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Use of oxytocin to attain cervical dilation for transcervical embryo transfer in sheep

Vanessa M. B. dos Santos[1] | Pedro Henrique N. Pinto[1] | Mario Felipe A. Balaro[1] | Juliana D. R. Santos[1] | Augusto R. Taira[1] | Caroline G. do Espirito Santo[1] | Fernanda M. Gonçalves[1] | Jeferson F. da Fonseca[2] | Felipe Z. Brandão[1]

[1]Faculdade de Veterinária, Universidade Federal Fluminense, Niterói, Brazil

[2]Embrapa Caprinos e Ovinos, Núcleo Regional Sudeste, Coronel Pacheco, Brazil

Correspondence
Felipe Z. Brandão, Faculdade de Veterinária, Universidade Federal Fluminense, Rua Vital Brazil Filho 64, 24230-340, Niterói, RJ, Brazil.
Email: fzbrandao@id.uff.br

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Abstract

The aim of this work was to determine whether a cervical dilation protocol (CDP) composed of only oxytocin can be used to perform transcervical (non-surgical) embryo transfer in sheep (NSET) without affecting the viability of the corpus luteum (CL). Likewise, we evaluated whether a cervical transposing test with a Hegar dilator (CT Hegar test), performed at oestrous time, could be used to screen ewes for NSET (greater or lower chances to transpose the cervix). For that, oestrous and ovulation synchronization was performed in 25 Santa Inês ewes to induce the dioestrous condition. Animals went through the following CDP in a crossover design: E + OX, oestradiol benzoate (100 μg intravenously [IV]) and oxytocin (100 IU IV); OX, oxytocin (100 IU IV); and SAL, saline solution (IV). Using a Hegar dilator, cervical transposing attempts were performed at oestrous (D0) and dioestrous time (D8). The viability of the CL (morphology, luteal blood flow and progesterone values) was evaluated by ultrasonography (colour Doppler and B-mode) and by serum progesterone measurement from D7 to D13. The cervical transposing rate was lower for the SAL group (64%; 16/25; $p < .05$) and did not differ between the E + OX (88%; 22/25, $p > .05$) and OX (84%; 21/25, $p > .05$) groups. No treatment affected the CL viability. The CT Hegar test showed a high sensitivity (85.7%–93.3%), satisfactory accuracy (72%–84%), low false-negative rate (6.7%–14.6%), but high false-positive rate (46%–66.7%). In conclusion, a CDP protocol composed exclusively of oxytocin can lead to good cervical transposing rates and does not affect the viability of the CL. In addition, a screening test (CT Hegar) performed at oestrus can identify ewes for which cervical transposing will likely not occur at NSET.

KEYWORDS

dilatation, embryo transfer, luteal phase, oxytocin, sheep

1 | INTRODUCTION

Because of the features of the ovine cervix, including a narrow lumen and misaligned folds, access to the uterine lumen through the cervical canal is particularly challenging in this species (Halbert, Dobson, Walton, & Buckrell, 1990). Therefore, surgical procedures are commonly applied in assisted reproductive technologies in sheep when access to the uterine lumen is needed (Candappa & Bartlewski, 2011, 2014). Surgical intervention is not desired in assisted reproductive technologies because it can lead to the formation of adhesions in the reproductive tract (up to 30%) (Bruno-Galarraga et al., 2014; Pinto et al., 2020) and, consequently, reproductive impairment.

One strategy to overcome this difficulty in sheep is multiple ovulation and embryo transfer (MOET), in which exogenous hormones analogs (e.g. prostaglandin, oxytocin and oestradiol benzoate) are used to induce cervical dilation before attempting to transpose the cervical folds (Fonseca et al., 2016; Khalifa, Sayre, & Lewis, 1992; Leite et al., 2018). Such drugs are used because their natural analogs are involved in the physiological cascade of events that leads to natural cervical dilation, such as at oestrus and parturition. This approach has resulted in cervical transposing (CT) rates (i.e. reaching the uterine lumen through cervical folds) as high as 80%–90% (Fonseca et al., 2019a; Khalifa et al., 1992).

