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FOLLICULAR AND OOCYTE DEVELOPMENT IN GILTS OF DIFFERENT AGE

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The aim of the present study was to estimate follicular and oocyte development of the same gilts in three phases of their reproductive life – prepuberal gilt (6 months old), cycling gilt (9.5 months old) and primiparous sow. Follicular development was induced by injections of 1000 IU PMSG followed by 500 IU hCG 72 h later. Cumulus-oocyte-complexes (COCs) were recovered from preovulatory follicles of the left ovary, and follicular fluid (FF) from the right ovary always 34 h after hCG by endoscopy. Altogether, 19 gilts were used in the prepuberal (P) and cycling (C) trials and 12 of them in the primiparous trial (S). Altogether 168, 190 and 82 follicles were aspirated from the left ovary and 106, 125 and 42 COCs recovered (recovery rate 60.5 ± 26.9, 62.7 ± 20.9 and 52.9 ± 21.8%). The average number of follicles was higher in C compared to P (19.7 ± 6.8 vs. 15.7 ± 6.8, $p = 0.06$) and to S (14.2 ± 4.0, $p < 0.05$), respectively. More uniform expanded COCs were aspirated from prepuberal and cycling gilts as compared to sows (89.7 and 78.4% vs. 46.3%, $p < 0.05$). Furthermore, the meiotic configuration in oocytes differed ($p < 0.05$) between these groups (55.5 and 61.7% vs. 0% Telo1/Meta2). Concentrations of progesterone in FF decreased ($p < 0.05$) from 590.0 ± 333.6 (P) to 249.1 ± 72.6 (C) and 161.4 ± 75.2 ng/ml (S). FF concentrations of oestradiol-17β were different between gilts and sows (9.3 ± 2.9, 21.9 ± 10.6 and 94.0 ± 15.9 pg/ml, $p < 0.05$). The progesterone/oestradiol ratio was 72.1, 15.2 and 4.7. Results indicate a different follicular and oocyte development during the investigated lifetime periods. Cycling gilts should preferably be used in IVF and breeding programs. The lower reproductive potential of primiparous sows is taken into consideration at breeding. Prediction of lifetime performance based on individual ovarian reaction of prepuberal gilts is unsuitable.

Key words: Follicle, oocyte morphology, follicular fluid, steroid hormones, swine

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In *in vitro* fertilisation (IVF) programs oocytes are derived usually from prepuberal gilts. However, oocyte quality, fertilisation rates and blastocyst formation are lower compared to postpuberal animals (Menino et al., 1989; French et al., 1991; Koenig and Stormshak, 1993; Marchal et al., 2000), especially if they are recovered from slaughtered gilts. Use of *in vivo* matured oocytes from puberal gilts and sows could benefit IVF and other assisted reproductive technologies. Limited information is available on oocyte development according to their origin from prepuberal, cycling gilts and primiparous sows and according to the age differences during periods of life in the same animal. Menino et al. (1989) compared *in vitro* development of oocytes of gilts at first and third oestrus, and Smith et al. (1992) analysed steroid and plasminogen activator concentrations in follicular fluid (FF), respectively. However, repeated laparotomy in the same gilts restricted their experiment. Endoscopic Ovum Pick Up and FF aspiration can overcome the disadvantages of surgical recovery (Brüssow and Rátky, 1994; Rátky et al., 1998).

The present study was conducted to compare morphology and maturation of oocytes, and steroid concentrations of FF recovered from preovulatory follicles of the same animals in three phases of their reproductive life – prepuberal gilt, cycling gilt and primiparous sow. Results could contribute evidence not only to the improvement of IVF but also to the selection of gilts for early breeding and possibly to predict lifetime performance.

Materials and methods

A total of 19 crossbred Landrace gilts were used each in three phases of their reproductive life – as prepuberal gilts (6 months old), as cycling gilts (9.5 months old) and as primiparous sows. Ovum Pick Up was carried out in cycling gilts and in primiparous sows in November and next September, respectively. Farrowing occurred in March and April.

