CSIRO PUBLISHING

Animal Production Science
https://doi.org/10.1071/AN18740

Successive *in vivo* embryo production in Santa Inês sheep

Pedro Henrique Nicolau Pinto[A,C], *Mario Felipe Alvarez Balaro*[A], *Helena Fabiana Reis de Almeida Saraiva*[A], *Viviane Lopes Brair*[A], *Vivian Angélico Pereira Alfradique*[A], *Luana Rangel Côrtes*[A], *Isabel Oliveira Cosentino*[A], *Joanna Maria Gonçalves Souza-Fabjan*[A], *Jeferson Ferreira da Fonseca*[B] and *Felipe Zandonadi Brandão*[A]

[A]Faculdade de Veterinária, Universidade Federal Fluminense, Rua Vital Brazil Filho. 64, 24230-340, Niterói, RJ, Brazil.
[B]Embrapa Caprinos e Ovinos, Núcleo Regional Sudeste, Rodovia MG 133 Km 42, 36155-000, Coronel Pacheco, MG, Brazil.
[C]Corresponding author. Email: pedropintoufpr@gmail.com

Abstract

Context. *In vivo* embryo production, also called multiple ovulation and embryo transfer, can accelerate genetic gain, and thus improve animal production. However, there are issues limiting a wider use of this biotechnology in sheep livestock.

Aims. This study aimed to determine (1) whether a previous response to superovulation (SOV) can be used as a criterion to select ewes for *in vivo* embryo production, (2) whether the intensity of the SOV response (number of corpora lutea, CL) can affect the embryo recovery rate, and (3) whether the number of CL quantified by colour Doppler ultrasonography can be used to calculate the recovery rate.

Methods. Twenty-five Santa Inês ewes underwent SOV three times (SOV1, SOV2 and SOV3), with 200 mg FSH and natural mating. The number of CL after each SOV was determined by laparoscopy and by colour Doppler ultrasonography.

Key results. The number of CL significantly decreased ($P < 0.05$) after SOV1 (7.5 ± 4.8) to 3.0 ± 5.0 at SOV 2 and 2.2 ± 3.5 at SOV3. Strong correlations were observed between SOV2 and SOV3 in terms of numbers of CL ($r = 0.86$, $r^2 = 0.74$; $P < 0.0001$) and viable embryos ($r = 0.79$, $r^2 = 0.63$; $P < 00001$). However, no correlations were observed between SOV1 and SOV2 or between SOV1 and SOV3. Recovery rate did not differ with the intensity of the SOV response ($\leq$6, 7–10, >10 CL) or between the methods used to quantify CL.

Conclusions. Ewes did not show the same pattern of response when submitted to successive FSH-based SOV. The intensity of the SOV response did not affect the recovery rate, and the number of CL estimated by colour Doppler ultrasonography can be used to calculate the recovery rate.

Implications. Selecting sheep embryo donors by a previous SOV response is not always feasible. The recovery rate is homogeneous and it is not affected by the intensity of the SOV response. A nonsurgical technique can be used to assess the recovery rate, improving animal welfare in MOET programs.

Additional keywords: corpus luteum, MOET, ovarian superstimulation.

Received 18 December 2018, accepted 20 June 2019, published online 22 January 2020

Introduction

In sheep production, assisted reproductive technologies are used to diminish reproductive inefficiencies and accelerate genetic gain. One such technology that stands out, owing to its capability to accelerate genetic gain, is multiple ovulation and embryo transfer (MOET) (Cognié *et al*. 2003), also known as *in vivo* embryo production. Recently, our research group demonstrated that it is possible to select ewes with better response to superovulation (SOV) based on the antral follicular count and the plasmatic concentration of anti-Müllerian hormone (Pinto *et al*. 2018*a*). However, it has not been established whether animals with a superior response to SOV usually repeat this performance in successive treatments.

In goats and cattle, satisfactory performance in SOV is highly repeatable in consecutive protocols (Taneja *et al*. 2000; Monniaux *et al*. 2011). In wool sheep, moderate to high correlations have been reported among ovulation rates in consecutive SOV treatments (Bari *et al*. 2001; Bruno-Galarraga *et al*. 2014). This feature has great applicability because the results of screening tests such as antral follicular count, anti-Müllerian hormone or even a first MOET program could be used to select animals that will produce more embryos in subsequent SOV. To the best of our knowledge, there are no reports evaluating the repeatability of responses to *in vivo* embryo production in tropical hair sheep such as Santa Inês ewes.

