the remaining 22.2% it was 3.3 ± 3.0 ng/mL. For anestrous gilts, there were no differences in progesterone concentration among IVD treated gilts (IVD100 – 26.1 ± 16.6 ng/mL; IVD200 – 34.5 ± 13.6 ng/mL; IVD400 – 46.3 ± 16.6 ng/mL; $P = 0.61$).

4. Discussion

The oral administration of altrenogest for synchronization of estrus in gilts (Martinat-Botté et al., 1990, 1995; Estienne et al., 2001; Horsley et al., 2005; De Rensis et al., 2015) and sows (Knox et al., 2017) is well established. Even though there were satisfactory reproductive outcomes, the oral use of altrenogest is laborious due to the individual daily oral administration regimen. Studies investigating MPA supplementation by the intravaginal route to synchronize the stage of the estrous cycle among gilts or sows are scarce when compared to the use of MPA administered via the oral route (Nellor, 1960; Dziuk and Baker, 1962; First et al., 1963; Pond et al., 1965). In the present study, IVDs with different dosages of MPA were utilized and found to be effective in inhibiting expression of estrus during the period of IVD treatments. The effectiveness of the IVD–MPA was evident as a result of the absence of gilts expressing estrus during the IVD treatment period and by the greater interval between estrous cycles in MPA-treated gilts than in the control gilts.

The interval between progestagen withdrawal and the onset of standing estrus (4–5 days) was similar to that reported in studies using altrenogest treatment for 14 days (5.4 ± 1.1; De Rensis et al., 2015) or 18 days (5.6 ± 1.0; de Jong et al., 2013) in gilts or

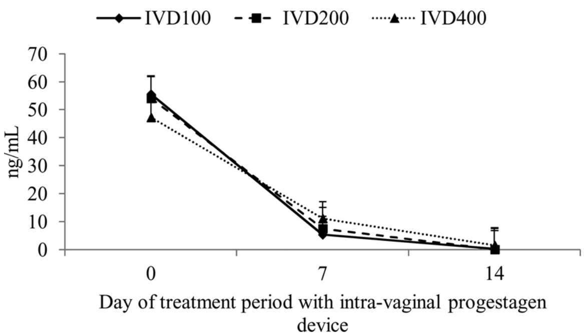

Fig. 4. Plasma progesterone concentration during the period of treatment with the intravaginal device containing different dosages of medroxyprogesterone acetate (MPA) in gilts.
D0 – day of insertion of the intravaginal device.
Treatment: $P = 0.99$; Day: $P < 0.01$; Treatment × Day: $P = 0.59$.

using IVDs containing MPA in sows (Gasperin et al., 2011). Using an IVD containing 200 mg of MPA for 12 days in sows after weaning of piglets, Gasperin et al. (2011) reported that this device had efficacy for controlling the estrous cycle. All sows were detected in estrus 5.3 ± 0.2 days after IVD removal. The subsequent reproductive performance and follicular dynamics, however, were not evaluated in this previous study. In gilts, the use of MPA contained in IVDs had efficacy in controlling the estrous cycle when there were different durations of treatment with the IVDs (Hirayama et al., 2011); however, only a small number of gilts were used in this study.

After altrenogest treatment of pubertal gilts for 14 or 18 days, the percentage of females in standing estrus (until 10 days after the last administration) was greater than 90% (Martinat-Botté et al., 1995; Horsley et al., 2005; De Rensis et al., 2015) and most of the gilts (85.7%) expressed estrus 6 to 8 days after altrenogest withdrawal (Knox et al., 2017). In the present study, all gilts in the control group expressed estrus, but there was a numeric reduction in the percentage of gilts expressing estrus after the IVD treatment period. The presence of vaginal discharge was observed in a large percentage of gilts in the IVD-treated groups. Gilts inoculated with *E. coli* in the uterus during the luteal phase have large amounts of vaginal discharge and considerable disturbances in the reproductive hormonal patterns (Jana et al., 2004). The secretion of LH and estradiol are reduced around the time of estrus in animals with vaginal discharge. Additionally, signs of estrous behavior are less distinct with there being some redness and slight swelling of the vulva without standing estrus in the bacteria-treated group (Jana et al., 2004). In the present study, it cannot be excluded the possibility of an ascending infection of the uterus and consequences on estrous expression, even considering the cervix is tightly closed during the period of IVD treatment. It is also speculated that there were occurrences of ovulations without expression of estrus as a consequence of impairment of follicle quality and steroidogenic function, considering that follicle growth was observed after the end of treatment in the present study. Additionally, there is no knowledge about the clearance rate of MPA after IVD withdrawal from the vagina. Further investigations, therefore, are necessary concerning these issues to evaluate the effectiveness of IVDs for synchronization of estrus in gilts or sows.

