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ULTRASONOGRAPHIC ASSESSMENT OF EARLY PREGNANCY IN ANIMALS

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Summary

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The study was performed on 22 cows, 23 mares, 97 sows and 19 bitches. The animals were divided in groups: the cows to multiparous and heifers, the other species – according to the breed. The possibilities for assessment of the early pregnancy through some parameters using the ultrasonic unit Aloka-SSD 500 with 5 MHz linear transducer (probe) in accordance with the animal species were studied. The last insemination was used as a criterion for the beginning of pregnancy. The following parameters were described: uterine horn diameter, chorionic cavity diameter, dimensions of the embryo, uterine lumen diameter and gravid corpus luteum diameter. The data showed that the average chorionic cavity and gravid corpus luteum diameters in cows at gestation days 28, 35 and 40 were significantly bigger than those in heifers ($p<0.001$). In mares, the ultrasonography showed a precision in the detection of pregnancy of 85.96% between gestation days 20–25; 94.74% between gestation days 30-35 and 100% between gestation days 40–50. In sows, the average uterine lumen diameter at gestation days 28 and 35, and the dimensions of the embryo at gestation day 35 were bigger in the Landrace breed compared to the Big Danubian White breed ($p<0.001$). In large canine breeds, the uterine lumen diameter at gestation days 20, 25 and 30 and the dimensions of the embryo at gestation day 35 were statistically significantly bigger than in small breeds.

Key words: bitches, cows, mares, pregnancy, sows, ultrasonography

INTRODUCTION

Ultrasound diagnostics of pregnancy has several advantages to the other routine methods: it coud be used in the early stages of pregnancy, the technique is non-invasive and harmless for both the patient and the operator, it does not require a special environment (Ginther and Person, 1983; Valocky et al., 1997a). At present, the ultrasonography is a very useful method for early and reliable detection of pregnancy in all animal species (Gerwing, 1996). For the purposes of routine diagnostics, the 5 MHz transducer is suitable for the detailed assessment of all structures at a 15-cm distance. The small portable units are appropriate for herd diagnostics in farm stables or even at the pastures (Gerwing, 1996; Grunert and De Kruif, 1999).

The diagnostics of pregnancy in cows is possible prior to the 20th day after the insemination, when the chorionic sac is visualized (most early between days 12 and 18). The different studies showed that practically, the reliable differentiation of pregnant and non-pregnant cows is possi-

ble after the post insemination days 24–26 with sensitivity of 95-97% and specificity of 75-79% (Piatrese et al., 1990; Kähn, 1991; Szenci et al., 1995; Grunert and De Kruif, 1999).

The studies of some authors (Palmer and Draincourt, 1980; Squires et al., 1983) upon the efficacy of ultrasonography for detection of pregnancy in mares after the 15th day of the ovulation with a 3 MHz transducer showed results with exceptional accuracy (over 97.4%). Later, McKinnon and Squires (1988) obtained a more precise information about the early development of the embryo with a 5MHz probe.

The most important things in the ultrasonographic assessment of swine is the tranquility of animals and thus, the evaluation is advised to be performed after or during the morning feeding. Furthermore it is necessary to ensure a good contact of the transducer with the soft abdominal wall.

At gestation age of 23-25 days in bitches, apart the gestation sacs, the uterine lumen and the chorionic cavity, the movements of the embryo and the heartbeat could also be distinguished. The fetal skeleton is imaged about days 35–39 (Valocky et al., 1997a) and is the most clearly seen by days 40–42 (Concannon, 2000).

Despite the numerous studies, the problem of establishment of precise criteria for early detection of pregnancy is still open.

The aim of the present study was to investigate the possibility of early detection of pregnancy in different animal species through specific parameters – using ultrasonography in heifers and multiparous cows, in mares, to determine the earliest period for detection of pregnancy in different porcine breeds and to determine criteria for detection of early pregnancy in small and large canine breeds.

MATERIALS AND METHODS

The echographic studies were performed in pregnant animals: 22 cows, 23 mares, 97 sows and 19 bitches.

