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Ultrasonographic assessment of the ovarian response in eCG-treated goats

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Abstract

A study was conducted to evaluate the accuracy of ultrasonographic evaluation of ovulation rate during the early luteal phase of does and to estimate the reliability of ultrasonic scanning to differentiate between the *corpus luteum* and luteinized follicles. Both ultrasonographic and laparoscopic observations of ovarian structures and radioimmunoassays of progesterone in plasma were performed in 47 goats on day 7 after estrus synchronization using intravaginal FGA sponges and eCG. Ultrasonographic scanning allowed the determination of the presence or absence of ovulation. Total efficiency in detecting the occurrence of multiple ovulations was 87.5%. However, accuracy to establish the number of *corpora lutea* decreased to 23.5% in goats with three or more ovulations, due to an increasing underestimation as the number of *corpora lutea* in the ovary increased ($p<0.001$). Ultrasonographic assessment of ovulation rate can be used to determine if a goat has ovulated or if multiple ovulations have occurred, but not to establish the precise number of *corpora lutea*. Evaluation of the ratio between cavity diameter and total luteal-tissue diameter is useful in distinguishing between *corpora lutea* and luteinized follicles, since the diameter differed between the two (0.36 ± 0.21 vs. 0.64 ± 0.16, respectively; $p<0.005$).

Keywords: Goats; Luteal-tissue; Ovulation; Ultrasound

1. Introduction

Embryo transfer in goats has developed mainly due to the international trade of genetic material, in order to avoid disease transmission (Chemineau et al., 1996). Embryo flushing can be done by either well-developed surgical or laparoscopical techniques, because the small body size and the characteristics of the genital tract of goats do not allow for the practical use of a non-surgical procedure (Flores-Foxworth et al., 1992). The same procedures may be used for the assessment of the ovulatory response in both donor and recipient goats. However, these techniques have the inconveniences of an invasive handling since exteriorization of the reproductive tract can induce post-operative adhesions, which decrease the life-span of the animal in an embryo transfer programme (Torres and Sevellec, 1987).

Transrectal ultrasonography is a non-invasive technique, firstly developed for ovarian scanning in ewes (Schrick et al., 1993) and thereafter in goats (Ginther

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and Kot, 1994), that allows one to image the *corpus luteum* (CL) in small ruminants, as routinely done in heifers. Accuracy of detection for the presence and number of CL during the oestrous cycle in poly-ovular ewes became 100% and 88.8%, respectively. This accuracy depended on the day of cycle, being higher during the midluteal phase (González de Bulnes et al., 1994).

Ultrasonography has not been previously used to assess the ovulatory response in embryo transfer programs in goats. Therefore, the aim of this study was to establish the adequacy of ultrasonography to evaluate ovarian response of both donor and recipient goats involved in superovulation and embryo transfer protocols. In this way, the reliability of ultrasonographic imaging to identify the number of CL's and differentiate between CL and luteinized follicles during the early luteal phase were evaluated in does induced to display either single or multiple ovulations ($\geq$3 CL) with an injection of eCG.

2. Material and methods

The trial was developed during the breeding season (July) using 24 Cashmere does (mean weight $\pm$ S.E.M.: 37.2$\pm$5.9 kg) and 24 Spanish does (mean weight $\pm$ S.E.M.: 45.7$\pm$6.8 kg), from the experimental herd of El Carbayal-Illano (Asturias, North Spain). Goats were selected as adult (four to six years old), multiparous, cycling and nonlactating.

Estrus was synchronized with the insertion of intravaginal progestagen sponges (45 mg fluorogestone acetate, Chronogest®, Intervet International, Boxmeer, Holland). Because these does were involved in another experiment, progestagen was maintained during 11 days in 12 does of each breed and during 14 days in the remaining 24 goats, and the first group received an intramuscular injection of 125 μg of cloprostenol (Estrumate®, Pitman–Moore, Friesoythe, Germany) 48 h before sponge withdrawal. A single intramuscular dose of 400 I.U. of eCG (Foligon®, Intervet International, Boxmeer, Holland) was injected 48 h before progestagen removal to induce ovulation. The dose of eCG was chosen in relation with the live weight and estimated adequate for Spanish goats but high for Cashmere does (Ritar et al., 1989), which allowed the obtaining of multiple ovulations. One Spanish female lost the sponge and was taken out of the experiment. Estrus was detected in all the remaining goats using vasectomized bucks every 3 h from 20 to 52 h after progestagen withdrawal.

