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

Dedicated to Prof. Dr. habil. Peter Glodek on the occasion of his 70th birthday

Abstract

Since the morphology of the reproductive organs could be a wherefore of reproductive performance of Mangalica, this study was conducted to get some more information about the number of corpora lutea and the size of reproductive tract both in cycling and 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 ovario-hysterectomy 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.

In Experiment 1, 58 gilts (88 %) were cycling ones with mean number of ovulation of 10.6 ± 3.1. In first estrous gilts (n = 8) it was 11.1± 2.5. No differences were observed both between left and right ovaries, and first estrous 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 breed*day of pregnancy interaction regarding the number of ovulation was found. 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 Landrace but not in Mangalica sows ($p<0.01$). Uterine weight as well increased earlier (day 1 to day 12) in Landrace in comparison to Mangalica (day 12 to day 24).

Results of this study support the concept that in Mangalica besides 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.

Key Words: ovary, oviduct, uterus, Mangalica, swine

Zusammenfassung

Titel der Arbeit: **Organometrische Daten des Reproduktionstraktes zyklischer und frühträchtiger Ungarischer Mangalitza Schweine**

Da die Morphologie der Reproduktionsorgane ursächlich für die Fortpflanzungsleistungen der Mangalitza mitverantwortlich sein kann, wurden Daten zur Anzahl der Gelbkörper und zu den Größen des Genitaltraktes bei zyklischen und trächtigen Mangalitza-Sauen in zwei Experimenten erhoben. Im Experiment 1 wurden die Reproduktionsorgane von 66 Jungsauen (Alter 12 – 15 Monate) unmittelbar nach der Schlachtung gewonnen, um die Ovarstrukturen, das Gewicht und den Durchmesser der Eierstöcke, sowie Länge und Gewicht der Eileiter und Uteri zu erfassen. Im Experiment 2 wurden 22 puberale Mangalitza (M) und 34 Deutsche Landrasse (L)

Jungsauen nach Brunstsynchronisation besamt. Der Genitaltrakt wurde mittels Ovariohysterektomie an den Trächtigkeitstagen TT1 (n = 8 M; 10 L), TT12 (n = 8 M; 10 L) und TT24 (n = 6 M; 2 L) entnommen und die Anzahl corpora lutea, das Gewicht und die Größe des Ovars sowie Gewicht und Länge von Eileiter und Uterus bestimmt. Die Eileiter und Uterushörner wurden zur Embryonengewinnung am TT1 und TT12 gespült bzw. geöffnet (TT24).
Im Experiment 1 waren 58 Jungsauen (88 %) zyklisch mit einer mittleren Anzahl Ovulationen von 10,6 ± 3,1. Erstmalig östrische Tiere (n = 8) wiesen 11,1 ± 2,5 Gelbkörper auf. Es gab keine Unterschiede zwischen dem rechten und linken Ovar sowie erst-östrischen und zyklischen Jungsauen. Die Anzahl der corpora lutea war mit dem Ovargewicht korreliert (r = 0,35, p<0,05). Die Eileiterlänge (24,4 ± 1,4 und 24,3 ± 0,5 cm), Eileitergewicht (4,3 ± 0,4 und 3,2 ± 0,2 g) sowie Uterushornlänge (143 ± 13 und 143 ± 5 cm) unterschieden sich nicht zwischen erst-östrischen und zyklischen Jungsauen. Das Uterusgewicht war bei den erst-östrischen Tieren höher (358 ± 32 vs. 250 ± 12 g, p<0,05).
Bei den trächtigen M- und L-Jungsauen wurde keine Interaktion Rasse*Trächtigkeitstag hinsichtlich Anzahl Gelbkörper ermittelt. Die mittlere Gelbkörperanzahl betrug 17,2 ± 1,2 (M) und 18,6 ± 1,3 (L). Die Länge der Uterushörner war bei den trächtigen M im Vergleich zu L reduziert (124 ± 5 vs. 188 ± 6 cm, p<0,01). Es erfolgte ein kontinuierliches Uteruswachstum zwischen den Trächtigkeitstagen 1 und 24 bei den Landrasse-, nicht jedoch bei den Mangalitza- Sauen (p<0,01). Auch das Uterusgewicht erhöhte sich bei L früher (TT1 - TT12) als bei M (TT12 - TT24).
Die Ergebnisse der Untersuchungen bestätigen die Hypothese, dass bei den Mangalitza neben einer geringeren Follikel- und Eizellenentwicklung, die Bedingungen im Uterus, insbesondere das Uteruswachstum, die frühe Trächtigkeit beeinflussen und somit eine weitere Ursache für die geringere Fruchtbarkeit sein können.