In prepuberal gilts oestrus was induced by application of 1000 IU PMSG (Folligon®, Intervet, The Netherlands) followed by an injection of 500 IU hCG (Choriogonin®, Richter, Hungary) 72 h post PMSG. At the age of 9.5 months, oestrus of gilts was synchronised by feeding altrenogest (daily, 16 mg Regumate®/animal, Serum-Werk Bernburg, Germany) for a 15-day period. Gilts were given injection of 1000 IU PMSG 24 h after the last Regumate® feeding, and 500 IU hCG 72 h thereafter. During the next oestrous cycle all gilts were mated. Altogether 12 gilts became pregnant. After parturition and a suckling period of 28 days piglets were weaned, and oestrus of primiparous sows was synchronised by 1000 IU PMSG 24 after weaning and 500 IU hCG 72 h after PMSG.

Cumulus-oocyte-complexes (COCs) and follicular fluid (FF) were always recovered 34 h after hCG by endoscopic Ovum Pick Up as described by Brüssow

and Rátky (1994). Endoscopy was carried out in general anaesthesia (15 ml ketamine, Ursotamin®, Serumwerk Bernburg, Germany, and 5 ml xylazine, Xylavet®, Lavet Pharmaceuticals Ldt., Hungary). COCs aspiration was carried out via a two-way cannula (40 mm length, 16-gauge) and an electric aspiration pump (model 3014, Labotect, Göttingen) with an initial vacuum of 100 mm Hg corresponding to a volume of 17 ml/min. The tip of the aspiration cannula was inserted into a follicle, the content aspirated, and the follicle was refilled and aspirated twice with heparinized PBS. COCs were recovered in each case from follicles of the left ovary. FF was sucked off from follicles of the right ovary via a one-way cannula, respectively, without flushing the follicles. Only macroscopically healthy follicles, well vascularised and translucent, and with a diameter of ≥ 5 mm were punctured.

The morphology of freshly recovered COCs was determined using an inverted microscope at × 60 magnification. COCs were classified as compact, expanded or denuded (Torner et al., 1998). Following the classification COCs were immediately prepared for evaluation of nuclear configuration. Removal of cumulus cells was accomplished in PBS containing 100 IU/ml hyaluronidase (Hylase, Impfstoffwerk Dessau, Germany) followed by repeated pipetting with a fine-bore glass pipette. Oocytes were mounted on slides and fixed for > 24 h in a mixture of acetic acid/alcohol/chloroform (3:6:1) before staining with 2% orcein in 60% acetic acid. The nuclear configuration of oocytes was examined using phase-contrast optics at × 250 to × 630 magnification. Based on their nuclear status the oocytes were classified as 1) immature – germinal vesicle (GV), with diplotene chromatin; 2) meiosis resumed – GV breakdown, diakinesis, M-I to A-I; or 3) mature – T-I and M-II.

FF concentrations of progesterone were determined using a direct, single-antibody ^{3}H-radioimmunoassay (Blödow et al., 1988) with some modifications. The [1,2,6,7-^{3}H] progesterone (tracer) was purchased from Amersham-Buchler (Germany). The antibody was raised in rabbits by immunization with 11α-hydroxyprogesterone conjugate (Steraloids Newport, USA). As a modification the antiserum was further purified by affinity chromatography on protein A superose (Pharmacia, Sweden), and used in a final titre of 1:200,000. The range of the standard curve was between 12.5 and 800 pg. From each follicle fluid 5 µl were diluted with 200 µl of phosphate buffer (pH 7.0). The progesterone analysis was performed in 25 µl of this dilution. The B/F separation of this assay was performed by the dextran-charcoal method. Counting of radioactivity was made by an LSC with integrated RIA program (Rackbeta 1219, Wallace). The intra-assay coefficient of variation (CV) was between 7 and 10%, the inter-assay CV between 9 and 12%. Oestradiol-17β was measured in 50 µl duplicates of diluted follicle fluid with a ^{3}H-radioimmunoassay (Blödow et al., 1988). The antibody raised in rabbits was further purified and used at a titre of 1: 55,000. Incubation, B/F separation and counting were accomplished as described for progesterone.