Despite the encouraging results of this method, there remain concerns about the use of oestradiol benzoate in food-producing animals because of the steroidal residues. Moreover, scientific evidence indicates that exposure to oestrogen is an important risk factor for breast cancer (Yager & Davidson, 2006). However, such concern is not a new topic of debate; since 2006, the European Union has restricted the use of oestrogenic compounds for oestrous synchronization in cattle, and its use is also restricted in the United States (Food & Drug Administration–European Commission, 2020; Lane, Austin, & Crowe, 2008). In Dorper ewes, the reduction of oestradiol dose or its absence in protocols for transcervical uterine flushing has been reported to have no significant effect on fluid recovery or structure recovery rates (Dias et al., 2020), indicating that oestradiol can be eliminated from cervical dilation protocols (CDPs) for non-surgical embryo recovery (NSER).

In fact, CDPs are more often evaluated for their use in artificial insemination or embryo recovery. In both scenarios, there are no concerns of luteolysis (Fonseca et al., 2019a; Sayre & Lewis, 1997; Stellflug et al., 2001). However, at embryo transfer (dioestrous time) in recipients, the corpus luteum (CL) is susceptible to luteolysis, and its viability is crucial for progesterone (P4) maintenance and embryo survival. Previous studies have reported that both oxytocin and oestrogen are involved in the cascade of events that lead to luteolysis (McCracken, Custer, & Lamsa, 1999; Silvia, Lewis, McCracken, Thatcher, & Wilson, 1991). In this sense, it is reasonable to investigate whether the administration of oxytocin, oestradiol or their association could trigger luteolysis and affect the viability of the CL.

The selection of ewes for NSER depends on assessing whether previous access to the uterine lumen was obtained and the degree of difficulty in achieving CT (i.e. very easy to impossible) in the ewes. These assessments include the evaluation (attempt to transpose the cervix using a Hegar dilator) of ewes at oestrus (Fonseca et al., 2019b; Fonseca et al., 2019a; Santos et al., 2019) or immediately before NSER (Prellwitz et al., 2019), and these studies have shown that more than 80% of ewes with a previous successful CT had a subsequent successful NSER. These screening tests appear to be useful for selecting ewes for NSER. However, these strategies have been evaluated only for selecting embryo donors and not for embryo recipients.

Considering the importance of CDP for uterine access in sheep, the carcinogenic effect of oestradiol, and the necessity of ensuring CL viability after CDP, this experiment aimed to evaluate whether a CDP based only on oxytocin could achieve satisfactory CT rates without affecting the CL viability in sheep. In addition, we evaluated the usefulness of the CT Hegar test for screening recipient ewes for transcervical embryo transfer.

2 | MATERIALS AND METHODS

All procedures described in this experiment were approved by the Ethical Committee for Animal Use of the Universidade Federal Fluminense (protocol 9500240418), followed the guidelines of the Conselho Nacional de Controle de Experimentação Animal (CONCEA), and were conducted under the ethical principles of the Sociedade Brasileira de Ciência em Animais de Laboratório.

2.1 | General experimental conditions

We conducted the experiment at the Unidade de Pesquisa Experimental em Caprinos e Ovinos in Cachoeiras de Macacu (22°27′45″S latitude), Rio de Janeiro, Brazil, during the breeding season (March–May). A total of 25 pluriparous Santa Inês ewes (4.3 ± 0.7 years old; 3.1 ± 0.2 BCS [1–5 scale]; 52.9 ± 4.8 kg live weight), with a minimum of 6 months and a maximum of 8 months postpartum, were kept in pens and fed twice a day with chopped grass (*Pennisetum purpureum*) and concentrate according to their maintenance requirements (16% crude protein; 3.13 Mcal/kg of metabolizable energy; NCR, 2007). Water and mineral salt (Ovinofós®, Tortuga) were provided ad libitum. All animals underwent a gynaecologic examination, and only ewes without reproductive abnormalities detected by ultrasonography (ovarian cysts, endometritis, cystic structures or echogenic masses around the ovaries, uterus and cervix) or clinical assessment (mastitis, vaginal discharge, hyperaemia and vaginal oedema) were used in this experiment. Selection of animals was not based on any kind of cervical morphology evaluation before the experiment.

The experiment evaluated two CDPs and a control group for transcervical embryo transfer in recipient ewes as well as their biological effect on CL morphology and viability. We used a crossover design, so all animals were submitted to each CDP tested with intervals of 21 days among treatments. In addition, we evaluated the efficiency of a test performed during oestrus for screening ewes for NSET (more or less suitable for non-surgical embryo transfer). A schematic representation of the experiment design is presented in Figure 1.

