Comparison of Follicular and Oocyte Development and Reproductive Hormone Secretion during the Ovulatory Period in Hungarian Native Breed, Mangalica, and Landrace Gilts

Jozsef RÁTKY1), Klaus-Peter BRÜSSOW2), Istvan EGERSZEGI1), Helmut TORNER2), Falk SCHNEIDER2), Laszlo SOLTI3) and Noboru MANABE4)

1)Research Institute for Animal Breeding and Nutrition, 2053 Herceghalom, Hungary, 2)FBN Research Institute for the Biology of Farm Animals, 18196 Dummerstorf, Germany, 3)Department of Obstetrics and Reproductive Biology, Faculty of Veterinary Science, Szent Istvan University, 1400 Budapest, Hungary, and 4)Research Unit of Animal Life Sciences, Animal Resource Center, The University of Tokyo, Ibaraki-Iwama 319–0206, Japan

Abstract. Only a very small amount of physiological data is available about the low fertility (mean litter size is 5.7 ± 0.8) of Hungarian native breed, Mangalica (M), sows. The aim of the present paper is to reveal the differences in preovulatory follicle development and intrafollicular oocyte maturation between M and Landrace (L) gilts, with special reference to the peri- and postovulatory secretion and peripheral concentrations of estradiol-17β (E2), progesterone (P4), and luteinizing hormone (LH). The number of preovulatory follicles was 6.8 ± 1.4 and 19.6 ± 6.6 in M and L gilts, respectively. A lower degree of cumulus expansion and a lower percentage of mature oocytes (TI/M II) was noted in M. Higher LH and E2 peak levels, a longer E2 to LH peak interval, and lower embryo survival was confirmed. Interestingly, despite the lower number of corpora lutea, a higher peripheral blood level of P4 was shown in M than in L gilts. Both diminished follicular development and protracted oocyte maturation may be involved in low fecundity in M, and the present findings may explain these reproductive phenomena.

Key words: Follicular development, Hungarian native Mangalica sow, Oocyte maturation, Porcine ovary

In the recent decade, European swine breeding has become somewhat more varied. While large companies are dominating the production of the crossbred Landrace pig, small (and sometimes large) farms and independent breeders are trying to compete by breeding special races or by slaughtering overweight pigs, which are suitable for high quality meat products. However, these products contain a large amount of fat, and they hardly meet the demands of typical dietetic science, an area in which consumers are definitely demonstrating a growing interest. Among the special breeds, we have identified the Hungarian Mangalica, with its three types (i.e. Red, Blonde and Swallow Belly) as attracting the attention of the market as well as physiologists (e.g. reproductive biologists).

Mangalica pigs appeared in the first half of the 19th century and were the most typical breed of swine in Hungary with the early 1950s. At that...
time it was replaced by modern breeds and quickly disappeared from the pig industry reaching a critical level of population in the 1980s. The reason for their fast decline and near disappearance was that the Mangalica could not fulfill the requirements of modern industrial swine production (i.e. it had a clean meat yield of between 30.5–35% compared to more than 50% in modern white breeds and the fattening time was much longer [1]) and before cc. 16 months of age, there was no sense in slaughtering them. Luckily, authorities and businessmen considered the danger to the breed and its potential, and, at the last minute, recognized its national and commercial values. Now, there is a population numbering more than 3000 sows, and tens of thousands of hams are exported to the European Union yearly.

While the economic interest is due to the delicious food products that are made from Mangalica meat and that are quite comparable to those of the world famous Iberian pigs, scientific interest is connected to the physiological parameters that differ from those of white, commercial breeds. According to the literature from the 19th and early 20th century, and following a few years of our own work with Mangalica pigs, we have experienced that it’s reproductive characteristics are not the same as those of the Landrace pigs that are generally used in experiments. Puberty is attained at 9–12 months of age. The mean age at first farrowing is $572 \pm 96$ days, $1.5 \pm 0.1/\text{year}$ can be achieved and the mean litter size is $5.7 \pm 0.8$ [2, 3]. In addition to the 6–7 week long rearing period, sows come into heat usually at the end of spring and at the beginning of autumn. If there is any estrous in other periods of the year, both symptoms and fertility are weak.