Schlüsselwörter: Ovar, Eileiter, Uterus, Mangalitza, Schwein

Introduction

Litter-size is an important determinant of production performance and economy in pig industry, and it is influenced by breed, ovulation and fertilization rates, uterine capacity and early embryo mortality (WU et al., 1989; WHITTENMORE, 1993; VAN DER LENDE et al., 1994; FORD, 1997; FARMER and ROBERTS, 2003). In Mangalica, an indigenous swine breed from Hungary (for review see EGERSZEGI et al., 2003a), the average litter-size is 5 - 7 piglets only. Although this breed was the most common of the country till the end of the Second World War, it nearly disappeared due to altered consumer requests and low reproductive performance. Nowadays however, there is revived interest on Mangalica, its stock is increasing continuously and numerous special meat products are in demand on the market. For the propagation of this breed special knowledge about its behavior, physiology and reproduction is requested. Recently there are some results on follicular, oocyte and early embryonic development, and on sexual hormones pattern during the estrous cycle in Mangalica (RÁTKY et al., 2001, EGERSZEGI et al., 2001, EGERSZEGI et al, 2003a,b). Organometric data of the reproductive tract are missing in Mangalica except the report of BULATOVICI (1932), who analyzed ovarian features and the number of fetuses of Mangalica sows at slaughter. Since the morphology of the reproductive organs could be a wherefore of reproductive performance of Mangalica, this study was conducted to get some more information about the number of corpora lutea and the size of reproductive tract both in cycling and pregnant Mangalica pigs.

Materials and Methods

Two experiments were carried out to investigate the reproductive tract of female Mangalica pigs. In Experiment 1, reproductive organs of 66 gilts (age: 12 – 15 month, mean carcass weight: 117 ± 9 kg) 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. Ovarian features (corpora lutea, corpora albicans, follicles) were counted and evaluated as described by SCHNURRBUSCH et al. (1981). Ovaries were cut and weighed. The oviducts were trimmed free from bursa ovarica and mesovarium, weighed and their length was measured using a ruler. Uterine horns were cut off at the bifurcation and the weight of each horn was recorded. Thereafter the length of uterine horns was measured. Measurement was done by placing one end of a cord at the utero-tubal junction and threading the cord along the anti-mesometrial border up to the uterine body. The length of the uterine horn was calculated placing the cord on a tape measure.

In Experiment 2, altogether 22 puberal Mangalica (M) gilts (12-13 month of age, 110-115 kg) and 34 puberal German Landrace (L) gilts (8.5 month of age, 120-130 kg) were used. The estrous cycle of the gilts was synchronized by 15 days long Regumate® feeding (16 mg altrenogest / day / animal; Serumwerk Bernburg, Germany). Follicular growth was stimulated by 1,000 IU PMSG (Folligon, Intervet) on day 16 and ovulation was induced by 750 IU hCG (Choriogonin, Richter Gedeon) on day 19 of the treatment. All animals were inseminated twice 24 and 36 hours after the hCG application. Gilts underwent laparatomy under ketamin/xylazin anaesthesia on day 1 (n = 8 M and 10 L), on day 12 (n = 8 M and 22 L) and on day 24 (n = 6 M and 2 L) of pregnancy, respectively. The gravid uteri were identified and ovario-hysterectomy was made lege artis. After removing the reproductive tract, the ovaries were cut off, ovarian features, and the ovarian diameter and weight were determined. Thereafter oviducts were removed, prepared free from bursa ovarica and mesosalpinx, and oviduct weight and length were measured. Oviducts of gilts from day 1 of pregnancy were flushed with 20 ml PBS medium and the flushing was analyzed for the presence of embryos under stereo microscope. The weight of each uterine horn was recorded and thereafter from each uterine horn the mesometrium was trimmed. The uterine horn length was measured along the anti-mesometrial border using a tape measure. Uterine horns of gilts from d 12 of pregnancy were flushed with 50 ml PBS medium and flushing was analyzed for embryos. On day 24 respectively, the uterine horns were opened with scissors and the number of fetuses recorded.

