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Pathogenesis and pathophysiology of ovarian follicular cysts in mammals

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Abstract

Ovarian cysts remain to be one of the most common and serious problems in reproduction of farm animals, as well as humans. Apart from causing the fall in reproductive potential of the ovaries, occupying the place in which folliculogenesis and oogenesis occur, they also cause hormone imbalances, by preventing corpus luteum formation, hence lowering the amount of steroid hormone production. While singular cysts rarely affect fertility, hormone fluctuations that are associated with their presence promotes their multiplication, which usually has more adverse effects. While the cysts are easily detectable in humans, possessing distinct echography while examined by ultrasound, multiple factors prevent widespread use of effective detection methods among large herds of farm animals. Because of lack of noticeable symptoms of early stages of such malignancies, they rarely get detected before the animal stops to exhibit symptoms of heat. That causes scientific research to be focused on not only methods of detection, but also the ways to negate the effects of ovarian cysts and bring the affected specimen back to reproductive potential. Despite that, high costs of diagnosis and treatment, cause them to be uncommon on commercial farms. As lack of fertility eliminates animals from breeding purposed herds, ovarian cysts persist as a cause of large losses of the animal husbandry business. Continuous research, focused on natural examples of ovarian cysts should be conducted, in order to improve methods of detection, prevention, treatment and recovery from the effects of ovarian cysts.

Running title: Ovarian cysts formation

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Physiology and pathophysiology of ovarian follicles

Ovarian cysts are a serious problem in the reproduction of animals and humans. This malignancy is commonly recognized as a cause of increased infertility in commonly bred animals. Cystic ovarian degeneration (COD) is primarily a disorder of the neuroendocrine processes in the female organism [1–4].

Ovarian cysts, in pigs, are determined as ovarian follicles having a diameter of 11 to 50mm. There are several types of cysts in the sow. These follicular and luteal cysts that may appear singly, or in large numbers, on the ovary [5, 6]. Follicular cysts are derived from ovarian follicles that have not undergone ovulation. They constantly increase in size, reaching diameter of more than 11mm. In ultrasonography, these structures are characterized by large size, lack of echogenicity and regular circular shape [7]. In addition, follicular cysts vary in the level of wall luteinisation. The wall is made of granulosa cells and follicular sheath and does not show signs of luteinisation. The cystic wall undergoes degeneration and fibrosis with time, leading to the formation of sheath-luteal cysts. They are characterized by follicular growth and luteinisation of the inner cortex, as well as occasional overgrowth of basal granulosa layer. In luteinized follicular cysts, luteinisation occurs in both the granulosa layer and the inner sheath, which results in an increase in the thickness of their walls [5].

Lutein cysts arise from ovulatory follicles or corpus luteum, with the possibility of detecting them with the help of ultrasonography appearing only in the non-heat period of the next cycle [8] [8]. This type of cyst is a result of hyperplasia and serum-filled vesicle formation. The cyst walls are fibrotic and degenerative [5]. In the ultrasound image, thick walls resemble ovarian stroma, which makes it difficult to diagnose them properly.

Single or multiple cysts can occur on one or both ovaries. They are a common occurrence in pig farms. In the inseminated pig population, this problem affects 1.1 - 37% of individuals. Single cysts do not significantly affect the maturation and ovulation of normal follicles. There are also not the cause of estrous cycle disorders in pigs. Only the presence of numerous cysts, with the absence of normal ovarian follicles and corpus luteum, results in periodic or permanent sterility of females [4].

The lifespan surveillance of ovarian cysts, using USG, on breeding farms is still not widespread. It is because single cysts do not significantly affect the functioning of the ovaries and do not cause disorders in the reproduction of the sow. This causes ovarian ultrasonography to be rarely performed. Observations of pathologic lesions on ovaries are usually performed *post-mortem* and refer only to the group of individuals eliminated from the breeding. Therefore, they do not indicate how widespread the problem of the presence of ovarian cysts in a given breeding population [9, 10].

Ovarian cysts are more common in the group of multiparous sows compared to females giving birth for the first time or sexually mature gilts. Most cases of this ovarian pathology involve sows after 6-7 births, which may indicate a close correlation between the occurrence of cysts and the age of females. Females whose lactation lasts for less than 17 days are particularly vulnerable, to that malignancy, as well as those whose heat has occurred 3 days after weaning [11, 12].