Only a small amount of physiological data is available about the low fertility in the Mangalica. Our research group demonstrated that follicular development and ovulation could be stimulated with exogenous gonadotropins in the Mangalica as in Landrace gilts [4]. The aim of the present paper is to summarize the possible differences in preovulatory follicle development and intrafollicular oocyte maturation between Mangalica and Landrace gilts with special reference to the peri- and postovulatory secretion and peripheral concentrations of estradiol-17$\beta$, progesterone, and luteinizing hormone (LH).

### Material and Methods

#### Experiment 1

Altogether 18 puberal Mangalica (M) and 19 Landrace (L) gilts aged 8.5 to 9 month, and with a body weight of 120 to 125 kg, were involved. Their estrous cycles were synchronized by feeding Regumate® (16 mg altrenogest/animal daily, Serumwerk Bernburg, Germany) for 15 days. Follicular growth was stimulated with an intramuscular injection of 1,000 IU equine chorionic gonadotropin (eCG, Folligon®; Intervet, Budapest, Hungary) 24 h after the last Regumate® feeding (08:00 h). The LH peak was initiated by 50 $\mu$g gonadotropin releasing hormone agonist (GnRH, Depherelin®, Veyx Pharma, Schwarzenborn, Germany) 80 h after eCG injection.

Cumulus-oocyte-complexes (COCs) were recovered 34 h after GnRH by endoscopic ovum pick up, as described by Brüssow and Rátky [5]. Follicular puncture and aspiration were carried out via a two-way cannula (40 mm length, 16-gauge) and an electric aspiration pump (model 3014; Labotect, Göttingen, Germany) with an initial vacuum of 100 mm Hg corresponding to a volume of 17 ml/min. The tip of the cannula was inserted into a follicle, the follicular content was aspirated, and the lumen was refilled with heparinized PBS and aspirated repeatedly. Only macroscopic healthy follicles with a diameter of larger 5 mm were punctured.

Follicular fluids were pooled for the different follicles of each ovary. The morphology of freshly recovered COCs was evaluated under an inverted microscope at $\times 60$ magnification. COCs were classified as compact, expanded, or denuded [6]. After classification, COCs were prepared for observation of the nuclear configuration. Cumulus cells were removed in PBS containing 100 IU/ml hyaluronidase (hylase; Impfstoffwerk Dessau, Germany) by repeated pipetting with a fine-bore glass pipette. Oocytes were mounted on slides, fixed for 24 h in a mixture of acetic acid, alcohol and chloroform (3:6:1), and then stained with 2 % orcein in 60% acetic acid. The nuclear configuration of oocytes was examined using a phase-contrast microscope at $\times 250$ to $\times 630$ magnification. Based on their nuclear status, the oocytes were classified as follows: immature—germinal vesicle (GV), with diplotene chromatin; meiosis resumed—GV breakdown, diakinesis, M I to A I; or mature—T I
and M II.

**Experiment 2**

Six 10–12 month-old puberal Ms with a body weight of 100–120 kg and four 9-month-old L gilts with a body weight of 110–120 kg were used. Estrus synchronization and follicle stimulation were the same as in Experiment 1.

On day 12 of Regumate® feeding, chronic jugular vein catheters were surgically fixed in all gilts [7]. Blood samples of 10 ml in volume were collected three times daily, and intensively in a 16 h period at 2 h-intervals following GnRH application. The samples were centrifuged, and the plasma was stored at −20°C until analyses.

Ovaries were observed by endoscopic surgery [8], 14 days after the last Regumate® feeding. Concentrations of LH, estradiol-17β (E2), and progesterone (P4) were determined by electrochemiluminescence immunoassay (ECLIA) and by radio-immunoassay (RIA), respectively. The methodology of the assays were described in details previously [9, 10].

Data were represented as the mean ± SD, and analyzed by t-test, Chi-square, Tukey-test, and one-way variance analysis. P<0.05 was considered to be significant.

