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Use of ultrasonography to detect ovarian response in goats submitted to multiple ovulation and embryo transfer program

Juraj Grizelj[1]*, Silvijo Vince[1], Marko Samardžija[1], Antonio Gonzalez de Bulnes[2], Toni Dovenski[3], Luigj Turmalaj[4], and Branimira Ževrnja[1]

[1]*Clinic for Obstetrics and Reproduction, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain*

[3]*Faculty of Veterinary Medicine, Ss. Cyril and Methodius University, Skopje, Macedonia*

[4]*Veterinary Medicine Faculty, Agricultural University, Tirana, Albania*

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ABSTRACT

The aim of this research was to establish the importance and accuracy of ultrasonic examination of goats in order to estimate the superovulatory response. For this reason, 28 donor goats were systematically examined by real time ultrasonography to count the preovulatory follicles at observed oestrus onset and to confirm respective ovulation(s) 48 h later. The accuracy of ultrasound exams was analysed comparing the number of preovulatory follicles with number of corpora lutea (CL′s) counted during embryo collection. The total relative undervalue of ultrasound prediction of multiple ovulation was found to be 19.4%. In all ovaries examined, the total number of preovulatory follicles minus number of unovulated follicles was lower than the number of CL′s. The relationship between the predicted ovulation rate and number of CL's was positive and significant (CL 3.98 + 0.75 follicle; r^2 0.93), with significantly higher intercept (1.45) for the right ovary. Also, the accuracy of ultrasound to establish the ovulating and non-ovulating goats was 100.0%. However, determination of the exact number of multiple ovulations on ovaries was 45.0%. In conclusion, the ultrasonography of donor goats is an useful tool to monitor follicular dynamics and success of superovulation procedures. Counting the number of follicles >4 mm in diameter at the oestrus onset and confirming the ovulation is a good method for selection of responding goats prior to flushing of uterus.

Key words: ultrasound, superovulation, ovulation, follicle, corpus luteum, goats

*Corresponding author:

Juraj Grizelj, Assoc. Prof., DVM, Clinic for Obstetrics and Reproduction, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: +385 1 2390 323, Fax: +385 1 2441 390, E-mail: jgrizelj@vef.hr

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Introduction

The improvement of goat industry involves multiplication of genetically superior females using multiple ovulation and embryyo transfer (MOET) programs. To optimize the results of these programs, the use of transrectal ultrasonography is necessary as it provides repeated, direct, non-invasive monitoring of ovarian structures and determing the number and size of follicles, regardless of their depth within the ovary (GINTHER et al., 1989). The ultrasound exams allow early diagnosis of non-responding females that can be removed from the MOET program maintaining the high level of reproductive efficiency. Avoiding unnecessary surgeries on animals that did not respond to the protocol of superovulation increase the efficiency, reduce costs and prevent early culling of embryo donors (BRANDAO et al., 2010). Also, there are considerable welfare implications if animals are spared of invasive surgical procedures (laparotomy, laparoscopy) with general anaesthesia or deep sedation (VIÑOLES et al., 2004).

Ultrasound exams allow the monitoring of growth and development of small antral follicles (2-3 mm), visualized as spherical anechoic structures with a thin wall (GONZÁLEZ de BULNES et al., 1994). Small follicles begin to rise immediately after the superovulatory stimulus, reaching maximum size during late proestrus or oestrus (RIESENBERG et al., 2001). MENCHACA et al. (2002) indicated that the number of follicles ≥5 mm at the time of the oestrus onset correlates with the number of CL′s counted when laparotomy was carried out. Moreover, this relationship could be used to anticipate the ovulation rate within superovulatory protocols (MENCHACA et al., 2001). The correlation coefficient between data obtained by ultrasound and slicing technique of the ovary for the number of follicles ≥3 mm was high (SIMOES et al., 2005), which highlights the use of ultrasonography as a potential methodology to study the follicular dynamic of goats.

The aim of this study was to establish the importance and accuracy of ultrasonic scanning of does in order to estimate the superovulatory response.

Materials and methods

Experimental animals. The study was conducted during the breeding season, which in temperate regions lasts from July till March. Twenty-eight Boer goats with an average age of 1.5 year were used. The mean ± SD body weight was 39.0 ± 9.09 kg. The animals were held outdoors and placed indoors at the beginning of the study, in the facilities of the Faculty of Veterinary Medicine Zagreb, Croatia at 45 °N latitude. Animals were fed with hay and concentrate twice a day and water was available ad libitum.