The sensitivity of the assay was about 2 pg/ml. Intra-assay and interassay CV were 8.5 and 12%, respectively.

Statistical calculations were done with Statistical Analysis System (SAS®). The number of follicles and the number of aspirated follicles were analysed by the GENMOD procedure with identity link function and Poisson distribution. All possible differences of LS-means, standard errors were computed and tested by a Wald chi-square test.

The recovery rate, serum steroid concentrations and the progesterone/oestradiol-17β ratio were subjected to GLM repeated measurement analysis of variance (repeated factor: Group) using the GLM procedure. The differences between groups due to 'cumulus expansion' and 'oocyte maturation' (immature, resumption of meiosis and mature) were analysed by contingency table analysis.

Results

Differences in the number of follicles were obtained between prepuberal gilts (P), cycling gilts (C) and primiparous sows (S). The mean number of follicles was increasing from P (15.7 ± 6.8) to C (19.7 ± 6.8; $p = 0.06$) and then decreasing in S (14.2 ± 4.0; $p < 0.05$), respectively. However, not all gilts pursued this trend. In some animals there was an increase in the number of follicles from P to C and a decrease from C to S (Fig. 1A), while others showed a continuous increase or decrease from P to S (Fig. 1B). The mean size of aspirated follicles was similar in all groups (6.3 ± 0.3; 7.2 ± 0.9 and 6.5 ± 0.7 mm for P, C and S, respectively), but their shape was different. Follicles of P and C were vaulted over the ovarian surface and the follicular wall was weaker, whereas those of S were flat, embedded into the depth of the ovary and the follicular wall was tight and firm.

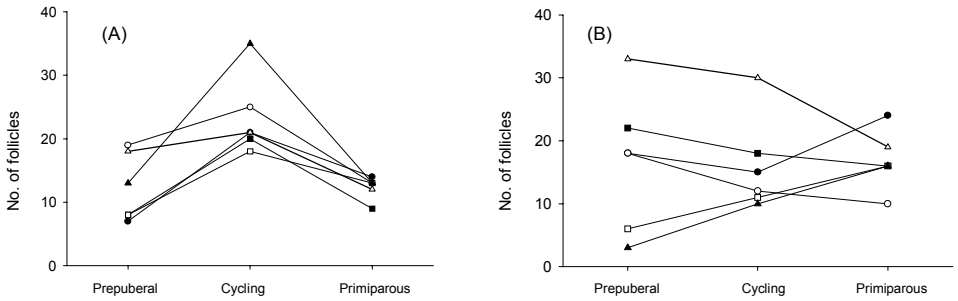

Fig. 1. Trends in the follicular development of gilts at different age (n = 12)

Altogether 440 follicles were aspirated from follicles of the left ovary for COC recovery. Results of COC recovery, COC morphology and of oocyte maturation are presented in Table 1. The recovery rate was similar in each group and ranged between 53 and 63%. Cumulus morphology was more uniform in prepuberal gilts. The percentage of oocytes with expanded cumulus decreased ($p < 0.05$) with progressing age.

Table 1

Results of oocyte recovery, cumulus-oocyte-complex morphology and chromatin configuration in gilts and sows of different age

		Group		
		Prepuberal gilts	Cycling gilts	Primiparous sows
No. of gilts/sows	n	19	19	11
No. of aspirated follicles	n	168	190	82
No. of recovered COCs	n	106	125	42
Recovery rate (%)	mean ± SD	60.5 ± 26.9	62.7 ± 20.9	52.9 ± 21.8
Oocytes with expanded cumulus	%	89.7[a]	78.4[b]	46.3[c]
No. of evaluated fixed oocytes	n	92	112	37
Oocyte maturation				
Immature	%	4.3[a]	6.2[a]	48.6[b]
Resumption of meiosis	%	40.2[a]	32.1[a]	51.4[a]
Mature	%	55.5[a]	61.7[a]	0[b]

Values with different superscripts within a row denote significant differences ($p < 0.05$; χ^2-test)

Different oocyte maturation was found in gilts at different age. P and C had less oocytes with immature chromatin configuration and on the other hand more mature oocytes compared to S ($p < 0.05$).

