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DETERMINATION OF FOETAL SEX IN BUFFALOES THROUGH A SINGLE SONOGRAPHIC EXAMINATION

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Summary

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The goal of the present study was to establish the possibility for determining the foetal sex in buffaloes through a single sonographic examination. The experiment included 39 female Bulgarian Murrah buffaloes raised and fed uniformly, inseminated by natural mating during the oestrus period. All animals were subjected to ultrasonographic examinations for pregnancy via transrectal approach. The accuracy of this method to determine the sex of foetuses was established. The foetal gestation age and position were found to play a key role for the precise diagnosis. The period between gestation weeks 10–13 was the most suitable for determining the foetal sex in buffaloes of the examined breed through a single sonography exam. It is recommended to perform the evaluation in frontal position of the foetus.

Key words: buffaloes, foetal sex, ultrasonography

INTRODUCTION

The development of genitalia in domestic mammals starts during the embryonic period with the migration of the primordial germ cells in the caudal area and the formation of the gonadal ridge – undifferentiated gonads (Dyce *et al.*, 2002). They are formed from the mesenchyme of the ventral abdominal wall, between the root of the tail, the hindlimbs, and the umbilical cord (Ziltschmann & Krölling, 1955). Cloacal folds, originating from the cloacal membrane, are divided into anal and urogenital parts, with the so-called genital tubercle occurring in their periphery (Noden & De Lahunta, 1985). These processes are the basis of determining the foetal sex during the embryonic development.

In animals, the primary and practically applicable method in this regard is ultrasonography (Curran & Ginther, 1989; Ali, 2004; Yotov *et al.,* 2008). The criteria for identifying male or female sex are the genital tubercles and rudiments of external genitalia. In males, the tubercles are localized directly behind the umbilical cord and appear as hyperechoic points. Female foetuses are identified by an echogenic formation (genital swelling) located in the tail area (Curran, 1992). According to Santos *et al.* (2005) tubercles are vital parameters and are prone to change. This could lead to wrong identification of male foetuses as female. Bürstel *et al.* (2002), accept that a foetus is male when the presence of a penis and scrotum is estab-

lished, and female, when there are rudimentary teats.

A number of attempts for ultrasonographic determination of the foetal sex have been performed in cows (Beal *et al.,* 1992), horses (Mari, 2002), and small ruminants (Sendag *et al.,* 2007; Freitas Neto *et al.,* 2010). According to the authors, the factors with the highest impact over the method's accuracy are the gestation age and position of the foetus, sex, the probe's frequency, as well as age- and breed-related peculiarities. Most results are based on multiple sonographic examinations in animals after oestrus and ovulation synchronization with a fixed insemination time.

The available data on foetal sex determination in buffaloes are insufficient and contradictory. There are isolated reports of experiments with 28 Italian buffaloes (Presicce *et al.,* 2001) and 12 Indian buffaloes, subjected to the Ovsynh protocol (Ali & Fahmy, 2008).

The goal of the present study was to establish the possibility of determining the foetal sex through a single ultrasonographic examination of female Bulgarian Murrah buffaloes after natural mating.

MATERIALS AND METHODS

The experiments were carried out with 39 female buffaloes at 4.5–9 years of age, weighing 480–500 kg, from the Bulgarian Murrah breed. The animals were kept under the same regime of feeding and caretaking. All buffaloes were inseminated by a male breeder during the natural oestrus period.

A single ultrasonographic pregnancy examination was performed via transrectal approach and at that point non-pregnant buffaloes were excluded from the experiment. After retrospective analysis of the data after birth, foetal gestation age ranged between 31–138 days.

Ultrasonographic examination was carried out using Mindray DP-2200 Vet apparatus (Shenzhen, Mindray Biomedical Electronics, Co. Ltd. China) and a linear probe of 4/6 MHz frequency. Findings were documented using the apparatus's software package.

Sex identification was based on the visualization and location of genital tubercles or the external genital structures (vulva and scrotum), depending on the foetal gestation age.