2.2 | Oestrous synchronization protocol

To simulate physiological dioestrous conditions at embryo transfer, ewes were previously submitted to an oestrous synchronization protocol according to Balaro et al. (2016). Briefly, a sponge impregnated with 60 mg of medroxyprogesterone acetate (Progespon®,

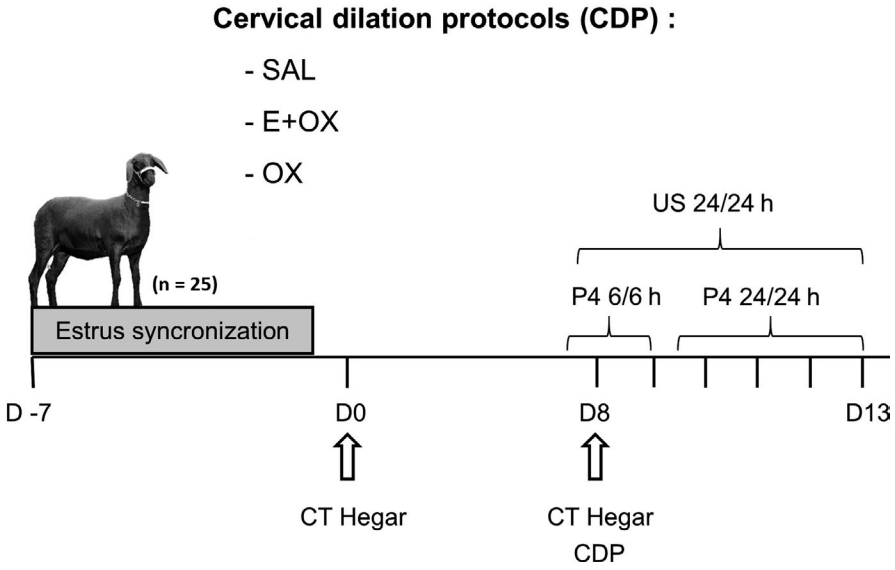

FIGURE 1 Schematic representation of the experiment design with indication of the ultrasonography evaluation (US); cervical transposing attempt with a Hegar dilator (CT Hegar) and serum progesterone measurement (P4). D0, day of oestrus; CDP, cervical dilation protocol; SAL, administration of saline solution; E + OX, CDP associating oestradiol benzoate and oxytocin; OX, CDP using oxytocin

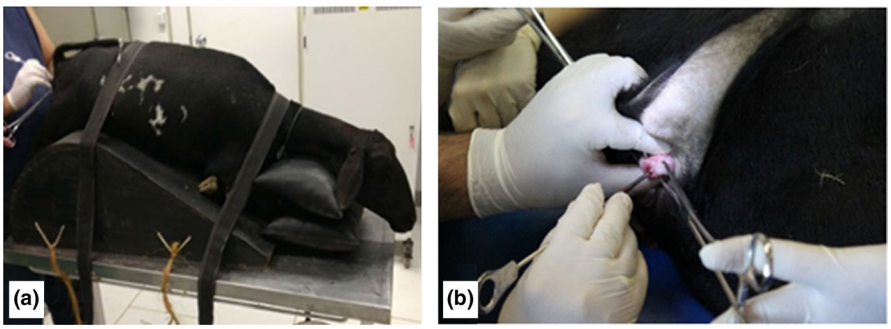

FIGURE 2 (a) Santa Inês ewe sedated and restrained to allow access to the perineum region. (b) Cervix of a Santa Inês ewe that was exposed and fixed during a transcervical transposing attempt with a Hegar dilator

Schering Plough) was inserted for 6 days. One day before sponge removal, ewes were injected with 300 IU of (intramuscular [IM]) eCG (Novormon 5000®, Schering Plough) and 120 μg of cloprostenol sodium (Estron®, Tecnopec). Thirty-six hours after sponge removal, 0.025 mg of lecirelin IM (Gestran Plus®, Tecnopec) was administered to induce ovulation. The beginning of the oestrous synchronization protocol was considered as D7 and the moment of oestrous manifestation as D0. Considering the crossover design (three treatments) and 25 experimental animals, a total of 75 oestrous synchronization protocols were performed.