Donor goat procedure. Standard hormonal treatment of donor goats consisted of progestagen vaginal sponge insertion (Day 0) for 11 days (Chrono-gest®, 40 mg fluorogestone acetate FGA), PGF2α (Estrumate®, Day 9: 50 μg); and porcine follicle-stimulating hormone (pFSH; Folltropin®-V, Vetrepharm, Canada) divided into 6 parts of

decreasing concentration and applied at 12 h intervals (Day 9: 50 mg/50 mg; Day 10: 25 mg/25 mg; Day 11: 25 mg/25 mg; 200 mg total).

Detection of oestrus was performed 24 h after vaginal sponge withdrawal (Day 12). Does were «hand-mated» same day if the buck was accepted. If rejection occurred, hand-mating was attempted a few more times every 4 h. The next morning (Day 13), hand-mating was repeated if the doe was still in oestrus. In general, each female was mated one or two times.

Seven days after the first mating, laparotomyc flushing of the uterus was performed. Donor goats were subjected to an alimentary and hydric fasting 24 h before flushing. The anaesthetic procedure consisted of applying xylazine 0.11 mgkg-1 i/m (Xylapan®, Vetoquinol AG, Belp, Switzerland), and 10 min later, ketamine 5.5 mgkg-1 i/m (Narketan®, Vetoquinol AG, Belp, Switzerland). Donor does were positioned in dorsal recumbency, inclinated anteroposterior from 30 to 45º; medioventral laparotomy was performed with a 10 cm incision after laparoscopic verification that early luteal regression did not occur. The uterus and ovaries were exteriorised, and the number of corpora lutea was counted before flushing. According to the number of CL's, the goats were allocated in three groups: non-responding donors (<5 ovulations), good responding donors (5-15 ovulations) and excellent responding donors (>15 ovulations). The flushing of each uterine horn was performed with 40 mL of phosphate buffered saline (PBS) supplemented with 2 % of bovine serum albumine (BSA).

Ultrasonic exams. The goats were submitted to ultrasonic exams once a day during the superovulatory treatment. Additional exams were performed at the day of oestrus detection and at the expected moment of ovulation (48 h after the onset of oestrus). To perform the ultrasonic exam, the goats were placed in dorsal recumbence and 7.5 MHz lubricated linear array ultrasound probe was introduced into the rectum (Sonovet 2000, Medison Co., Ltd., South Korea). In general, probes with outer dimensions of 10 cm length, 3 cm height and 2 cm width can be introduced into the rectum without difficulties (KASPAR, 1988). It is necessary for the probe to be rotated laterally. If the probe to be used is not attached to a sufficiently stiff cable, which can advance the probe into the rectum, a pipe or concave rod should be used to stiffen the probe (KÄHN, 2004). The anaesthetic lubricant was applied in the rectal ampulla 5 min before introducing the rectal probe (Lidocaine 2.0% diluted in ultrasound gel in 1:10 rate). After the probe was placed into the rectum, with the transductor directed ventrally, it was pushed cranially to find the urinary bladder, which represents the orientation point. After reaching it, the probe was turned a few centimetres cranially and then laterally on both directions in order to localise the ovaries, which are situated within the curves of the uterine horns. The ultrasound scanning of each ovary lasted at least 5 minutes for precise measuring and counting the number of follicles (>4 mm) at the oestrus onset and the number of unovulated follicles

(≥5 mm) or luteinized follicles 48 h after oestrus onset. The ultrasonograms were saved on internal memory of ultrasound device (several different projections of ovary) and later transferred to computer for further examination using Adobe Photoshop software. In that way, pictures were adjusted with more sharpness and contrast for detailed inspection.

Statistical analysis. Statistical analyses were performed using SAS 9.1.3. software (SAS Institute Inc., 2002-2003). Simple descriptive statistics was done by Proc Means and Proc Freq (oestrus onset, duration of oestrus, body weight, number of follicles, number of corpora lutea). Data for the number of CL's (dependent variable) was analyzed by Proc Mixed in the model where number of follicles was considered as covariate, position of the ovarium as fixed effect and individual goats as random variable. In addition, we used Proc GLM to obtain approximate coefficient of determination. Number of counted CL's was a reference point to establish the level of ultrasound accuracy depending on the goats responsivity. Goats with early luteal regression were not included in the statistical analysis. To anticipate the ovulation rate, unovulated and luteinized follicles from each ovary were omitted from the number of preovulatory follicles before comparing with number of CL's.

