Article

Follicular dynamics and ovulation time in gilts and post-weaning sows

Sara I. Williams, R. Luzbel de la Sota

Abstract — Ultrasonography was used to study follicular dynamics from the beginning of estrus to ovulation in pubertal gilts and post-weaning sows. Ultrasound turned out to be a useful tool to determine patterns of growth of preovulatory follicles, to predict ovulation time, and to design protocols for fixed time insemination.

Résumé — Dynamique folliculaire et moment de l'ovulation chez les cochettes et les truies en période post-sevrage. On a utilisé l'échographie pour étudier la dynamique folliculaire du début de l'œstrus jusqu'à l'ovulation chez les cochettes post-pubertaires et les truies en période post-sevrage. L'échographie s'est avérée un outil utile pour déterminer les tendances de croissance des follicules pré-ovulatoires, prédire le moment de l'ovulation et concevoir des protocoles pour une insémination à un moment fixe.

(Traduit par Isabelle Vallières)

Can Vet J 2017;58:65–69

Introduction

During follicular development in the estrous cycle, 3 features appear in all species. These features are: i) sequence of events (recruitment, selection and dominance); ii) need for sequential gonadotropins, follicle-stimulating hormone (FSH) for recruitment and luteinizing hormone (LH for dominance); and iii) range of requirements (number of waves per cycle, follicles by wave number) as well as temporary requirements (time of selection and duration of dominance). When comparing the pattern of follicular development among animal species, follicular waves are described in bovine, ovine, and equine, while only observed during the prepubertal period in swine (1,2).

The process of follicular development in the sow involves 2 of the events mentioned above as the first feature: recruitment and selection. Both in prepubertal and pubertal gilts and in sows, there is a pool of approximately 50 follicles of 1 to 6 mm on the surface of the ovary (3).

In pigs, the ovaries are noteworthy for having a large number of follicles compared with other species (4). During the luteal phase, 30 to 90 small follicles of 1 to 2 mm, and 30 to 50 medium-sized follicles of 2 to 7 mm can be seen in each ovary (3,5,6). Conversely, during the follicular phase, the number of small and medium-sized follicles decreases dramatically in both ovaries, leaving a total of approximately 20 follicles, most of which are ovulatory follicles (5), which reach 7 to 10 mm in diameter prior to ovulation (7).

At the beginning of the luteal phase, immediately after ovulation, pig ovaries have only small antral follicles. However, following decreases in concentration of Inhibin and E_2 after ovulation, negative feedback on FSH disappears, FSH concentrations increase, and a wave of synchronized follicle development is initiated. Afterwards, Inhibin production from this wave of follicles reduces FSH production and increasing concentrations of progesterone (P_4) from developing corpora lutea suppress gonadotropin secretion (6,8). Hence, there is a continuous growth and atresia of ovarian follicles during the rest of the luteal phase (days 7 to 15 of the estrous cycle), without evidence of waves or follicular dominance (5,7,9). This lack of dominance is associated with no changes in plasma FSH and E_2 concentrations for the remainder of the luteal phase (1,9). Ovulatory follicles start to grow between days 14 to 16 of the estrous cycle (10). Ovulatory follicles are recruited from the pool of antral follicles, which develop during the luteal phase of the cycle, and reach about 5 mm (11). Towards the end of the luteal phase, P_4 levels fall and the increasing FSH and LH produce follicle recruitment. Small follicles that do not have enough FSH and LH receptors are not recruited and became atretic (12).

Ultrasonography has been available for the last 2 decades to study follicular development and time of ovulation in swine production (12). Initially, transabdominal ultrasonography was used to study follicular development and time of ovulation (13). More recently, it was demonstrated that transrectal ultrasonography is an appropriate method to study pig follicular dynamic because it allows for real-time studies of follicle development and time of ovulation without interfering with the processes (12).

Cátedra y Servicio de Reproducción Animal, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Calle 60 y 118, La Plata, B1900AVW, Argentina (Williams, de la Sota); CONICET, Godoy Cruz 2290, CABA, C1425FQB, Argentina (de la Sota).