The cows were from the Black and White breed, artificially inseminated and divided into 2 groups: multiparous (n=13) and heifers (n=9). They were examined by ultrasonography everyday between days 14 and 27 after the insemination, detecting the first appearance of the embryonic vesicle and the embryo. At days 28, 35 and 45 after the insemination, the heartbeat of the embryo was detected. At gestation days 28, 35 and 45, morphometric measurements for determination of the dimensions of the embryo, chorionic cavity diameter (CD) and the gravid corpus luteum diameter (GCLD) were performed. The parameters were compared between both groups. The percentage of negative findings by day 28 and afterwards in cases of pregnancy with detected heartbeat was recorded. Ninety days after the artificial insemination all cows were submitted to rectal examinaiton in order to confirm or reject the pregnancy.

The mares were aged between 4 and 9 years and were divided into 2 groups according to the breed: a group of the Hannoverian breed (n=15) and a group of the Haflinger breed (n=8). They were scanned three times in the periods between days 20-25, 30-35 and 45-50 as well as examined rectally by day 90 after the last fertilization. During the ultrasonography, the dimensions of the embryo, the CD and the uterine horn diameter (UHD) were determined.

The sows were divided into two breed groups: Landrace sows (n=48) and Big Danubian White sows (n=49). They were

scanned at days 21, 28 and 35 after the artificial insemination. The dimensions of the embryo and the uterine lumen diameter (ULD) were determined. The measured parameters were compared between groups.

The bitches were divided according to the size of the breed: a group of females from large breeds, weighing over 15 kg (n=11) and a groups of bitches from small breeds (<15 kg, n=8). The first day after the last insemination was accepted as onset of the pregnancy. All bitches were submitted to ultrasonography between days 20 and 30. The values of ULD and CD were determined at gestation days 20, 25 and 30 whereas the dimensions of the embryo - at gestation days 25 and 30.

All scans were done using a portable ultrasonic unit Aloka SSD–500 with 5 MHz frequency linear transducer.

The data were statistically processed according to Student's t-test.

RESULTS AND DISCUSSION

Cows

In the animals from the multiparous group, the embryonic vesicle was seen 18.77 ± 0.35 days, while in the heifer group – 18.11 ± 0.56 days after the insemination. The first detection of the embryo occurred after 25.55 ± 0.34 and 25.13 ± 0.30 days, respectively but the difference between groups was not statistically significant. The heartbeat determination tests revealed that the rpegnancy was evident by day 28 in all animals from both groups. Our data corresponded with those, obtained by a 5 MHz frequency transducer, reported in the literature, i.e. that the earliest demonstration of the embryo could be performed after days 22–26. The heartbeat of the embryo could be perceived between days 25–30 and after the 28th day; the embryo could be clearly seen along with the amniotic vesicle (Peters, 1996; Grunert and De Kruif, 1999).

The results from the diagnostics of pregnancy in cows and heifers at various gestation ages up to day 90 after the artifiial insemination are presented in Table 1 and on Figs. 1, 2, 3 and 4. They showed that by day 28, pregnancy was proved in all animals. In 2 multiparous cows and one heifer, the pregnancy detected by this interval was not confirmed thereafter. Most probably, it was due to an occult abortion. This assumption corresponds to data reported by Chaffaux et al. (1986), Hanzen and Laurent (1991), Peters (1996) that the pregnancy discovered between

Table 1. Data from the early diagnostics of gestation in cattle

Gestation age	Findings	Group I (multiparous cows)		Group II (heifers)		Total	
		n	%	n	%	n	%
28 days (Aloka SSD 500)	Positive	13	100	9	100	22	100
	Negative	-	-	-	-	-	-
35 days (Aloka SSD 500)	Positive	11	81.82	8	77.5	19	84.22
	Negative	2	18.18	1	12.5	3	15.78
45 days (Aloka SSD 500)	Positive	11	81.82	8	77.5	19	84.22
	Negative	2	18.18	1	12.5	3	15.78
90 days (rectal examination)	Positive	11	100	8	100	19	100
	Negative	-	-	-	-	-	-

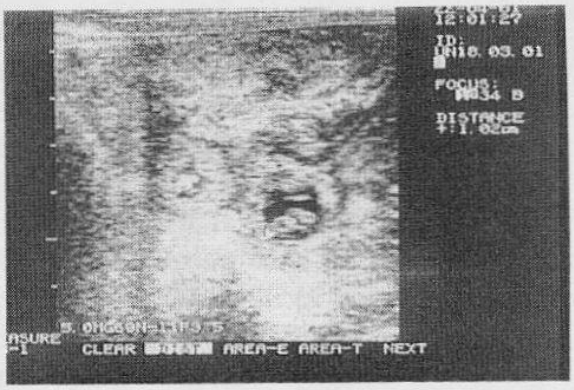

Fig. 1. Gestation in a heifer; 28th day – dimensions of the embryo.

