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Follicular Development in Lactating, Post Weaning and Anoestrous Primiparous Sows

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Rojanasthien, S., S. Einarsson and I. Settergren: Follicular development in primiparous lactating, post weaning and anoestrous sows. Acta. vet. scand. 1987, 28, 421–427. – The ovarian follicular system was studied in 4 lactating sows (6 or 8 weeks lactation period), 4 post weaning sows (2 or 4 days after weaning) and 5 post weaning anoestrous sows (22 days after weaning) by macroscopical and microscopical examinations. Blood sampling was performed daily in the post weaning anoestrous sows. The results showed that none of the sows had ovulated during lactation and after weaning. Only small and medium sized follicles were present in the ovaries of the sows. The blood levels of oestradiol-17β and progesterone were low throughout the post weaning period in the anoestrous sows. Microscopical examination showed that all sows had more normal than atretic follicles. In the lactating sows all follicles were below 5 mm in size, the majority being small (1.00-2.99 mm in diameter). The post weaning sows had follicles up to 5.00-5.99 mm and 23-24% of the follicles were medium sized (3.00-5.99 mm in diameter). The post weaning anoestrous sows had no follicles above 5 mm in diameter but many normal medium sized follicles.

follicular system; pig.

Introduction

Sows are normally anoestrus during lactation (*Burger* 1952). However, some lactating sows may exhibit signs of oestrus within a few days after parturition but without follicular growth or ovulation (*Warnick et al.* 1950). After a lactation length of 4 to 8 weeks, oestrus usally occurs within a week after weaning, but the interval is longer in primiparous sows than in multiparous sows (*Einarsson & Settergren* 1974, *King* 1978, *Karlberg* 1980). The weaning oestrus interval is also affected by several other factors such as: length of lactation period (*Self & Grummer* 1958), weight loss during lactation (*Maclean* 1969) and season (*Linde et al.* 1984).

Post weaning anoestrus is a common problem in breeding sows. It is more obvious in primiparous sows than in multiparous sows (*Einarsson & Settergren* 1974, *Radev et al.* 1976, *Linde et al.* 1984). The size of the ovarian follicles in lactating sows increased as the lactation period progressed (*Palmer et al.* 1965, *Kunavongkrit et al.* 1982) with concomittant increasing proportion of normal follicles (*Kunavongrit et al.* 1982). Only a few studies are available concerning the ovarian follicular system after weaning and particularly in post weaning anoestrous sows (*Cox & Britt* 1982, *Cox et al.* 1983). *Cox & Britt* (1982) showed there was an increasing number of follicles >5 mm in size and a gradually increase in follicular size with pro-

gressive day after weaning. The ovaries of sows, anoestrus for more than 35 days after weaning had few medium- to large-size follicles (*Cox et al.* 1983). The ovarian follicles of the post weaning anoestrous sows could respond and ovulation be induced by various exogenous hormones (*King et al.* 1982, *Andersson-Dalin* 1984, *Dial et al.* 1984, *Armstrong & Britt* 1985). No information is available about the quality of follicular system expecially in post weaning anoestrous sows. The purpose of this investigation was to study the ovarian activity with particular reference to number and quality of follicles ≥ 1 mm in size in (1) lactating sows, (2) post weaning sows and (3) post weaning anoestrous sows.

Materials and methods

Thirteen crossbred (Swedish Yorkshire X Swedish Landrace) primiparous sows were used in this study. They were divided into 3 groups. The clinical data of the sows are shown in Table 1. Nine pregnant sows (nos. 1-4 and 9-13) were purchased from commercial herds and brought to the Department of Obstetrics and Gynaecology 3-4 weeks before expected farrowing. They were housed in individual pens and kept there throughout the experimental period. The lactation periods of the sows nos. 1 and 2 (group IA) and sows nos. 3 and 4 (group IB) were 6 and 8 weeks when they were slaughtered, respectively. Four post weaning sows, nos. 5-8 (group II) belonged to a farm close to Uppsala with very good fertility and good ability to show post weaning oestrus (the majority of the primiparous sows on the farm showed oestrus within 1 week after weaning). Sows nos. 5 and 6 (group IIA) and sows nos. 7 and 8 (group IIB) were slaughtered on the farm 2 and 4 days after weaning, respectively. Five post weaning anoestrous sows nos. 9-13 (group III) which belonged to another experimental study at the Department of Obstetrics and Gynaecology were used in this study. Their lactation period was 5 weeks. They had not shown standing oestrus during the experimental period and were slaghtered 22 days after weaning.