The male sex was determined by the presence of an echogenic formation located near the umbilical cord, in front of the hindlimbs, or when a penis and a scrotum were observed. The female sex was determined by the visualization of an irregularly shaped echogenic formation behind the hindlimbs near the tail, or by rudimentary teats. The imaging results were compared to what became evident at birth.

The possibility to establish the sex of a foetus through ultrasonography was estimated as percentage of the number of foetuses with observed criteria for sex determination vs the total number of examined foetuses. The method's accuracy (percentage of correct diagnoses vs total number of diagnoses) was calculated as well.

The data were processed statistically with the Stat Soft software (Microsoft Corp. 1984-2000 Inc.). Non-parametric analysis was used to compare the relative values, based on Student's *t* criterion.

RESULTS

The sonographic examination of all 39 animals showed that 35 of them were pregnant (positive diagnosis) and 4 were not (negative diagnosis). While estimating the possibility for the used method to

identify the foetal sex, out of 35 buffaloes (100%) with positive diagnoses, criteria evidencing the sex were registered in 63% (22/35), whereas in the other 37% (13/25) this was impossible. Up to the 8th gestation week there was no clear identification of signs allowing a precise diagnosis. Precise criteria for ultrasonographic identification of the foetal sex were observed for the first time on the 68th day of gestation.

The highest number of foetuses with unidentified sex, 69% (9/13), was registered in the period between the 4th and 9th gestation week.

Out of 22 (100%) foetuses whose sex was determined by ultrasonography, 41% (9/22) were diagnosed as male and 59% (13/22) as female (Fig. 1 & 2). The data from the births confirmed 3 and 11 of diagnoses for male and female sex, respectively. A higher accuracy percentage of 85% (11/13) was registered when identifying the female foetuses, compared to 33% (3/9) for males.

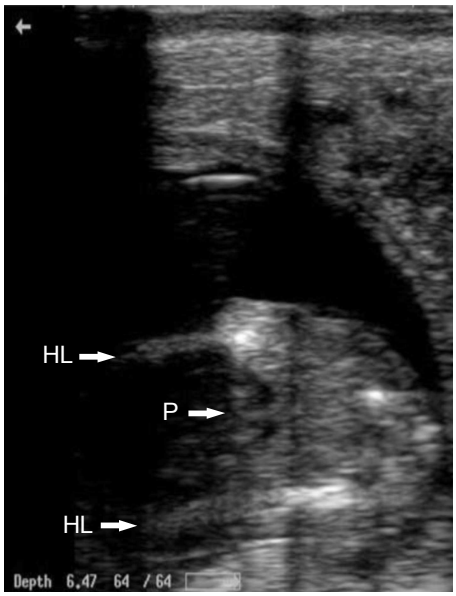

Fig. 1. Male foetus, 12th gestation week: HL – hindlimb; P – penis.

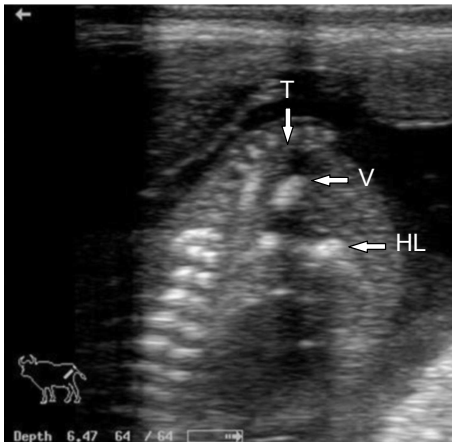

Fig. 2. Female foetus, 13th gestation week: HL – hindlimb; V – vulva; T – tail.

The overall accuracy of the method was estimated as 64% (14/22) (Table 1). The accuracy of 64% (9/14) measured in the period between the 10th and 13th gestation week was significantly ($P<0.05$) higher than those estimated in the 14th–16th week – 29% (4/14) and during the 9th gestation week – 7% (1/14).

Of all confirmed diagnoses, 71.4% (10/14) were made in frontal and 28.6% (4/14) in sagittal position of the foetus.

DISCUSSION

Foetal sex determination is one of the modern trends in reproduction science (Ali, 2004; Moraes *et al.,* 2009; Freitas Neto *et al.,* 2010), yet information about its application in buffaloes is insufficient. The present study could help improve reproductive management of these animals in Bulgaria.