Fig. 2. Gestation in a heifer; 35th day – the diameter of the gravid corpus luteum is shown.

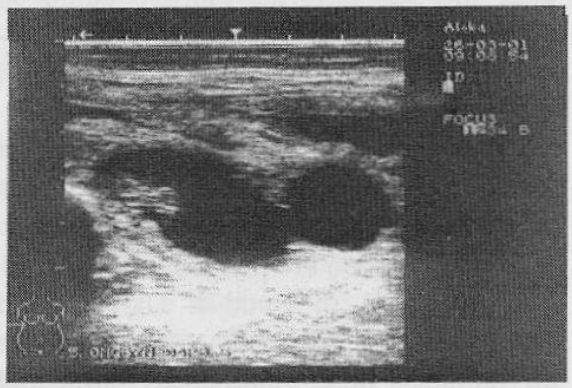

Fig. 3. Gestation in a heifer; 35th day – segments from the cross-section of uterine horns with the embryo.

Fig. 4. Gestation in a heifer; 45th day, dorsolateral view. The dimension of the embryo is 2.14 cm.

days 20–42 after the insemination could not be confirmed later in 12-16% of animals because of the embryonal death. By means of transrectal ultrasonography, the early diagnostics of the late embryonal or the early fetal death is possible via the detection of lack of heartbeat, few amount of allantoic fluid, lack of amniotic fluid, desintegration of fetal membranes (Chaffaux et al., 1986; Hanzen and Laurent, 1991; Peters, 1996). Because of the larger intervals of our study and the relatively early period, we did not succeeded to determine the moment of embryonic death occurrence.

The biometric indexes – dimensions of the embryo, CD and GCLD at gestation days 28, 35 and 45 in cows revealed that they were significantly different between groups ($p<0.001$) (Table 2). This could be explained by the presence of permanent changes in the birth tract of multiparous cows due to previous gestations, parturitions etc.

Mares

The examination of 23 mares for pregnancy, made within the period of days 20–25 after the fertilization resulted in positive diagnosis in 12 Hannoverian and 8 Haflinger mares and negative diagnosis in 3 Hannoverian mares (Table 3). During that period, the embryo was within the embryonic vesicle, most oftenly in its ventral part (Fig. 5). After the 22nd day,

Table 2. Dimensions of the embryo, chorionic cavity diameter and gravid corpus luteum diameter in multiparous cows and heifers during the different periods of gestation (mean ± SEM)

Gestation age	Parameter	Group I (multiparous cows)	Group II (heifers)
28 days		n = 13	n = 9
	Dimensions of the embryo, mm	6.30 ± 0.06	6.35 ± 0.14
	Chorionic cavity diameter, mm	15.31 ± 0.07	14.9* ± 0.06
	Gravid corpus luteum diameter, mm	30.67 ± 0.54	26.31* ± 0.58
35 days		n = 11	n = 8
	Dimensions of the embryo, mm	11.01 ± 0.14	10.92 ± 0.17
	Chorionic cavity diameter, mm	26.22 ± 0.06	25.98* ± 0.04
	Gravid corpus luteum diameter, mm	30.49 ± 0.58	26.22* ± 0.57
45 days		n = 11	n = 8
	Dimensions of the embryo, mm	25.00 ± 0.09	25.12 ± 0.17
	Chorionic cavity diameter, mm	42.33 ± 0.05	41.90* ± 0.04
	Gravid corpus luteum diameter, mm	30.35 ± 0.55	26.11* ± 0.57

* $p<0.001$ vs group I.