2.3 | Cervical dilation protocols

The experimental protocols for cervical dilation were performed at D8 as follows: (a) E + OX, 100 μg intravenous (IV) injection of oestradiol benzoate (Estrogin®, Biofarm), diluted within 2.5 ml absolute ethyl alcohol and 2.5 ml saline solution, administered 12 hr before the CT attempt, and 100 IU of IV oxytocin (Ocitocina, Biofarm) administered 15 min before the CT attempt ($n = 25$); (b) OX, 5.1 ml of IV saline solution 12 hr prior to the CT attempt and 100 IU of IV oxytocin (Ocitocina, Biofarm) administered 15 min before the CT attempt ($n = 25$); and (c) SAL, 5.1 ml of IV saline solution 12 hr before the embryo transfer and 10 ml of IV saline solution (corresponding volume to the oxytocin application) 15 min before the CT attempt ($n = 25$).

2.4 | Cervical transposing

CT attempts were performed at oestrous (D0, 32 hr after sponge removal, without administering CDP) and dioestrous time (D8, with CDP). Before both CT attempts, ewes were sedated by IV injection with 0.1 mg/kg of acepromazine maleate (Acepran®, Vetnil) and 0.2 mg/kg of diazepam (Diazepam, Teuto). Only at dioestrous time, ewes received an epidural anaesthesia with 0.2 mg/kg of ketamine (Cetamin®, Syntec). Ewes were then restrained on a table (1.2 m tall) using a rounded, rubberized wooden device that allowed the perineum to be exposed (Figure 2a). Cervix visualization, traction and restraint were performed as previously described by Santos et al. (2019). Briefly, after the cervix was located with a speculum and a light source, the cervical os was clipped with Allis forceps and exteriorized by caudal traction using two Pozzi forceps inserted on each side of the cervical os in the fornix region. The Hegar dilator was inserted into the cervical os, and oscillatory and progressive movements were performed, aided by a two-finger manipulation of the cervix (Figure 2b). Once the technician could not feel resistance towards the progression of the Hegar dilator, the cervix was

considered transposed. A maximum of three attempts per animal to insert the Hegar dilator through the cervix lumen were made, with a maximum duration of five minutes per attempt and intervals between attempts of 10, 20 or 40 min after the pre-anaesthetic medication (test at oestrus) or after epidural anaesthetic (test at the embryo transfer time), respectively.

2.5 | Ultrasound evaluations

Ultrasound assessments of the ovaries were performed every 24 hr, from D7 PM (before the first dose, CDP protocol) to D13, by the transrectal via, using portable equipment (Sonoscape S6) with a 7.5 MHz linear transrectal transducer suitable for use in small ruminants. The reproductive tract was scanned using the B-mode to locate the ovaries bearing one or more CL. After location of the ovaries, the CL and the ovary were scanned by rotating the transducer to the right and left, and a subjective luteal assessment was performed using a scale (ranging from 1 to 3) according to the echogenicity and echotexture adapted from Simões et al. (2007). Briefly, a score of 1 indicated near anechoic, heterogeneous and coarse granulation; a score of 2, hypoechoic, homogeneous and fine granulation; and a score of 3, echogenic, homogeneous and fine granulation (CLs with score ≥ 2 were considered functional). Freeze mode was activated; using the cineloop function, the image with the CL's wider sagittal section was selected. With the still image on the screen, the diameter and area were assessed using the equipment tools 'distance' and 'oval selection', respectively. In this sense, data for the CL diameter and area were calculated by the equipment software after making the selections as described above. Thereafter, colour flow mode was activated, and another complete scan of the CL was performed. Luteal blood flow was evaluated using a subjective score (ranging from 1 to 4); briefly, 1:0%–25%; 2:26%–50%; 3:51%–75%; and 4:76%–100%, as proposed by Arashiro et al. (2018) (CLs with a score ≥ 2 were considered functional). All examinations were performed by the same technician, and B-mode and colour Doppler settings were standardized and kept constant throughout the experiment (frames per second: 23; depth: 6.7 cm; gain: 255; colour flow mode frequency: 6.0 MHz; pulse repetition frequency: 1.0 kHz; and wall filter: 75 kHz).