Statistical analyses were done with the SAS System for Windows, release 8.02 (SAS Institute, 1999). Experiment 1 and 2 data were evaluated by analysis of variance (ANOVA) using the GLM procedure of SAS/STAT software in the SAS System. The analysis-of-variance model of the Experiment 1 data contained the fixed factors site of ovary (left, right) and cycling (first estrous gilt, cycling gilt) and their interactions. The ANOVA model for the traits in Experiment 2 included the fixed factors breed (Landrace, Mangalica) and day of pregnancy (days 1, 12, 24) and the breed*day of pregnancy interactions. In addition, least-squares means (LSMeans) for all levels of the factors and their interactions and the standard errors of the LSMeans (SE) were estimated and tested. Spearman rank-order correlation coefficients (r) were estimated and tested vs. 0 with the CORR procedure in SAS/BASE software of the SAS System.

The results are presented as LSMeans ± SE. The correlation coefficients shown, are only significant ones (p = 0.05).

Results

In Experiment 1, 58 gilts (88 %) were recorded as cycling ones presenting corpora albicans. The mean number of ovulation of their previous estrous cycle was 11.7 ± 3.5.

The average ovulation number of the actual estrus was 10.6 ± 3.1. In first estrous gilts (n = 8) it was 11.1±2.5, respectively. Results of ovarian parameters recorded are presented in Table 1.

No differences were observed regarding the number of ovulation both between left and right ovaries, and first estrous and cycling gilts. As well ovarian diameter and weight were alike. There was a significant correlation between the number of corpora lutea and ovarian weight (r = 0.35).

Table 1
Number of ovulation, ovarian diameter and weight in slaughtered first estrous and cycling Mangalica gilts (LSMeans ± SE) (Anzahl Ovulationen, Ovardurchmesser und -gewicht bei erst-östrischen und zyklischen Mangalitza-Jungsauen)

Animals (n)	Number of CL * per ovary		Number of CA * per ovary		Ovarian diameter (cm)		Ovarian weight (g)	
	left	right	left	right	left	right	left	right
First estrous gilts (8)	6.0 ± 0.8	5.1 ± 0.8	-	-	3.4 ± 0.2	3.2 ± 0.2	8.4 ± 0.9	7.7 ± 1.0
Cycling gilt (58)	5.7 ± 0.3	4.8 ± 0.3	6.2 ± 0.4	5.6 ± 0.4	3.2 ± 0.1	3.1 ± 0.1	6.8 ± 0.3	6.3 ± 0.3

* CL- corpora lutea; CA – corpora albicans

Organometric data of the uterus in Mangalica gilts are shown in Table 2.

Table 2
Length and weight of oviducts, and uterine horns in slaughtered first estrus and cycling Mangalica gilts (LSMeans ± SE) (Länge und Gewicht der Eileiter und Uterushörner bei erst-östrischen und zyklischen Mangalitza-Jungsauen)

Animals (n)	Length of oviduct (cm)		Weight of oviduct (g)		Length of uterine horn (cm)		Weight of uterine horn (g)	
	left	right	left	right	left	right	left	right
First estrous gilts (8)	24.4 ± 1.4	23.6 ± 1.4	4.2 ± 0.4	4.3 ± 0.4	144 ± 13	142 ± 13	334 ± 32[a]	372 ± 32[c]
Cycling gilt (58)	24.3 ± 0.5	24.2 ± 0.5	3.2 ± 0.2	3.2 ± 0.2	144 ± 5	141 ± 5	253 ± 12[b]	247 ± 12[d]

[a:b] =0.09, [c:d] <0.05

No differences were observed between oviduct length and weight, and uterine length in first estrous and cycling gilts. However the uterine horn weight was always higher ($p = 0.09$ and $p < 0.05$; left and right horn, respectively) in first estrous gilts. Generally, uterine weight was correlated with uterine length (r = 0.24).