Results

The oestrus was detected in all animals at 24.96 ± 0.73 h after vaginal sponge withdrawal. The duration of oestrus was 26.30 ± 0.67 h. The ovarian response of donor goats to FSH treatment (at least one CL) was 75.0% (21/28) and the mean ± SD number of corpora lutea, 14.8 ± 6.84. Three goats did not ovulate and four goats had early luteal regression. In all the examined ovaries the total number of preovulatory follicles minus number of unovulated follicles was lower ($P<0.05$) than the number of CL′s. The total relative undervalue of ultrasound prediction of ovulation was 19.46%; the total ultrasound accuracy of all donor goats was 80.54% (Table 1).

Table 1. Descriptive statistics of ultrasound and laparoscopic findings; number of follicles[#], number of corpora lutea and relative deviation[$] (Rdev) per ovary

Variable	N	Mean	Median	Range	SD
Follicles	42	5.52[a]	5.0	1.00; 14.00	2.83
Corpora lutea	42	7.40[b]	7.0	1.00; 16.00	3.76
Rdev	42	-19.46	-15.47	-75.00; 0.00	22.71

[#]The number of unovulated follicles (≥5 mm) and luteinized follicles found 48 h after oestrus onset was deducted from number of follicles (>4 mm) found at the beginning of oestrus. [$] Rdev 100 x (Follicles - Corpora lutea) / Corpora lutea. Values with different superscripts letters (a, b) within the same column ($P<0.05$).

Estimated individual (goat) and residual variances from the mixed model analysis were 3.12 (SE ± 2.05; P 0.065) and 3.00 (SE ± 1.16; P 0.005), respectively. The linear relationship between the number of CL′s and follicles obtained by ultrasound was positive and significant (CL 3.98 + 0.75 follicle; P<0.001) with significantly higher intercept (1.45; P<0.05) for the right ovary (Fig. 1).

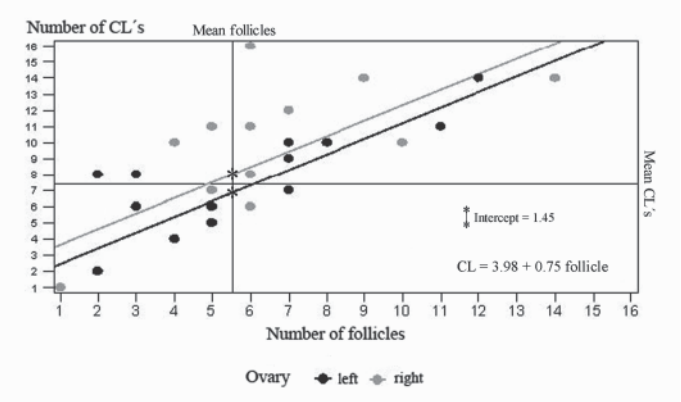

Fig. 1. Relationship between number of follicles (counted with ultrasound) and number of corpora lutea (counted at laparotomy) on the left and right ovary, respectively.

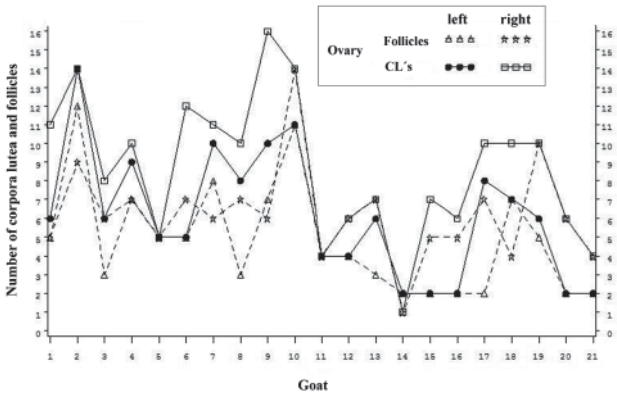

Fig. 2. Difference between the left and right ovary in number of CL′s and follicles for each goat

When we used the General Linear Model with SS1 and goat as random effect we obtained determination coefficient equal to 92.7% (P<0.001). The above-mentioned difference between the left and right ovary in the number of CL′s after laparotomy was also found in the number of follicles after ultrasound examinations (Fig. 2).

The accuracy of ultrasound to establish non, good and excellent responding donors was 100.0%, 53.0% and 20.0%, respectively (Table 2).