2.6 | Progesterone assay

Blood was collected by jugular venipuncture into vacuum tubes (5 ml) and centrifugated at 1,500 *g* for 15 min. Serum was aspirated and stored at −20°C until hormone determination. Starting at D7 PM (before the first dose, CDP protocol) and continuing until D9 PM, samples were taken every six hours. From D10 AM to D13 AM, samples were collected every 24 hr. Progesterone values were calculated using solid-phase radioimmunoassay kits (ImmuChem, MP Biomedicals). We used the following for controls: serum of animal in oestrus (0.68 ng/ml; CV: 8.0%) and serum of pregnant sheep (5.4 ng/ml; CV: 9.5%); control samples and samples for setting the curve were evaluated in duplicate. Samples of the trial underwent a single evaluation. The assay sensitivity and intra-assay coefficient of variation were 0.05 ng/ml and 13.0%, respectively. All data were within the maximum and minimum points of the curve.

2.7 | Statistical analysis

The data were analysed using the statistical program BioEstat 5.3 (Universidade Federal do Pará). The Lilliefors test and Bartlett's test were used to verify the normality and homoscedasticity of the variables, respectively. Categorical data (CT positive or negative) were assessed by Fisher's exact test. Paired ordinal data (CL score by B-mode [1–3] and colour Doppler [1–4]) were evaluated by the Friedman test. Progesterone values were analysed by an analysis of variance for repeated measures and Tukey's test for means comparison. For all tests, $p < .05$ was considered significant, and values ranging from $p = .051$ to $p = .06$ were considered as tendency.

The CT rate (%) was calculated by the following formula: (positive CT/ewes submitted to the procedure) × 100. The performance of the CT Hegar test as a tool to identify ewes eligible for the non-surgical embryo transfer was evaluated based on the sensitivity (SENS = TP/(TP + FN)), specificity (SPEC = TN/(FP + TN)), positive predictive value (PPV = TP/(TP + FP)), negative predictive value (NPV = TN/(FN + TN)), accuracy (Ac = (TP + TN)/n) and kappa index (κ). The following were considered: true positive (TP; animals with positive CT in both attempts [oestrus and dioestrus]), true-negative (TN; animals with negative CT in both attempts [oestrus and dioestrus]), false-positive (FP; animals with a positive CT at oestrus but a negative CT at dioestrus) and false-negative (FN; animals with a negative CT at oestrus but a positive CT at dioestrus).

3 | RESULTS

3.1 | CT rate and CT Hegar test

SAL treatment had a lower CT rate (64%; 16/25) when compared with E + OX (88%; 22/25; $p < .05$) and when compared with OX (84%; 21/25; $p = .06$). However, the CT rate did not differ between E + OX and OX. The CT Hegar test had good values for sensitivity, accuracy and PPV, and a low number of FNs. The role efficiency of the CT Hegar test, as a diagnostic test, is presented in Table 1.

3.2 | Luteal assessment and progesterone concentration

The luteal assessment revealed that area, diameter and blood flow were not affected by any CDP treatment. For all experimental

groups (SAL, E + OX and OX), from D7 (just prior to the CDP onset), an increase in area and luteal diameter was observed, followed by a plateau around D11 that lasted until D13 (Figure 3). The frequency of viable CLs evaluated by B-mode or by colour Doppler mode did not differ between the moments of assessment. Likewise, serum progesterone values did not differ among CDP treatments and remained between 2 and 5 ng/ml throughout the experimental days (D7–D13), as presented in Figure 4.

TABLE 1 Overall performance of the cervical transposing test using a Hegar dilator (CT Hegar) to select Santa Inês ewes (embryo recipients) for non-surgical embryo transfer in a MOET programme

	SAL	E + OX	OX
Sensitivity (%)	93.3	91	85.7
Specificity (%)	40	33.3	54.6
Accuracy (%)	72	84	72
Positive predictive value (%)	70	91	71
Negative predictive value (%)	80	33.3	75
False-positive (%)	60	66.7	46
False-negative (%)	6.7	9.1	14.6
Kappa index	0.36	0.24	0.42

Note: SAL, administration of saline solution; E+OX, cervical dilation protocol with oestradiol benzoate and oxytocin; OX, cervical dilation protocol using oxytocin.