In pregnant Mangalica (M) and Landrace (L) gilts no breed*day of pregnancy interaction regarding the number of ovulation was found. Such interaction was observed with relation to ovarian weight increasing in Mangalica during early pregnancy between day 1 and 12 ($p<0.01$) and differing on day 12 of pregnancy between Landrace and Mangalica sows ($p<0.05$; Table 3).

Table 3
Number of ovulation and ovarian weight in pregnant M and L gilts on different days (1, 12 and 24) of pregnancy (LSMeans ± SE) (Anzahl der Ovulationen und Ovargewichte bei Mangalitza- und Landrasse-Jungsauen an den Trächtigkeitstagen 1, 12 und 24)

Breed	Animals (n)	Day of pregnancy	Number of CL	Ovarian weight (g)
Mangalica	22	total	17.2 ± 1.2	21.6 ± 3.0
Landrace	34		18.6 ± 1.3	18.4 ± 2.7
Mangalica	8	1	16.4 ± 2.0	10.8 ± 4.2[A]
Landrace	10		18.2 ± 1.8	11.3 ± 3.8
Mangalica	8	12	13.4 ± 2.0	36.8 ±4.2[a,B]
Landrace	22		18.2 ± 1.2	21.1 ± 2.5[b]
Mangalica	6	24	21.8 ± 2.4	17.3 ± 6.9
Landrace	2		19.3 ± 3.3	22.7 ± 6.9

[A:B] $p<0.01$; [a:b] $p<0.05$

The mean oviduct length was 29.1 ± 0.8 cm in Landrace and 23.0 ± 1.2 cm in Mangalica sows ($p<0.01$). Oviduct weight was significantly increased in Landrace sows on day 1 of pregnancy compared to days 12 and 24 (8.8 ± 0.8 g vs. 4.5 ± 0.5 and 4.3 ± 1.4 g, $p<0.05$) but not in Mangalica (5.8 ± 0.9 vs. 3.5 ± 0.9). Landrace gilts (124 ± 5 vs. 188 ± 6 cm, $p<0.01$). Table 4 provides uterine length and weight parameters on different days of pregnancy.

Table 4
Uterine horn length and weight in pregnant M and L gilts, and on different days (1, 12 and 24) of pregnancy (LSMeans ± SE) (Uterushornlängen und -gewichte bei Mangalitza- und Landrasse-Jungsauen an den Trächtigkeitstagen 1, 12 und 24)

Breed	Animals (n)	Day of pregnancy	Total length of uterine horns (cm)	Total weight of uterine horns (g)
Mangalica	8	1	122 ± 8	360 ± 63[g]
Landrace	10		132 ± 7[a]	515 ± 57[e]
Mangalica	8	12	126 ± 9[A]	428 ± 63[A,h]
Landrace	22		165 ± 5[B,b]	861 ± 38[B,f]
Mangalica	6	24	123 ± 9[C]	1221 ± 73[h]
Landrace	2		268 ± 16[D,c]	928 ± 179

[A:B; C:D] between breeds $p<0.01$; [a:b:c; e:f; g:h] between days of pregnancy $p<0.01$

From this data is becomes evident that uterus grows in length continuously between day 1 and 24 of pregnancy in Landrace but not in Mangalica sows. Uterine weight as well increased earlier (day 1 to day 12) in Landrace in comparison to Mangalica (day 12 to day 24). The Figure presents the dynamic of these processes.

Discussion

In Hungarian Mangalica the average litter-size is 5 - 7 piglets only (EGERSZEGI et al., 2003a) and its physiological reason(s) are not fully brightened, yet. Already in 1932 BULATIVICI supposed that first the lower number of ovulation compared to other breeds (9.9 in Mangalica vs. 12.4 and 12.8 in Berkshire and Yorkshire sows) and respective lower number of matured oocytes ovulated are a wherefore. This supposition was supported by previous results (EGERSZEGI et al., 2001), where Mangalica gilts have shown both a smaller number of preovulatory follicles (6.8 ± 1.4 vs. 19.6 ± 6.6) and lower rate of oocytes with mature chromatin configuration (27 vs. 62 %) compared to Landrace gilts. Although follicular and oocyte development are involved, thereby the lower fecundity in Mangalica cannot be explained, only. Besides others, uterine development, uterine capacity and placental efficiency have a significant impact (WU et al., 1989; CHRISTENSON, 1993; FORD, 1997; VALLET and CHRISTENSON, 2004). Due to lacking information on organometric data of the reproductive tract in Mangalica, we aimed to obtain respective parameters of cycling and pregnant Mangalica gilts.