**Results**

**Experiment 1**

The average number of preovulatory follicles was 6.8 ± 1.4 in M and 19.6 ± 6.6 in L gilts (P<0.05), respectively. The size of punctured preovulatory follicles ranged between 5 to 7 mm in M and 6 to 9 mm in L. Altogether, 298 follicles were punctured and 183 COCs were recovered. In M gilts a lower recovery rate of COCs was achieved. There were differences in regard to the morphology of recovered COCs between breeds (Table 1).

Nevertheless, the rate of oocytes with a compact cumulus was higher in M gilts compared to L gilts (P<0.05), most COCs (62 and 78%, P<0.05), aspirated from the preovulatory follicles, possessed an expanded cumulus. Although only macroscopically healthy follicles were aspirated, 6 to 7% of the COCs were denuded, but there was no difference between breeds.

As shown in Table 2, the meiotic configuration of oocytes differed between M and L gilts. Since the rate of oocytes with a mature chromatin configuration was higher (P<0.05) in L sows, the proportion of immature oocytes and resumption of meiosis was lower compared to M.

**Experiment 2**

Peripheral concentrations of hormones, intervals from GnRH application, and from peak concentration of E2 to LH peak, and ovulation rates are shown in Table 3. An E2 peak (46.5 ± 5.7 vs. 26.0 ± 6.8 pg/ml; P<0.05) was observed in M and L on day 2 and day 4 after the last Regumate® feeding, respectively. LH peaks were found until 6 h following GnRH injection and concentrations were similar in M and L (11.5 ± 4.1 vs. 6.5 ± 2.5 ng/ml). P4 concentrations increased from day 6 after the last Regumate® feeding (from 0.6 ± 0.3 to 0.7 ± 0.4 ng/ml to maximal level of 14.0 ± 2.4 and 11.3 ± 2.1 ng/ml in M and L, respectively). While the mean P4 secretion was higher in M between days 10 and 15 (12.9 ± 2.6 vs. 9.3 ± 2.2 ng/ml; P<0.05), the number of corpora lutea was lower in M than L (10.3 ± 1.5 vs. 17.8 ± 5.0, P<0.05).

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**Table 1.** Number of punctured follicles and aspirated COCs, and the morphology of COCs in M and L gilts (n=37)

<table>
<thead>
<tr>
<th>M</th>
<th>L</th>
</tr>
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<tbody>
<tr>
<td>No. of aspirated follicles n</td>
<td>108</td>
</tr>
<tr>
<td>No. of recovered COCs n</td>
<td>58</td>
</tr>
<tr>
<td>Recovery rate %</td>
<td>53.7a</td>
</tr>
<tr>
<td>COC morphology Compact %</td>
<td>31a</td>
</tr>
<tr>
<td>Expanded %</td>
<td>62a</td>
</tr>
<tr>
<td>Denuded %</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 1. P<0.05.

**Table 2.** Chromatin configuration of oocytes recovered from M and L gilts (n=37)

<table>
<thead>
<tr>
<th>M</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of evaluated oocytes n</td>
<td>44</td>
</tr>
<tr>
<td>Chromatin configuration Immature (GV) %</td>
<td>18a</td>
</tr>
<tr>
<td>Resumption of meiosis (GVBD-AI) %</td>
<td>55a</td>
</tr>
<tr>
<td>Mature (TI / MII) %</td>
<td>27a</td>
</tr>
</tbody>
</table>

Table 2. P<0.05.
Discussion

The most typical breed of pig in Hungary until the early 1950s, the M quickly disappeared from the pig industry. However, in recent years, its population is increasing. Preservation and propagation of native pig breeds has attracted growing public interest. The exceptional taste of the meat, and their robustness and motherliness support these efforts. Nevertheless, low prolificacy and marked seasonality remain a problem. The aim of the present paper was to find the possible implications of the physiological basis of low fecundity with special regard to preovulatory follicle and oocyte development and peripheral concentrations of ovarian steroids and LH. Since differences have been shown in follicular development and oocyte maturation between European white breeds and the highly prolific Chinese Meishan [11], the present study was also carried out using this method, i.e. by comparing the parameters in M and L gilts. Except for our previous results, there is no data available on the number of follicles in M [4]. According to the results, M gilts grow only 6.8 ± 1.4 follicles. A significant higher number of follicles were stimulated in Landrace gilts (19.6 ± 6.6, p<0.05). It must be mentioned that usually, M only express spontaneous heat at the end of spring and the at beginning of autumn, and that the present experiment was conducted in November.