The assessment of the number of corpora lutea (CL) after SOV, traditionally performed using laparoscopy, is a well-established proxy to determine which animals are worth submitting to embryo recovery (Cordeiro *et al*. 2003; Bruno-Galarraga *et al*. 2014). Such a procedure screens out animals for which there are low expectations for embryo production. Pinto *et al*. (2018*b*) and Oliveira *et al*. (2018) demonstrated that CL count in sheep donors can be performed by using colour Doppler ultrasonography (US), but it has not been evaluated whether the number of CL determined by colour Doppler US would be useful for estimating the recovery rate. Besides, there are no reports evaluating whether the intensity of the SOV response (low, medium or high) could influence the recovery rate. If significant, this information could define new parameters for determining which animals will be submitted to embryo recovery. Therefore, this study aimed to determine (1) whether Santa Inês ewes maintain similar embryo production patterns when subjected to three successive SOV treatments, (2) whether the intensity of the SOV response affects the embryo-recovery rate, and (3) whether the number of CL quantified by colour Doppler US can be used to calculate the recovery rate.

Material and methods

This research was approved by the Ethical Committee for Animal Use of Universidade Federal Fluminense (protocol 699/15) and conducted under the ethical principles of the Sociedade Brasileira de Ciência em Animais de Laboratório.

Experimental location, animals, and design

The experiment was conducted at the Unidade de Pesquisa Experimental em Caprinos e Ovinos (UniPECO) in Cachoeiras de Macacu, Rio de Janeiro, Brazil (22°S). We used nulliparous Santa Inês ewe lambs ($n = 25$) that had been approved in clinical evaluations and presented good body condition scores (2.8 ± 0.3, on a 1–5 scale where 1 is emaciated to 5 is obese). Ewes were kept in a confined system and fed with chopped Napier grass (*Pennisetum purpureum* cv. Cameron) and concentrate to meet maintenance requirements. The same flock, under the same rearing conditions, was submitted to three successive MOET protocols (SOV1, SOV2, SOV3) for data collection.

Superovulation protocol and mating

Ewes underwent the SOV protocol after a short oestrus-synchronisation protocol (Balaro *et al*. 2016), following the 'Day 0 protocol' concept (Menchaca *et al*. 2009). In brief, a sponge impregnated with 60 mg medroxyprogesterone acetate (Progespon; Schering Plough (now Merck Co.), Kenilworth, NJ, USA) was maintained for 6 days. One day before sponge removal, 300 IU equine chorionic gonadotrophin (eCG) (Novormon; Schering Plough) and 0.24 mg cloprostenol sodium (Estron; Tecnopec, São Paulo) were administered intramuscularly (IM). Thirty-six hours after sponge removal, 0.025 mg lecirelin (Gestran Plus; Tecnopec) was administered IM. The SOV was started 80 h after sponge removal, using 200 mg follicle-stimulating hormone (FSH) (Folltropin-V; Bioniche Animal Health, Belleville, ON, Canada) administered every 12 h in a tapering dose (50/50, 30/30, 20/20 mg). At the first FSH dose, a new sponge was inserted and maintained until the fifth dose (González-Bulnes *et al*. 2005). Together with the last FSH dose, 0.24 mg cloprostenol sodium was administered, and 24 h later, 0.025 mg lecirelin was administered, both IM. All FSH was from the same batch. Ewes were naturally mated with fertile Santa Inês rams every 12 h, from the last FSH dose to the end of oestrus. We used an interval of 21 days from the end of one protocol (embryo recovery) to the beginning of the next (first sponge insertion) (Fig. 1).

Ultrasonographic evaluation

Transrectal B-Mode US was performed to quantify the number of small (<3 mm), medium (3–5 mm) and large (≥5 mm) follicles during each one of the three SOV protocols. A portable device (SonoScape S6; SonoScape, Shenzhen, China) equipped with a 7.5 MHz linear transducer was used. The follicular population was assessed every 24 h from the first FSH dose (Day 9, afternoon) until the last FSH dose (Day 12, morning). Six days after the last FSH dose (12 h before embryo recovery), ewes were submitted to new US evaluation for CL count. For that, each ovary was first located by using B-Mode, colour Doppler mode was activated, and the number of functional CL was determined, as previously described (Pinto *et al*. 2018*a*). Ewes were assigned to one of three categories according to the number of CL counted in colour Doppler mode ($CL_{DOPPLER}$): ≤6, 7–10, or >10. We used the following Doppler settings: 20% colour gain, 1.0 kHz pulse repetition frequency, 7 cm depth, and a 75 kHz wall filter. The same technician performed all of the US evaluations.

