



Szent István University

**Endocrinological and morphological changes
in the ovaries and uteri of cycling and
pregnant Mangalica gilts**

Ph. D. Thesis

Egerszegi István

Gödöllő

2005

Nowadays zoo- and biotechnical methods have an important role in farm animal breeding. For the better results we have to get much more information about the reproductive biology of these animals. Mangalica is an indigenous swine breed of Hungary, which was the most typical during over 100 years. The breeders created a very robust and resistant race. In the middle of the last century the population decreased and nearly disappeared because of its too much lard and lower reproductive parameters than modern commercial breeds. The number of registered breeding animals was under 200 in the middle of 1990-ies. The real escape from extinction came with the new economic exploitation of the Mangalica and with growing interest to endangered animals. The modern zoo- and biotechnological methods could be useful for the propagation of Mangalica. But at first we must improve our knowledge about the reproductive characteristics of this race to elaborate the most adequate techniques.

Aims of the present thesis are the following:

- Comparison of intrafollicular oocyte development and maturation in Mangalica and Landrace gilts.

- Analysis of luteinizing hormone and steroid hormones concentration during the cycle in Mangalica and Landrace gilts
- Collection and description of some organometric data about the genital tracts from cycling and pregnant Mangalica sows.

Hopefully the results of these investigations can increase our knowledge about reproductive physiology of female swine and all these can be used in practical breeding and in research of in vitro technologies. At least these information can improve the efficiency of Mangalica's propagation.

Materials and methods

Comparison of intrafollicular oocyte development and maturation in Mangalica and Landrace gilts on day 20 of the estrus cycle

18 Blonde and Swallow-Belly Mangalica and 19 Landrace gilts were involved in the first treatment. The estrus cycle was synchronized by Regumate feeding and the endoscopic ovum pick up was performed on day 20 of the cycle as described by Brüssow and Rátky (1994).

The morphology of the cumulus-oocyte-complexes (COCs) was determined under stereo microscope. The COCs were classified as compact, slightly expanded, expanded, oocyte with corona radiata or denuded oocyte (Torner et al., 1998).

The chromatin configuration of the COCs was evaluated after orcein staining. We differentiated these groups: (1) immature; (2) resumption of meiosis; (3) mature or (4) degenerated.

Data were analyzed by Chi test.

Analysis of luteinizing hormone (LH), oestradiol-17 β (E₂) and progesterone (P₄) concentrations during the peri- and postovulatory period in Mangalica and Landrace gilts

6 Mangalica and 4 Landrace gilts were used in this experiment. The estrus cycle was synchronized by Regumate feeding. Three days before the last Regumate feeding animals were catheterized in v. jugularis as described by Rodriguez and Kunavongkrit (1983).

Blood samples were collected three times a day (08:00; 12:00; 16:00) and after the GnRH injection during a 16 hours period in every two hours. The ovulation rate was estimated by endoscopic method (Rátky et al., 1998).

LH concentration was determined by ECLIA, oestradiol-17 β and progesterone by RIA. Data were analyzed by one way ANOVA and Tukey-test.

Organometric data of the reproductive tract in cycling and early pregnant Mangalica pigs

Two experiments were carried out to investigate the reproductive tract of Mangalica gilts. In Experiment 1 reproductive organs of 66 gilts (12 – 15 month of age) were recovered immediately after slaughter to determine the number of ovarian features, the weight and diameter of the ovaries, and the weight and length of oviducts and uteri, respectively. In Experiment 2, 22 puberal Mangalica (M) gilts and 34 German Landrace (L) gilts were used following estrus synchronization and artificial insemination. Genital tracts were recovered by ovariectomy on day 1 (n = 8 M; 10 L), day 12 (n = 8 M; 22 L) and day 24 (n = 6 M; 2 L) of pregnancy and the number of corpora lutea, ovarian weight and diameter, and the weight and length of oviducts and uteri evaluated. Oviducts and uterine horns (day 1 and 12, respectively) were flushed or uterine horns were opened (day 24) for embryo recovery. For data analysis Windows SAS

System 8.02-vel (SAS Institute, 1999) was used (ANOVA, GLM).