Pathogenesis of cysts formation

The problem of ovarian cysts has not been fully explained. Causes of ovarian cyst formation are seen in disorders of the control of normal development of ovarian follicles and ovulation. Mammalian reproductive activity and associated ovarian function are influenced by hypothalamic-pituitary-ovary hormones, ovarian steroid hormones, and local auto- and paracrine-based protein factors. The term “disturbance in hormonal balance” is used when describing this kind of pathologies [13]. Endocrine dysfunctions in the transition period after the completion of lactation, in particular the inhibition luteotropic secretion (LH) from the pituitary gland are considered a major cause of ovarian cyst formation. It was found that the LH content of pituitary glands of sows with ovarian dysfunction was 13.6 mg / g dry weight, compared to the normal with 8.8 mg / g dry weight. Disturbances in the pulsatile GnRH secretion from the hypothalamus can also lead to dysfunction in the appropriate secretion of LH, and consequently, to a decrease in pre-ovulatory discharge of this hormone. Endocrine dysfunctions cause e.g. disorders in the development of ovarian follicles, ovulation inhibition, and eventually ovarian cyst formation. A “vicious pathogenic cycle” occurs, through negative hormonal feedback on the ovary-pituitary-hypothalamus axis [4].

Stress adversely affects the proper functioning of the reproductive organs, especially the process of follicular formation and ovulation. Under the influence of stress, activation of the hypothalamic-pituitary-adrenal axis occurs, which leads to the release of ACTH, which stimulates the secretion of glucocorticoids from the adrenal glands. Cortisol is the dominant glucocorticoid in the pig. Its high level may be one of the causes of ovarian cyst formation. In the case of females, that showed no heat symptoms after weaning, elevated blood levels of cortisol were observed, coupled with low levels of LH., ACTH, administered during the follicular phase, caused the inhibition of heat symptoms and development of cysts on the ovaries [9, 13]. In females with removed adrenal glands, on the other hand, ovarian cysts were absent [14, 15]. This fact led to the hypothesis that the direct effect on the for-

mation of ovarian cysts in the sow is not caused by ACTH, but above all by high levels of cortisol. The glucocorticoids, secreted during stress, interfere with the feedback mechanism of estrogen on the hypothalamus and pituitary gland, which inhibits the secretion of GnRH and LH from the anterior pituitary gland. Cortisol causes an increase in androgen synthesis by the sheath cells, which leads to an increase in their level in blood and tissues. During stress, in the cells of the granulosa cell layer of ovarian follicles, inhibition of aromatase enzyme activity and estrogen synthesis occur simultaneously [16].

The formation of ovarian cysts is also supported by the administration of exogenous progesterone during the follicular phase of the estrous cycle of the sows. The experimental administration of ACTH, during lactation and in heat, caused not only the increase in the level of cortisol, but also progesterone of adrenal origin. Progesterone inhibited the anterior pituitary gland, leading to disturbances in the release of FSH and LH, and inhibited ovulation [1]. In addition, the use of exogenous gonadotropins such as PMSG, hCG, LH and FSH may lead to disturbances in the process of folliculogenesis [16].

Restrictive nutrition may be an important reason for changes in hormone secretion in the hypothalamus-pituitary-ovary axis. Excess of leguminous plants and goitrogenic (antithyroid) feeds in the diet, may have a negative effect on the course of estrus cycles and reduce the fertility of sows. It was found, that this diet reduces the secretion of LH and release of GnRH, which leads to impaired ovarian activity. It is assumed, however, that there is no high correlation between the development of ovarian cyst and feeding of the animals [11].

Thyroid hormones are also one of the factors controlling the course of the estrus cycle, the development of ovarian follicles, secretory functions of the granulosa layer and ovulation. These hormones regulate the actions of the pituitary gland and the production of gonadotropin receptors in the ovaries. Disorders of the thyroid-ovary axis result in a decrease in the level of sex hormones in blood and inhibit the rise of estradiol levels in the pre-estrus period [16].