Embryo recovery

At 6–7 days after the last mating, ewes were submitted to embryo recovery. For that, females were deprived of food for 24 h and water for 12 h, and then were submitted to general anaesthesia (Lima *et al*. 2015). Immediately before embryo recovery, CL and ovarian cysts were counted by laparoscopy, as already described (Bruno-Galarraga *et al*. 2015); ewes were submitted to embryo recovery only if they had three or more functional CL. Embryos were surgically recovered via longitudinal ventral laparotomy. After uterus exposure, an 18-gauge IV catheter (BD, Franklin Lakes, NJ, USA) was inserted near the utero-tubal junction, and the uterine lumen received an injection (40 mL) of warmed (37°C) buffered phosphate solution (DMPBS; Biodux, São Paulo) supplemented with 10% adult bovine serum (Nutricell, São Paulo). This flushing medium was recovered by using a Foley catheter (size 08 Fr) inserted at the external bifurcation of the uterine horns. Flushing content was recovered in 50-mL Falcon tubes and sent for evaluation. During the recovery procedure, the genital tract was washed constantly with heparinised saline solution (5 IU/mL) (Liquemine; Roche, Basel, Switzerland) at 37°C. Embryo morphologies were evaluated under a stereomicroscope (Nikon, Tokyo) using 20–40× magnification.

End-points

The following end-points were determined: oestrus response after SOV ((no. of ewes in oestrus/no. of treated ewes) × 100); time to oestrus onset (interval from sponge removal to first

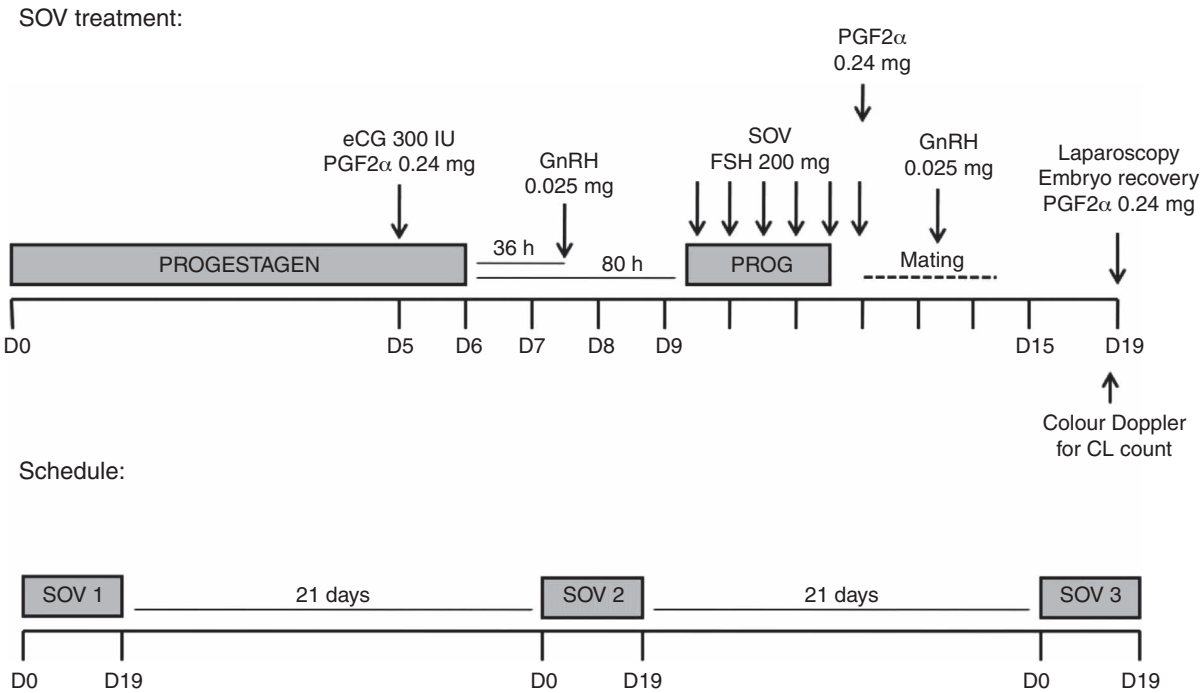

Fig. 1. Schematic representation of the FSH superovulation (SOV) protocol, intervals among SOV treatments, and time of corpora lutea (CL) count by colour Doppler ultrasonography and laparoscopy. PROG, Progestogen (60 mg of medroxyprogesterone acetate).

