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SHORT COMMUNICATION

WILEY Reproduction in Domestic Animals

Colour-Doppler ultrasound imaging as a laparoscopy substitute to count corpora lutea in superovulated sheep

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Contents

This study evaluated colour-Doppler ultrasound imaging (UI) as a substitute for laparoscopy to count the corpora lutea (CL) in superovulated sheep. Twenty-five Santa Ines ewes were superovulated three times at 21-day intervals. Corpora lutea were counted by colour-Doppler UI ($CL_{DOPPLER}$) 6 days after each superovulation and confirmed by laparoscopy (CL_{LAP}) 12 hr later. The mean number of CL was similar for both techniques (2.1 ± 2.5 vs. 2.1 ± 2.7 for $CL_{DOPPLER}$ and CL_{LAP}, respectively) with a significant positive correlation (r = .94; r^2=.89). Colour-Doppler UI effectively evaluated the ovarian response in superovulated ewes and efficiently identified animals that did not respond to superovulation.

1 | INTRODUCTION

Despite efforts to improve superovulatory protocols (SOV) in sheep, variability in ovarian response remains the major limitation in multiple ovulation and embryo transfer (MOET) programs (Bartlewski et al., 2016). Thus, the CL are counted by laparoscopy to determine ovarian response and whether a surgical embryo recovery procedure is justified. Although it is a less invasive surgery, laparoscopy requires care, such as fasting and anaesthesia (Fonseca et al., 2016) similar to other surgical procedures.

Compared to laparoscopy, ultrasound imaging (UI) is a non-invasive, safe procedure that does not require previous preparation (Ginther, 2014). In ewes, B-Mode and colour-Doppler UI are used for CL evaluation (Dickie, Paterson, Anderson, & Boyd, 1999; Figueira et al., 2015). However, most previously published studies evaluated non-superovulated ewes, and, to the best of our knowledge, colour-Doppler UI has never been used to screen responding ewes in MOET programs. Thus, the present study evaluated colour-Doppler UI as a laparoscopy substitute for evaluating superovulation in sheep.

2 | MATERIALS AND METHODS

This research was approved by the Ethical Committee for Animal Use of the Universidade Federal Fluminense (protocol 699/15).

2.1 | Experimental location, animals and design

The experiment was performed at Unidade de Pesquisa Experimental em Caprinos e Ovinos, in Cachoeiras de Macacu, Rio de Janeiro, Brazil. Nulliparous Santa Ines ewes (n = 25) were superovulated three times at 21-day intervals, for a total of 75 SOV. Six days after each SOV, the number of CL per ovary was counted by colour-Doppler UI and confirmed by laparoscopy 12 hr later.

2.2 | Superovulation and mating

Eighty hours after ovulation, SOV was performed using 200 mg of FSH (Folltropin-V®, Bioniche Animal Health, Ontario, Canada) administered in six decreasing doses every 12 hr (50/50, 30/30, 20/20 mg). A sponge containing 60 mg of medroxyprogesterone acetate

(Progespon®, Zoetis, São Paulo, Brazil) was inserted with the first FSH dose and removed at the fifth dose. Cloprostenol sodium (0.24 mg; Estron®, Tecnopec, São Paulo, Brazil) was administered with the last FSH dose, and 0.025 mg of lecirelin (Gestran Plus®, Tecnopec, São Paulo, Brazil) was administered 24 hr later. After the last FSH dose, ewes were bred with fertile Santa Ines rams every 12 hr until the end of oestrus.

2.3 | Corpora lutea count

Six days after the last FSH, the CL number in each ovary was counted by colour-Doppler UI ($CL_{DOPPLER}$) using a portable device (SonoScape S6®, China) equipped with a 7.5-MHz transrectal linear transducer. After locating the ovary using B-Mode, colour-Doppler mode was activated, and the CL number and vascularization were determined. Luteal vascularization was subjectively assessed using an increasing score scale of 1–4 (Figure 1). Only functional CL (score ≥ 2) in each ovary were counted. The Doppler settings used were as follows: 20% colour gain, 1.0 kHz pulse repetition frequency, 7 cm depth and a 75 kHz wall filter.

The CL number was confirmed by laparoscopy (CL_{LAP}) 12 hr after the ultrasound examination. The laparoscopy procedure was performed per the method of Bruno-Galarraga et al. (2015) under sedation with i.v. acepromazine maleate (0.1 mg/kg, Acepran® 1%, Vetnil, São Paulo, Brazil) and i.v. diazepam (0.3 mg/kg, Uni-Diazepax®, União Química, São Paulo, Brazil). Only red-coloured CL were considered functional and counted.