All sows were fed according to the Swedish breeding stock standard. Oestrous detection was performed once or twice daily in the presence of a boar throughout the lactation period (group I and III) and after weaning until slaughter (groups II and III). Jugular vein catheterization was performed in group III sows during the first or the third week of lactation period (*Rodriguez & Kunavongkrit* 1983). Blood collection was performed daily via the permanent jugular vein catheter at 9.00 a.m. from the day of weaning until 22 days after weaning of the sows in group III. Blood samples were centrifuged immediately after collection and plasma was separated and stored into plastic tubes and kept at -20°C until radioimmunoassays for oestradiol -17β (*Boilert et al.* 1973) and progesterone (*Bosu et al.* 1976) were performed. These radioimmunoassays have priveously been validated in the porcine species (*Kunavongkrit et al.* 1983).

At slaughter, the reproductive organs were removed and examined macroscopically within 1 h after slaughter. The ovaries were fixed in Bouin's fluid (about 50 ml for each ovary) for 30 h. They were dehydrated according to the standard method and embedded in histowax at 56°C. The tissues were serially sectioned in 10 μm thick sections. Every twentieth section was stained with hemalum eosin. The stained sections were projected onto photographic paper with 5X magnification.

In the photographs the number and size of the follicles ≥ 1 mm were determined by using the same methods as described by *Rajakoski* (1960) for heifers. The follicles were

Table 1. Clinical data of sows slaughtered at different stages of lactation and post weaning periods.

Sow no.	Group	Litter size	Lactation length (days)	Post weaning period (days)
1	IA	11	42	-
2	IA	11	42	-
3	IB	10	56	-
4	IB	5	56	-
5	IIA	7	40	2
6	IIA	7	38	2
7	IIB	8	48	4
8	IIB	8	45	4
9	III	10	35	22
10	III	11	35	22
11	III	11	35	22
12	III	14	35	22
13	III	8	35	22

divided into the following classes according to the diameter: 1.00-1.99, 2.00-2.99, 3.00-3.99, 4.00-4.99 and 5.00-5.99 mm. The mean diameter of the follicles (d) was obtained from the formula:

$$d = \frac{d1 + d2 + d3}{3}$$ in which

d1 = the greatest diameter of the follicle
d2 = the diameter at right angle to dl
d3 = the diameter perpendicular to the plane of sectioning obtained by counting the total number of sections in which the follicle was seen.

The identification of normal and atretic follicles was carried out microscopically according to the method described by *Kunavongkrit et al.* (1982). The atretic follicles were characterized by degeneration of the granulosa cells with pycnosis of their nuclei. During early stages, degenerated granulosa cells became detached from the follicular wall and pycnotic nuclei floated in the follicular cavity. During later stages of atresia the granulosa cells disappeared completely and the thecal connective tissue started to grow and began to fill up the lumen.

Results

Post mortem and hormonal findings

No abnormality of the reproductive organs of any sow was observed at the post mortem examination. Their ovaries contained no visible corpora lutea but many follicles of different sizes (<6 mm). The peripheral concentrations of oestradiol -17β and progesterone of the anoestrous sows (group III) were below the detection limits of the assays throughout the post weaning period until slaughter (22 days after weaning).

Microscopical findings

The average number and percentage of total follicles of different sizes per sow in each group are presented in Table 2. In the lactating sows (group I) all follicles were below 5 mm in size. The majority of the follicles were small (1.00-2.99 mm in diameter). The post weaning sows (group II) had follicles up to 5.00-5.99 mm. The percentage of small follicles (1.00-2.99 mm in diameter) were 76-77 while 11-13% of the follicles were 4.00-5.99 mm in diameter. The post weaning anoestrous sows (group III) had no follicles abo-

Table 2. Average number, range of number and percentage of total number of follicles of different size (mm) in each group.

Group	1.00-1.99		2.00-2.99		3.00-3.99		4.00-4.99		5.00-5.99		Total
	no	%	no	%	no	%	no	%	no	%	no
IA	122.5 (62-183)	66	44.5 (0-89)	24	18.5 (0-37)	10	0.5 (0-1)	0.3	-		186
IB	120.0 (88-152)	54	67.0 (77-87)	30	32.0 (6-58)	15	2.0 (0-4)	1	-		221
IIA	96.0 (58-134)	54	40.5 (21-60)	22	19.0 (8-24)	11	14.5 (13-16)	8	8.0 (3-13)	5	175
IIB	102.0 (86-118)	57	34.5 (16-53)	20	21.0 (11-31)	12	17.5 (17-18)	10	1.5 (0-3)	1	176.5
III	80.2 (34-144)	44	49.6 (6-101)	28	25.0 (5-36)	14	24.4 (9-34)	14	-		179.2

ve 5 mm in diameter. The percentage of small follicles (1.00-2.99 mm in diameter) was 72 and the rest of the follicles were equally distributed in the size classes 3.00-3.99 and 4.00-4.99 mm in diameter.