mating); oestrus length (interval from the first to the last time that the ewe accepted the male) (sexual behaviour evaluated as reported by Souza-Fabjan *et al*. 2017); ewes that responded to the SOV protocol (≥3 CL at laparoscopy); percentage of ovarian cysts at laparoscopy; number of CL at laparoscopy; number of CL at colour Doppler US; total recovered structures (oocytes, zona pellucida, degenerated and viable embryos); numbers of viable, non-fertilised and degenerated embryos. We also calculated the rates of recovery ((total recovered structures/CL counted) × 100), viability ((viable embryos/total recovered structures) × 100), non-fertilisation ((unfertilised structures/total recovered structures) × 100), and degeneration ((degenerated embryos/total recovered structures) × 100).

Statistical analyses

Data were tested for normality by the Lilliefors test. Normally distributed variables were compared by analysis of variance (ANOVA) and differences between means were evaluated by the Student's *t*-test. Fisher's exact test or a chi-square test was used to compare among protocols the proportions of ewes that displayed oestrus, responded to SOV, and presented an ovarian cyst. Nonparametric variables were compared by the Kruskal–Wallis test followed by Dunn's test. Statistical analyses of the rates of recovery, viability, fertilisation and degeneration were performed after transformation of each percentage to the arcsine square root. Pearson correlation coefficient, simple linear regression and intra-class correlation coefficient (ICC) were determined to evaluate the repeatability of response between SOV protocols. The Statistical Analysis System program (SAEG 9.0, Universidade Federal de Viçosa, Viçosa-MG, Brazil) was used, and we considered significance when $P < 0.05$ for all tests.

Results

The follicular population (small, medium and large) did not differ ($P > 0.05$) at Day 9 (first FSH dose) among the three repetitions of SOV (Fig. 2). There was an increase in populations of medium and large follicles throughout FSH administration (Day 9 to Day 12) for all SOV protocols, and at Day 12 (last FSH dose) the population of medium follicles was the same for all SOV protocols (Fig. 2). There were fewer large follicles at Day 12 in SOV3 (1.4 ± 1.9), but a similar number in SOV1 (3.3 ± 2.6) and SOV2 (3.0 ± 2.1) (Fig. 2). None of the oestrous parameters differed between successive SOV protocols (Table 1). The percentage of ewes showing ovarian cysts after FSH treatment was higher ($P < 0.05$) at SOV3 than SOV1, and showed a non-significant ($P > 0.05$) trend to be higher at SOV3 than SOV2 (Table. 1).

The number of CL at SOV2 and SOV3 showed strong positive correlations ($r = 0.86$, $r^2 = 0.74$; $P < 00001$) and good agreement (ICC 0.74; $P < 00001$). However, the number of CL declined ($P < 0.05$) after SOV1 (Table 1), and the number of CL at SOV1 did not correlate with the number of CL at SOV2 or SOV3. No premature CL regression was observed. Viable embryo data followed the same pattern, with correlation found only between SOV2 and SOV3 ($r = 0.79$, $r^2 = 0.63$; $P < 00001$), and with fair agreement (ICC 0.56; $P < 0.01$).

Recovery rates were lower ($P < 0.05$) at SOV2 and SOV3 than SOV1 (Table 1). Recovery rate did not differ among females with different degrees of response to the SOV protocol (stratified by number of CL: ≤6, 7–10, >10) (Table 2). Also, there was no difference in recovery rate recorded between the two methods used for CL count (laparoscopy and colour Doppler US) (Table 2). Post-surgical adhesions were observed in the second and third embryo recovery procedures. At the second