The number and morphological characteristics of CL and luteinized follicles were assessed both by ultrasonography and laparoscopy performed on day 7 after estrus. Ultrasonographic scanning was performed as described by Schrick et al. (1993), using an Aloka 500 SSD (Ecotron, Madrid, Spain) fitted with a 7.5 MHz linear-array probe. Each ovary was scanned several times in different planes to obtain both the number of CL and luteinized follicles and their ultrasonographic patterns. Thereafter, the largest total luteal-tissue diameter and the largest cavity diameter was determined. Total luteal-tissue diameter was determined by measuring with electronic calipers in the interface of luteal-tissue with ovarian stroma, whereas the calipers were placed in the interface between luteal-tissue and cavity to measure the cavity diameter.

Samples of 5 ml jugular blood were collected, concurrently with the ultrasonographic and laparoscopic observations, using vacuum blood evacuation tubes with heparine (Vacutainer® Systems Europe 5 ml, Becton Dickinson, Meylan Cedex, France). Plasma progesterone concentrations were used as reference value, considering plasma progesterone levels above 0.5 ng/ml as indicative of a functional CL. Blood samples were centrifuged at 3500 rpm for 15 min and plasma was stored at -20°C until assayed. After thawing samples, duplicates of 200 μl of plasma were extracted in 3 ml hexano (N-Hexano, Merck, Darmstadt, Germany) for 20 min. Vials were frozen at -20°C and the liquid phase was decanted into a new vial and evaporated in nitrogen stream to obtain a dry residue with the progesterone. Plasma progesterone concentration was determined by radioimmunoassay as described by Lopez Sebastian et al. (1984). Extraction efficiency was 84.3%, detection limit was 0.06 ng/ml and inter- and intra-assay variation coefficients were 10.4% and 13.6%, respectively.

Statistical analysis was used to evaluate the ultrasonographic findings. Using data from laparoscopic observations and progesterone assays as reference, to determine the accuracy or predictive value of ultrasonographic evaluation of ovulation rate and the effect of the number of *corpora lutea* on the level of accu-

racy. Predictive values were established in terms of sensitivity (percentage of goats correctly diagnosed as bearing a certain number of CL's), specificity (percentage of goats correctly diagnosed as not bearing a certain number of CL's) and total efficiency (total percentage of individuals correctly classified as bearing or not a certain number of CL's). Relationship between predictive value of ovulation rate and number of *corpora lutea* was assessed by Pearson correlation procedures (BMDP Statistical Software) considered to be statistically significant at $p<0.05$. Reliability of ultrasonic scanning to assess differences between CL and luteinized follicles were also evaluated, using 24 goats in which only one CL or luteinized follicle had been detected by laparoscopy in at least one of the two ovaries. Only those goats were used to avoid error when several luteal structures were present in the same ovary. Both ultrasonographic morphology and measurements of total diameter, cavity diameter and relationship between total and cavity diameter of luteinized follicles and CL were determined. Differences in measurements were evaluated by analysis of variance of BMDP statistical software. Results were expressed in mean ± S.E.M. and differences were considered to be statistically significant at $p<0.05$.

3. Results and discussion

Oestrous behaviour was detected in 44 of the 47 goats (93.6%) at 23.6±3.3 h after the sponge withdrawal. The use of ultrasonographic scanning allowed to determine if a goat had ovulated or not in all cases (Table 1). There were 40 does with at least one CL (89.3%) and seven without ovulation by laparoscopy; all of them were well recognized by ultrasound and confirmed as positive by concentration of progesterone.

The laparoscopic examination showed that 23 of the 40 ovulated goats had one or two CL's whereas the other 17 had three or more. The sensitivity of the ultrasonographic observation to determine the presence of multiple ovulations in a doe only reached 70.5%, but the specificity remains 100%. The total efficiency was 87.5%. However, the accuracy of determination of the number of CL in goats with multiple ovulations was very low, only 23.5%. This was an accumulative error, because the total efficiency determining the ovulation rate in every ovary was 74.4% for all the ovaries and 64.7% for the ovaries with at least one ovulation. This was probably due to underestimation when the CL number was higher than three or because of mistakes in distinguishing between CL's and luteinized follicles. Underestimation in highly responsive females was also described in ewes (González de Bulnes et al., 1994; Schrick et al., 1993). Causes of underestimation might be the results of a lack of discernment between the luteal-tissue of detected and non-detected CL, since efficiency of detection of CL number in current study was highly related to the number of CL in the ovary ($p<0.001$), as depicted in Fig. 1.