The results showed that ultrasonographic pregnancy examination could start from the 4th week after insemination, as pointed out by Glatzel *et al.* (2000). The foetal sex could only be determined after the 9th week of pregnancy. These data do

Table 1. Results from the ultrasonographic examination, birth, and the method's accuracy depending on the foetal sex and position

	Diagnoses							
	Made by ultrasonography		Confirmed		Unconfirmed		Accuracy	
	n	%	n	%	n	%	n	%
Foetal sex								
Male	9	100	3	33	6	67	3/9	33
Female	13	100	11	85	2	15	11/13	85
Total	22	100	14	64	8	36	14/22	64
Position of the foetus								
Frontal	12	100	10	83	2	17	10/12	83
Cross-sectional	3	100	–	–	3	100	0/3	–
Sagittal	7	100	4	43	3	57	4/7	43
Total	22	100	14	64	8	36	14/22	64

not correspond to the findings of Presicce *et al.* (2001) for Mediterranean buffaloes. They reported a visualization of the genital tubercles as early as the 50th day. Similarly to our results, Ali & Fahmy (2008) registered a 100% possibility to determine the sex during the 10th gestation week.

The high percentage (6%) of foetuses with unidentified sex in the period between the 4th and 9th weeks is probably due to the absence or unclear visualization of the sex indicators and their locations. The differentiation of genital tubercles in buffalo foetuses begins between the 51st and 60th day, and the external genitalia (scrotum and vulva) are formed after the 10th week of pregnancy, whereas the ossification of the skeleton takes place between the 8th and 10th week (Abdel-Raouf & El-Naggar, 1970).

The difference in the accuracy of determining of the male and female foetal sex could be explained with the clearer visualization and location of the indices for identifying female foetuses and the absence of foetal structures in the scanned area, which could impede the examination. In males, the ultrasonographic image of the limbs or the umbilical cord is sometimes overlaid on the genital tubercle image, which could lead to diagnostic error. Such problems have been encountered in sheep by Santos *et al.* (2005).

The overall accuracy in this report (64%) was different from what was established (97.1%) in other studies (Ali & Fahmy, 2008). Unlike the present experiment, they performed serial ultrasonographic examinations on the same animals with synchronized oestrus and ovulation. This is the cause for the higher accuracy, yet, in practice, multiple examinations are difficult to perform and economically unjustified.

The most accurate (64%) determination of sex in buffaloes could be achieved when the foetus was between the 10th and 13th week of gestation. At earlier stages of pregnancy, the image is harder to interpret, due to the poorer visualization of the ultrasonographic criteria. After this period, the foetus's growth and the change of its location cause a reduction in accuracy. Ali & Fahmy (2008) also reported

such dependencies in Indian buffaloes.

Another factor having an influence on the method's accuracy is the foetus's position at the time of scanning (Presicce *et al.,* 2001). The present study indicates that the sex is identified more accurately (71.4 %) in frontal rather than in sagittal or cross-sectional position. These results are similar to the findings of Ali & Fahmy (2008), who achieved better results with frontal scanning of the foetus. The time span for examining the foetus in this position is very large and provides sufficient clues for proving the localization of genital tubercles, the penis or the vulva.

Scanning the foetus in the sagittal position can be used at a later stage, when there is a clearly observable scrotum or rudimentary teats. However, the dislocation of the pregnant uterus limits the possibilities for establishing these criteria.

In conclusion, it may be summarized that in female Bulgarian Murrah buffaloes, the period between the 10th and 13th gestation weeks is the most suitable for determining the sex of the foetus through a single ultrasonographic examination. In order to make a precise diagnosis, it is recommended for diagnostics to take place with the foetus in frontal position.

REFERENCES

Abdel-Raouf, M. & M. A. El-Naggar, 1970. Further study of the biometry and development of the Egyptian buffalo foetus. *United Arab Republic Journal of Veterinary Science,* **7**, 125–140.