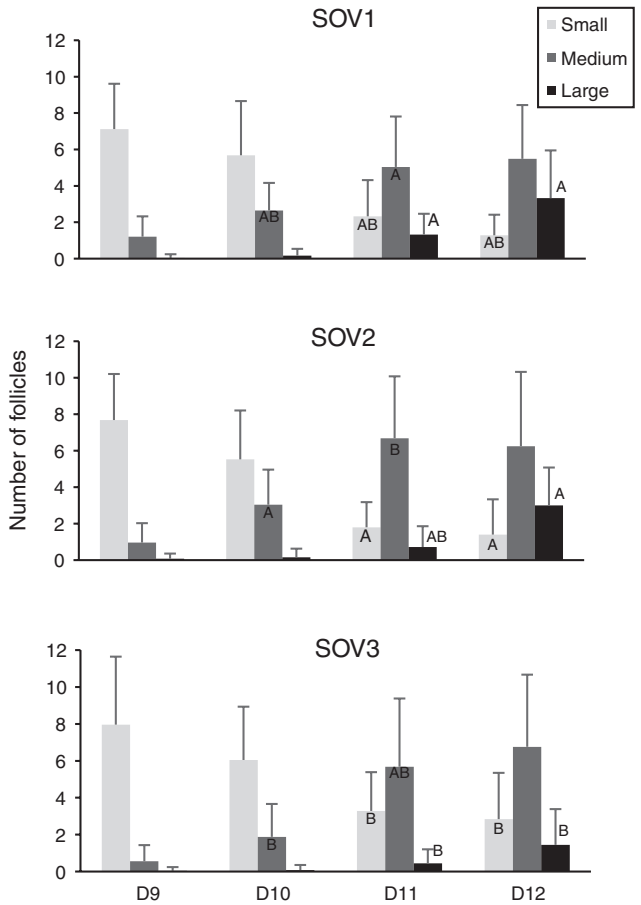

Fig. 2. Mean (± s.d.) population of small (<3 mm), medium (3–5 mm) and large follicles (>5 mm) as assessed by transrectal ultrasonography in Santa Inês ewes at three successive FSH-based superovulation protocols (SOV1, SOV2 and SOV3), with a 21-day interval between each SOV. FSH (200 mg) was administered in six decreasing doses (12 h apart) from Day 9 to Day 12 of the protocol (Days 9, 10, 11 and 12): first dose Day 9 (afternoon) and last dose Day 12 (morning). The categories of follicular population (small, medium or large) were compared only at the same time among SOV repetitions (e.g. medium follicles at Day 11 on SOV1 were statistically evaluated with medium follicles at Day 11 on SOV2 and with medium follicles at Day 11 on SOV3); therefore, within a column, means with the same letter (or no letter) are not significantly ($P > 0.05$) different.

embryo-recovery procedure, four ewes showed adhesions between the omentum and the abdominal wall (right below the incision). At the third embryo-recovery procedure, six ewes showed adhesions between the omentum and the abdominal wall (right below the incision), and two ewes presented adhesions between the uterine horns.

Discussion

Responses at SOV1 were not correlated with subsequent treatments. This finding deviates from other results in the SOV literature. Goats submitted to successive SOV treatments showed highly repeatable responses for the number of CL and the number of collected and transferable embryos (r^2 varying between 0.67 and 0.68; $P < 0.001$) (Monniaux *et al.* 2011). In wool sheep, significant correlations have been reported for ovulation rate ($r = 0.55$, Bari *et al.* 2001; $r = 0.84$, Bruno-Galarraga *et al.* 2014) and for the number of embryos recovered ($r = 0.38$) (Bari *et al.* 2001). Two previous studies submitted the same flock of tropical hair sheep as used in the present study to successive SOV protocols but did not evaluate the repeatability of response, only the total number of embryos produced (Cordeiro *et al.* 2003; Lima *et al.* 2015). Nevertheless, it was reported that ewes failing the first SOV treatment subsequently failed in the second trial (Cordeiro *et al.* 2003). Despite the published data indicating that ovulation rate and embryo yield can be repeatable, under our experimental conditions, no consistent pattern of response was observed for successive SOV protocols. The severe decline in SOV response after the first treatment may have contributed to the lack of correlations between SOV1 and SOV2 or SOV3.

The percentage of non-responding ewes at SOV2 and SOV3 exceeded the number usually reported for Santa Inês sheep (Cordeiro *et al.* 2003; Souza-Fabjan *et al.* 2017). Refractoriness to FSH in successive SOV treatments has not been reported in sheep (Bari *et al.* 2001; Cordeiro *et al.* 2003; Lima *et al.* 2015), and our data did not suggest that either. The increases in the number of medium and large follicles throughout the three SOV protocols show that there was responsiveness to the FSH; however, ovulation did not occur. We hypothesise that the low ovulation rates at SOV2 and SOV3 occurred because the *luteinising hormone* (LH) surge was not enough to induce ovulation. Chakraborty *et al.* (1974) showed that continuous infusion with synthetic LH-releasing hormone diminishes pituitary LH content and concentration. In addition, we found an increase in the number of ovarian cysts at SOV3, indicating that follicular growth was induced but ovulation failed. Besides, our protocol included oestrus synchronisation before each SOV treatment, demanding successive LH surges at short intervals. In studies that used longer periods between SOV treatments, ewes did not show lower ovulation rates in successive treatments (Bari *et al.* 2001; Cordeiro *et al.* 2003, Lima *et al.* 2015). These longer periods between successive treatments may have allowed LH reserves to replenish. Thus, different approaches should be taken to evaluate whether short intervals between induced ovulations can drain the LH reserve to an extent that will hamper subsequent ovulations.

