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SHORT COMMUNICATION

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Early resynchronization protocols for goats: Progestogens can be used prior to an early pregnancy diagnosis without affecting corpus luteum functionality

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Abstract

This study aimed to evaluate the exogenous progesterone (P4) effect on the luteal function from Day 16 to Day 21 of the oestrous cycle in inseminated goats with unknown pregnancy status. A total of 54 does passed through a short progestin-based synchronization protocol and, on Day 16 of the following oestrous cycle, 27 does received a new P4 device which was retained until Day 21. Blood samples were collected daily from all does during this period, as well as on Day 24. Pregnancy diagnoses were performed on Day 30. Serum P4 values from 26 animals (G_{NPSP}: Group of non-pregnant does with second sponge: $n = 8$; G_{NPNSP}: Group of non-pregnant does without second sponge: $n = 6$; G_{PSP}: Group of pregnant does with second sponge: $n = 5$; G_{PNSP}: Group of pregnant does without second sponge: $n = 7$) were determined by radioimmunoassay commercial kits. No P4 differences were found between groups (G_{NPSP}: 3.1 ± 2.8; 1.7 ± 1.8; 0.4 ± 1.0; and 0.0 ± 0.0 vs. G_{NPNSP}: 4.4 ± 1.8; 3.0 ± 2.2; 0.8 ± 0.8; and 0.0 ± 0.0 or G_{PSP}: 4.2 ± 1.0; 3.4 ± 0.6; 3.3 ± 1.6; 3.2 ± 0.9; 3.6 ± 1.2; 3.5 ± 1.3; 2.7 ± 1.3 vs. G_{PNSP}: 4.4 ± 1.6; 3.6 ± 1.5; 3.7 ± 1.5; 3.8 ± 1.4; 3.2 ± 1.2; 3.1 ± 1.2; 3.6 ± 1.1; D16, D17, D18, D19, D20, D21, D24, respectively) or for the interaction of group and time. In conclusion, a second progestogen device had no effect on luteolysis or early pregnancy in the following oestrous cycle.

KEYWORDS

medroxyprogesterone acetate, serum P4, ultrasound

1 | INTRODUCTION

Resynchronization protocols have been studied and successfully performed in cattle since the beginning of the century (Bartolome et al., 2005; Sani et al., 2011; Stevenson et al., 2003). With variations in timing and procedures, such protocols are primarily used to reduce the calving interval by inducing more synchronic ovulations during the breeding season. Hence, early resynchronization protocols are being studied to allow fixed time artificial insemination (FTAI) at intervals similar to the natural oestrous cycle (Palhão et al., 2020; Pugliesi et al., 2019), for which a new progesterone (P4) device should be inserted before the pregnancy diagnosis.

The corpus luteum (CL) is a temporary endocrine gland of high importance in the maintenance of pregnancy, especially for goats, since ewes and cows can sustain late pregnancy without it (Casida & Warwick, 1945; Drummond-Robinson & Asdell, 1926; Estergreen

et al., 1967; Meites et al., 1951). Knowledge of its functionality has led to the implementation of new hormonal protocols. However, studies in which exogenous P4 administration is used indicate concerns about its effect on CL in early pregnancy: a P4 device inserted as early as three days after oestrous manifestation in cows may lead to both better conceptus development and early luteolysis (O'Hara et al., 2014; Pugliesi et al., 2014). However, when it is used at the end of the oestrous cycle, aiming at a resynchronization protocol, no effect on CL has been detected (Sani et al., 2011; Stevenson et al., 2003).

In ewes, even though Miranda et al. (2018) demonstrated a negative effect on P4 production, Cosentino et al. (2019) did not. Both studies concluded that the use of a second progestogen device from Day 12 to Day 17 of the cycle had no effects on pregnancy or luteolysis, enabling its use in the early resynchronization protocol. However, it has not been studied for goats, and we hypothesize that, as with ewes, a new P4 device inserted early in does would not have a negative effect on CL viability and functionality. Therefore, this study aimed to evaluate the effect of a second progestogen device inserted late in the oestrous cycle on goats' CL functionality, and consequently in their not yet diagnosed pregnancy or sub-sequential luteolysis.