Address all correspondence to Dr. Sara I. Williams; e-mail: swilliams@fcv.unlp.edu.ar

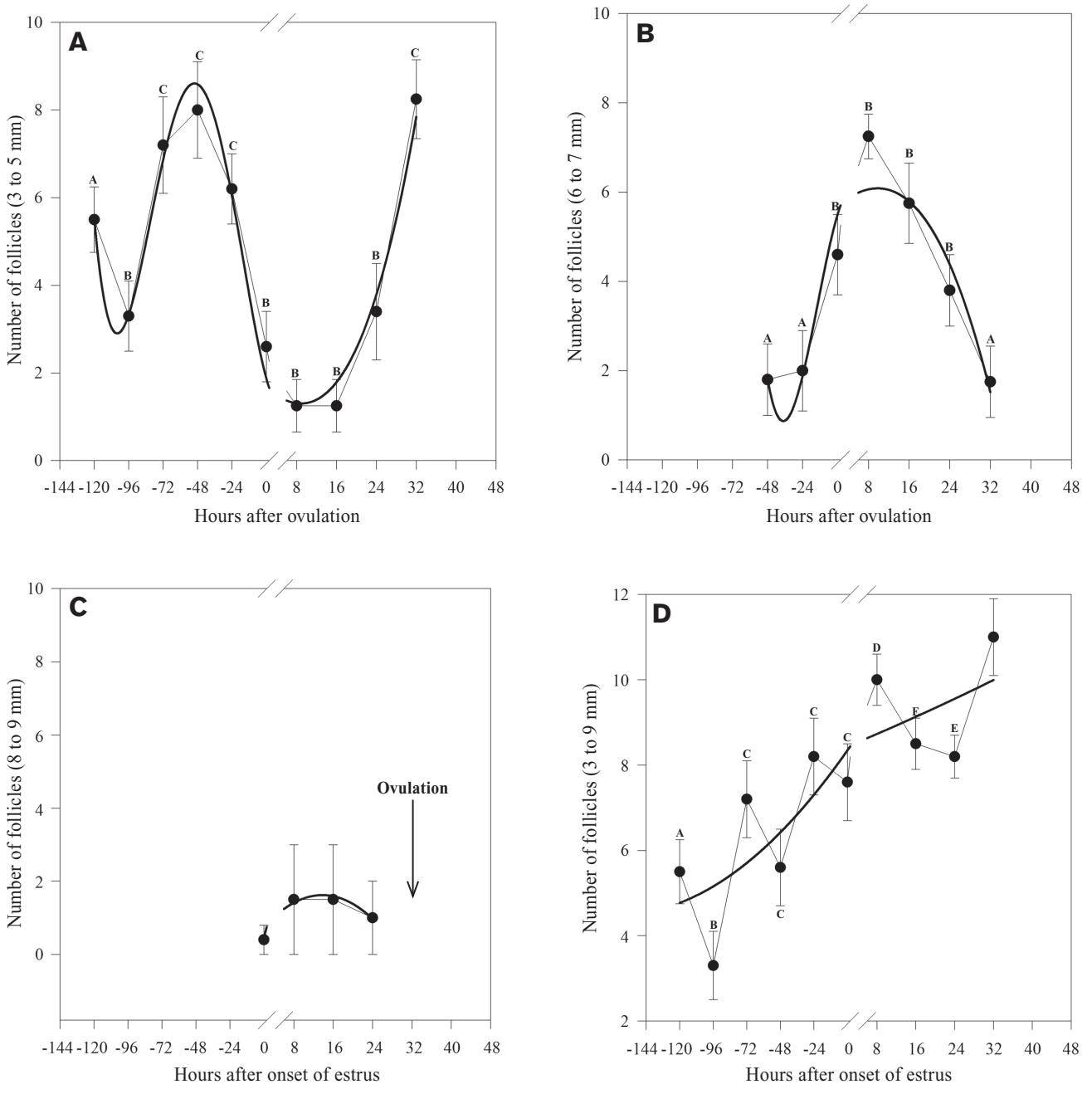

Figure 1. Mean [± standard error (SE)] number of follicles from 120 h before to 32 h after the onset of estrus [3 to 5 mm (A), 6 to 7 mm (B), 8 to 9 mm (C), and 3 to 9 mm (D)] in pubertal gilts. Values with different letters differ at $P < 0.05$.