2.4 | Statistical analysis

The outcome variables, $CL_{DOPPLER}$ and CL_{LAP} (mean ± SD), were determined using Kolmogorov–Smirnov's test. Means were compared by a paired Student's *t* test. Associations were determined by Pearson's correlation (*r*) and simple linear regression (r^2). Considering CL_{LAP} as the "standard method," the performance of $CL_{DOPPLER}$ as a diagnostic method to determine the number of CL was evaluated by calculating the sensitivity (SENS), specificity (SPEC), positive (PPV) and negative (NPV) predictive values and area under the ROC curve (AUC) at different cut-off points (CL per ovary, 1–9). The kappa coefficient and the area under the ROC curve were determined using the Statistical Package for the Social Sciences, Inc. A p value < .05 was considered significant.

3 | RESULTS

The CL number counted by colour-Doppler ultrasonography ($CL_{DOPPLER}$) was not significantly different from the CL number counted by laparoscopy (CL_{LAP}) (2.1 ± 2.5 vs. 2.1 ± 2.7, respectively). In 70% of the evaluations (105/150), the CL count was the same for both methods. In 16% of the evaluations (24/150), $CL_{DOPPLER}$ was higher than CL_{LAP}, and in 14%, CL_{LAP} was higher than $CL_{DOPPLER}$ (21/150). A positive correlation between CL_{LAP} and $CL_{DOPPLER}$ (r = .94; r^2 = .89; p < .05, Figure 2) was observed. Ewe ovaries demonstrating both low and high responses, visualized by colour-Doppler UI, are illustrated in Figure 3 (a and b, respectively). The performance of $CL_{DOPPLER}$ as a tool for CL count at different cut-off points (1–9 CL per ovary) is presented in Table 1.

4 | DISCUSSION

The results demonstrated a strong correlation between both CL counting methods. The kappa index remained above 91%

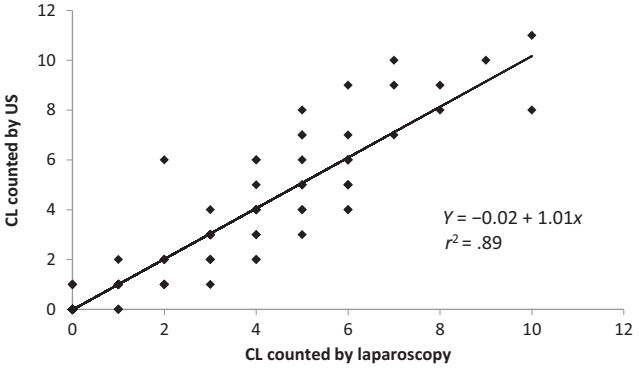

FIGURE 2 Scatter plot showing the correlation (p < .05) between the corpora lutea (CL) counted by colour-Doppler ultrasound imaging ($CL_{DOPPLER}$) and by laparoscopy (CL_{LAP}) in superovulated sheep

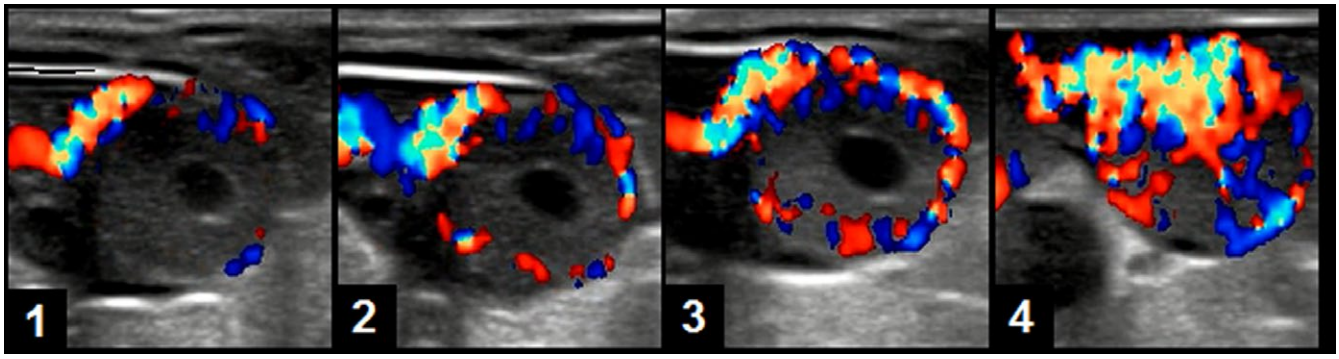

FIGURE 1 Subjective corpora lutea vascularization scores (1–4) based on the luteal area percentage (0%–100%) with coloured pixels. Score 1 (0%–25%), Score 2 (25%–50%), Score 3 (50%–75%) and Score 4 (75%–100%)