The average number of normal and atretic follicles of different sizes per sow in each group are presented in Table 3. Approximately 60% of the follicles of the lactating sows were normal (64 and 59% for groups IA and IB, respectively). In group IA the sows had almost the same amount of normal and atretic follicles 3.00-4.99 mm in diameter while in group IIB the sows had higher number of normal follicles 3.00-4.99 mm in diameter than atretic ones (19.5 and 12.5 follicles, respectively). Seventyfive % of the follicles in the sows slaughtered 2 days after weaning (group IIA) were atretic. The majority of the follicles 1.00-3.99 mm in diameter were atretic while approximately ⅔ of the larger follicles (4.00-5.99 mm in diameter) were normal. In the sows slaughtered 4 days after weaning, 55% of the follicles were of normal appearance. All follicles 4.00 mm or more in diameter were normal in these sows. The post weaning anoestrous sows (group III) had more normal (56%) than atretic follic-

Table 3. Average and total number and range of normal (N) and atretic (A) follicles of different size (mm) in each group.

Group	1.00-1.99		2.0-2.99		3.00-3.99		4.00-4.99		5.00-5.99		Total	
	N	A	N	A	N	A	N	A	N	A	N(%)	A(%)
IA	82 (52-112)	40.5 (10-71)	27.5 (0-55)	17 (0.34)	9.5 (0-19)	9 (0-18)	0	0.5 (0-1)	-	-	119 (64%) (52-186)	67 (36%) (10-124)
IB	66 (44-88)	54 (44-64)	43.5 (25-62)	23.5 (22-25)	19.5 (6-33)	12.5 (0-25)	1.5 (0-3)	0.5 (0-1)	-	-	130.5 (59%) (75-186)	115 (41%) (66-115)
IIA	27.5 (16-39)	68.5 (42-95)	1 (0-2)	39.5 (19-60)	0	16 (8-24)	7 (3-11)	7.5 (5-10)	7.5 (3-12)	0.5 (0-1)	43 (25%) (31-55)	132 (75%) (127-137)
IIB	55.5 (43-68)	46.5 (43-50)	10 (0-20)	24.5 (16-33)	13 (6-20)	8 (5-11)	17.5 (17-18)	0	1.5 (0-3)	0	97.5 (55%) (70-125)	79 (45%) (64-94)
III	44 (27-57)	36.2 (7-87)	24.2 (0-52)	25.4 (6-49)	17.4 (5-32)	7.6 (0-14)	15.2 (6-23)	9.2 (6-11)	-	-	100.8 (56%) (59-125)	78.4 (44%) (33-150)

les. Of the follicles 3.00-3.99 and 4.00-4.99 mm in diameter, 70 and 62% respectively were normal.

Discussion

Very little information is available about the ovarian activity in sows after weaning and particularly in post weaning anoestrous sows. It was therefore of importance to study quality and quantity of the ovarian follicular system of anoestrous sows and compare them with lactating and post weaning sows. In the present study the sows in group I represented normal lactating sows with 6 or 8 weeks lactation period. Furthermore, the sows in group IB (8 week lactation period) were slaughtered at about the same time after parturition as the anoestrous sows in group III (Table 1). The litter sizes of all sows in the present study were within the normal range for primiparous sows of the breeds to which they belonged (Table 1). The lactating periods of the sows in group IIA and IIB were 48 and 45 days respectively. The majority of the sows on the farm to which those sows belonged showed oestrus within 1 week after weaning. As the sows in group IIA and IIB also were in good nutritional status at weaning, they were expected to come in oestrus soon after weaning. Thus, the ovaries of the group II sows are supposed to represent the ovarian activity in normal sows.

The macroscopical and the microscopical examinations of the ovaries showed that none of the sows used in the present study had ovulated during lactation or after weaning. Only small and/or medium size of follicles were present in the ovaries of the sows. The oestradiol-17β and progesterone levels of the sows in group III were lower than the detecting limit of the assay throughout the post weaning period until slaughter. This finding is in agreement with some earlier studies (e.g. *Ash & Heap* 1975; *Kunavongkrit* 1984), and indicates that the ovaries of anoestrous sows produced low amount of oestradiol-17β after weaning. The blood level of oestradiol-17β in the utero-ovarian vein of anostrous sows however, is high (40-50 pmol/1) indicating a significant follicular activity (*Rojanasthien,* unpublished data).