Follicular fluid was aspirated altogether from 245 follicles of the right ovary. Results of follicular fluid steroid concentrations are presented in Table 2.

Table 2

FF concentrations of oestradiol-17 β and progesterone in gilts and sows at different age

		Group		
		Prepuberal gilts	Cycling gilts	Primiparous sows
No. of gilts/sows	n	19	19	11
No. of aspirated follicles	n	86	114	45
Concentration of oestradiol (pg/ml)	(mean ± SD)	9.3 ± 2.9[a]	21.9 ± 10.6[a]	94.0 ± 15.9[b]
Concentration of progesterone (ng/ml)	(mean ± SD)	590.0 ± 333.6[a]	249.1 ± 72.6[b]	161.4 ± 75.2[b]

Values with different superscripts within a row denote significant differences ($p < 0.05$)

Follicles of prepuberal gilts had a higher concentration of progesterone and lower oestradiol-17β as compared to cycling gilts and primiparous sows. Likewise the progesterone/oestradiol-17β ratio was varying at different ages (Fig. 2). In the present experiment a direct comparison of steroid concentrations and of oocyte maturation from the same follicle was not possible. However, comparing maturation of the pool of oocytes recovered from follicles of the left ovary to steroid concentrations within follicles of the right ovary, it can be assumed that higher concentrations of FF progesterone and lower concentrations of oestradiol-17β are associated with a higher incidence of mature chromatin configuration (Fig. 3). No differences between the left and right ovary were obtained in gilts and sows regarding the total number of follicles and follicles aspirated (data not shown).

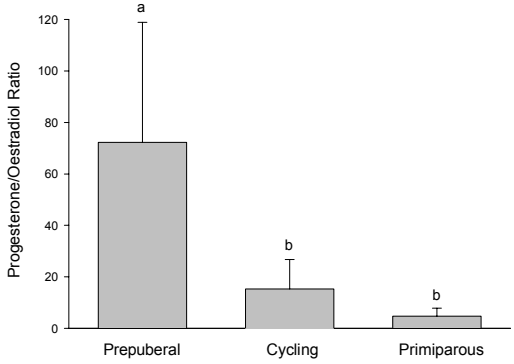

Fig. 2. Progesterone/Oestradiol-17β ratio in FF of gilts at different ages (a,b $p < 0.05$)

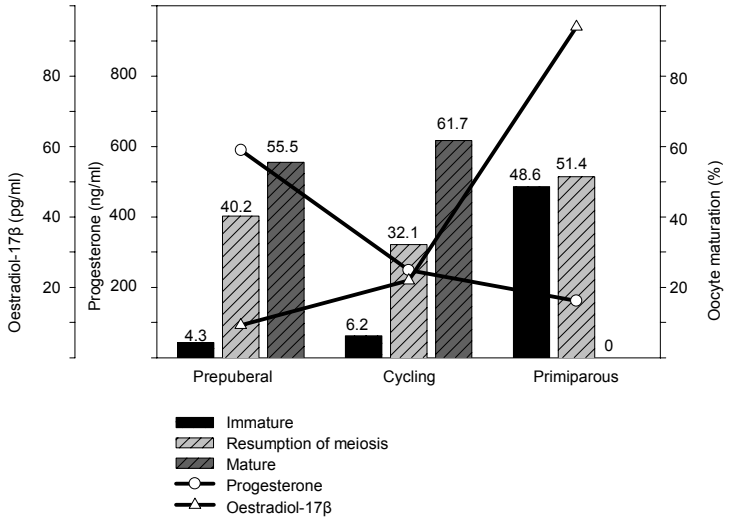

Fig. 3. FF steroid concentrations and stage of oocyte maturation in gilts at different ages