Bergstein-Galan *et al.* (2019), evaluating MOET efficacy in successive programs, did not find an association between the number of times that sheep were submitted to embryo collections and embryo output in subsequent MOET procedures. In their experimental design, the manifestation of at least one physiological oestrous cycle was allowed between MOET protocols, and an eCG dose was administered at the end of the FSH treatment. Such procedures might have a beneficial impact and improve the feasibility of performing successive MOET protocols in sheep. By contrast, owing to the decline in embryo production observed after SOV1, our data suggest that the hormonal protocol and the 21-day interval between SOV treatments applied in our experiment are not suitable for successive MOET programs in young sheep.

We observed post-operative adhesions after successive surgical embryo recovery procedures, which are well

Table 1. Oestrous parameters, number of corpora lutea (CL) and embryo yield of Santa Inês ewes submitted to three successive FSH-based (200 mg total) superovulation (SOV) protocols, naturally mated, and submitted to surgical embryo recovery

Data expressed as percentage, or as mean ± s.d. (range of values or fraction presented as applicable in parentheses). Within a row, means followed by the same letter (or no letter) are not significantly ($P > 0.05$)

	SOV1	SOV2	SOV3	Total
Rate of oestrous response after SOV (%)	88.0 (22/25)	72.0 (18/25)	64.0 (16/25)	74.6 (56/75)
Time to oestrous onset (h)	33.3 ± 7.3 (24–48)	41.3 ± 13.2 (24–72)	41.3 ± 12.4 (24–72)	38.1 ± 11.5
Oestrus length (h)	47.5 ± 19.4 (24–84)	39.3 ± 14.1 (12–60)	42 ± 18.6 (12–72)	43.3 ± 17.7
Rate of ewes that responded to SOV (≥3 CL) (%)	76.0A (19/25)	36.0B (9/25)	24.0B (6/25)	45.3 (34/75)
No. of CL	7.5 ± 4.8A (0–16)	3.0 ± 5.0B (0–20)	2.2 ± 3.5B (0–13)	4.2 ± 5.0
Rate of ovarian cysts (%)	28.0A (7/25)	36.0AB (9/25)	60B (15/25)	41.3 (31/75)
Total no. of recovered structures	5.4 ± 4.4A (0–16)	1.8 ± 4.0B (0–17)	1.2 ± 2.3B (0–9)	2.8 ± 4.1
No. of viable embryos	4.0 ± 3.5A (0–10)	1.2 ± 3.0B (0–12)	1.1 ± 2.1B (0–9)	2.1 ± 3.2
No. of non-fertilised structures	0.2 ± 0.5 (0–2)	0.04 ± 0.2 (0–1)	0	0.1 ± 0.3
No. of degenerated structures	0.8 ± 1.5 (0–6)	0.2 ± 1.2 (0–6)	0	0.3 ± 1.1
Recovery rate (%)	75.5A (0–100)	52.1B (0–100)	48.4B (0–69.2)	64.5
Viability rate (%)	73.1 (0–100)	74.7 (0–100)	89.3 (75–100)	77.1
Non-fertilisation rate (%)	5.0 (0–50)	1.0 (0–5.9)	0	3.1
Degeneration rate (%)	12.9 (0–50)	16.7 (0–100)	0	10.7

Table 2. Recovery rate (%) determined using two methods of corpora lutea (CL) count in Santa Inês ewe lambs submitted to three successive FSH-based superovulation (SOV) protocols

Means followed by the same letter are not significantly different: lowercase letters are for comparison among CL categories (≤6, 7–10, >10) within method of CL count; upper case letters are for comparison between methods of CL count within CL category. Owing to the low number of observations in each CL category at SOV2 and SOV3, statistical analysis was performed only for SOV1. n.a., not available (no ewe in this category)

