

THE STUDY OF THE OVARIES' STATE IN LOCAL BULGARIAN EWE'S BREEDS BY REAL-TIME ULTRASONOGRAPHY**

D. Kacheva^{1*}, E. Kistanova¹, B. Georgiev¹, E. Rycheva², N. Metodiev², B. Grigorov², E. Kipriotis³

¹Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, 73 Tzarigradsko shosse, 1113 Sofia, Bulgaria

²Institute of Animal Science, 2232 Kostinbrod, Bulgaria

³Institute of Animal Science, Komotini, Greece

* corresponding author : dikacheva@hotmail.com

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Abstract: The aim of this study was the characterization of the ovaries' state in local Bulgarian ovine breeds at the moment of ovulation by real-time transrectal ultrasonography. It was observed 32 ewes of Bulgarian local breeds Karakachanska and West-Oldmountainous between 1,5 and 4 years old, housed under closely to their natural conditions. Ovaries were examined ultrasonically after estrus synchronization with the implantation of intravaginal sponges containing 45 mg of Cronolone over 14 days. The effect of age and breed on the variability of the ovary's size and preovulatory follicle's size was studied.

Key words: ovaries, ovulatory follicles, local sheep breeds, ultraconography

Introduction and literature review

According to the requirements of Amsterdam Protocol "Animal protection and welfare" on protecting and providing favourable conditions for animals in their breeding and performing experiments, adopted by the European Community, the ultrasonography is the most suitable method for investigations of both the female reproductive organs and a pregnancy course. This method is a faster and less stressful one for the animals in comparison to laparoscopy and laparotomy (*Schrick and Inskip, 1997*), what is underlined from some authors provided comparative analyse between these methods (*Haresign et al., 1995; Simoes et al., 2004; Slosarz et al., 2003*). Moreover, they point out the high correlation coefficients between results regarding the size of ovaries, the

number and size of the big follicles, corpora luteum and the early diagnostic of pregnancy obtained by different methods (laparoscopy, morphometry, ultrasonography). The same authors noted that the accuracy of ultrasonic method goes down (until 80%) by investigation of the small follicles (*Simoes et al., 2004*). Also an accuracy of ultrasonic measuring of these parameters enhances with the experience of the person taking the measurements (*Slosarz et al., 2003*).

Undoubtedly, that the ultrasonography is the congenial method for the study of the reproductive potency of the local animal breeds. The special attention to these animals is due to necessity to preserve the genetic resources and breed's diversity in the country. The finding the animals with the reproductive problems as rudimentary ovaries, cysts, pathological uterus, has a big importance for a practice. It gives the possibility for the timely veterinary help or for an early decommission of animals, what will increase the reproductive potency and will have a great economic impact on sheep breeding.

The ultrasonic investigations by *Ginther et al. (1995)*, *Slosarz et al. (2003)* and *Souza et al. (1997)* had shown the breeds differences as in a number and size of follicles as well as in a number and structure of corpora luteum. The accumulation of the database from different ovine breeds is very important and necessary for the precise estimation of the ultrasonic results about ewe's ovaries. It is no literature dates about ultrasonic investigations of ovaries in the Bulgarian local ewe's breeds like Karakachnaska and West-Oldmountainous.

The aim of this study was the characterization of the ovaries' state in Karakachanska and West-Oldmountainous ewe's breeds at the moment of ovulation by real-time transrectal ultrasonography.

Material and methods

Experiment was designed with 32 ewes of Bulgarian local breeds Karakachanska and West-Oldmountainous herd in the experimental base "Zlatusha" of Institute of Animal Science – Kostinbrod. All animals were between 1,5 and 4 years old, housed under closely to their nature conditions. The investigations carried out during the September. It is the most active breeding time for these breeds. For the examination during of the necessary stage of sexual cycle the ewes were subjected to estrus synchronization with the implantation of intravaginal sponges containing 45 mg of Cronolone over 14 days (Chrono-Gest® - INTERVET International B.V.) During the removal of the intravaginal sponges a stimulating dose of Folligon (500 units, INTERVET International B.V.) was injected intramuscularly. Ovaries were observed ultrasonically at the second day after injection.

For investigations the ultrasound device “PIA Medical SCANNER 100” with a 5 and 7,5 MHz linear probes was used. This device is suitable as for laboratory as for field conditions. It is harmless for both an operator and a “patient”. Ultrasound examinations were conducted through the rectum in standing position of sheep. First the *ampula recti* from faecal was clean, after that the ultrasound probe with gel was covered and inserted into the rectum. The use of high range of sound waves (7,5 MHz) gives the possibility to obtain the clear ultrasound images. The size of ovaries and preovulatory follicles was measured at the monitor screen. Moreover, the ultrasound images were recorded using a 3,5 floppy disk for further analysis (Fig. 1).