Discussion

Data on oocyte maturation and on follicular fluid hormone concentrations in the same gilts from prepuberal to adults are missing in the literature. Only Menino et al. (1989) and Smith et al. (1992) reported on *in vitro* development of embryos, and on steroid and plasminogen activator concentrations recovered from the same first and third oestrous gilts. Application of minimal-invasive oocyte and FF aspiration (Brüssow and Rátky, 1994) enables the repeated recovery of substrates without disturbing subsequent physiological processes of follicle growth and ovulation. To our knowledge, this is the first report describing ovum characteristics (cumulus morphology, nuclear configuration) and FF steroid concentrations in the same gilts at three different phases of their reproductive life – prepuberal gilt, cycling gilt and primiparous sow.

Well aware of some differences between spontaneously oestrous and PMSG/hCG-primed gilts and sows (for review see Hunter and Wiesak, 1990), in the present experiment all animals were treated with PMSG and hCG to ensure concurrent follicular and oocyte development, and oocyte and FF recovery at a comparable moment.

The number of preovulatory follicles was lower in prepuberal gilts compared to cycling ones but similar to primiparous sows. However, such uniform trend was not observed in all animals. Thus a prediction of lifetime performance of follicular growth and ovulation based on ovarian response to PMSG in prepuberal gilts is not possible. Results from the literature are various too. Ovulation rate increased at second oestrus compared to the first one (9.5 vs. 11.1 vs. 13.1, Andersson and Einarsson, 1980). However, Smith et al. (1992) did not find differences in the number of mature preovulatory follicles of gilts between first and third oestrus (10.3 ± 0.4 vs. 9.0 ± 0.9). In PMSG (1000 IU) primed prepuberal and cycling gilts the mean follicle number ranged between 19.6 to 25.5 and 18.6 to 24.3, respectively (Hunter, 1964; Baker and Coggins, 1968; Philippo, 1968; Kruff, 1985).

The mean size of follicles was similar between gilts and sows, but their shape was different. Follicles of primiparous sows were less vaulted, deeper embedded under the ovarian surface, flat and less vascularised. Such morphological criteria could indicate a lower degree of follicle maturation (Schnurrbusch et al., 1981).

Cumulus cell expansion is related to nuclear oocyte maturation (Cran, 1985; Torner et al., 1998). Even though cumulus expansion precedes nuclear maturation, it may be a predictor of developmental competence and maturation of oocytes (Torner et al., 1998; Guthrie and Garrett, 2000). The percent of oocytes with expanded cumulus was higher in prepuberal compared to cycling gilts and primiparous sows. So in prepuberal gilts the population of PMSG/hCG-stimulated follicles and their maturation was apparently more homogeneous.

More oocytes of gilts resumed meiosis or were mature compared to primiparous sows (95%, 94% vs. 50%). Koenig and Stormshak (1993) found a

lower percentage of mature ova at first compared to third oestrus (66.7 vs. 75.9%). In the present study the absence of mature oocytes in sows was unexpected. However, it is known that follicular growth, oocyte maturation and subsequent ovulation in primiparous sows are influenced more by lactation and nutrition, presumably by altering follicular secretions (Zak et al., 1997; Hunter, 2000; Guedes and Nogueira, 2001). Evidently the used follicular population of our primiparous sows was less mature at the time of recovery. Not only the shape and vascularisation of the follicles were different from those of gilts, but also their steroid concentrations.