2 | MATERIALS AND METHODS

All procedures performed in this study were given prior approval by the Ethical Committee for Animal Use of the Universidade Federal Fluminense (Protocol 1021) and were carried out under the ethical principles of the Sociedade Brasileira de Experimentação Animal. Moreover, this manuscript followed the guidelines laid out in Animal Research: Reporting of In Vivo Experiments (Kilkenny et al., 2010).

This study was carried out in a dairy goat farm located in the state of Rio de Janeiro, Brazil (22°07′50.2″S, 42°47′47.1″W) during the transition season (January–February/2019). According to Köppen (1948), the local climate is tropical hot-humid (Aw). For this study, a total of 54 primiparous and multiparous Saanen goats [2.7 ± 0.5 years old; body condition score: 2.9 ± 0.3 [scale 1–5; (Suiter, 1994)]; bodyweight 60 ± 5 kg; 7.7 ± 3.6 months post-partum (mean ± *SD*)] were used. All animals had previously undergone a gynecological examination and only does without reproductive abnormalities detected by ultrasonography (US) or clinical examination were used. Throughout the study, does were kept confined in collective pens and fed twice a day with corn silage, as well as concentrate according to their maintenance requirements [16% crude protein; National Research Council (NCR), (2007)]. Water and mineral salt for goats (Caprinofós) were provided ad libitum.

Intravaginal sponges containing 60 mg of medroxyprogesterone acetate (MAP: Progespon; Schering Plough) were used for 6 days. One day before sponge withdrawal, 200 IU of equine chorionic gonadotropin (eCG, Folligon, MSD) and 0.24 mg of cloprostenol sodium (Estron, Agner União) were administered intramuscularly (i.m.). Thirty-four hours after sponge withdrawal, the females received 0.025 mg of lecirelin (GnRH: Gestran Plus, Tecnopec) and 52 hr after the sponge withdrawal, they were inseminated with a 0.25 ml straw of commercial frozen semen (5.0×10^7 alive spermatozoa after defrost). From Day 16 to Day 21 of the following oestrous cycle, half the does received a new MAP device. General proceedings are described in Figure 1.

Ovarian US was conducted every 8 hr after sponge withdrawal until the moment of ovulation, which was taken to be Day 0 (55.0 ± 8.5 hr after sponge withdrawal) of the oestrous cycle. US scans were performed using a portable device (Sonoscape S6) with a 7.5 MHz linear rectal transducer adapted for use in small ruminants, with does in a standing position. All does were re-examined