Results

Comparison of intrafollicular oocyte development and maturation in Mangalica and Landrace gilts on day 20 of the estrus cycle

Mangalica (M) gilts had significantly lower ovulation rate compared to Landrace (L) ($p < 0,05$). The number of preovulatory follicles was $6,8 \pm 1,4$ in M and $19,6 \pm 6,6$ in L gilts. Altogether 298 follicles were aspirated and 183 COCs were recovered. We obtained higher recovery rate in L than M. The morphology of the COC was different too, the number of the oocytes with compact cumulus was two fold higher in M. Most of the oocytes had expanded cumulus in both breeds (62 és 78%, $p < 0,05$). We recorded 6-7 % of denuded oocytes and similar results of nuclear development. Mangalica had higher rate of immature and meiosis resumed oocytes in this experiment ($p < 0,05$).

Analysis of luteinizing hormone (LH), oestradiol-17 β (E₂) and progesterone (P₄) concentrations during the peri- and postovulatory period in Mangalica and Landrace gilts

Typical LH and ovarian steroid secretion pattern were found during the peri- and postovulatory period in Mangalica and Landrace gilts. Preovulatory E₂ peak was observed on day 2 (M) and day 4 (L) after the last Regumate feeding. E₂ peak was different between M and L (46.5 ± 5.7 vs. 26.0 ± 6.8 pg/ml, $p < 0.05$). LH maximum levels were found up to 6 h after GnRH and were not different between M and L (11.5 ± 4.1 vs. 6.5 ± 2.5 ng/ml). Starting on day 6 after Regumate® P₄ concentrations significantly increased from 0.6 ± 0.3 and 0.7 ± 0.4 ng/ml to maximal 14.0 ± 2.4 and 11.3 ± 2.1 ng/ml in M and L, respectively. Mean P₄ secretion was higher in M on days 10 – 15 (12.9 ± 2.6 vs. 9.3 ± 2.2 ng/ml; $p < 0.05$). However the number of corpora lutea was lower in M compared to L (10.3 ± 1.5 vs. 17.8 ± 5.0 , $p < 0.05$).

Organometric data of the reproductive tract in cycling and early pregnant Mangalica pigs

In Experiment 1 58 gilts (88 %) were ovulated already with mean number of ovulation of 10.6 ± 3.1 . In first estrous gilts (n = 8) it was 11.1 ± 2.5 . No difference was observed both between left and right ovaries, and first estrus and cycling gilts. The number of corpora lutea were correlated with ovarian weight ($r = 0.35$, $p < 0.05$). Oviduct length (24.4 ± 1.4 and 24.3 ± 0.5 cm) and weight (4.3 ± 0.4 and 3.2 ± 0.2 g), and uterine horn length (143 ± 13 and 143 ± 5 cm) were not different in first estrous and cycling gilts. Uterine weight was higher in first estrous compared to cycling gilts (358 ± 32 vs. 250 ± 12 g, $p < 0.05$).

In pregnant Mangalica (M) and Landrace (L) gilts no interaction was found between day of pregnancy and number of ovulation in the two breeds. Mean number of corpora lutea was 17.2 ± 1.2 (M) and 18.6 ± 1.3 (L). Length of uterine horns was reduced in pregnant M compared to L gilts (124 ± 5 vs. 188 ± 6 cm, $p < 0.01$). Uterus grows in length continuously between day 1 and 24 of pregnancy in L but not in M sows ($p < 0.01$). Uterine weight increased earlier (day 1 to day 12) in L in comparison to M (day 12 to day 24).