The aim of the present work was to study the growth pattern of follicles from the beginning of estrus to ovulation in post-weaning sows using daily transrectal ultrasonography, and from day 15 of the estrus cycle until ovulation time in pubertal gilts, using trans-abdominal ultrasound. The information in this report warrants the study of size differences of ovulatory follicles between gilts and sows and the possibility of daily scans of the ovary by transrectal ultrasonography. Consequently, this information can be used to design protocols for fixed time insemination.

Materials and methods

Pubertal gilts [crossbred Landrace (LD) × Large white (LW) 90 to 100 kg body weight (BW), aged 160 to 180 d)] were used. Gilts (n = 7) were housed in individual pens (Veterinary Teaching Hospital, Faculty of Veterinary Sciences, National University of La Plata, Argentina), fed a commercial gestation diet, and treated with gonadotropins (400 IU of eCG + 200 IU of hCG; Duogestal, Syntex SA, Buenos Aires, Argentina), 5 mL, IM, on the day of arrival. Estrus was observed for 96 h after

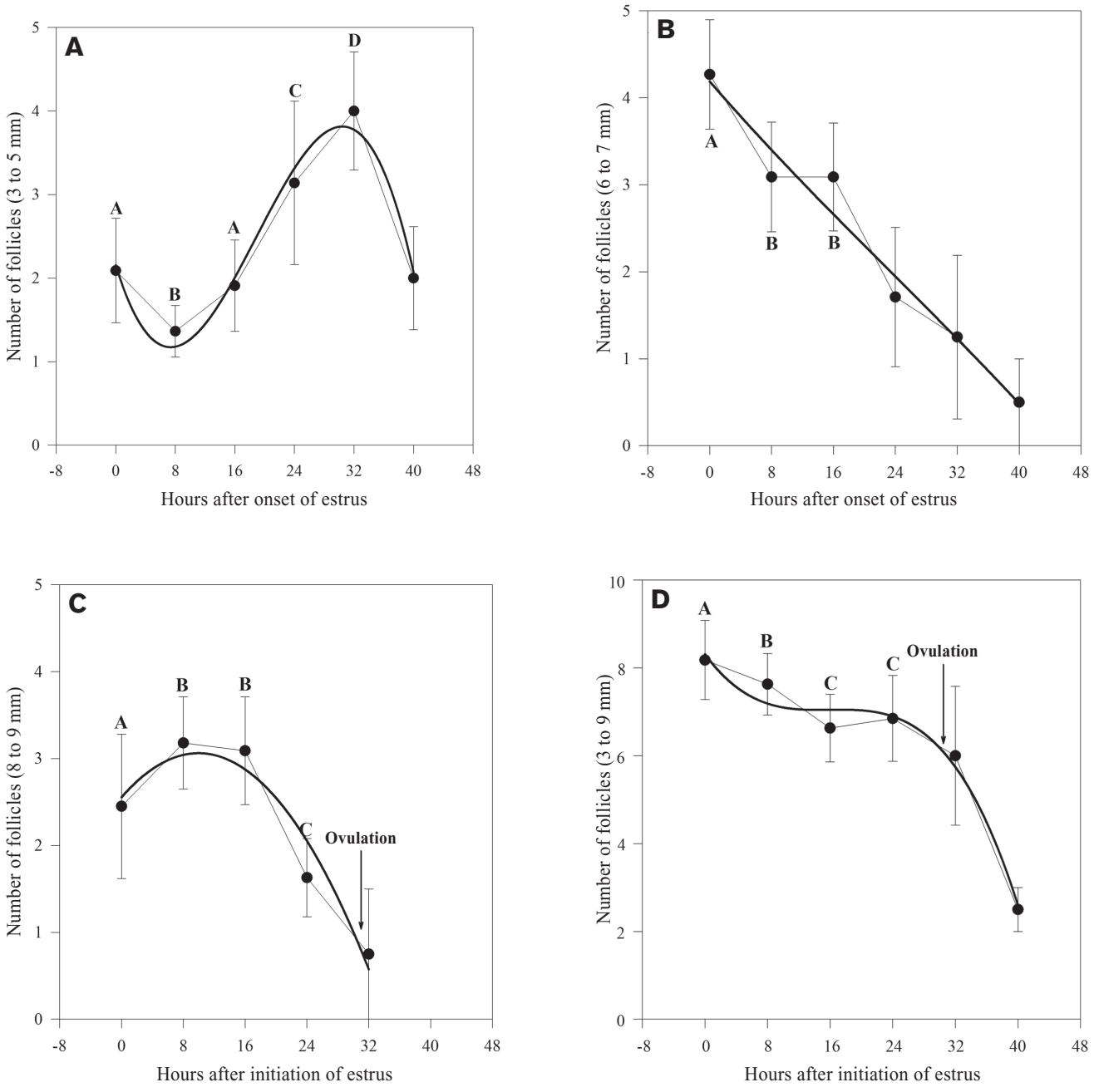

Figure 2. Mean (± SE) number of follicles from 8 h before to 40 h after the onset of estrus [3 to 5 mm (A), 6 to 7 mm (B), 8 to 9 mm (C), and 3 to 9 mm (D)] in post-weaning sows. Time of ovulation 29 ± 2 h of onset of estrus. Values with different letters differ at $P < 0.05$.

treatment. All experimental procedures were in compliance with the EC guidelines for animal experimentation and were approved by the local animal care committee, the Graduate School and the Laboratory Animal Care and Use Committees of the Faculty of Veterinary Sciences at National University of La Plata.