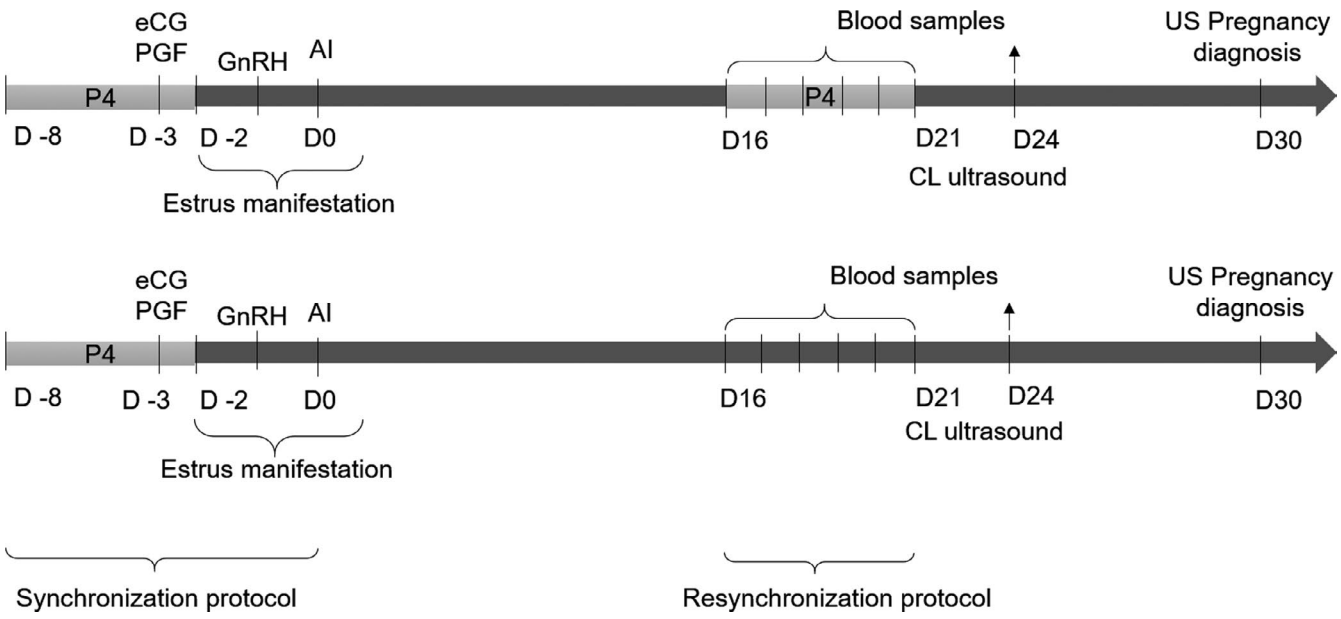

FIGURE 1 Experimental proceedings timeline

on Days 16, 21 and 24 for assessment of luteal morphology (score 1: near anechoic, heterogeneous and coarse granulation; 2: hypoechoic, homogeneous and fine granulation; and 3: echogenic, homogeneous and fine granulation) and blood flow (score 1: 0% to 25% vascularization; 2: 25% to 50%; 3: 50% to 75%; and 4: 75% to 100%), with scores above 2 being related to pregnancy (Cosentino et al., 2018). The first evaluation (Day 16) aimed to count the viable CLs before the second P4 device; the second evaluation (Day 21) was for early pregnancy diagnosis at time of sponge removal; and the third evaluation (Day 24) was to evaluate possible early pregnancy losses and further effects of the second P4 device. The last US scan was performed on Day 30 for pregnancy diagnosis by evaluation of the embryo vesicle and heartbeat. After the Day 30 pregnancy diagnosis, the does were classified in four groups: G_{NPSP}: Group of non-pregnant does with second sponge; G_{NPNSP}: Group of non-pregnant does without second sponge; G_{PSP}: Group of pregnant does with second sponge; and G_{PNSP}: Group of pregnant does without second sponge.

Blood samples were collected daily from Day 16 to Day 21 and on Day 24 by jugular venipuncture using tubes (without anti-coagulant) with a vacuum system. Blood samples were centrifuged at 1,000 *g* for 15 min; serum was separated and stored at −20°C until analysis. The serum P4 concentration from 26 animals (G_{NPSP} = 8; G_{NPNSP} = 6; G_{PSP} = 5; G_{PNSP} = 7) was determined by radioimmunoassay using commercial kits (MP Biomedicals, LLC, Diagnostics Division). Sensitivity and intra-assay coefficient of variation were 0.05 ng/ml and 12%, respectively. All data were within minimum and maximum points of the curve. CL were considered functional when production was above 1.0 ng/ml.

Data were analysed using a statistical program for statistical analyses (BioEstat®). Lilliefors and Bartlett tests were used to verify the normality and homoscedasticity of variables, respectively. P4 values were analysed using a mixed model procedure including the group (treated or not treated), time (from Day 16 to Day 21, and Day 24) and their interaction (groups vs. time) as main effects in the model. Fisher's LSD test was used for individual mean comparisons. Score data (CL evaluation) were assessed by Fisher's exact test. For all tests, $p < .05$ was considered significant.