	Determined by laparoscopy				Determined by colour Doppler ultrasonography			
CL count:	≤6	7–10	>10	Total	≤6	7–10	>10	Total
SOV1	73.3 (5)aA	75.7 (7)aA	76.9 (7)aA	75.5 (19)	60.4 (8)aA	70.8 (7)aA	87.2 (6)aA	71.5 (21)
SOV2	43.3 (5)	85.7 (1)	55.6 (3)	52.1 (9)	60.0 (2)	60.7 (4)	100.0 (1)	66.1 (7)
SOV3	33.5 (2)	51.4 (3)	69.2 (1)	48.4 (6)	47.2 (3)	59.0 (4)	n.a. (0)	53.9 (7)
Total	54.2	70.0	70.4	64.5	57.3	65.0	89.0	66.9

documented to lead to lower recovery rates (Torres and Sevellec 1987; Bruno Galarraga *et al*. 2014). Adhesions can even make it infeasible to flush uterine horns in some ewes (Forcada *et al*. 2011; Bruno Galarraga *et al*. 2014). In our study, despite the decrease in recovery rate, even the ewes that were submitted to three surgical interventions were still able to have the uterine horns exposed and flushed. No statistical difference was observed in the recovery rates among groups with different intensities of SOV response (i.e. CL count ≤6, 7–10, >10) or between the two methods for CL count (laparoscopy and colour Doppler US). It therefore is possible to estimate the number of embryos that will be recovered using the number of CL determined by colour Doppler US or by laparoscopy, regardless of the intensity of the SOV response. Reliable estimation of the number of recoverable embryos can contribute to the organisation of procedures and inform the destiny of embryos. Such logistic support is especially important in embryo technology research.

Conclusions

We could not determine the circumstances in which the repeatability of response will occur. The decline in embryo production observed after the first SOV may indicate that the hormonal protocol used, associated with a 21-day interval between SOV treatments, is not suitable for successive MOET programs in young sheep. The intensity of the SOV response did not affect the recovery rate, and the number of CL estimated by colour Doppler US can be used to calculate the recovery rate.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank the Federal Fluminense University, Infra-Laboratory Pesq/PROPPI, Embrapa (Project 02.13.06.026.00.02), FAPERJ, and CNPq (400785/2016-1) for funding the project. FZB is fellow of the CNPq, JMGSF of CAPES, and EKNA of FAPERJ.

References

Balaro MFA, Fonseca JF, Barbosa TGB, Souza-Fabjan JMG, Figueira LM, Teixeira TA, Cavalheira LR, Brandão FZ (2016) Potential role for GnRH in the synchronization of follicular emergence before the superovulatory Day 0 protocol. *Domestic Animal Endocrinology* **54**, 10–14. doi:10.1016/j.domaniend.2015.07.002

Bari F, Khalid M, Wolf B, Haresign W, Murray A, Merrel B (2001) The repeatability of superovulatory response and embryo recovery in sheep. *Theriogenology* **56**, 147–155. doi:10.1016/S0093-691X(01)00550-7

Bergstein-Galan TG, Weiss RR, Kozicki LE (2019) Effect of semen and donor factors on multiple ovulation and embryo transfer (MOET) in sheep. *Reproduction in Domestic Animals* **54**, 401–407. doi:10.1111/rda.13381

Bruno-Galarraga MM, Cueto M, Gibbons AE, Pereyra-Bonnet F, Catalano R, González-Bulnes A (2014) Repeatability of superovulatory response to successive FSH treatments in Merino sheep. *Small Ruminant Research* **120**, 84–89. doi:10.1016/j.smallrumres.2014.04.002

Bruno-Galarraga M, Cueto M, Gibbons A, Pereyra-Bonnet F, Subiabre M, González-Bulnes A (2015) Pre-selection of high and low ovulatory responders in sheep MOET programs. *Theriogenology* **84**, 784–790. doi:10.1016/j.theriogenology.2015.05.011

Chakraborty PK, Adams TE, Tarnavsky GK, Reeves JJ (1974) Serum and pituitary LH concentrations in ewes infused with LH-RH/FSH-RH. *Journal of Animal Science* **39**, 1150–1157. doi:10.2527/jas1974.3961150x

Cognié Y, Baril G, Poulin N, Mermillod P (2003) Current status of embryo technologies in sheep and goat. *Theriogenology* **59**, 171–188. doi:10.1016/S0093-691X(02)01270-0