As can be seen from Table 2, there was a marked variation in the number of different size of follicles between animals. With the limited number of sows available no statistical analysis was made for the firm comparison between groups. However, the ovaries of the sows in group IIA and IIB had the largest sized follicles of all sows in the present study. Futhermore, the group III sows had an average of 24.4 follicles 4.00-4.99 mm in diameter, while the sows belonging to groups IA and IB had 0.5 and 2.0 follicles of this size, respectively. These results indicate that weaning normally provides hormonal changes (*Shaw & Foxcorft* 1985) resulting in a prompt resumption of ovarian activity with increasing of the follicular size (*Cox & Britt* 1982). The larger follicles in the ovaries of the group II sows compared to the group III sows reflect a higher ovarian activity through a better response of the hypothalamo-hypophyseal-ovarian axis to weaning.

As is evident from Table 3, approximately 60% of the counted follicles were of normal appearance except in group IIA sows, which had a lot of small atretic follicles (<3 mm in diameter). Concerning medium sized follicles (3-6 mm in diameter), groups IIB and III sows had almost the same proportion of normal follicles (about 70%) while group IIA had about 37% follicles of normal appearance. The reason for the very high proportion of atretic follicles in group IIA sows is not known. One possible explanation might be a heavy gonadotrophic stimulation of the ovaries after weaning resulting in a rapid

growth of follicles of different sizes, followed by atresia of the smallest sized follicles. However, the small number of animals in the group would not allow any definite conclusions.

The results of the present study clearly show that the ovaries of the lactating sows nursing a normal litter had less activity than the ovaries of the post weaning sows. Eventhough the ovaries of the post weaning anoestrous sows were more active than those of the lactating sows, they were still less active than the ovaries of the weaned sows approaching their first oestrus. *Almond et al.* (1986) suggested that the ovaries of the post weaning anoestrous sows exert sufficient inhibitory feed back through an unknown mechanism to inhibit normal hypothalamo-hypophyseal axis control of follicular development of the ovaries. *Armstrong & Britt* (1985) on the other hand demonstrated that hourly pulsatile treatment of GnRH induced ovulatory oestrus in seasonally anoestrous primiparous sows. A changes environment such as transportation and relocation of post weaning anoestrous sows seems in some cases to cause enough stimulation to induce oestrus within 1 week (*Rojanasthien* unpublised data).

Conclusion

It can be concluded that lactating sows had a high numer of follicles but less follicular growth than post weaning sows. The post weaning anoestrous sows had many normal medium sized follicles. However, their ovaries were less active than in the normal post weaning sows.

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References

Almond G M, Dial G D, Pressing A L, Hilley H D: LH response of seasonally anestrous sows to estradiol. Proc. 9th Int. Pig. Vet. Soc. Congr. Barcelona, Spain. July 15-18, 1986, p. 48.

Andersson-Dalin A-M: The effect of a PMSG/HCG preparation in anoestrous sows and gilts. Nord. Vet. Med. 1984, *36,* 201-206.

Armstrong J D, Britt J H: Pulsatile administration of gonadotropin-releasing hormone to anestrous sows: Endocrine changes accociated with GnRH-induced and spontaneous estrus. Biol. Reprod. 1985, *33,* 375-380.

Ash R W, Heap R B: Oestrogen, progesterone and corticosteroid concentrations in peripheral plasma of sows during pregnancy, parturition, lactation and after weaning, J. Endocr. 1975, *64,* 141-154.

Boilert B, Edqvist L-E, Johansson E D B, Lindberg P, Martinsson K: The influence of conjugated estrogens in radioimmunoassays using different antibodies against oestraidiol-17β. Steroids 1973, *22,* 891-894.

Bosu W T K, Edqvist L-E, Lindberg P, Martinsson K, Johansson E D B: The effect of various dosages of lynesterol on the plasma levels of oestrogens and progesterone during the menstrual cycle in the rhesus monkey. Contraception 1976, *13,* 677-684.

Burger I F: Sex physiology of pigs. Onderstepoort J. Vet. Res. 1952, Suppl. 2, 3-218.

Cox N M, Britt J H: Relationships between endogenous gonadotropin-releasing hormone, gonadotropins, and follicular development after weaning in sows. Biol. Reprod. 1982, *27,* 70-78.