Table 2. Estimation of superovulatory response by ultrasonography for different donor's responsivity level

	Ultrasound prediction	Laparoscopy findings	Accuracy (%)
Not-ovulating goats	3/24[a]	3/24[a]	100.0
Not-responding goats (<5 ovulations)	2/24[a]	2/24[a]	100.0
Good responding goats (5-15 ovulations)	17/24[a]	9/24[b]	53.0
Excellent responding goats (>15 ovulations)	2/24[a]	10/24[b]	20.0

Values with different superscripts (a, b) within the same row (P<0.05)

Also, the accuracy of ultrasound to establish the ovulating and non-ovulating goats was 100.0%. Determination of the exact number of multiple ovulations on ovaries was 45.0% by comparing with laparotomy findings.

Discussion

Our results show that ultrasound accuracy to determinate superovulatory response in all donor goats by comparing the number of preovulatory follicles with number of corpora lutea counted during embryo collection was very satisfied (80.54%) with coefficient of determination equal to 92.7% (P<0.001). The previous studies have attempted to quantify ovulatory response by ultrasonic exam of ovaries after ovulation by counting the number of corpora haemorraghica (TEIXEIRA et al., 2008) or number of corpora lutea during the early luteal phase (GONZÁLEZ de BULNES et al., 1999b). TEIXEIRA et al. (2008) found positive correlation coefficient between the number of Corpora haemorragica detected by ultrasound and number of CL's counted at laparotomy (r^2 0.71; P<0.001), whereas the sensitivity for the multiple ovulation detection was 25.0 %. GONZÁLEZ de BULNES et al. (1999b) had a total efficiency in multiple ovulations detection of 87.5 % and accuracy to determine the exact number of CL's decreased to 23.5 % in goats with three or more ovulations due to increasing underestimation as the number of CL's increased (P<0.001).

The difficulties of Corpora haemorragica detection are due to their very similar ultrasound pattern with ovarian parenchyma (PIERSON and GINTHER, 1988; GONZÁLEZ de BULNES et al., 2004). GONZÁLEZ de BULNES et al. (1999a) point out the problem with

counting CL's that might be the result of a lack of discernment between the luteal-tissue of CL′s. The inabilities to differentiate the CL′s, one from each other, are due to their merged outlines, despite hyperechoic pattern attained. That is why technique of counting follicles at the oestrus onset and the number of unovulated follicles 48 h after oestrus onset, as presented in this study, seems more appropriate for prediction of superovulatory response.

The main problem of method used in our research is underestimation of ultrasound accuracy in highly responsive donor goats, similar to those described by TEIXEIRA et al. (2008) when trying to predict ovulation by counting corpora haemorragica with ultrasound. The explanation for this underestimation is follicular superposition. The follicles placed one next to another could mask each other and consequently provoke mistakes (Fig. 3).

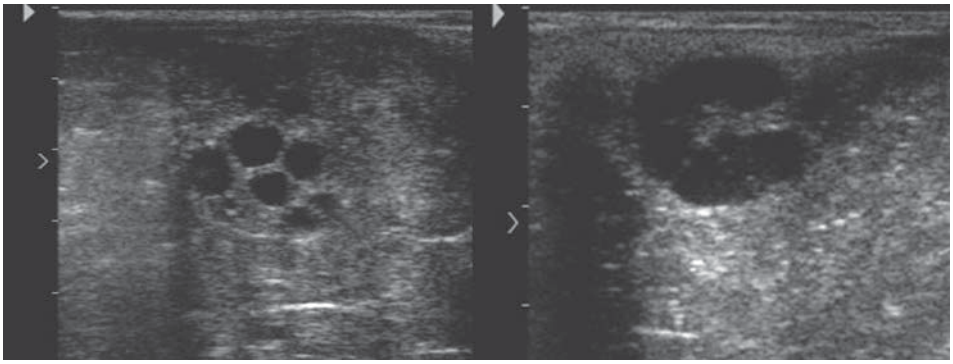

Fig. 3. Ultrasonography images showing an ovary without follicular superposition (left) and another ovary with follicular superposition (right). Note the difficulty to determine the number of follicles.