Ali, A., 2004. Effect of gestational age and fetal position on the ultrasonographic fetal gender determination in dairy cattle. *Reproduction in Domestic Animals,* **39**, 190–194.

Ali, A. & S. Fahmy, 2008. Ultrasonographic fetometry and determination of fetal sex in buffaloes (*Bubalus bubalis*). *Animal Reproduction Science*, **106**, 90–99.

Beal, W. E., R. C. Perry & L. R. Corah, 1992. The use of ultrasound in monitoring reproductive physiology of beef cattle. *Journal of Animal Science,* **70**, 924–929.

Bürstel, D., S. Meinecke-Tillmann & B. Meinecke, 2002. Ultrasonographic diagnosis of fetal sex in small ruminants bearing multiple fetuses. *The Veterinary Record*, **23**, 635–636.

Curran, S, 1992. Fetal sex determination in cattle and horses by ultrasonography. *Theriogenology,* **37**, 17–21.

Curran, S. & O. J. Ginther, 1989. Diagnosis of equine fetal sex by location of the genital tubercle. *Journal of Equine Veterinary Science*, **9**, 77–83.

Dyce, K. M., W.O. Sack & C. J. G. Wensing, 2002. Textbook of Veterinary Anatomy, 3rd edn, Elsevier Science, Philadelphia.

Freitas Neto L. M., M. H. B. Santos, C. R. Aguiar Filho Almeida-Irmão, J. M. Santos Junior, E. L. C. Caldas, P. F. Lima & M. A. L. Oliveira, 2010. Reliability of ultrasound for early sexing of goat fetuses derived from natural mating and from fresh, frozen and vitrified embryo transfer. *Reproduction, Fertility and Development*, **22**, 489–493.

Glatzel, P. S., A. Ali, M. Gilles & C. Fiedlak, 2000. Zur Feststellung der Frühträchtigkeit bei 30 Wasserbüffelfärsen. (*Bubalus bubalis*) durch die transrektale Palpation mit und ohne Ultrasonographie. *Tierärztliche Umschau*, **55**, 329–332.

Mari, G., C. Castagnetti & S. Belluzzi, 2002. Equine fetal sex determination using a single ultrasonic examination under farm conditions. *Theriogenology,* **58**, 1237–1243.

Moraes, E. P. B. X., L. M. Freitas Neto, C. R. Aguiar Filho, F. Q. G. Bezerra, M. H. B. Santos, J. P. Neves, P. F. Lima & M. A. L. Oliveira, 2009. Mortality determination and gender identification of conceptuses in pregnancies of Santa Ines ovine by ultra-

sound. *South African Journal of Animal Science*, **39**, 307–312.

Presicce, G. A., G. De Santis, R. Stecco, E. Senatore, G. J. De Mauro & G. M. Terzano, 2001. Foetal gender determination by ultrasound in the Mediterranean Italian buffalo (*Bubalus bubalis*). *Theriogenology*, **55**, 532.

Santos, H., P. Moraes, D. Moura, P. F. Lima, H-D. Reichenbach & M. A. L. Oliveira, 2005. Early identificationof the fetal sex in small ruminants by ultrasonography. *Acta Scientiae Veterinariae,* **32**, 131–134.

Sendag, S., H. Celik, I. Aydin, D. Dinc, A. Wehrend & M. Gurkan, 2007. Ultrasonic determination of fetal sex in ewes under field conditions by a single examination. *Tierärztlische Praxis,* **35**, 197–201.

Noden, D. M & A. De Lahunta, 1985. The embryology of domestic animals. In: *Developmental Mechanisms and Malformations.* Williams and Wilkins, Baltimore, pp. 330–342.

Yotov, S., A. Wehrend, N. Vassilev, P. Georgiev, S. Lottner, A. Antonov & A. Atanasov, 2008. Ultrasonographic fetal sex determination in sheep depending on gestational age and fetal position. *Reproduction of Domestic Animals*, **43** (Suppl. 1), Abstract, p. 35.

Zietschnmann, O. & O. Krölling, 1955. Lehrbuch der Entwicklungsgeschichte der Haustiere. 2. Aufl., Verlag Paul Parey, Berlin und Hamburg, 400–413.

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