The effect of age and breed on the variability of the investigated parameters was studied. Basic analysis of obtained data was performed using the methods of mathematical statistics as one-way and regression analysis by STATISTICA Ver.6.0 of the Stat Soft Inc.

Results and discussion

The effect of the age on ovaries' and preovulatory follicles' size in both breeds are shown in Table 1. There aren't significant differences among these parameters as inside one age group as well as between age's groups during the investigated period from 1,5 to 4 years old.

Table 2 reflects the effect of the breed on the investigated parameters. In Karakachanska breed we observed that the right ovaries are significantly smaller than the lefts. There are tendency that preovulatory follicles from right ovaries are smaller than from the lefts, too, but this difference is not significant. In West-Oldmountainous breed aren't significantly differences between sizes of ovaries and preovulatory follicles from right and left sites. We can speak only about tendency that the right ovaries and right follicles are bigger than lefts one.

Table 1. Effect of the ewe's age on the size of ovaries and preovulatory follicles in the investigated breeds**Tabela 1. Uticaj uzrasta ovce na veličinu jajnika i preovulatornih folikula ispitivanih rasa**

Parametrs/ Parametri	n	Before 3 years old/malđe od 3 godine			n	Over 3 years old/starije od 3 godine			Significance/ Signifikantnost
		\bar{x}	Sx^-	Sd		\bar{x}	Sx^-	Sd	
Right ovary (RO)/Desni jajnika, sm	9	2,23	0,254	0,673	9	2,30	0,134	0,381	NS
Left ovary(LO)/Levi jajnika, sm	8	2,12	0,235	0,577	10	2,30	0,145	0,458	NS
Follicles from RO/Folikuli iz desnog j.,mm	9	7,3	0,63	1,91	9	7,3	0,49	1,21	NS
Follicles from LO/Folikuli iz levog j.,mm	5	7,3	0,44	0,88	6	6,9	0,66	1,32	NS

Legend: \bar{x} - average mean/prosečna srednja vrednost; Sx^- - standard error/standardna greška; Sd – standard deviation/standardno odstupanje; n- number of observation/broj opservacija; NS – not significant/nije signifikantno

Table 2. Effect of the breed of ewes on the size of ovaries and preovulatory follicles**Tabela 2. Uticaj rase ovaca na veličinu jajnika i preovulatornih folikula**

Parametrs/ Parametri	n	Karakachanska breed/ Karakačanska rasa			n	West- Oldmountainous breed/Zapadno staroplaninska rasa			Significance/ Signifikantnost
		\bar{x}	Sx^-	Sd		\bar{x}	Sx^-	Sd	
Right ovary (RO)/Desni jajnik, sm	9	1,95	0,200	0,635	9	2,39	0,137	0,434	NS
Left ovary (LO)/Levi jajnik, sm	9	2,54	↕ 0,167	0,528	9	2,05	0,106	0,335	P<0,05
Follicles from RO/Folikule iz desnog j., mm	8	6,2		0,49	1,39	10	8,1	0,46	1,47
Follicles from LO/Folikule iz levog j.,mm	5	7,6	0,43	1,41	6	7,1	0,46	1,03	NS

Legend: \bar{x} - average mean/prosečna srednja vrednost; Sx^- - standard error/standardna greška; Sd – standard deviation/standardno odstupanje; n- number of observation/broj opservacija; NS – not significant/nije signifikantno

We determined that the left ovaries of Karakachanska breed have a bigger size than left one from West-Oldmountainous breed ($P < 0.05$). The next parameter with significant difference ($P < 0.01$) between breeds is the size of the preovulatory follicles from right ovaries. Characteristic of Karakachanska breed is the wide range of variability of preovulatory follicles size with maximum 6.3 ± 0.49 mm. In the right ovaries of West-Oldmountainous breed is observed the compact distribution the follicles by size with maximum 8.1 ± 0.46 mm. The close correlation between ovary's and preovulatory follicle's size from right as well as from left sites in both groups was established. The correlation coefficients are high and significant (table 3)

Table 3. The correlation coefficients between sizes of ovaries and follicles
Tabela 3. Koeficijenti korelacije između veličine jajnika i folikula

	Correlation coefficient/Koeficijent korelacije - r		Significance/ Signifikantnost
	KKB	WOMB	
RO: RF	0,66	0,61	$P < 0,05$
LO: LF	0,77	0,68	$P < 0,05$

Legend: RO,LO – right and left ovaries/desni i levi jajnici; RF,LF –follicles from right and left ovaries/folikule iz desnih i levih jajnika, KKB – Karakachanska breed/Karakačanska rasa; WOMB - West Old-Mountainous breed/zapadno-Staroplaninska rasa