New scientific results

1. Organometric data of Mangalica gilts (weight, length and diameter of ovaries, oviducts, uteri) were determined
2. Data were collected from 110 female Mangalica swine. The mean ovulation rate was $10,6\pm 3,1$ and $11,7\pm 3$ (n=66) in spontaneous estrus. In synchronized animals ovulation rate varied between $6,8\pm 1,4$ and $17,2\pm 1,2$, which is indicated Mangalica's different response to hormonal treatment.
3. The morphology and the chromatin configuration of the preovulatory cumulus-oocyte-complexes differed between the two breeds. It can be concluded that the lower ovulation rate and diminished oocyte development are involved in low prolificacy of Mangalica pigs.
4. No significant difference was observed in the hormone secretion pattern during the estrus cycle between the two breeds. However progesterone secretion was higher in M despite of lower ovulation

rate. Further investigation is necessary to elucidate this phenomenon

5. It was found that growth restricted uterine development compared to modern pig breeds, may influence the initial process of early pregnancy and can be another reason of lower fecundity of Mangalica pigs.

Suggestions

The lower preovulatory follicle development and diminished oocyte maturation are involved in moderate prolificacy of Mangalica pigs. The diminished and delayed oocyte development can affect fertilization, so it should be kept in mind by timing artificial insemination. Further investigations must be carried out to clarify relationship between intra follicular hormone contents and oocyte maturation in Mangalica

The results of the Experiment 2 did not support the theory that different hormonal secretion can be play a role in lower reproductive ability of Mangalica. New experiment can elucidate that this hormone concentration are typical for this

breed (different hormone synthesis, enzyme activity, steroid turnover).

Mangalica gilts reach puberty till the age of 11-12 month. The mean ovulation rate is 10-14, but the ovary has a biological capacity to develop more follicles on it (superovulation, embryo donation).

Results of Experiment 3 support the concept that beside diminished ovarian and oocyte development, uterine conditions, especially growth restricted uterine development may influence the initial process of early pregnancy and can be another reason of lower fecundity in Mangalica. However, other experiments must highlight biological processes of early embryo/fetal development of this native pig breed.

In conclusion due to these results breeders must find the balance between requestes of the Mangalica pigs and production for the market without decreasing valuable characteristics of this breed.

List of publications

Scientific papers in reviewed journals

1. Rátky, J., Brüssow, K-P., **Egerszegi, I.**, Torner, H., Solti, L.: Mangalica - an old pig breed with actual interest and its propagation by means of biotechnology. AETE Newsletter 2000. 12. 6-10.
2. **Egerszegi, I.**, Torner, H., Rátky, J., Brüssow K-P.: Follicular development and preovulatory oocyte maturation in Hungarian Mangalica and Landrace gilts. Arch.Tierzucht, 2001. (44) 4. 413-419.
3. Brüssow K-P., Torner, H., Kanitz, W., Rátky, J., **Egerszegi, I.**, Noboru, M., Solti, L.: A sertésembrió-átültetés biotechnológiai vonatkozásai. Állattenyésztés és Takarmányozás, 2001. (50) 6. 481-493.
4. Brüssow, K.-P., Rátky, J., Torner, H., **Egerszegi, I.**, Schneider, F., Solti, L. and Tuchscherer, A.: Follicular and oocyte development in gilts of different age. Acta Veterinaria Hungarica, 2002. (50) 101-110,
5. **Egerszegi, I.**, Rátky, J., Solti, L., Brüssow K-P.: Mangalica - an indigenous swine breed from Hungary (Review). Arch.Tierzucht, 2003. (46) 3. 413-419.

6. **Egerszegi, I.**, Schneider, F., Rátky, J., Soós, F., Solti, L., Manabe, N., Brüssow K-P.: Comparison of luteinizing hormone and steroid hormone secretion during the peri- and post-ovulatory periods in Mangalica and Landrace gilts. *Journal of Reproduction and Development*, 2003. (49) 4. 291-296.
7. Brüssow, K. P., **Egerszegi, I.**, Rátky, J., Soós, F., Casado, P. G., Tuchscherer, A., Tóth, P.: Organometric data of the reproductive tract in cycling and early pregnant Hungarian Mangalica pigs. *Arch. Tierzucht*, 2004. (47) 6. 585-594.
8. Brüssow, K. P., **Egerszegi, I.**, Rátky, J., Torner, H., Tóth, P., Schneider, F.: Reproduction in the Hungarian Mangalica pig – a review. *Pig News and Information*, 2005. 1. 23N-28N.
9. Rátky, J., Torner, H., **Egerszegi, I.**, Schneider, F., Sarlós P., Manabe N., Brüssow, K. P.: Ovarian activity and oocyte development during follicular development in pigs at different reproductive phases estimated by the repeated endoscopic method. *Journal of Reproduction and Development*, 2005. (51) 1. 109-115.
10. **Egerszegi, I.**, Hazeleger W., Rátky, J., Sarlós, P., Kemp B., Bouwman, E., Brüssow K.-P., Solti L.: Eltérő takarmányozási szinteken tartott, különböző fajtájú nőivarú