Ultrasound examination started on day 15 of the estrous cycle. Animals were monitored once a day for ovarian mapping and to record the number, location, and size of follicles > 3 mm. From day 18 of the estrus cycle, estrus detection was performed twice a day, and from the time that gilts showed signs of estrus (0 h), ultrasound examinations were performed every 8 h. The time of ovulation was defined as the time of the first ultrasound images without suspected ovulated follicles, less 4 h. Ovulation was confirmed with a subsequent ultrasound examination. Follicular development was monitored by trans-abdominal ultrasonography using a Tringa S50 (Pie Medical, Maastricht, The Netherlands) ultrasound machine with a sectorial probe and a frequency of 5 to 7.5 MHz. The number of follicles according to size category (3, 4, 5, 6, 7, 8, and 9 mm) and the total number of follicles, from the first scan until ovulation time were recorded.

The study in post-weaning sows was conducted on a commercial farm and crossbred LD × LW multiparous sows were

used (n = 11). After weaning, sows were observed twice a day for the onset of estrus (according to the routine work on the farm, consisting of introducing a mature boar in the pen and registering the immobile response of sows and swollen vulvas). The onset of estrus (0 h) was defined as the time at which sows were first observed in estrus. From 0 h onwards, both ovaries of each sow were monitored every 8 h and the number, location, and size of follicles > 3 mm, were registered to make ovarian maps. Time of ovulation was defined as the time of the first ultrasound images without suspected ovulated follicles, less 4 h. Ovulation was confirmed with a subsequent ultrasound examination.

Transrectal ovarian ultrasonography was performed with a Pie Medical S100 ultrasound (Maastricht, Netherlands) with a sectorial probe (5.0 to 7.5 MHz) placed in a guide of polyvinyl chloride (PVC), with a fixed angle of 25°. The number of follicles from 3 to 5 mm, 6 to 7 mm, 8 to 9 mm, and the total number of follicles, from time −8 h to 48 h of observed estrus were recorded.