The development of ovarian cysts and the occurrence of polycystic ovary syndrome (PCOS) in sows, may be influenced by other factors, other than hormonal. One of them may be the overexpression of the IGF-1 growth factor, which acts as a paracrine modulator, affecting gonadotrophic hormones in the ovary [17]. In humans, mutations in the INSR insulin receptor gene have also been demonstrated in patients with polycystic ovary syndrome. When disturbances occur in the INSR gene, abnormal ovulation and, as a result, improper maturation of the oocyte occurs [18]. An interesting aspect, that requires testing, is the INHA gene, as its mutation leads to ovarian failure and other abnormalities in the function of these gonads [19].

The main clinical symptoms of ovarian cysts

The lack of specific symptoms characteristic for the occurrence of ovarian cysts is a difficulty in recognizing this pathology in pigs. In individuals with ovarian cysts lack of heat, regular or irregular repetition of heat and extended estrus cycles were observed. In the case of irregular cycles, the breaks were 25 days and more [9, 20]. In addition, sows with several cysts (<10 cysts / animal) had regular estrus cycles because both "healthy" (ovulatory) and pathologically altered ovarian follicles coexisted within their ovaries. However, these animals were characterized by reduced fertility and reduced number of piglets in litters. In contrast, in sows with numerous, abnormal follicles (> 10 cysts / animal) disturbances in the course of folliculogenesis and oogenesis were observed [9].

The presence of cysts on the ovary leads to a reduction in the number of ovulating follicles in a given estrous cycle, as well as changes in the behavior of the sows. Lutein cysts inhibit heat behavior, while follicular cysts increase it [9].

Occurring ovarian cysts can undergo spontaneous atresia during the period of 15-18 days. Sometimes, however, they can stay on the ovary for 50 days or longer. After ovarian cyst atresia, delayed heat, ovarian follicle ovulation and formation of the corpus luteum occurs. However, the return to normal ovarian activity is hampered by persistent disturbances in the hormonal balance of females that may contribute to ovarian cyst regression [21].

The occurrence of pathological changes on the ovarian surface is also important for scientific research, as it was noted, in the ovaries of bitches, that the presence of ovarian cysts correlates with collection of less COCs less than the physiologically normal ovarian [22].

Ovarian cysts diagnostic and treatment in mammals

The lack of typical symptoms of ovarian cysts and the limited possibilities of clinical examination of reproductive organs in sows on breeding farms make it difficult to diagnose the disease on a lifetime basis. Diagnosis of cystic ovarian degeneration syndrome (COD) is carried out on post-mortem or during life. Intravital methods used to determine disorders in ovarian activity include rectal ovarian examination and ultrasound [9]. For experimental purposes, laparoscopy and laparotomy are also used. Palpation of the ovaries and uterus by the rectal method is not widely used due to the porcine pelvis being too narrow [23].

The most useful way to diagnose ovarian pathologies appears to be transrectal ultrasound and more often used - abdominal ultrasound examination. In the ultrasound image, ovarian cysts are characterized by different shape, wall thickness and size. Follicular cysts are smooth-walled structures with thin

walls. Lutein cysts, however, have clearly thickened walls. Disturbances in the ultrasound image in regards of quantity and type of ovarian cyst occurring may result due to excessive fat. However, this does not change the fact that ultrasonography is a good diagnostic method, useful for the early detection of this disorder. However, the widespread use of ultrasound examination for monitoring of disturbances in ovarian activity in sows in field conditions remains negligible [24, 25].

Diagnosis of ovarian cysts only on the basis of blood tests is not very reliable. It has been shown that high hormone levels including progesterone, cortisol and estradiol in the blood are similar in sows with ovarian cysts and in females with normal ovarian follicles in the *diestrus* phase. However, with the help of the assessment of blood progesterone concentration and ultrasound examination, the presence of cysts in the ovaries of the sows can be confirmed [16].