sertések tüszőfejlődése. Állattenyésztés és Takarmányozás, 2005 (in press)

Book, chapter in books:

1. Rátky, J., Brüssow, K.-P., Torner, H., **Egerszegi, I.** and Solti, L.: Minimal invasive surgery in pig reproductive studies. In: Reproductive biotechnology. Eds.: Miyamoto, H. and Manabe, N., Hokuto Shobo, Kyoto, Japan, 2001.

Abstracts

1. Rátky J, Brüssow K-P, **Egerszegi I**, Torner H, Toth P, Solti L. Role of Biotechnology in the Propagation of Mangalica Pigs. Save-DAGENE. International Meeting of Mangalica Breeders. Budapest, 21st August 2001
2. **Egerszegi, I.**, Brüssow, K.-P., Torner, H. and Rátky, J.: Comparison of follicular development in Mangalica and Landrace gilts. 17th Scientific Meeting of the European Embryo Transfer Association. 2001. Lyon, France, 222.
3. **Egerszegi, I.**, Brüssow, K.-P., Rátky, J., Schneider, F., Solti, L.: LH and steroid hormone secretion during the peri- and

- postovulatory period in Mangalica gilts. Wiener Tierärztliche Monatsschrift 2003. (90) Suppl. 1. 10.
4. Rátky J, Brüssow K-P, **Egerszegi I**, Sarlos P: Az embrióátültetés korlátai sertésben 10. Szaporodásbiológiai Talaálkozó Kiskunmajsa 2003.
 5. Brüssow K-P, Rátky J, **Egerszegi I**, Solti L: Physiological aspects of in vivo fertilization in pigs. Proc. 15th European A.I. Vets Meeting 8.-11. October 2003, Budapest, Hungary, Pig Session 7-12.
 6. **Egerszegi, I.**, Hazeleger W., Schneider, F., Rátky, J., Kemp, B., Brüssow, K.-P.: Comparison of follicular development and oocyte quality in Mangalica and Landrace gilts after feeding with different energy levels. 20th Scientific Meeting of the European Embryo Transfer Association. 2004. Lyon, France, 146.
 7. Rátky, J., Brüssow, K. P., **Egerszegi, I.**, Torner, H., Schneider, F., Solti, L.: Comparison of follicular and oocyte development and reproductive hormone secretion during the ovulatory period in Mangalica and Landrace gilts. The Journal of Reproduction and Development, Vol. 50. Suppl. August 2004; 152.
 8. **Egerszegi, I.**, Hazeleger W., Rátky, J., Kemp, B., Brüssow, K.-P., Solti, L.: Különböző energia tartalmú takarmányok hatása mangalica kocasüldők tüsző- és petesejtérésére. 11.

Szaporodásbiológiai Találkozó Dobogókő 2004.
nov.19-20.

Other

1. Rátky, J., **Egerszegi, I.**, Brüssow, K-P.: A koca ciklikus nemi működésének élettani alapjai. A Sertés, 2000 (5) 2. 4-9.
2. Rátky, J., **Egerszegi, I.**, Brüssow, K-P., Torner, H., Szabó, P., Tóth, P., Fésüs, L., és Solti, L.: Mangalica - régi sertésfajta új jelentőséggel. A Sertés, 2001. (6) 1. 16-19.
3. Brüssow K-P., Kanitz, W., Rátky, J., **Egerszegi, I.**: Embryotransfer Schwein und assoziierte Biotechniken. Rekasan[®]-Journal, 2003. (10) 19/20. 73-79.