Cordeiro MF, Lima-Verde JB, Lopes-Junior ES, Teixeira DIA, Farias LN, Salles HO, Simplício AA, Rondina D, Freitas VJF (2003) Embryo recovery rate in Santa Inês ewes subjected to successive superovulatory treatments with pFSH. *Small Ruminant Research* **49**, 19–23. doi:10.1016/S0921-4488(03)00059-2

Forcada F, Ait Am.-Meziane M, Abecia JA, Maurel MC, Cebrián-Pérez JA, Muiño-Blanco T, Asenjo B, Vázquez MI, Casao A (2011) Repeated superovulation using a simplified FSH/eCG treatment for *in vivo* embryo production in sheep. *Theriogenology* **75**, 769–776. doi:10.1016/j.theriogenology.2010.10.019

González-Bulnes A, Veiga-Lopez A, Garcia P, Garcia-Garcia RM, Ariznavarreta C, Sanchez MA, Tresguerres JAF, Cocero MJ, Flores JM (2005) Effects of progestagens and prostaglandin analogues on ovarian function and embryo viability in sheep. *Theriogenology* **63**, 2523–2534. doi:10.1016/j.theriogenology.2004.10.013

Lima JTM, Fonseca JF, Balaro MFA, Esteves LV, Ascoli FO, Leite CR, Ribeiro ACS, Delgado KF, Souza-Fabjan JMG, Torres Filho RA, Brandão FZ (2015) Effect of natural mating or laparoscopic artificial insemination in superovulated Santa Inês ewes on superovulatory response, fertility and embryo viability. *Animal Production Science* **56**, 1463–1468. doi:10.1071/AN14827

Menchaca A, Vilariño M, Pinczak A, Kmaid S, Saldaña JM (2009) Progesterone treatment, FSH plus eCG, GnRH administration, and Day 0 Protocol for MOET programs in sheep. *Theriogenology* **72**, 477–483. doi:10.1016/j.theriogenology.2009.04.002

Monniaux D, Baril G, Laine AL, Jarrier P, Poulin N, Cognié J, Fabre S (2011) Anti-Mullerian hormone as a predictive endocrine marker for embryo production in the goat. *Reproduction (Cambridge, England)* **142**, 845–854. doi:10.1530/REP-11-0211

Oliveira MEF, Ribeiro IF, Rodriguez MGK, Maciel GS, Fonseca JF, Brandão FZ, Bartlewski PM (2018) Assessing the usefulness of B-mode and colour Doppler sonography, and measurements of circulating progesterone concentrations for determining ovarian responses in superovulated ewes. *Reproduction in Domestic Animals* **53**, 742–750. doi:10.1111/rda.13165

Pinto PHN, Balaro MFA, Souza-Fabjan JMG, Ribeiro LS, Bragança GM, Leite CR, Arashiro EKN, Silva KM, Fonseca JF, Brandão FZ (2018*a*) Anti-Müllerian hormone and antral follicle count are more effective for selecting ewes with good potential for *in vivo* embryo production than the presence of $FecG^{E}$ mutation or eCG pre-selecting tests. *Theriogenology* **113**, 146–152. doi:10.1016/j.theriogenology.2018.02.018

Pinto PHN, Bragança GM, Balaro MFA, Arashiro EKN, Santos GB, Souza GN, Souza-Fabjan JMG, Fonseca JF, Brandão FZ (2018*b*) Colour-Doppler ultrasound imaging as a laparoscopy substitute to count corpora lutea in superovulated sheep. *Reproduction in Domestic Animals* **53**, 266–269. doi:10.1111/rda.13089

Souza-Fabjan JMG, Rosa RM, Balaro MFA, Pinto PHN, Santos GB, Arashiro EKN, Fonseca JF, Ungerfeld R, Brandão FZ (2017) Effect of different hormonal combinations on follicular wave emergence and superovulatory response in sheep. *Theriogenology* **103**, 24–29. doi:10.1016/j.theriogenology.2017.07.036

Taneja M, Bols PEJ, Velde AV, Ju JC, Schreiber D, Tripp MW, Levine H, Echelard Y, Riesen J, Yang X (2000) Developmental competence of juvenile calf oocytes *in vitro* and *in vivo*: influence of donor animal variation and repeated gonadotropin stimulation. *Biology of Reproduction* **62**, 206–213. doi:10.1095/biolreprod62.1.206

Torres S, Sevellec C (1987) Repeated superovulation and surgical recovery of embryos in the ewe. *Reproduction, Nutrition, Development* **27**, 859–863. doi:10.1051/rnd:19870612

Handling editor: Rodolfo Ungerfeld