In cows, disorders in the secretion of GNRH and LH are the reason for the formation of ovarian cysts. In therapy, it seems natural to use preparations containing GnRH to stimulate the pituitary gland and the secondary release of LH. Drugs containing human chorionic gonadotropin (hCG) are also characterized by high reliability, but the high price limits their widespread use. Exogenous GnRH stimulates the secretion of LH and FSH from the pituitary gland, which in turn leads to the luteinisation of ovarian cysts under the influence of LH and recruitment of a new wave of follicles (effect of FSH). Ovulation, formation of a functional corpus luteum at the surface of the ovary, and in the next step, development of the dominant follicle occur due to that processes. After application of GnRH the animals show symptoms of heat in 13-23 days. In order to shorten the period of reproductive recovery, it is recommended to administer PGF2α approximately 7-9 days after GnRH. Cow under the influence provoked luteolysis, begins heat after 3-5 days. In order to suppress recurrences of ovarian cysts after treatment with GnRH and PGF2α, ovulation should be induced with GnRH or hCG again. Studies confirm the high effectiveness of this program in the treatment of ovarian cysts in cows [26–28]. PGF2α is also used in the therapy of lutein cysts, where its administration results in luteolysis of cysts and reduction of high levels of progesterone. This enables re-growth and ovulation of the dominant follicle. However, this model of hormone therapy is "reserved" only for cases of recurrent ovarian cysts or for cows with high genetic value.

In sows with experimentally induced ovarian cysts, different doses of GnRH were used in therapy. However, the effect was not entirely satisfactory. Attempt of corpus luteum lysis by hCG, turned out to not be useful in practice. The use of chorionic gonadotropin only gave a positive effect after previous laparotomy. PGF2α was also used for the treatment of ovarian cysts, but a positive result was obtained only in the case of large follicles. The action of PGF2α is related to the degree of luteinisation of the cystic wall, hence the effects of therapy were unsatisfactory in small follicular cysts [5, 21].

Influence of ovarian cysts on animal health – economic features

In recent years, there has been an increase in disturbances in cyclical ovarian activity, leading to, among others, the occurrence of ovarian cysts. In cattle breeding herds, the scale of occurrence of this complication is estimated at 15% [29], in sows 2.4-40% [4]. In women, this disorder affects about 6-8% of the population [30]. The presence of single cysts on the ovaries does not lead to disturbances in ovarian activity. In sows with a single ovarian cyst, a comparable number of corpora luteum was found, as in females with normal ovarian follicles [31]. However, sows with polycystic ovary syndrome (PCOS) usually remained sterile. In sows with numerous ovarian cysts in 68% of cases, no heat symptoms were recorded, 19% showed regular heat and irregular estrus cycles were reported in 13% of females [32]. In addition, it has been shown that as the number of cysts on the surface of the ovaries increases, the number of corpora luteum decreases as well. Disorders in ovarian activity may lead to a decrease in the number of births, and piglets in a litter, which effectively reduces the profits from breeding [7, 31, 33]. The constantly increasing number of animals removed from the herd for breeding defects reasons, resulting from the still ineffective method of treatment of ovarian cysts, causes higher economic losses. There is still a small amount of information on the etiology and pathogenesis of this disease in swine [34, 35]. Most of the research so far has been carried out on gilts with experimentally induced cysts. In farm conditions, however, the disease affects mainly older multiparous sows [4].

Conclusions

While there knowledge about the ovarian cysts and their effects on the reproductive processes is constantly growing, there is still no solution to the problems associated with this disease, that affect especially animal husbandry industry. Rapid ultrasound diagnosis facilitates early diagnosis of females affected by this disorder, but it is not being widely performed, due to the lack of non-expensive methods that lead to elimination of the malignancy and fast recovery. However, the development of research on the causes of ovarian cyst formation may in the future lead to the development of an effective therapy method and significantly reduce the percentage of animals eliminated from breeding.

Ethical approval

The conducted research is not related to either human or animal use.

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Conflict of interest statement
The authors declare they have no conflict of interest.