Another source of error in detecting CL could be due to confusion of a CL with a central cavity and that of an anovulatory luteinized follicle, because presence of a central cavity was detected in a 79.6% of the CL. This finding is in agreement with experiments describing the presence of a non-pathological central cavity in 18–33% of the CL's in ewes (González de Bulnes et al., 1994, 1995 and in 37–79% of CL's in heifers (Kastelic et al., 1990b and Kito et al., 1986, respectively), but without any effect on luteal function

Table 1
Sensitivity, specificity and predictive value following transrectal ultrasonography to detect the occurrence of ovulation and identify goats bearing 1 to 2 CL's or ≥3CL's

	Occurrence of ovulation		Occurrence of multiple ovulations	
Total number of goats used	–	47	–	40
Number of goats	Ovulating	40	1–2CL	23
	Non-ovulating	7	≥3CL	17
Sensitivity	–	100% (40/40)	–	70.5% (12/17)
Specificity	–	100% (7/7)	–	100% (23/23)
Total efficiency		100% (47/47)	–	87.5% (35/40)

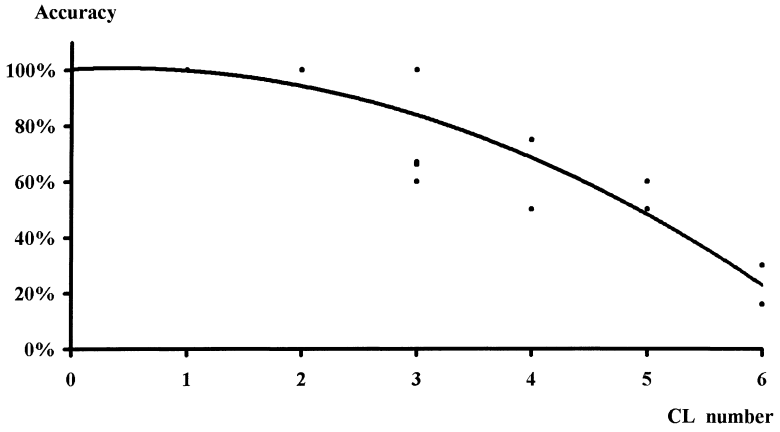

Fig. 1. Predictive value following ultrasonographic estimation of ovulation rate in individual ovaries based on the number of *corpus luteum* detected by laparoscopy.

(Kastelic et al., 1990a; Son et al., 1995). The CL cavity was usually hypoechoic, but occasionally exhibited a slightly increased and diffuse pattern or the presence of echogenic lines. Analysis performed in heifers showed that the former were the ultrasonic image of accumulations of hemolyzed blood, whereas the latter were fibrin-like strands (Pierson and Ginther, 1987).

There were no differences in the echogenic patterns or in the outlines of luteal-tissue between luteinized follicles and CL's in the study, but former showed a thicker wall and a larger cavity than the latter (Figs. 2 and 3). Mean diameter was larger in luteinized follicle than in CL (15.8±1.4 mm vs 14.13±1.7 mm; $p<0.05$), as was cavity diameter (10.2±3.0 mm vs 5.31± 3.37 mm; $p<0.001$) and ratio between cavity diameter and total diameter (0.64±0.16 vs 0.36±0.21; $p<0.005$).

In conclusion, ultrasonographic evaluation of the ratio between cavity and luteal tissue diameters is useful to distinguish between CL's and luteinized follicles. Assessment of ovulation rate by transrectal ultrasonography can be used to select responding donors and recipients or to evaluate if a donor has multiple ovulations but not to establish the exact CL number.

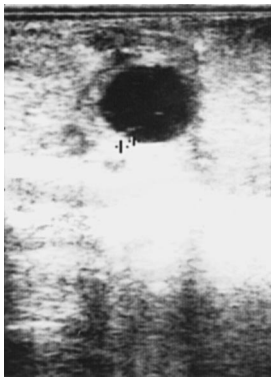

Fig. 2. Ultrasonographic image of a luteinized follicle, with a thick luteal wall and large cavity.

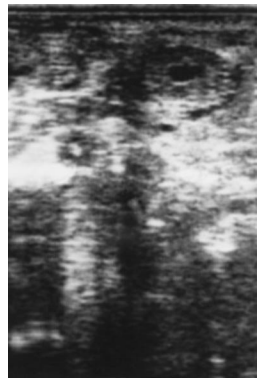

Fig. 3. Ultrasonographic image of a *corpus luteum*, with luteal-tissue larger than cavity.

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