Table 3. Percentage of positive and negative findings in mares, examined for being pregnant with Aloka SSD 500

Gestation age	Findings	Group I (Hannoverian breed)		Group II (Haflinger breed)		Total	
		n	%	n	%	n	%
20–25 days	Positive	12	80.00	8	100	20	85.96
	Negative	3	20.00	-	-	3	14.04
30–35 days	Positive	11	91.67	8	100	19	94.74
	False positive	1	8.33	-	-	1	5.26
40–45 days	Positive	11	100	8	100	19	100
	Negative	-	-	-	-	-	-
90 days	Positive	11	100	8	100	19	100
	Negative	-	-	-	-	-	-

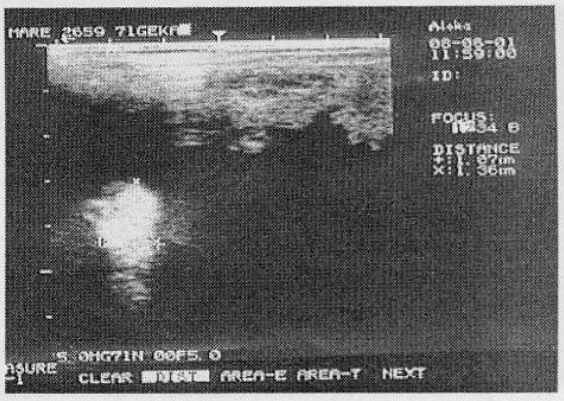

Fig. 5. Gestation in a mare; 20th day – the dimension of the embryo is 1.36 cm.

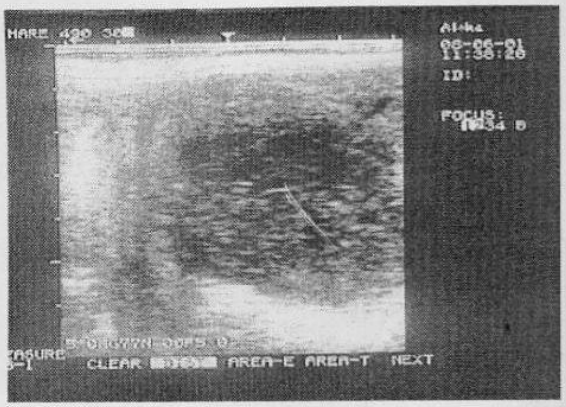

Fig. 6. Gestation in a mare; 30th day – gravid corpus luteum.

Table 4. Dimensions of the embryo, chorionic cavity diameter and uterine horn diameter in pregnant mares (mean ± SEM)

Gestation age	Parameter	Group I (Hannoverian breed)	Group II (Haflinger breed)
20–25 days		n = 12	n = 8
	Days	22.10 ± 0.62	22.90 ± 0.67
	Dimensions of the embryo, cm	1.83 ± 0.05	1.92 ± 0.04
	Chorionic cavity diameter, cm	2.90 ± 0.06	3.04 ± 0.05
	Uterine horn diameter, cm	3.97 ± 0.05	4.12* ± 0.05
30–35 days		n = 11	n = 8
	Days	33.0 ± 0.60	32.90 ± 0.67
	Dimensions of the embryo, cm	2.17 ± 0.08	2.17 ± 0.04
	Chorionic cavity diameter, cm	4.00 ± 0.04	4.38** ± 0.08
	Uterine horn diameter, cm	5.20 ± 0.06	5.80** ± 0.08
40–45 days		n = 11	n = 8
	Days	48.00 ± 0.60	47.90 ± 0.67
	Dimensions of the embryo, cm	3.35 ± 0.09	3.46 ± 0.05
	Chorionic cavity diameter, cm	7.92 ± 0.07	8.46** ± 0.09
	Uterine horn diameter, cm	9.28 ± 0.11	10.41** ± 0.20

* $p<0.05$; ** $p<0.001$.

the heartbeat – a primary finding in the diagnostics of the embryonal development, could be perceived. The second scan, made within the 30th and the 35th gestation days showed that 11 Hannoverian and 8 Haflinger mares were pregnant (Fig. 6). An animal from the first group was determined as being non-pregnant and the data of its first scan was a false positive finding, most probably due to the early embryonal death. Similar facts are reported by other investigators (Villahos et al., 1985; Ball and Woods, 1987) too. The third scan and the subsequent rectal examination at gestation day 90 confirmed the pregnancy in the ani-

mals from both groups, determined during the previous scan (Fig. 7). The data for the precision of diagnostics for pregnancy for all three periods were 85.96%, 94.74% and 100%, respectively. Our results are similar to those reported by Giovanni (1997), McKinnon et al. (1988) obtained with a 5MHz transducer but differ from the observations of Squires et al. (1983) using a 3.5 MHz transducer.