All data were analyzed with SAS® (Cary, North Carolina, USA) PROC MIXED and PROC REG procedures. To compare the number of follicles per class during the study period, multiple comparison test PDIFF of PROC MIXED was used.

Results

In pubertal gilts, only 3 to 4 mm follicles were observed at the beginning of scans (day 15 of the estrous cycle), and between 120 h and 48 h before the onset of estrus. Between 96 h before and the onset of estrus, the number of 3 to 5 mm follicles increased rapidly to peak at 72 h before and then decrease at 24 h before the onset of estrus (Figure 1A; $P < 0.05$), and was explained by a quartic polynomial function ($P < 0.01$). At the onset of estrus (0 h), the number of 3 to 5 mm follicles decreased and was followed by an increase in the number of 6 to 7 mm follicles, peaking at 8 h after estrus and decreasing thereafter (Figure 1B; $P < 0.05$), and was explained by a cubic polynomial function ($P = 0.05$). Concomitantly, after the onset of estrus, there was an increase in 8 to 9 mm follicles (Figure 1C), that was explained by a cubic polynomial function ($P < 0.10$). Ovulation occurred at 36.5 ± 1.8 h [mean ± standard deviation (SD)] from the onset of estrus and the size of the preovulatory follicles was 5.43 ± 0.06 mm. After ovulation, the number of 3 to 5 mm follicles increased (Figure 1A; $P < 0.05$). Lastly, the number of follicles increased with time (Figure 1D) following a cubic polynomial function ($P = 0.02$).

In post-weaning sows, the number of 3 to 5 mm follicles had a continuous increase from 8 to 32 h and then decreased sharply by 40 h after the onset of estrus (Figure 2A; $P < 0.05$) and was explained by a cubic polynomial function ($P = 0.04$). The number of 6 to 7 mm follicles decreased from 0 to 40 h after the onset of estrus (Figure 2B; $P < 0.05$) and was explained by a linear polynomial function ($P < 0.01$). The number of 8 to 9 mm follicles increased from 0 to 8 h, then plateaued, and then started to decrease 16 h after the onset of estrus (Figure 2C; $P < 0.05$) and was explained by a 3rd degree polynomial function ($P = 0.05$).

Transrectal ultrasonography allowed visualization of only 1 face of the ovary, and this could explain the total number of follicles reported in this study. Ovulation occurred at 29.1 ± 2.9 h from the onset of estrus and the size of the preovulatory follicles was 7.0 ± 1.0 mm, with a weaning-to-estrus interval close to 6 d [131.6 ± 11.3 h; 95% confidence interval (CI): 106.3 to 156.8].

Discussion

To our knowledge, this is the first study on follicular dynamics from the beginning of estrus to ovulation in pubertal gilts and in post-weaning sows. In pubertal gilts, follicles observed at the beginning of scans (towards the end of the luteal phase) were only 3 to 5 mm. After luteolysis, small and medium-sized follicles quickly disappeared, while the > 6.5 mm appeared and increased the number near the time of ovulation. Selection of follicles in pubertal gilts is a unique process that takes place in the presence of corpus luteum (CL). Previous data demonstrate that the presence of CL alters the blood supply and can indirectly influence the growth of follicles (14). In addition, the population of follicles differs dramatically before and after the formation of the CL, and before and after luteolysis.

The decrease in the number of ovarian follicles during the period studied was mainly due to a reduction in the number of follicles of 6 to 9 mm from the onset of estrus to the time of ovulation (Figure 2D). Follicles 6 to 7 mm in size grew to 8 and 9 mm, and thus the number of medium-sized follicles decreased and the number of large-sized follicles increased. The decrease in number of 6- to 7-mm follicles occurred between 0 and 24 h from the start of estrus and the increase in number of follicles of 8- and 9-mm took place from 24 to 32 h from the beginning of estrus. These 2 events made it possible to form a pool of preovulatory follicles. Some 6- to 7-mm follicles were atretic, became reduced in size and constituted the pool of follicles of 3 to 5 mm. The enlargement of the 3- to 5-mm follicle pool could be due to the atretic follicles, since the number of 6- to 7-mm follicles decreased.