The effectiveness of IVDs containing MPA in inhibiting the reproductive axis was also evident because of the greater follicle size observed on day 18 of the estrous cycle in the control group (which corresponds to day 6 of the IVD treatment period). In addition, the differences in follicle growth between days 16 and 18 of the estrous cycle were observed only in the control group. The blood samples were collected at treatment onset, which coincided with day 12 of the estrous cycle, when the concentrations of progesterone are greatest. The progesterone concentrations detected were about 50 ng/mL, similar to that reported for gilts by Estienne et al. (2001). During the period of IVD treatment, the progesterone concentrations were decreasing, and were basal on day 14 of the treatment period, indicating that luteolysis had occurred. Progesterone analogs are active compounds for the suppression of the release of gonadotropins from the anterior pituitary, preventing the development of ovulatory follicles (Knox, 2014). There was follicular development, however, during the period of MPA treatment but without estrous expression in gilts of all treatment groups. Similar findings were reported by Soede et al. (2007), where it was observed that the size of the follicles were 0.5 mm larger during altrenogest treatment, indicating that the suppression of gonadotropin secretion by progesterone analogs is less efficacious than by endogenous progesterone. Another hypothesis is that treatment for 14 days might be too long for adequate MPA release, suggesting that device replacement would be necessary for the concentrations used in the present study to be maintained throughout the MPA treatment period. In ewes treated for 14 days with a single IVD, the LH pulse frequency was greater compared to those animals having replacement of the devices every 5 days (Flynn et al., 2000). Furthermore, a decrease of 63% in MPA serum concentrations was noted from day 2 to day 13 during an IVD treatment period in sheep (Greyling et al., 1994), indicating the decrease in progestagen as the treatment period advanced.

After IVD removal, all gilts in the IVD400 group had follicular growth with an average size of 5.1 ± 0.8 mm. This follicle size corresponds to that observed in the control group at the time of estrous onset. Considering the follicle size, it was expected that there would be estrous expression in these gilts; however, a significant proportion (5/10) of gilts did not express estrus until 10 days after IVD removal. Interestingly, these five gilts were detected in estrus 27.6 ± 2.2 days after IVD removal, indicating these gilts had ovulations without estrous expression after the IVD withdrawal from the vagina. Additionally, it is also noteworthy that one gilt in IVD100 and four gilts in IVD200 treatment group had an inter-estrus interval of 21 and 27 days, which could indicate there was a previous estrous expression closer to the time of IVD removal from the vagina. The luteal phase progesterone concentrations present in these gilts on day 10 after IVD removal reinforce this hypothesis. The reason why these gilts were not detected in standing estrus within 10 days after IVD removal remains unclear.

Exogenous gonadotropins (eCG or eCG + hCG) have been used to stimulate follicular growth in gilts (Estienne et al., 2001), and the use after altrenogest feeding (14 days) resulted in 91.7% of gilts in standing estrus during the 7 days after cessation of the PG600 treatment (Estienne and Crawford, 2015). Based on the responses of follicle growth and estrus onset after IVD removal in the present study, in a pilot study with 12 gilts there was use of 400 IU of eCG and 200 IU of hCG (Duogestal®, MSD Saúde Animal, Brazil) administered 24 h after IVD removal. The gilts (four in each group) were treated with three dosages of MPA (100, 200 and 400 mg) and the IVDs were maintained for 11 days. The overall percentage of gilts in estrus (7/12; 58.3%), however, was not increased by eCG + hCG administration compared to results of the present study, in which gonadotropins were not used. Even considering the absence of effects of the gonadotropins in the pilot study, it is an alternative approach that should be considered in further studies concerning the development of protocols for the use of IVD in gilts and/or sows.

The large percentage of gilts with persistent follicles in IVD100 and IVD200 treatment groups could be attributed to the follicle growth during the IVD treatment period. Furthermore, this could be a reason for the aging of follicles, which might affect the response to preovulatory LH surge release. Using different dosages of MPA (50 to 400 mg) via the oral route of administration in gilts and sows, First et al. (1963) observed an average of 58% of persistent follicles after the withdrawal of treatments. The greater occurrence of persistent follicles in the IVD100 group in the present study is consistent with the observations of First et al. (1963)

with dosages smaller than 240 mg of MPA. In a comparative study with two dosages of altrenogest (2.5 or 15 mg/day) administered for 18 days, there was a greater incidence of ovarian cysts in females treated with the smaller dosage and these conditions were also thought to be attributed to follicular development without ovulation (Redmer and Day, 1981). In pubertal gilts treated with altrenogest, the occurrence of cystic ovaries (14%) was associated with a lesser responsiveness to LH of the follicles (Ziecik et al., 2017). Additionally, it is likely that follicle aging associated with a lesser LH release from the anterior pituitary had a negative effect on the reproductive performance, which in turn is a plausible reason for fewer piglets being born in the MPA-treated females compared to the females of the control group. This could also explain why there was a lesser percentage of the total number of normal embryos (12.2%) in gilts treated with MPA by the IVD route for 16 days than what was present in the control females (Hirayama et al., 2011).

5. Conclusions

The use of 100, 200, or 400 mg of MPA released by an intravaginal device inhibited estrous expression in gilts. The use of the 400 mg dosage, however, was more effective in controlling the estrous cycle, based on the lesser follicular development observed during the period of IVD treatment and lesser numbers of persistent follicles. Further studies are needed to elucidate the reasons for the decreased number of gilts detected in estrus and the fewer piglets born after MPA treatment.

Declaration of Competing Interest

All authors involved in conducting this research declare there are no conflicts of interest.

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