According to Ginther and Pierson (1983, 1984) and Ginther (1986), the ultrasonic detection of equine pregnancy between gestation days 10–12 requires 5 or 7.5 MHz transducers, but from a practical point of view, the first scan is advised between the 18th and 20th days after the ovulation.The 5 MHz transducer is recommended for the determination of the shape and dimensions of the embryo.

The morphometry performed during the three scans showed that the dimensions of the embryo between groups were not significantly different: 1.83 ± 0.05 cm; 2.17 ± 0.08 cm and 3.35 ± 0.09 cm for the Hannoverian breed and 1.92 ± 0.04 cm, 2.17 ± 0.04 cm and 3.46 ± 0.05 cm in the Haflinger breed respectively (Table 4). The uterine horn diameter and CD were statistically different between groups: UHD during the three examined periods and CD during the last two periods.

Sows

Amniotic fluid was detected by day 21 (Fig. 8) and the embryo – by day 28 after the last insemination (Fig. 9) in 64% of sows. This percentage was 100% by day 35 (Fig. 10). According to Almoond and Dial (1987), the ultrasonic detection of pregnancy in sows is generally possible prior to the 16th day after the last insemination. The predominating opinions however state that the confident and precise differentiation of pregnant and non-pregnant animals is possible after the 21st day with a 5MHz transducer with a sensitivity of 96–98% and specificity of 80-97% (Bosc et al., 1975; Inaba et al., 1983). Our data also confirm those reports. The morphometry showed usually higher values of the studied parameters in Landrace embryos (Table 5). The differences in ULD at gestation days 28 and 35 and in embryo dimensions at gestation day 35 were statistically significant (Fig. 11).

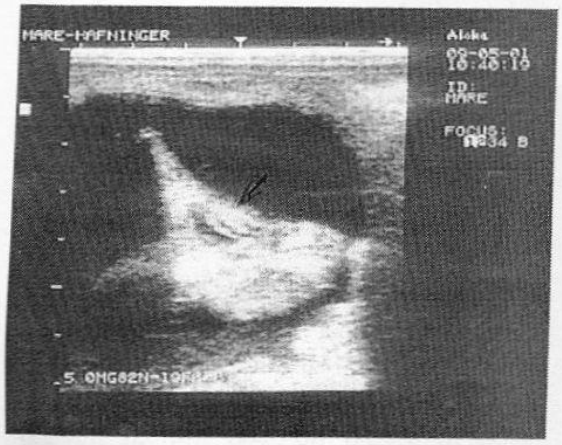

Fig. 7. Gestation in a mare; 46th day – ultrasonic appearance of the embryo.

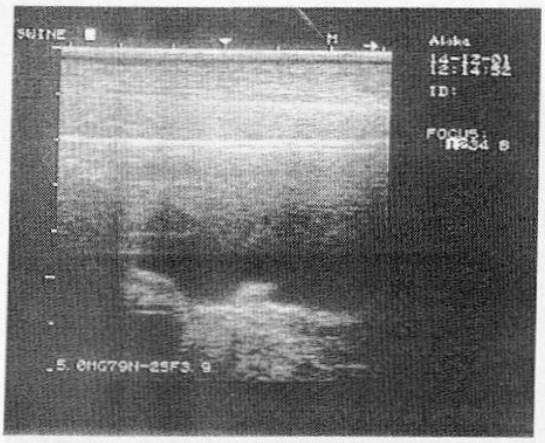

Fig. 8. Gestation in a Big Danubian White sow; 21th day – amniotic fluid.