Non-responding donors (<5 ovulations) (sheep: 20.0%; goat: 10.0%) are not valuable for embryo recovery (BREBION et al., 1992). Ultrasound detection of animals that did not respond to the treatment or did not ovulate was 100.0% successful in this research, and presents the largest advance of this method. Although the ultrasonic exams could not determine the exact number of follicles in highly responsive donor goats, it is a great tool to follow up the follicle growth during superovulatory procedure and timely occurred ovulations (Fig. 4).

In the study of LEHLOENYA et al. (2008) the mean number of CL′s, after superovulation of Boer goats (during and out of breeding season), was also higher on right ovary but there are no data about significance. The increased functional activity of the right ovary compared with the left ovary in cattle is probably caused by an increased local temperature

due to the nearness of the rumen on the left side (CUSHMAN et al., 2005). That could also explain the different findings between the ovaries in this trial.

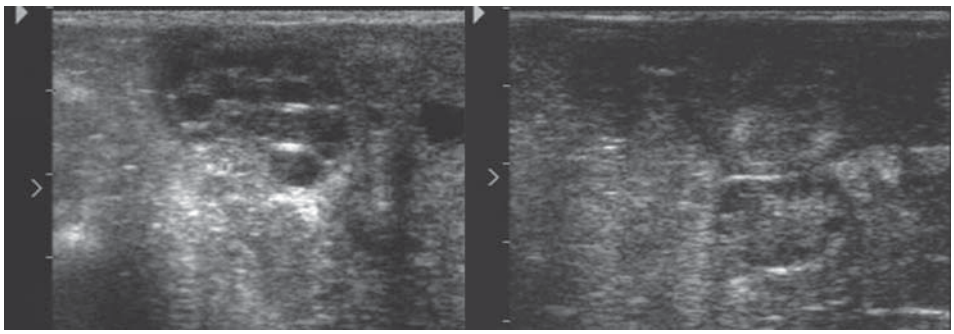

Fig. 4. Ultrasonography images of the ovary 48 h after the beginning of the superovulatory treatment (left) and after ovulation (right). All anechoic structures larger than 4 mm (follicles) observed in the right image are not seen in the left one.

In conclusion, although impossible to determine the exact number of follicles developed after hormonal treatment of donor goats, the ultrasonic exams are found to be very useful tool to estimate the tendency of follicular development, to eliminate non-ovulating and non-responding donor goats, as well as, to approximately predict the ovulatory rate. In that purpose relative deviation report can be used to predict the success of superovulation in a group of donor goats, provided that ultrasound scanning is performed always by the same person.

Combination of two ultrasonic exams (counting of follicles at oestrus onset and 48 h after ovulation was expected) could save time and money by avoiding unnecessary surgeries and preventing early culling of embryo donors due to elimination of animals that did not respond to the treatment or did not ovulate before collection of embryos. Also, it had positive effect on issues concerning animal welfare.

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SAŽETAK

Cilj ovog istraživanja bio je ustanoviti važnost i točnost ultrazvučnog pregleda koza u procjeni uspješnosti postupka superovulacije. U tu svrhu je 28 koza davateljica bilo sustavno pregledavano „real-time" ultrazvučnom metodom kako bi se izbrojali preovulacijski folikuli na početku estrusa i potvrdile ovulacije 48 sati kasnije. Provjerena je točnost ultrazvučnih nalaza i uspoređena s brojem žutih tijela utvrđenih prilikom ispiranja maternice. Ukupna relativna podcijenjenost ultrazvučnog predviđanja multiple ovulacije iznosila je 19,4%. Na svim pregledanim jajnicima, ukupni broj preovulacijskih folikula umanjen za broj neovuliranih folikula bio je manji nego broj žutih tijela. Odnos između predviđene stope ovulacije i broja žutih tijela bio je pozitivan i značajan (CL 3,98 + 0,75 folikula; r^2 0,93), sa značajno višim interceptom (1,45) za desni jajnik. Također, točnost utvrđivanja ovulirajućih i neovulirajućih koza ultrazvukom iznosila je 100,0%. Međutim, određivanje točnog broja multiplih ovulacija na jajnicima bilo je na razini od 45,0%. Iz navedenog se može zaključiti kako je ultrazvučno pregledavanje koza davateljica korisna metoda za praćenje folikularne dinamike i uspjeha superovulacije. Brojenje folikula promjera >4 mm na početku estrusa i potvrda ovulacije je dobra metoda za odabir koza koje su dobro reagirale na superovulacijske postupke prije ispiranja maternice.

Ključne riječi: ultrazvuk, superovulacija, ovulacija, folikul, žuto tijelo, koza