Our results showed that the preovulatory follicles in Karakachnska breed during the breeding season were from 6.2 ± 0.49 to 7.6 ± 0.43 mm an average, in West-Oldmountainous breed were from 0.71 ± 0.46 to 0.81 ± 0.46 mm. According to dates from authors the average size of the ovulatory follicles is following: in the Jezersko Solchavska – Romanov cross-breed is 5.52 ± 1.12 mm (*Slosarz et al., 2003*); in the Polypay breed - 5.9 ± 0.2 mm (*Ginther et al., 1995*); in the Finnish and Merino cross-breed it is 6.8 ± 0.5 mm (*Souza et al., 1997*); in the Finnish breed - 5.6 ± 0.2 mm, in the Western white faced breed it is 6.7 ± 0.2 mm (*Bartlewski et al., 2001*) and in the Merino del Pais breed it is 6.4 ± 0.3 mm (*Lopez-Sebastian et al., 1997*).

The size of the ovulatory follicles of the Karakachnska breed is comparable with the Finnish and Merino cross-breed, Merino del Pais and West white head breeds. The West-Oldmountainous breed has the biggest size of the ovulatory follicles between investigated breeds. As noticed some authors, the size of the ovulatory follicles is connected with the concentration of progesterone (*Ginther et al., 1995; Scaramuzzi and Downing, 1999*) and the ovulation rate (*Bartlewski et al., 1999; Campbell et al., 2003; Driancourt et al., 1991; Webb et al., 1989*). The Karakachnska and West-Oldmountainous are mono ovulated breeds.

Moreover, this parameter depends on breeding season. *Bartlewski et al. (1999a)* point out the average size of ovulatory follicles in Western white faced breed 6.7 mm during the mid-breeding season, but in transition to an anoestrus this size is not more 5 mm. Our results about the big size of preovulatory follicles could be explained with the fact that the ewes were observed at the most active time of their breeding season.

We found out that the number of preovulatory follicles was more in right ovaries than in left in both investigated breeds. By *Strmsnik et al. (2002)* and *Lopez-Sebastian et al. (1997)* there is an equal number of follicles in the right and left ovaries characteristic of Jezersko Solchavska – Romanov cross-breed and on Merino del Pais breed. Although the preovulatory follicles were equally distributed between the right and the left ovaries, significantly more corpora luteum appeared in the right ovaries of the pregnant animals (*Strmsnik et al., 2002*), what means that more follicles in the right ovaries ovulate (*Dickie et al., 1999*). Our results regarding more functional activity of right ovaries in local Bulgarian ovine breeds consist with these literature dates.

Conclusion

In the investigated group of animals, included ewes of age between 1.5-4-years old, we did not determine aged differences between sizes of ovaries, nor between sizes of ovulatory follicles. Although that the following breed's differences were established. Inside group Karakachanska breed was determined that the left ovaries of ewes are significantly bigger than the right one. By the same token the left ovaries of Karakachanska breed ewes are reliably bigger than these of the West-Oldmountainous breed, too. It wasn't significant difference between sizes of right ovaries in two breeds, but the size of ovulatory follicles from right ovaries of West-Oldmountainous ewes was significantly bigger than this of Karakachanska breed

The high positive correlation coefficients between the size of ovaries and the size of ovulatory follicles in the left and right ovaries characteristic of two breeds were determined.

Istraživanje stanja jajnika lokalnih bugarskih rasa ovaca korišćenjem ultrasonografije u realnom vremenu

D.Kacheva, E.Kistanova, B.Georgiev, E.Rycheva, N.Methodiev, B.Grigorov, E.Kipriotis

Rezime

Cilj ovog istraživanja je bio karakterizacija stanja jajnika lokalnih rasa ovaca u Bugarskoj u momentu ovulacije korišćenjem transrektalne ultrasonografije u realnom vremenu.

U ogled su bile uključene 32 ovce iz zapata bugarskih lokalnih rasa karakačanska i zapadno-staroplaninska u eksperimentalnoj stanici "Zlatusha" Instituta za stočarstvo - Kostinbrod. Sve životinje su bile uzrasta od 1,5 do 4 godine, držane u uslovima koji su veoma slični prirodnim uslovima. Istraživanje je izvedeno tokom meseca septembra. To je najaktivniji priplodni period za ove zapate. U cilju obavljanja neophodnih ispitivanja tokom seksualnog ciklusa ovce su podvrgnute sinhronizaciji estrusa i to korišćenjem intravaginalnih sundera koji su sadržavali 45 mg preparata Cronolone u periodu od 14 dana (Chrono-Gest® - INTERVET International B.V.) Prilikom odstranjivanja intravaginalnih sundera stimulativna doza preparata Folligon (500 jedinica, INTERVET International B.V.) je injektovana intramuskularno. Jajnici su posmatrani ultrazvučno drugog dana nakon injekcije.