Cumulus expansion was lower in M (62 vs. 78%). Cumulus morphology dramatically changes during preovulatory maturation. The uncoupling between oocyte and cumulus starts in vivo about 22 h after LH peak or hCG, respectively [6, 12]. In L gilts, nearly all COCs (98%) expressed expanded cumulus 34 h after hCG [6]. The lower degree of cumulus expansion in M could be attributed to a more protracted follicle and oocyte maturation.

More oocytes with an immature chromatin configuration were found in M (18 vs. 6%). This result is rather reasonable because a higher portion of oocytes with compact cumulus was found in M. A relationship between cumulus morphology and oocyte meiotic maturation has been demonstrated, with most oocytes with a compact cumulus in the GV-stage [6]. However, the majority of oocytes for both breeds resumed meiosis (82 and 94%). Complete GV breakdown was also observed in gilts 24 to 36 h after the preovulatory LH surge [13]. The percentage of mature oocytes (T I/M II) was lower in M.

In the 2nd trial, also, a lower ovulation rate was found in M compared to L gilts (10.3 ± 1.5 vs. 17.8 ± 5.0). The pattern of LH and ovarian steroid secretion was similar in the two breeds during the estrous cycle. Previous investigations on prolific and less prolific pigs have demonstrated no marked difference in hormonal secretion [11, 14–16].

In the current study, LH peak occurred up to 6 hours after GnRH in all animals which is in accordance with earlier work [17–20]. The peak height of the LH surge ranged from 4.2 to 16.0 ng/ml and tended to be higher in M than in L (P=0.08). The LH value returned to the base level after the peak. The E2 peak occurred in M and L before LH surge two and four days after the last Regumate® feeding, which was one to two days (12 to 48 h) before the LH peak. Whereas this corresponds with previous findings [21, 22], it is worthwhile to note that some experts in L sows have recognized a relationship between higher E2 peak concentrations, longer E2 to LH peak intervals and lower embryo survival rates [23]. The exact opposite was reported in prolific Meishan pigs: higher E2 values, longer E2 to LH peak intervals,

Table 3. Results of hormone secretion and ovulation in M and L gilts

<table>
<thead>
<tr>
<th></th>
<th>M</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time from GnRH to LH peak (h)</td>
<td>2.5 ± 1.0a</td>
<td>6.5 ± 2.5p</td>
</tr>
<tr>
<td>Peak LH (ng/ml)</td>
<td>11.5 ± 4.1</td>
<td>6.6 ± 2.3</td>
</tr>
<tr>
<td>Highest E2 concentration (pg/ml)</td>
<td>46.5 ± 5.7a</td>
<td>26.0 ± 6.8p</td>
</tr>
<tr>
<td>Interval from peak E2 to peak LH (h)</td>
<td>13.5 ± 11.8</td>
<td>10.5 ± 3.0</td>
</tr>
<tr>
<td>Highest P4 concentration (ng/ml)</td>
<td>14.0 ± 2.4</td>
<td>11.3 ± 2.1</td>
</tr>
<tr>
<td>Number of corpora lutea</td>
<td>10.3 ± 1.5a</td>
<td>17.8 ± 5.0p</td>
</tr>
</tbody>
</table>

a,b P<0.05
and a typically outstanding survival rate [15]. Although M had a low ovulation rate and small litter size the estradiol secretion pattern was similar in both races. The same situation was found in the changes of P4 concentrations, i.e. M and L followed the usual pattern [22, 24]. Interestingly, despite the lower number of corpora lutea a higher concentration of P4 was measured in M than in L gilts.

Based on our findings, we assume that both diminished follicular development and protracted intrafollicular oocyte maturation may be involved in low fecundity in M, and that the slightly different LH and ovarian steroid secretions most likely have no role in the lower reproductive capability of this aboriginal Hungarian pig breed.

Acknowledgement

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