References

1. Bostedt H, Ebbert W. Syndrom torbieli jajnikowych u macior hodowlanych. Nowa Wet. 1999;04(3):14–7.
2. Close RW, Liptrap RM. Plasma progesterone levels in sows with induced cystic ovarian follicles. Res Vet Sci. 1975;19(1):28–34.
3. Isobe N, Yoshimura Y. Immunocytochemical study of cell proliferation in the cystic ovarian follicles in cows. Theriogenology. 2000;54(7):1159–69.
4. Szulanczyk-Mencel K, Bielas W. Torbiele jajnikowe jako przyczyna niepłodności loch. Zycie Wet. 2010;85(12).
5. Cech S, Dolezel R. Treatment of ovarian cysts in sows – a field trial. Veteri Med. 2007;52(9):413–8.
6. Kauffold J, Rautenberg T, Gutjahr S, Richter A, Sobiraj A. Ultrasonographic characterization of the ovaries in non-pregnant first served sows and gilts. Theriogenology. 2004;61(7-8):1407–17; DOI:10.1016/j.theriogenology.2003.08.007.
7. Szczebiot A, Janowski TM. Ultrasonographic examination of ovaries in swine. Med Wet. 2008;64(4):395–9.
8. Kempisty B, Jackowska M, Bukowska D, Antosik P, Woźna M, Jaśkowski JM. Mechanisms regulating oogenesis, folliculogenesis and fertilization in pigs. Med Wet. 2011;67(5):299–303.
9. Castagna CD, Peixoto CH, Bortolozzo FP, Wentz I, Neto GB, Ruschel F. Ovarian cysts and their consequences on the reproductive performance of swine herds. Anim Reprod Sci. 2004;81(1-2):115–23.
10. Vargas AJ, Bernardi ML, Bortolozzo FP, Mellagi APG, Wentz I. Factors associated with return to estrus in first service swine females. Prev Vet Med. 2009;89(1-2):75–80; DOI:10.1016/j.prevetmed.2009.02.001.
11. Fitko R. Etiologia i patogeneza torbieli w jajnikach swin. Nowa Wet. 1998;03(4):14–6.
12. Knox RV, Zas SL. Factors influencing estrus and ovulation in weaned sows as determined by transrectal ultrasound. J Anim Sci. 2001;79(12):2957–63.
13. Brandt Y, Lundeheim N, Madej A, Rodriguez-Martinez H, Einarsson S. Effects of ACTH injections during estrus on concentrations and patterns of progesterone, estradiol, LH, and inhibin alpha and time of ovulation in the sow. Domest Anim Endocrinol. 2007;32(2):122–37; DOI:10.1016/j.domaniend.2006.02.002.
14. Soede NM, Helmond FA, Kemp B. Periovulatory profiles of oestradiol, LH and progesterone in relation to oestrus and embryo mortality in multiparous sows using transrectal ultrasonography to detect ovulation. J Reprod Fertil. 1994;101(3):633–41.
15. Waberski D, Kunz-Schmidt A, Borchardt Neto G, Richter L, Weitze KF. Real-time ultrasound diagnosis of ovulation and ovarian cysts in sows and its impact on artificial insemination efficiency. J Anim Sci. 2000;77(E-Suppl):1; DOI:10.2527/jas2000.00218812007700ES0037x.
16. Fitko R, Jana B, Kucharski J. Correlations of hormone concentrations in blood plasma and their relations to ovarian structures in hypo- and hyperthyroid cyst-bearing gilts. Pol J Vet Sci. 1998;01(1).
17. Kranc W, Budna J, Kahan R, Chachula A, Bryja A, Ciesiolka S, Borys S, Antosik MP, Bukowska D, Brussow KP, Bruska M, Nowicki M, Zabel M, Kempisty B. Molecular basis of growth, proliferation, and differentiation of mammalian follicular granulosa cells. J Biol Regul Homeost Agents. 2017;31(1):1–8.
18. Kranc W, Celichowski P, Budna J, Khozmi R, Bryja A, Ciesiółka S, Rybska M, Borys S, Jeseta M, Bukowska D, Antosik P, Brüssow KP, Bruska M, Nowicki M, Zabel M, Kempisty B. Positive Regulation of Macromolecule Metabolic Process Belongs to the Main Mechanisms Crucial for Porcine Oocytes Maturation. Adv Cell Biol. 2017;5(1):15–31; DOI:10.1515/acb-2017-0002.
19. Kempisty B, Jackowska M, Woźna M, Antosik P, Piotrowska H, Zawierucha P, Bukowska D, Jaśkowski JM, Nowicki M, Brüssow KP. Expression and cellular distribution of INHA and INHB before and after in vitro cultivation of porcine oocytes isolated from follicles of different size. J Biomed Biotechnol. 2012;2012:742829; DOI:10.1155/2012/742829.
