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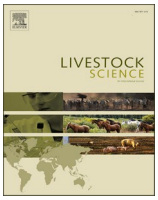

Occurrence of premature regression of corpus luteum in MOET programs in Dorper ewes under subtropical climate

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HIGHLIGHTS

- Premature regression of corpus luteum (PRCL) affected 25% of superovulated Dorper ewes;.
- PRCL occurrence was not associated to sheep donor age and body condition score;.
- PRCL occurrence was not associated to the season (breeding, transition, or non-breeding);.
- PRCL occurrence was not associated to the number of MOET procedures (one to five) within the same donor.

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ABSTRACT

Premature regression of corpus luteum (PRCL) is a functional alteration that occurs mainly in superovulated females hampering the widespread use of multiple ovulation and embryo transfer (MOET). This study evaluated the association of donor age (3–9 years old), body condition score (2 to 4.5), number of MOET (1 to 5) repetitions and season (breeding, transition, or non-breeding) with the occurrence of PRCL in ewes. Multiparous Dorper ewes ($n = 44$) were subjected to MOET during four consecutive years, with the same superovulatory treatment. Before embryo collection, ovaries were laparoscopically performed to assess the corpora lutea (CL). Overall, the total PRCL occurrence was 24.7% (26/105) and it had no association with the studied factors. Percentages of ewes that presented PRCL once or twice were 45.5% (20/44) and 6.8% (3/44), respectively, while the percentage of ewes that had no PRCL was 47.7% (21/44). Proportions of PRCL were similar ($P > 0.05$) among breeding (21%), transition (31%), and non-breeding (24%) season; and also ($P > 0.05$) in lower/thin (20%), average/good (24%), and higher/fat (29%) females. In conclusion, although this phenomenon had no association to the factors studied, the occurrence of PRCL affects ~25% of ewes subjected to MOET procedures and future alternatives to circumvent this obstacle need to be developed.

1. Introduction

The implementation of reproductive technologies has the potential to enhance the productive efficiency of sheep farming by accelerating genetic gain, increasing selective pressure, and decreasing the generation interval (Smith, 1988). Despite all these advantages, the use of multiple ovulation and embryo transfer (MOET) on a commercial scale is still impaired by the variability in the individual responses to gonadotropins, unsuccessful fertilization, and occurrence of premature regression of corpus luteum (PRCL) (Forcada et al., 2011). Females subjected exactly to the same treatment present different responses due to intrinsic or extrinsic factors as age, breed, climate, and interval between protocols (Bartlewski et al., 2016).

The PRCL is a luteal dysfunction characterized by low progesterone secretion (< 1.5 ng/mL) and occasionally alteration in the estrous cycle duration, commonly verified in the transition from anestrous to cyclic

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conditions, as in puberty, after seasonal or lactational anestrous (Baird, 1992). Of note, it is frequently observed in superovulated (SOV) sheep (Okada et al., 2000; Forcada et al., 2011), but its exact cause is not fully understood. The PRCL may occur partially or totally in the ovaries, affecting either some or all the CLs, respectively (Oliveira et al., 2018). Various studies have been carried out to elucidate the connection between PRCL during SOV programs and factors such as: use of different SOV treatments (Forcada et al., 2011), porcine follicle-stimulating hormone (pFSH) doses (Rodriguez et al., 2018), age of donor ewes (Lopes Júnior et al., 2006), breeds and season (Okada et al., 2000), or type of estrus, either natural, induced or in a random day (Quan et al., 2011).

Assessment of the CL morphology by laparoscopy before embryo collection allows the identification of reddish protruding and vascularized CL (normal/functional) or pale whitish small and avascular CL (abnormal/PRCL) (Oliveira et al., 2018). This CL evaluation is crucial in order to determine if the females will whether be subjected to the embryo collection, since it is well known that PRCL leads to low embryo recovery rate (Souza-Fabjan et al., 2017) and a reduction in embryo quality in terms of number of transferable embryos (Quan et al., 2011). This study evaluated the association of different intrinsic and extrinsic factors with the occurrence of PRCL in Dorper ewes subjected to MOET programs during four consecutive years, under subtropical climate.

2. Materials and methods

2.1. Ethics, period, location

This study was approved by the Animal Care Committee of the School of Veterinary Medicine and Animal Science of the University of São Paulo (# 2717181220). The study was performed over four years (2017–2020) in a commercial sheep herd in São Luís do Paraitinga (23°22′ S and 45°26′ W), state of São Paulo, Brazil. The ewes were kept in intensive management with corn silage and balanced feed in addition to mineral salt and water *ad libitum*.