3 | RESULTS

A total of 15/54 females did not present signs of oestrous, of which only four did not present mucus production and cervix dilatation at

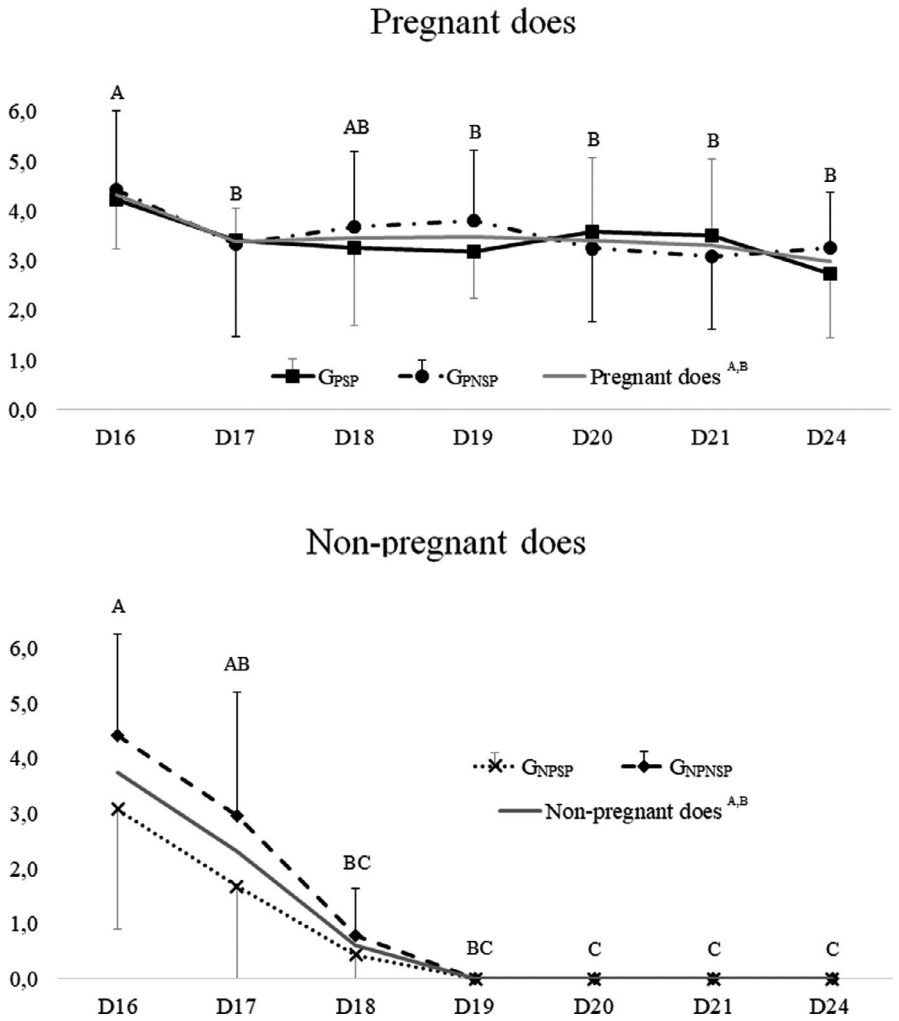

FIGURE 2 Serum progesterone concentration (ng/ml) from Day 16 to Day 21 and on Day 24, in pregnant (n = 12) and non-pregnant (n = 14) does. An intravaginal sponge impregnated with medroxyprogesterone acetate was inserted in does from the G_{NPSP} and G_{PSP} groups. G_{NPSP}: Group of non-pregnant does with second sponge: n = 8; G_{NPNSP}: Group of non-pregnant does without second sponge: n = 6; G_{PSP}: Group of pregnant does with second sponge: n = 5; G_{PNSP}: Group of pregnant does without second sponge: n = 7. ^{A,B}different letters over time when considering the treatments together (silver line) by Student's t test ($p < .05$)

the moment of FTAI. On Day 16, eight females did not present CL, of which only one did not present mucus at the moment of FTAI. Of the 14 does diagnosed pregnant on Day 21, 13 were confirmed on Day 24 (93%), and 12 on Day 30 (86%), both from the group without a second sponge (22% of pregnancy: 12/54). No differences were found between groups (G_{NPSP} vs. G_{NPNSP} or G_{PSP} vs. G_{PNSP}: $p < .05$) both for P4 values (Figure 2) and the presence of vascularized CL on Days 16, 21, 24 and 30. Pregnant does presented 100% of vascularized CL on all days (5/5 G_{PSP} and 7/7 G_{PNSP}); non-pregnant does G_{NPSP} presented 72.7% (16/22) on Day 16 and 0% on the others; and G_{NPNSP} presented 90% (18/20), 10% (2/20), 5% (1/20) and 0%, respectively. The CL vascularization was related to P4 values above 1.0 ng/ml for all does. Nor did the interaction between group and time show any differences for the P4 analysis. However, P4 values differ over time, when considering the treatments together (Figure 2).