FF of prepuberal gilts showed the highest content of progesterone and the lowest of oestradiol-17β. This result is comparable to those of Ainsworth et al. (1980; P_4: 901 ± 208 ng/ml, E_2: 14.4 ± 1.6 pg/ml) and Blödow et al. (1990; P_4: 675.1 ± 135.4 ng/ml, E_2: 20.8 ± 14.2 pg/ml). Regardless of whether they are from PMSG/hCG-primed or spontaneously ovulating gilts, late preovulatory follicles have a higher progesterone/oestradiol-17β ratio (Ainsworth et al., 1980; Blödow et al., 1990; Guthrie et al., 1993; Conley et al., 1994). This is due to the dramatic decrease of follicular capacity to produce oestradiol-17β, and the increased progesterone production after preovulatory LH surge or hCG administration (Downey and Driancourt, 1994). However, there was a notable biochemical follicular heterogeneity in gilts and sows. The variations in FF oestradiol-17β and progesterone concentrations amount to 31 and 56% (P), 48 and 29% (C), and 17 and 47% (S), respectively. It has been demonstrated that follicles are heterogeneous both in spontaneously ovulating and PMSG/hCG-primed gilts and sows. Morphological and biochemical asynchrony continues into the immediate preovulatory period and follicles respond differently to the LH/hCG surge signal, reflecting their maturational status at that time (Ainsworth et al., 1980; Grant, 1989; Hunter et al., 1989; Hunter and Wiesak, 1990).

Along with oocyte maturation and follicular morphology, higher oestradiol-17β and lower progesterone concentrations in FF of primiparous sows imply lower follicle maturation relative to prepuberal and cycling gilts when treated in the same manner. Because frequency of mature oocytes increases as follicular production of oestrogen decreases and synthesis of progesterone increases (Ainsworth et al., 1980), follicles of primiparous sows evidently need a longer time of maturation.

In summary, this study has shown a different follicular and oocyte development during investigated lifetime periods. Follicular heterogeneity was always present both in gilts and sows. *In vivo* matured oocytes of prepuberal and cycling gilts can be used for *in vitro* fertilisation procedures. However, cycling gilts should preferably be taken in IVF and breeding programs because of the more balanced intrafollicular milieu and oocyte maturation. The possibly lower reproductive potential of primiparous sows is taken into consideration at breeding. Individual ovarian reaction of prepuberal gilts is inappropriate for prediction of lifetime performance.

References

Ainsworth, L., Tsang, B. K., Downey, B. R., Marcus, G. J. and Armstrong, D. T. (1980): Interrelationship between follicular fluid steroid levels, gonadotropic stimuli, and oocyte maturation during preovulatory development of porcine follicles. Biol. Reprod. **23,** 621–627.

Andersson, A. M. and Einarsson, S. (1980): Studies on the estrus and ovarian activity during five successive estrous cycles in gilts. Acta Vet. Scand. **21,** 677–688.

Baker, R. D. and Coggins, E. G. (1968): Control of ovulation rate and fertilization in prepuberal gilts. J. Anim. Sci. **27,** 1607–1610.

Blödow, G., Bergfeld, J., Kitzig, M. and Brüssow, K.-P. (1990): Steroid hormone levels in follicular fluid of pigs with spontaneous oestrus and synchronised ovulation. Arch. exp. Vet. med. **44,** 611–620.

Blödow, G., Götze, M., Kitzig, M., Brüssow, K.-P. and Duschinski, U. (1988): Radioimmunological determination of steroid hormones in the follicular fluid of cattle and pigs. Isotopenpraxis **24,** 151–155.

Brüssow, K.-P. and Rátky, J. (1994): Repeated laparoscopical follicular puncture and oocyte aspiration in swine. Reprod. Dom. Anim. **29,** 494–502.

Conley, A. J., Howard, H. J., Slanger, W. D. and Ford, J. J. (1994): Steroidogenesis in the preovulatory porcine follicle. Biol. Reprod. **51,** 655–661.

Cran, D. G. (1985): Qualitative and quantitative structural changes during pig oocyte maturation. J. Reprod. Fertil. **74,** 237–245.