2.2. Experimental design

Multiparous Dorper ewes ($n = 44$) aging 3 to 9 years old and presenting body condition score (BCS) from 2 to 4.5 were subjected to MOET programs, with the same SOV protocol, over four years. The ewes were subjected to the procedure at least once and at maximum five times, with a minimum interval of four months between MOET, totalling 105 procedures. Before each session, the ewes had their age and BCS recorded as well as the season. The BCS was categorized as lower/thin (2 to 2.5), average/good (3 to 3.5) and higher/fat (4 to 4.5) and the season as breeding (February to July), transition (January and August), or non-breeding (September to December). The effect of age and MOET repetition within each animal were also assessed. The same technician performed the CL evaluation and embryo collection throughout the study.

2.3. Superovulation, artificial insemination, and CL evaluation

On a random day of the estrous cycle or anovulatory period (Day 0), the ewes received an intravaginal device containing 0.33 mg of progesterone (CIDR®, Zoetis, São Paulo, Brazil). On Day 7, the device was replaced for a new one and the ewes received 0.24 mg of cloprostenol (Sincrocio®, Ourofino, São Paulo, Brazil) intramuscularly (i.m.). The SOV treatment with 256 mg of pFSH (Folltropin®, Vetoquinol, São Paulo, Brazil) started on Day 12 and consisted in eight decreasing doses (20/20/15/15/10/10/5/5%) administered i.m. every 12 h for four days. On Day 14, 200 IU of eCG (Novormon®, Zoetis, São Paulo, Brazil) i.m. was given at device removal and 0.1 mg of GnRH (Fertagyl®, MSD, São Paulo, Brazil) i.m. on Day 15 (Fig. 1).

The laparoscopic AI was performed twice (36 and 42 h after removal of the progesterone device) on Day 16 using commercial cooled semen. Five days after AI, ovaries were assessed by laparoscopy to check the presence and morphology of CL, according to Oliveira et al. (2018). Ewes that had only small, pale, and pinkish to whitish color CL were classified with PRCL, while those presenting protruding, bright, and reddish color CL were categorized as having functional CL. Only the latter were submitted to the embryo recovery procedure.

2.4. Statistics

The association of different ages, categories of BCS, seasons and MOET repetition with occurrence of PRCL was assessed. Effect of age was checked by logistic regression, BCS by Fisher test, and season and MOET repetition by Kappa test. Statistical analyses were performed using the Bio Estat 5.0 software and values of $P < 0.05$ were considered significant.

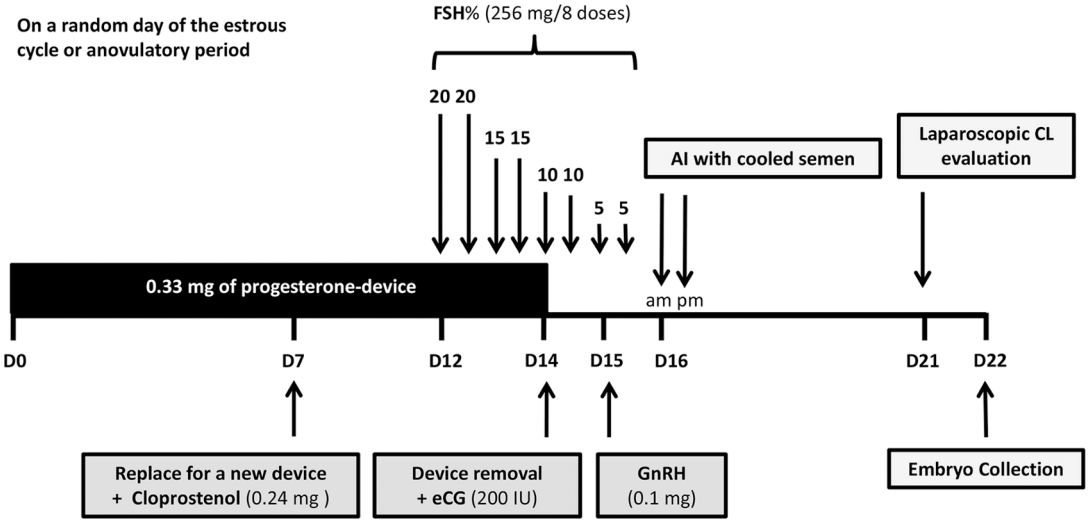

Fig. 1. Schematic presentation of superovulatory protocol in Dorper ewes submitted to artificial laparoscopic insemination followed by corpus luteum evaluation and surgical embryo collection. AI: artificial insemination; CL: corpus luteum; eCG: equine chorionic gonadotropin; FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing hormone.

Table 1
The occurrence of total premature regression of corpora lutea (PRCL) in Dorper ewes subjected to multiple ovulation and embryo transfer (MOET) programs during four consecutive years under subtropical climate.