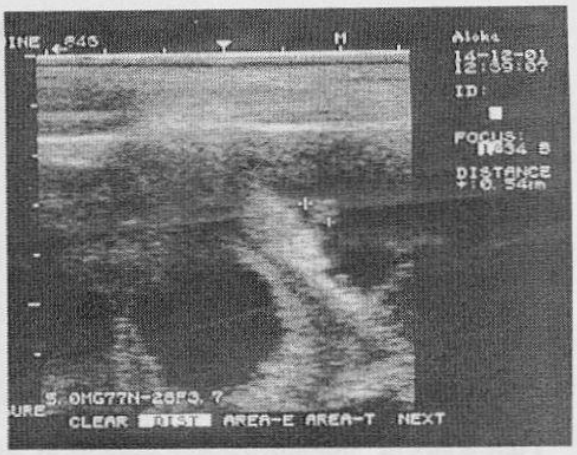

Fig. 9. Gestation in a Big Danubian White sow; 28th day – cross-section of uterine horns and dimension of the embryo.

Fig. 10. Gestation in a Landrace sow; 28th day – cross-section of uterine horns and dimension of the embryo.

Table 5. Uterine lumen diameter and dimensions of the embryo in swine (mean ± SEM)

Gestation age	Parameter	Group I (Landrace)	Group II (Big Danubian White)
		n = 48	n = 49
28 days	Uterine lumen diameter, cm	5.17 ± 0.05	4.92* ± 0.05
	Dimensions of the embryo, cm	0.98 ± 0.02	0.99 ± 0.02
35 days	Uterine lumen diameter, cm	5.72 ± 0.05	5.40*** ±0.06
	Dimensions of the embryo, cm	1.67 ± 0.01	1.51** ± 0.01

* p<0.05; ** p<0.01; *** p<0.001.

Bitches

The earliest period for detection of an embryo was by day 22 in large breed females and day 23 for small breed bitches (Fig. 12).The data of morphometry are presented in Table 6. The values of ULD were significantly higher in the large breed group at gestation days 20 (p<0.01), 25 (p<0.05) and 30 (p<0.001). The CD values were statistically different at days 25 and 30 (p<0.001). The dimensions of the embryo (Fig. 13 and 14) at days 25 and 30 were 10.99 ± 0.88 mm and 14.09 ± 0.76 mm for the large breed group and 8.05 ± 0.28 mm and 10.63 ± 0.40 mm in the small breed group, respectively (p<0.001). In dogs, 17–18 days after the ovulation, the gestation sacs are visualized in a row, as anechoic vesicles (Gary, 1998). Valocky et al. (1997b) reported ULD vales of 11 mm at gestation day 18 in large canine breeds and 10 mm at day 20 in small breeds. The respective values of CD were 3 mm in large breeds and 2 mm in small ones. According to Concannon (2000), ULD were about 10 mm at gestation day 24 and 30mm at gestation day 30; the embryo increased by 3–6 mm at the 20th day. Gary (1988) however states that 22 days after the LH peak the embryo is detected as a homogenous echogenic

Table 6. Dimensions of the embryo, chorionic cavity diameter and uterine lumen diameter in pregnant bitches (mean ± SEM)

Gestation age	Parameter	Group I (large breeds)	Group II (small breeds)
		n = 11	n = 8
20 days	Uterine lumen diameter, mm	12.03 ± 0.35	10.50** ± 0.21
	Chorionic cavity diameter, mm	2.46 ± 0.21	2.26 ± 0.09
25 days	Uterine lumen diameter, mm	18.36 ± 0.27	17.38* ± 0.21
	Chorionic cavity diameter, mm	16.52 ± 0.18	13.66*** ± 0.18
	Dimensions of the embryo, mm	10.99 ± 0.27	8.05*** ± 0.28
30 days	Uterine lumen diameter, mm	29.84 ± 0.48	25.29*** ± 0.37
	Chorionic cavity diameter, mm	21.42 ± 0.38	16.19*** ± 0.44
	Dimensions of the embryo, mm	14.09 ± 0.23	10.63*** ± 0.40

* p<0.05; ** p<0.01; *** p<0.001.

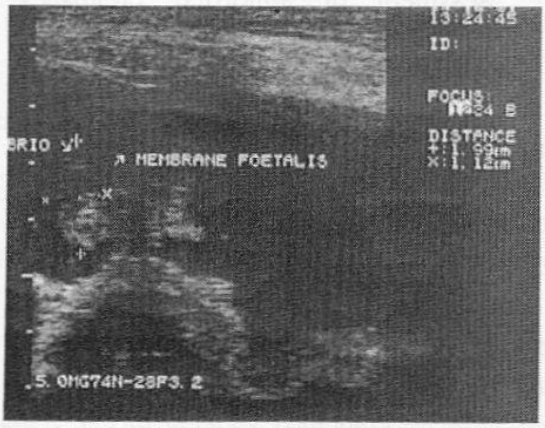

Fig. 11. Gestation in a Landrace sow; 35th day – segments of the cross-section of uterine horns and dimension of the embryo.