20. Kurzok J. Przypadek torbielowatego zwyrodnienia jajnikow u maciory. Zycie Wet. 2001;76(07).
21. Silvia WJ, Hatler TB, Nugent AM, Laranja da Fonseca LF. Ovarian follicular cysts in dairy cows: an abnormality in folliculogenesis. Domest Anim Endocrinol. 2002;23(1-2):167–77.
22. Kempisty B, Ziolkowska A, Ciesiolka S, Piotrowska H, Antosik P, Bukowska D, Nowicki M, Brussow KP, Zabel M. Association between the expression of LHR, FSHR and CYP19 genes, cellular distribution of encoded proteins and proliferation of porcine granulosa cells in real-time. J Biol Regul Homeost Agents. 2014;28(3):419–31.
23. Moriyoshi M, Sawamura T, Yasuda M, Nakao T, Kawata K. Using Ultrasound for Clinical Observation of the Porcine Ovary Through the Course of the Estrous Cycle and to Monitor Treatment of Ovarian Disease. J Reprod Dev. 1996;42(4):277–82; DOI:10.1262/jrd.42.277.
24. Kauffold J, Rautenberg T, Hoffmann G, Beynon N, Schellenberg I, Sobiraj A. A field study into the appropriateness of transcutaneous ultrasonography in the diagnoses of uterine disorders in reproductively failed pigs. Theriogenology. 2005;64(7):1546–58; DOI:10.1016/j.theriogenology.2005.03.022.
25. Kauffold J, Althouse GC. An update on the use of B-mode ultrasonography in female pig reproduction. Theriogenology. 2007;67(5):901–11; DOI:10.1016/j.theriogenology.2006.12.005.
26. Bartolome JA, Sozzi A, McHale J, Swift K, Kelbert D, Archbald LF, Thatcher WW. Resynchronization of ovulation and timed insemination in lactating dairy cows. Theriogenology. 2005;63(6):1643–58; DOI:10.1016/j.theriogenology.2004.07.018.
27. Bartolome J, Hernandez J, Sheerin P, Luznar S, Kelbert D, Thatcher WW, Archbald LF. Effect of pretreatment with bovine somatotropin (bST) and/or gonadotropin-releasing hormone (GnRH) on conception rate of dairy cows with ovarian cysts subjected to synchronization of ovulation and timed insemination. Theriogenology. 2003;59(9):1991–7.
28. Vries A de, Crane MB, Bartolome JA, Melendez P, Risco CA, Archbald LF. Economic Comparison of Timed Artificial Insemination and Exogenous Progesterone as Treatments for Ovarian Cysts. J Dairy Sci. 2006;89(8):3028–37; DOI:10.3168/jds.S0022-0302(06)72576-0.
29. Probo M, Comin A, Mollo A, Cairoli F, Stradaioli G, Veronesi MC. Reproductive performance of dairy cows with luteal or follicular ovarian cysts after treatment with buserelin. Anim Reprod Sci. 2011;127(3-4):135–9; DOI:10.1016/j.anireprosci.2011.07.019.
30. Sproul K, Jones MR, Mathur R, Azziz R, Goodarzi MO. Association study of four key folliculogenesis genes in polycystic ovary syndrome. BJOG. 2010;117(6):756–60; DOI:10.1111/j.1471-0528.2010.02527.x.
31. Heinonen M, Leppävuori A, Pyörälä S. Evaluation of reproductive failure of female pigs based on slaughterhouse material and herd record survey. Anim Reprod Sci. 1998;52(3):235–44.
32. Ebbert W, Bostedt H. Cystic Degeneration in Porcine Ovaries - First Communication: Morphology of Cystic Ovaries, Interpretation of the Results. Reprod Domest Anim. 1993;28(6):441–50; DOI:10.1111/j.1439-0531.1993.tb01021.x.
33. Pejsak Z. Torbiele jajnikowe - niedoceniana przyczyna zaburzeń w rozrodzie świń. Lecz Duzych Zwierzat. 2007;02(2).
34. Peters AR. Veterinary clinical application of GnRH—questions of efficacy. Anim Reprod Sci. 2005;88(1-2):155–67; DOI:10.1016/j.anireprosci.2005.05.008.
35. Toriumi H, Tsumagari S, Kuwabara Y, Takagi K, Ohba S, Takeishi M. Clinical Findings and Serum Steroid Hormone Concentrations in Sows with Post-Weaning Ovarian Disorders. J Reprod Dev. 1996;42(1):1–6; DOI:10.1262/jrd.42.1.