For post-weaned sows, the onset of estrus-ovulation interval observed in this study is less than in previous reports, in which ovulation occurred at 35 ± 8 h after estrus (12) or at an interval of 39 ± 12.4 h from the onset of estrus until ovulation ended (7).

In the present work, the average size of the preovulatory follicles (7.0 ± 1.0 mm) observed in post-weaning sows was similar to that reported previously (12) (7.1 ± 0.9 mm); however, in post-weaning sows the diameter of the largest follicles during ovulation has been reported to be 9.3 mm (15).

In conclusion, the number of follicles changed throughout the period studied since only selected follicles subsequently reached ovulatory size. Ultrasound turned out to be a useful tool to describe the pattern of growth of preovulatory follicles and to predict the time of ovulation. The differences observed between pubertal gilts and post-weaning sows in size of ovulatory follicles and time of ovulation highlight the importance of using ultrasound to predict the onset of ovulation. Furthermore, ovulatory size and time of ovulation could be used to design protocols for fixed time insemination.

Acknowledgments

This study was supported in part by UNLP grants V11/162 and V11/200 to SI Williams and RL de la Sota. CVJ

References

1. Driancourt MA. Regulation of ovarian follicular dynamics in farm animals. Implications for manipulation of reproduction. Theriogenology 2001;55:1211–1239.
2. Schwarz T, Murawski M, Wierzchoś A, Bartlewski P. An ultrasonographic study of ovarian antral follicular dynamics in prepubertal gilts during the expected activation of the hypothalamo-pituitary-ovarian axis. J Reprod Dev 2013;59:409–414.
3. Foxcroft GR, Hunter MG. Basic physiology of follicular maturation in the pig. J Reprod Fertil Suppl 1985;33:1–19.
4. Evans ACO. Characteristics of ovarian follicle development in domestic animals. Reprod Dom Anim 2003;38:240–246.
5. Dalin AM. Ovarian follicular activity during the luteal phase in gilts. J Vet Med 1987;34:592–601.
6. Guthrie HD, Grimes RW, Cooper BS, Hammond JM. Follicular atresia in pigs: Measurement and physiology. J Anim Sci 1995;73:2834–2844.
7. Dalin AM, Nanda T, Hultén F, Einarsson S. Ovarian activity at naturally attained oestrus in the sow. An ultrasonographic and LH study. Acta Vet Scand 1995;36:377–382.
8. Soede NM, Langendijk P, Kemp B. Reproductive cycles in pigs. Anim Reprod Sci 2011;124:251–258.
9. Guthrie HD, Cooper BS. Follicular atresia, follicular fluid hormones, and circulating hormones during the midluteal phase of the estrous cycle in pigs. Biol Reprod 1996;55:543–547.
10. Cox NM. Control of follicular development and ovulation rate in pigs. J Reprod Fertil Supp 1997;52:31–46.
11. Soede NM, Bouwman EG, Lanjendijk P, van der Laan I, Canora A, Kemp B. Follicular development during luteal phase and altrenogest treatment in pigs. Reprod Dom Anim 2007;42:329–332.
12. Soede NM, Hazeleger W, Kemp B. Follicle size and the process of ovulation in sows as studied with ultrasound. Reprod Dom Anim 1998; 33:239–244.
13. Weitze KF, Habeck O, Willmen T, Rath D. Detection of ovulation in the sow using transcutaneous sonography. Zuchthygiene 1989; 24:40–42.
14. Murphy BD, Gevry N, Ruiz-Cortes T, Côté F, Downey BR, Sirois J. Formation and early development of the corpus luteum in pigs. Reprod Supp 2001;58:47–63.
15. Mburu JN, Einarsson S, Dalin AM, Rodriguez-Martinez H. Ovulation as determined by transrectal ultrasonography in multiparous sows: Relationships with oestrus symptoms and hormonal profiles. J Vet Med 1995;42:285–292.