Repetitions *	Ewes**	Ewes with PRCL by repetition (%)	PRCL once (%)***	PRCL twice (%)***	Total PRCL	Total MOET
1	13	3 (23)	3 (23)	—	3	13
2	14	10 (71)	8 (57)	2 (14)	12	28
3	7	5 (71)	5 (71)	0 (0)	5	21
4	7	4 (57)	3 (43)	1 (14)	5	28
5	3	1 (33)	1 (33)	0 (0)	1	15
Total	44	23	20	3	26	105

* number of times each ewe was subjected to MOET procedure (1 to 5);.
** number of ewes subjected to each MOET repetitions (1 to 5);.
*** number of ewes presenting PRCL for the first (once) or second (twice) time through repetitions.

3. Results

From 105 laparoscopy evaluations, the PRCL was identified in 26 procedures, totalling 24.7% of occurrence. Percentages of ewes that presented PRCL once or twice were 45.5% (20/44) and 6.8% (3/44), respectively, while the percentage of ewes that had no PRCL was 47.7% (21/44) (Table 1). No ewes presented PRCL more than twice. There was no difference ($P > 0.05$) in the proportions of PRCL among breeding season (21%, 10/48), transition period (31%, 10/32) and non-breeding season (24%, 6/25). The BCS did not affect ($P > 0.05$) the occurrence of PRCL, being similar to lower/thin (20%, 2/10), average/good (24%, 16/67) and higher/fat (29%, 8/28) females. Similarly, there was no association ($P > 0.05$) between the PRCL occurrence and the age of donors.

4. Discussion

The present study evaluated the association of the occurrence of PRCL in ewes submitted to MOET programs during four consecutive years with factors such as age and BCS of donor female, season, and number of MOET repetitions. Overall, the total PRCL occurrence was 24.7% and it had no association with the studied factors. This percentage was similar to 22.6% of ewes of different breeds presenting abnormal CL (Okada et al., 2000) and slightly higher than the 12.5% reported in Santa Inês ewes (Oliveira et al., 2018) and 9.6% in Ojalada Soriana ewes (Forcada et al., 2011), all studies applying SOV/MOET. Importantly, the complete CL regression after SOV treatment impaired the *in vivo* production as it led to a low number of transferable embryos. In addition, regarding animal welfare and the possibility of abdominal adhesions (Quan et al., 2011), donors should not be subjected to surgical embryo collection without an assessment of the SOV response and CL functionality. Therefore, the PRCL is an obstacle seen on the efficiency of MOET programs in sheep.

In the current study, the occurrence of PRCL could not be associated with season and MOET repetition. Similarly, the prevalence of ewes presenting abnormal CL in spring and autumn did not differ (Okada et al., 2000). Quan et al. (2011) reported no effect of the season and in the first, second, or third SOV treatment on the number of transferable embryos per ewe, although repetition for three times affected surgical embryo recovery, probably to adherences found. Curiously, it was earlier demonstrated that the number of ewes with PRCL was different in those receiving distinct SOV protocols (Forcada et al., 2011), but, as described above, in the present study the same SOV treatment was applied over the four years, "blocking" this effect.

Both age and BCS did not affect the occurrence of PRCL in the present study. Similarly, no difference on the PRCL rate was reported between different age groups (1–2 vs. 3–4 years old) (Lopes Júnior et al., 2006) and in those SOV-ewes receiving normal diet *vs.* half energetic diet, although the number of transferable embryos was lower in undernourished ewes (Abecia et al., 2013). The absence of any effect in these variables in our study is probably related to the fact that all the donors were multiparous and their BCS did not involve extremes of undernutrition or obesity.

Considering that in the commercial system where the study was performed a historical average of eight viable embryos per MOET procedure are normally obtained (data not shown), in 26 procedures, we can estimate that approximately 208 viable embryos were not collected due to the relatively high PRCL occurrence, being a substantial financial loss for the producer.

5. Conclusion

Under the conditions of our study, the occurrence of PRCL affects ~25% of ewes subjected to MOET procedures and future alternatives to circumvent this obstacle need to be developed. This phenomenon had no association to the factors studied such as season, donor age, BCS, and number of MOET repetitions.

CRediT authorship contribution statement

Marcela S. Rocha: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft. **Ana Lucia R.S. Maia:** Methodology, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Paulo Sérgio C. Rangel:** Formal analysis, Investigation, Data curation, Writing – review & editing. **Maria Emilia F. Oliveira:** Formal analysis, Visualization, Writing – review & editing. **Jeferson F. Fonseca:** Formal analysis, Visualization, Writing – review & editing. **Cláudio A. Oliveira:** Supervision. **Joanna M.G. Souza-Fabjan:** Conceptualization, Supervision, Visualization, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition.

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