Utvrđene su sledeće razlike između rasa. Unutar grupe ovaca karakačanske rase utvrđeno je da su levi jajnici bili signifikantno veći od desnih. Takođe, levi jajnici ovaca karakačanske rase su bili veći od onih zapadno-staroplaninske rase. Razlika između veličine desnih jajnika kod ovaca ob erase nije bila signifikantna, ali veličina ovulatoernih folikula iz desnih jajnika zapadno-staroplaninskih ovaca je bila signifikantno veća nego kod karakačanske rase.

Visoki pozitivni koeficijenti korelacije između veličine jajnika I veličine ovulatoernih folikula u levim i desnim jajnicima, kao karakteristika ob erase su utvrđeni.

References

- BARTLEWSKI PM, BEARD AP, RAWLINGS NC,(1999). An ultrasonographic study of luteal function in breeds of sheep with different ovulation rates. *Theriogenology* 52,115-130.
- BARTLEWSKI PM, BEARD AP, RAWLINGS NC., (1999a.) Ovarian function in ewes during the transition from breeding season to anoestrus. *Anim Reprod Sci.*57(1-2):51-66.
- BARTLEWSKI PM, BEARD AP, RAWLINGS NC.,(2001). Ultrasonographic study of the effects of the corpus luteum on antral follicular development in

unilaterally ovulating western white-faced ewes. *Anim Reprod Sci.* 65(3-4) 231-244.

CAMPBELL BK, BAIRD DT, SOUZA CJ, WEBB R., (2003). The FecB (Booroola) gene acts at the ovary: in vivo evidence. *Reproduction* 126(1):101-111.

DICKIE AM, PATERSON C, ANDERSON JLM, BOYD JS., (1999). Determination of corpora lutea numbers in Booroola Texel ewes using transrectal ultrasound. *Theriogenology* (51)1209-1224.

DRIANCOURT MA, WEBB R, FRY RC., (1991). Does follicular dominance occur in ewes? *J Reprod Fert* (93)63-70.

GINTHER OJ, KOT K, WILTBANK MC, (1995). Associations between emergence of follicular waves and fluctuations in FSH concentrations during the estrous cycle in ewes. *Theriogenology* (43)689-703.

HARESIGN W., WILLIAMS R.J., KHALID M., RODWAY R., (1995). Heart rate responses and plasma cortisol and b-endorphin concentrations in ewes subjected to laparoscopy and its associated handling procedures. *Animal Science* (61) 77-83.

LOPEZ-SEBASTIAN A, BULNES AG, MORENO JS, BRUNET AG, TOWNSEND EC, INSKEEP EK, (1997). Patterns of follicular development during the estrous cycle in monovular Merino del Pais ewes. *Anim Reprod Sci* (48) 279-91.

SCHRICK NF, INSKEEP EK. ,(1993) Determination of early pregnancy in ewes using transrectal ultrasonography. *Theriogenology* (40) 295-306.

SCARAMUZZI RJ, DOWNING JA, (1999). Effect of progesterone on the GnRH-induced secretion of estradiol and androstenedione from autotransplanted ovary of the anoestrus ewe. *J Reprod Fert* (116)127-32.

SIMÕES J., J. POTES , J. AZEVEDO , J. C. ALMEIDA , P. FONTES , G. BARIL AND R. MASCARENHAS, (2004). Morphometry of ovarian structures by transrectal ultrasonography in Serrana goats . *Animal Reproduction Science*, 85(3-4): 263-273.

SLOSARZ P., A. FRANKOWSKA, M. MISH. (2003) Transrectal ultrasonography in diagnosing the ovulation rate in sheep. *Animal Science Papers and Reports*, 21 (3), 183-189.

Souza CJH, Campbell BK, Baird DT, 1997. Follicular dynamics and ovarian steroid secretion in sheep during the follicular and early luteal phases of the estrous cycle. *Biol Reprod.* 56 (2): 483-488.

STRMSNIK L., M. POGACNIK, N. CEBULJ KADUNC, M. KOSEC., (2002). Examination of oestrus cycle and early pregnancy in sheep using transrectal ultrasonography. *Slov Vet Res.*39 (1): 47-58.

WEBB R, GAULD IK, DRIANCOURT MA., (1989). Morphological and functional characterization of large antral follicles in three breeds of sheep with different ovulation rates. *J Reprod Fert* (87) 243-55.