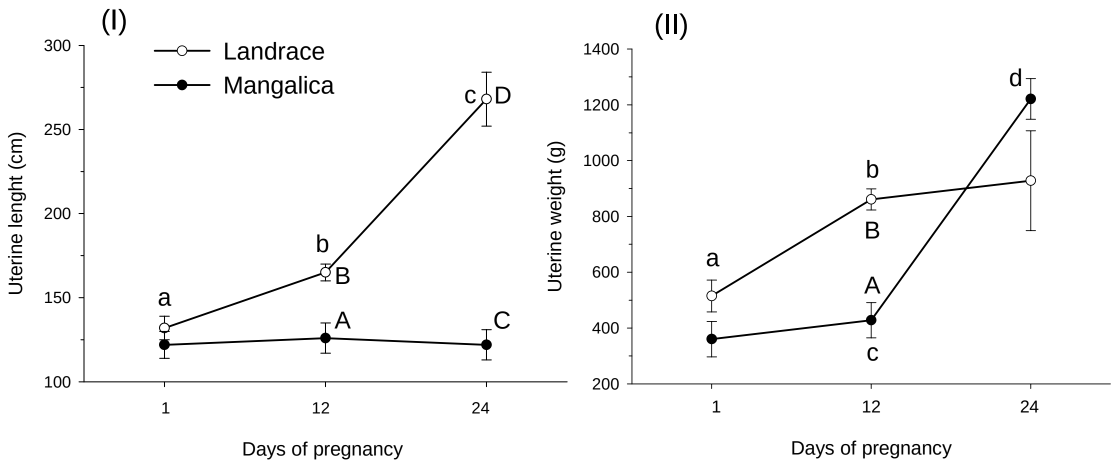

Fig.: Dynamic of uterine length (I) and uterine weight (II) development in Mangalica and Landrace gilts on days 1, 12 and 24 of pregnancy. (Data with different minuscules p<0.01 within breed; with different capital letters p<0.01 between breeds) (Dynamik der Uteruslängen- und Uterusgewichtsentwicklung bei Mangalitza- und Landrasse-Jungsauen an den Trächtigkeitstagen 1, 12 und 24)

In intensive breeding conditions Mangalica gilts attain puberty at the age of about 11 to 12 month (ENESEI DORNER, 1926; RÁCZ, 1932, GÁBOS, 1935). This was affirmed in Experiment 1 where 88 % of slaughtered gilts, which were at the age of 12 – 15 month, were cycling ones demonstrating corpora albicantia, and the remaining gilts were first estrous gilts. The mean number of corpora lutea (10.6 ± 3.1 and 11.7 ± 3.5) was comparable to the data reported by BULATOVICI (9.9; 1932) and RÁTKY and BRÜSSOW (11.5 ± 2.1; 1998). However, the number of corpora lutea is lower as in white crossbred or Meishan gilts (15.3 ± 0.4, 14.4 ± 0.6 and 15.1 ± 0.6; GAMA and JOHNSON, 1993; VALLET et al., 2003). On the other hand, the Mangalica ovaries have the biological potential to grow a higher number of ovulating follicles. Using 750, 1,000 and 1,250 IU PMSG as an additional gonadotropic source to stimulate follicular growth, the mean number of ovulated follicles was 13.7 ± 2.1, 24.2 ± 3.1 and 21.0 ± 2.9, respectively (RÁTKY and BRÜSSOW, 1998). Comparable results were observed in Experiment 2, where after stimulation with 1,000 IU PMSG the mean number of corpora lutea was 17.2 ± 1.2. However, not every time PMSG may stimulate a higher number of ovulation. EGERSZEGI et al. (2001, 2003b) found 6.8 ± 1.4 and 10.3 ± 1.5 corpora lutea following stimulation with 1,000 IU PMSG, which is in the range of non-stimulated ovaries. No differences were found always between left and right ovaries.