Downey, B. R. and Driancourt, M.-A. (1994): Morphological and functional characteristics of preovulatory follicles in Large White and Meishan gilts. J. Anim. Sci. **72,** 2099–2106.

Grant, S. A. (1989): Ovarian function in the gilt and the lactating sow. Ph.D. Thesis, University of Nottingham, U.K.

Guedes, R. M. and Nogueira, R. H. (2001): The influence of parity order and body condition and serum hormone on weaning-to-estrus interval of sows. Anim. Reprod. Sci. **67,** 91–99.

Guthrie, H. D. and Garrett, W. M. (2000): Changes in porcine oocyte germinal vesicle development as follicles approach preovulatory maturity. Theriogenology **54,** 389–399.

Guthrie, H. D., Bolt, D. J. and Cooper, B. S. (1993): Changes in follicular estradiol-17β, progesterone and immunoactivity in healthy and atretic follicles during preovulatory maturation in the pig. Dom. Anim. Endocrinol. **10,** 127–140.

French, A. J., Zviedrans, P., Ashman, R. J., Heap, P. A. and Seamark, R. F. (1991): Comparison of prepubertal and postpubertal young sows as a source of one-cell embryos for microinjection. Theriogenology **35,** 202.

Hunter, M. G. (2000): Oocyte maturation and ovum quality in pigs. Reviews of Reprod. **5,** 122–130.

Hunter, M. G. and Wiesak, T. (1990): Evidence for and implication of follicular heterogeneity in pigs. J. Reprod. Fert. Suppl. **40,** 163–177.

Hunter, M. G., Grant, S. A. and Foxcroft, G. R. (1989): Histological evidence for heterogeneity in the development of preovulatory pig follicles. J. Reprod. Fertil. **86,** 165–170.

Hunter, R. H. F. (1964): Superovulation and fertility in the pig. Anim. Prod. **6,** 189–194.

Koenig, J. L. F. and Stormshak, F. (1993): Cytogenetic evaluation of ova from pubertal and third-estrous gilts. Biol. Reprod. **49,** 1158–1162.

Kruff, B. (1985): Embryotransfer und Bestandssanierung. Tierzüchter **37,** 546–547.

Marchal, R., Feugang, J. M., Perreau, C., Venturi, E. and Mermillod, P. (2000): Developmental competence of prepubertal and adult swine oocytes: Birth of piglets from *in vitro*-produced blastocysts. Theriogenology **53,** 361.

Menino, A. R. Jr., Archibong, A. E., Li, J.-R., Stormshak, F. and England, D. C. (1989): Comparison of *in vitro* development of embryos collected from the same gilts at first and third estrus. J. Anim. Sci. **67,** 1387–1393.

Philippo, M. (1968): Superovulation in the pig. In: McLaren, A. (ed.) Advances in Reproductive Physiology. Logo Press, London, pp. 147–166.

Rátky, J., Brüssow, K.-P. and Solti, L. (1998): Endoscopic methods in swine reproductive research: A review. Acta Vet. Hung. **46,** 487–492.

Schnurrbusch, U., Bergfeld, J., Brüssow, K.-P. and Kaltofen, U. (1981): Diagram for ovarian assessment in swine. Mh. Vet.-Med. **36,** 811–815.

Smith, G. D., Menino, A. R. Jr., Rowe, K. E. and Stormshak, F. (1992): Steroids and plasminogen activator concentrations in follicular fluid of gilts at first and third estrus. J. Anim. Sci. **70,** 3838–3843.

Torner, H., Brüssow, K.-P., Alm, H. and Rátky, J. (1998): Morphology of porcine cumulus-oocyte-complexes depends on the stage of preovulatory maturation. Theriogenology **50,** 39–48.

Zak, L. J., Xu, X., Hardin, R. T. and Foxcroft, G. R. (1997): Impact of different patterns of feed intake during lactation in the primiparous sow on follicular development and oocyte maturation. J. Reprod. Fertil. **110,** 99–106.