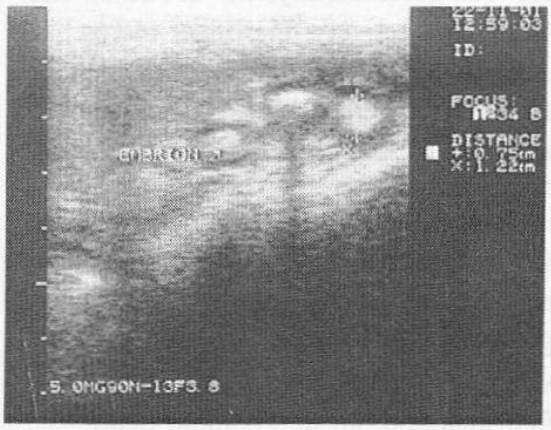

Fig. 12. Gestation in a bitch; 23th day – gestation sacs in a row, the uterine lumen and embryo are shown.

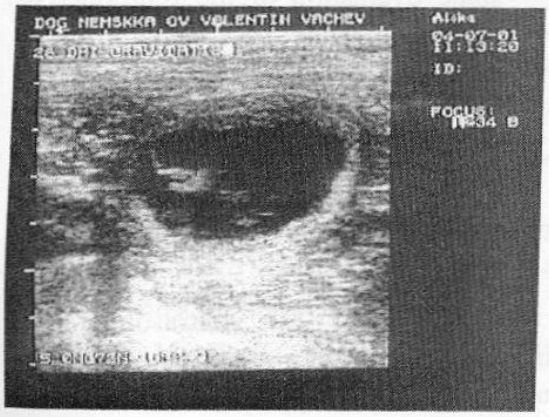

Fig. 13. Gestation in a bitch; 26th day. Uterine lumen and embryo - transversal cross-section, lateral view.

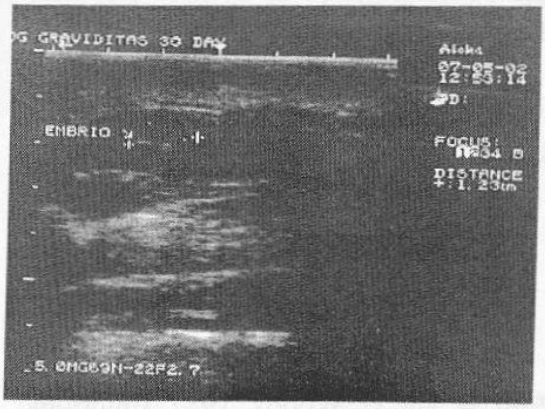

Fig. 14. Gestation in a bitch; 30th day. Uterine lumen and embryo – longitudinal cross-section – 1.23 cm.

structure with a diameter of about 7 mm and length of 15 mm. Our results are closer to those of Concannon (2000).

CONCLUSIONS

The chorionic cavity and gravid corpus luteum diameters in multiparous cows between gestation days 28 and 40, assessed by the ultrasonic unit Aloka SSD-500 and a 5 MHz linear transducer, were significantly bigger than those in heifers ($p<0.001$).

The pregnancy in mares could be ultrasonographically detected with a precision of 85.96 %, 94.74 % and 100 % between gestation days 20–25, 30-35 and 40–50 respectively. Haflinger mares had larger uterine horn diameters by days 25 an 30–35 and chorionic cavity diameters at days 30–35 compared to the Hannoverian breed ($p<0.001$).

Within gestation days 28 and 35 in sows, the average uterine lumen diameter and the dimensions of the embryo at gestation day 35 were bigger in the Landrace breed compared to the Big Danubian White breed.

In large canine breeds, the uterine lumen diameter and the dimensions of the embryo were statistically significantly higher than in small breeds ($p<0.001$) and could be used as criteria for early detection of pregnancy (days 20–30).

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