4 | DISCUSSION

To the author's knowledge, this is the first study performed in goats which aims to investigate the effect of a second P4 device on CL functionality for further application in early resynchronization protocols. Our current results show that the second P4 device has no effect on luteolysis or early pregnancy from the first FTAI in Saanen goats. Considering mucus production and cervix dilatation, it is possible to say that the majority of does responded to the synchronization protocol, even though not all of them presented signs of oestrous, which can be explained by social ranking (Zuñiga-Garcia et al., 2020) or a silent heat. However, when analysing the presence of CL on Day 16, it is not possible to say whether those females did not respond at all, or whether the absence of CL was due to early regression. Since none of the females without mucus at FTAI became pregnant, both are possible.

Miranda et al. (2018) proposed a negative effect of MAP devices on P4 production in ewes (5.8 ± 0.5 ng/ml for control group vs. 4.7 ± 0.5 ng/ml for MAP group on Day 15), although without negative effects on the final percentage of pregnant females. A later study found no effect on P4 values either on pregnancy or luteolysis in sheep species (Cosentino et al., 2019). The results of the present study indicate that the use of a second P4 device does not imply negative effects on ovarian activity, since no difference was found between P4 values in does that received the second device and those which did not (G_{NPSP} vs. G_{NPNSP} or G_{PSP} vs. G_{PNSP}). The differences found over time were observed in both groups for each category, indicating that these were physiological and not induced by the MAP device. Unlike in heifers, when the progestogen device is used during early metoestrous (Day 3 to 7 after ovulation), during which the presence of exogenous P4 is enough to affect the CL development and therefore decrease its size and lifespan (O'Hara et al., 2014), the use in mid/late dioestrus does not affect the functionality and viability of the mature CL (Burke et al., 1999). Neither does it enhance the conceptus development or τ-interferon production (Mann et al., 2006). It could be expected that the exogenous progestogen would downregulate LH release and therefore impact the P4 production, since its production is partially dependent on LH levels (Wiltbank et al., 2012). In this sense, we feared that, by the time of sponge withdrawal, the CL from pregnant does could have been affected and would suffer luteolysis. However, the Day 24 P4 serum concentration and Day 30 ultrasound suggest that the MAP device had no deleterious effect.

Additionally, both females diagnosed as pregnant on Day 21 by luteal evaluation and finally diagnosed as non-pregnant on Day 24 and Day 30 did not receive the second MAP device. In this case, the change in diagnosis may be explained by the fact that those does presented long lifespan CL or early foetal loss; however, neither was caused by the use of external progestogen. Also, the P4 value of above 1 ng/ml and vascularization above score 2 (Cosentino et al., 2018) confirm luteal functionality even after the second progestogen device. These results endorse the finding that the second P4 device had no negative effects on CL function and viability, which enables its use in resynchronization protocols.

In conclusion, a second P4 device can be used in goats with unknown pregnancy status without affecting early pregnancy, luteolysis or endogenous P4 production. Therefore, a second P4 device can be used for resynchronization protocols in goats.

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CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTIONS

IOC discussed the general study design, organized the experimental procedures, collected data, revised and worked on the preparation of the manuscript, and approved the final version. MFAB discussed the general study design, collected data, revised and worked on the preparation of the manuscript, and approved the final version. FSCL, LFCB, FMG, GFF and MMN collected data, revised and worked on the preparation of the manuscript, and approved the final version. FZB discussed the general design, revised and worked on the preparation of the manuscript, and approved the final version.

DATA AVAILABILITY

No additional data are available.

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