To our knowledge, no data were published on oviduct and uterine horn length and weight in Mangalica, yet. Oviduct length in Mangalica gilts varied between 23.6 ± 1.4 and 24.4 ± 1.4 cm, and is not different to those reported for Landrace gilts (25.0 ± 2.9, 23.5 ± 3.7 cm; BRÜSSOW, 1985; BRÜSSOW et al., 1987). RICKBY (1968) reported in Large White a mean oviduct length of 37.7 ± 8.4 cm. Oviduct weight did not differ between first estrous and cycling Mangalica gilts (4.2 ± 0.4 and 3.2 ± 0.2 g), and in pregnant gilts at day 1 and 12 of pregnancy (5.8 ± 0.9 and 3.5 ± 0.9 g). The slightly higher oviduct weight on day 1 in Mangalica and Landrace gilts (in Landrace $p<0.05$) could be due to increased ascitic and metabolic processes during fertilization.
In the pig up to 140th day of life, genital tract is growing in length and size with age and body weight increase. After the age of 140 days there are no considerable changes, however a drastic lengthening occur just prior to the first estrus (HADEK and GETTY, 1959; ERICES and SCHNURRBUSCH, 1979; DYCK and SWIERSTRA, 1983; BARTOL et al, 1993). WU and DZIUK (1995) measured at 150 days of age the length of one uterine horn to be 70 ± 14 cm. At day 10 after first estrus uterine horn length had increased to 141 ± 27 cm. RIGHBY (1968) found differences in uterine horn length between estrous (67 to 96 cm) and post-estrous sows (118 to 134 cm). In gilts selected for litter size the uterine horn length was between 150 and 157 cm (GAMA and JOHNSON, 1993). Similar uterine horn length was found in first estrous and cycling Mangalica gilts (143 ± 9 and 143 ± 3 cm, respectively). Thereby it has taken into account that in Experiment 1 all animal were almost at the luteal phase of the estrous cycle, i.e. post-estrous gilts.
Uterine horn weight of puberal Mangalica gilts (247 ± 12 to 372 ± 32 g) was always in the range reported of other breeds (white crossbreed gilts - 354 g, WU and DZUIK, 1995; Landrace gilts - 284 to 325 g; HEINZE et al., 1983; BERGFELD et al., 1990; WÄHNER, 2000).
The most surprising result of our experiments comes from data on uterine length and weight of pregnant Magalica in comparison to Landrace gilts. Overall, the length of each uterine horns was significantly shorter in Mangalica (124 ± 5 vs. 188 ± 6 cm). Furthermore, the uterus did not grow in length during early pregnancy (days 1 to 24), whereas in pregnant Landrace gilts uterus increased continuously. PERRY and ROWLANDS (1962) found in white breeds also an increase in uterine length throughout the first 18 days after mating. The elongation of the uterus was rapid within days 2 and 6 of pregnancy representing an increase of 50%, and the mean uterine length was 360 cm on day 18. Moreover, in our study uterine weight increased significantly later in Mangalica (day 12 to day 24) compared to Landrace (day 1 to day 12).
DAVIS et al. (1987) observed that on day 35 of pregnancy total uterine length was greater in Duroc than Yorkshire sows (411 vs. 375 cm) despite having fewer embryos (9.9 vs. 10.5). WU et al. (1989) argue that each fetus surviving to day 50 requires about 36 cm of initial uterine length. Considering the data of DAVIS et al. (1987), each Duroc and Yorkshire fetus occupies about 41.5 and 35.7 cm of uterine length. In Mangalica, fetuses had only 17.8 ± 2.7 cm of uterine horn length (5 to 10 fetuses per sow on day 24 of pregnancy; n = 5 sows).
Results of this study support the concept that in Mangalica besides 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. However, another experiments have to highlighted biological processes of early embryo/fetal development of this native pig breed.

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