Cox N M, Esbenshade K L, Britt J H: Treatment of longterm anestrous sows with estradiol benzoate and GnRH: Response of serum LH and occurrence of estrus. Theriogenology 1983, *20,* 499-507.

Dial G D, BeVier G D, Hixor J E, Gustafsson B K: Endocrine pathogenesis of postweaning anestrus in swine: Response of the persistently anestrous sow to hormonal stimuli. Amer. J. Vet. Res. 1984, *45,* 1737-1742.

Einarsson S, Settergren I: Fertility and culling in some pig breeding herds in Sweden. Nord. Vet. Med. 1974, *11,* 427-442.

Karlberg K: Factors affecting postweaning oestrus in the sow. Nord. Vet. Med. 1980, *32,* 185-193.

King G J: Occurrence of postweaning estrus in primiparous and pluriparous sows. Proc. 5th World Int. Pig Vet. Soc. Congr., Zagreb, Yugoslavia 1978, paper K.A. 12.

King R H, Williams I H, Barker I: Induction of oestrus in anoestrous, first litter sows with pregnant mare's serum gonadotrophin. Anim. Reprod. Sci. 1982, *5,* 41-45.

Kunavongkrit A: Clinical and endocrinological studies in primiparous post partum sows: Effect of lactation length and litter size. Acta vet. scand. 1984, *25,* 260-279.

Kunavongkrit A, Einarsson S, Settergren I: Follicular development in primiparous lactating sows. Anim. Reprod. Sci. 1982, *5,* 47-56.

Kunavongkrit A, Kindahl H, Madej A: Clinical and endocrinological studies in primiparous zero-weaned sows: 2. Hormonal patterns of normal cycling sows after zero-weaning. Zbl. Vet. Med. A. 1983, *30,* 616-624.

Linde C, Einarsson S, Petersson H: Reproductive performance in gilts through their first two parities. Nord. Vet. Med. 1984, *36,* 207-214.

Maclean C W: Observations on non-infectious infertility in sows. Vet. Rec. 1969, *85,* 675-682.

Palmer W M, Teague H S, Venzke W G: Macroscopic observations on the reproductive tract of the sow during lactation and early postweaning. J. Anim. Sci. 1965, *24,* 541-545.

Radev G, Andreev A, Kostov L: The influence of age and season on the weaning to oestrus period in sows. Commun. Abstr., 8th Int. Congr. Anim. Reprod. & A. I., Krakow 1976, p. 208.

Rajakoski E: The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variation. Acta Endocrinol. (Kbh.) 1960, Suppl. 52, 1-68.

Rodriguez H, Kunavongkrit A: Chronic venous catheterization for frequent blood sampling in unrestrained pigs. Acta vet. scand. 1983, *24,* 318-320.

Self H L, Grummer R H: The rate and economy of pig gains and reproductive behaviour in sows when litters are weaned at 10 days, 21 days or 56 days of age. J. Anim. Sci. 1958, *17,* 862-868.

Shaw H J, Foxcroft G R: Relationships between LH, FSH and prolactin secretion and reproductive activity in the weaned sows. J. Reprod. Fert. 1985, *75,* 17-28.

Warnick A C, Casida L E, Grummer R H: The occurence of estrus and ovulation in postpartum sows. J. Anim. Sci. 1950, *9,* 66-72.

Sammanfattning

Follikelutvecklingen hos ungsuggor under diperioden och efter avvänjningen

Follikelaktiviteten i äggstockarna studerades såväl makroskopiskt som mikroskopiskt hos 4 lakterande suggor (6 och 8 veckors diperiod), 4 avvandra suggor (2 och 4 dagar efter avvänjningen) och 5 suggor som inte visat brunst inom 22 dagar efter avvänjningen. Blodprov togs dagligen från de suggor som inte visade brunst. Resultaten visade att ingen sugga hade ovulerat under diperioden eller efter avvänjningen. Äggstockarna innehöll endast små och medelstora folliklar. Blodplasmanivåerna av 17β-östradiol och progesteron var låga under hela observationsperioden hos de suggor som inte visat brunst. Samtliga suggor hade fler normala än atretiska folliklar i äggstockarna (≥1 mm i diameter). Hos de lakterande suggorna var samtliga folliklar mindre än 5 mm i diameter (majoriteten 1,00-2,99 mm i diameter). Hos de nyligen avvanda suggorna innehöll äggstockarna folliklar som var upp till 5,00-5,99 mm i diameter och 23-24% av folliklarna var medelstora (3,00-5,99 mm i diameter). De suggor som inte visat brunst hade inga folliklar större än 5 diameter, men ett flertal medelstora folliklar.

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