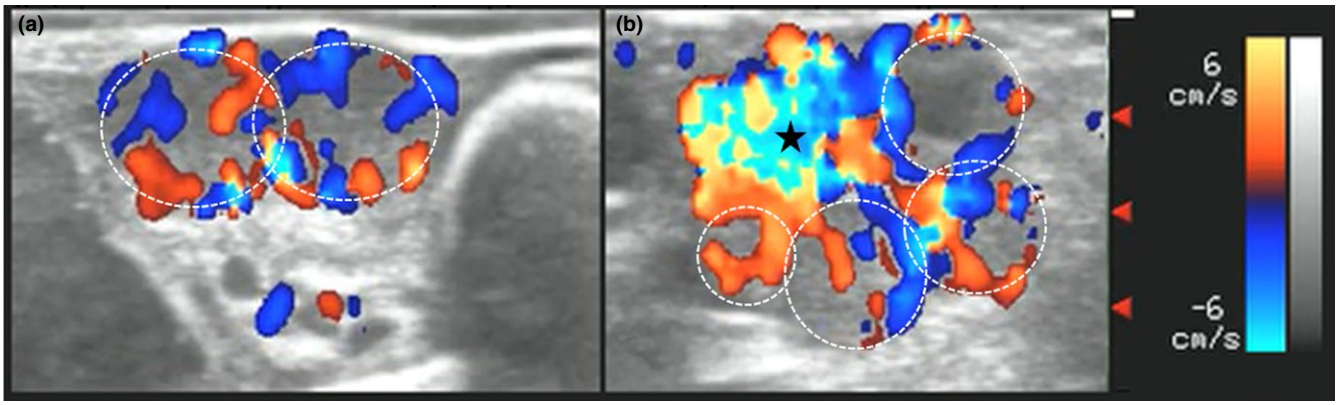

FIGURE 3 Colour-Doppler ultrasound imaging of superovulated ovaries in sheep: (a) poor response (two CL) and (b) good response (four CL). Black star indicates ovarian vessels at the hilum. White dashed circles indicate each CL

TABLE 1 Sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), negative predictive value (NPV), kappa index and area under the ROC curve (AUC) of the colour-Doppler UI used for CL count

CL	SENS	SPEC	PPV	NPP	Kappa	AUC
1	0.98	0.93	0.91	0.99	0.95	0.89
2	0.98	0.94	0.89	0.99	0.95	0.84
3	0.95	0.95	0.88	0.98	0.95	0.75
4	0.86	0.96	0.83	0.97	0.94	0.67
5	0.67	0.96	0.74	0.95	0.92	0.61
6	0.61	1.00	1.00	0.96	0.97	0.55
7	0.56	1.00	1.00	0.97	0.97	0.53
8	0.33	0.99	0.67	0.97	0.97	0.52
9	0.33	0.99	0.50	0.99	0.98	0.51

regardless of the CL number in the ovaries. Viñoles, Meikle, and Forsberg (2004) demonstrated that transrectal UI is as efficient as laparoscopy and laparotomy for detecting CL in non-superovulated sheep. Our results indicate that $CL_{DOPPLER}$ is also as effective as laparoscopy for assessing ovarian response in superovulated sheep.

Previous studies using non-superovulated animals reported that identifying individual CL using UI was difficult and would have limited use in SOV (Dickie et al., 1999; Viñoles et al., 2004). In the present study, the AUC and sensitivity values demonstrated that $CL_{DOPPLER}$ performance decreased when more than four CL were present. This was probably due to difficulty in distinguishing individual CL in high numbers. Despite this limitation, when the number of CL was <4, the performance parameters of $CL_{DOPPLER}$ were satisfactory (sensitivity ≥90% and AUC ≥ 75%). Moreover, $CL_{DOPPLER}$ accurately identified animals with a poor ovarian response, demonstrated by the high specificity and NPP values at all cut-off points. It is crucial that CL evaluation is performed by laparoscopy or laparotomy in ovine MOET programs (Fonseca et al., 2016); however, these procedures can injure and impair the animal's reproductive capacity (Dickie et al., 1999; Fonseca et al., 2016).

Real-world scenarios regarding assisted reproductive technology and animal well-being will likely lead to reducing or prohibiting successive surgical procedures in the same animal (Fonseca et al., 2013). Thus, colour-Doppler UI can be used to remove poorly responding ewes from MOET programs, avoiding unnecessary surgical procedures. Animal welfare is a growing concern in research, and livestock production practices and ultrasound examinations as "clean and ethical" techniques (Miyamoto et al., 2006) are an excellent laparoscopy substitute for evaluating SOV in sheep.

These results support the hypothesis that colour-Doppler UI can substitute laparoscopy to assess ovarian response in superovulated ewes, although the procedure's accuracy decreases when more than four CL are present.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

PHNP elaborated the hypothesis, discussed the experimental design, collected the data from the animals, analysed the data and wrote the first version of the manuscript. GMB, MAFB and GBS collected the data from the animals. ENA and JMGS-F discussed the design of the experiment and analysed the data. GNS elaborated and worked on the statistics. FZB and JFF discussed the design of the experiment and collected the data from the animals. In addition to the contributions already cited, all the authors contributed to the manuscript writing, revised and approved the final version of the manuscript.

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