4 | DISCUSSION

The main outcome of this study was that the use of a CDP consisting exclusively of oxytocin can lead to satisfactory CT rates at dioestrus (embryo transfer time) without affecting the viability of the CL in hair sheep. In addition, the CT Hegar test showed acceptable feasibility for selecting recipients for non-surgical embryo transfer.

The rates of CT reached in the treated groups were similar to CT rates from other studies (81%–95%) that associated prostaglandin, oestradiol benzoate and oxytocin (Figueira et al., 2020; Fonseca et al., 2019a; Leite et al., 2018). Despite the fact that we observed no difference between the treated groups, it was possible to achieve CT rates close to 85% using only oxytocin. Recently, Dias et al. (2020) reported CT rates close to 100% in Dorper ewes after administration of a CDP composed of PGF2α and oxytocin (50 IU). Khalifa et al. (1992), using a higher dosage (400 IU) and the same method of administration, also reported the efficiency of using only oxytocin for favourable CT outcome (83%). Thus, it is reasonable to consider the simplicity (one shot) and safety (not carcinogenic effect) of oxytocin CDP as a fair option for performing CT in sheep at dioestrus.

We believe that once oxytocin receptors were present in non-pregnant animals, despite the known decrease during gestation (Arthur, Taggart, Zielnik, Wong, & Mitchell, 2008), the activation of oxytocin receptors by the exogenous oxytocin administered would lead to the production of prostaglandin E2 (PGE_2), as occurs in response to natural oxytocin (Arrowsmith & Wray, 2014). PGE_2

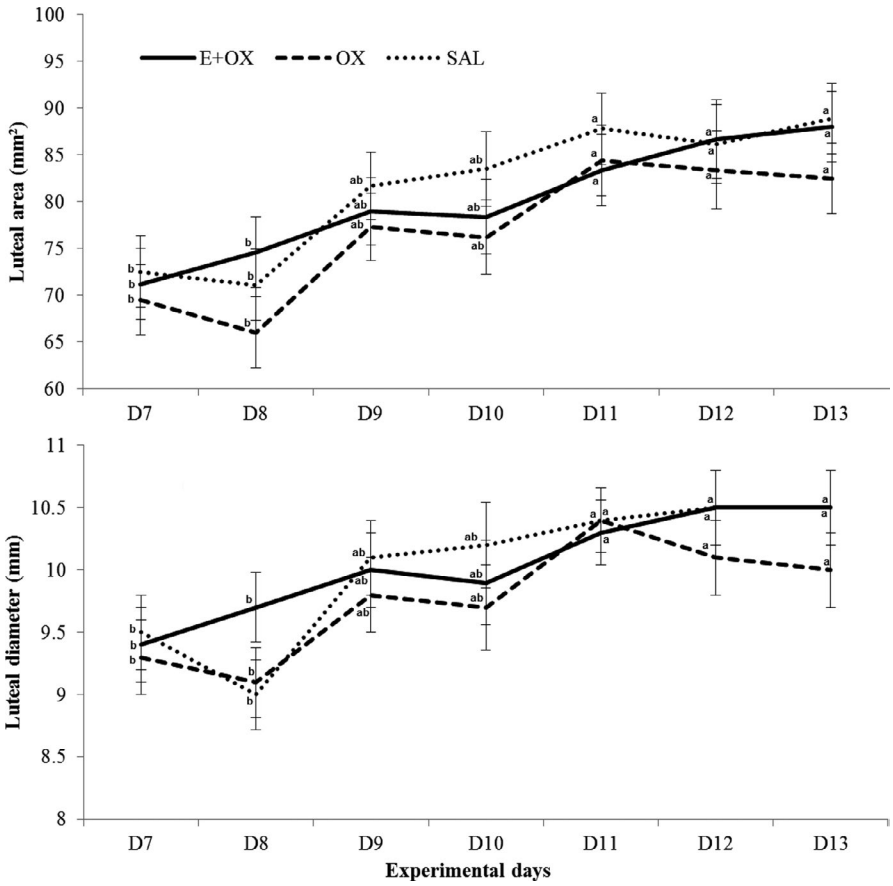

FIGURE 3 Area and diameter of the corpus luteum, as measured by transrectal ultrasonography in Santa Inês ewes submitted to three different cervical dilation protocols (CDP). SAL, administration of saline solution; E + OX, cervical dilation protocol associating oestradiol benzoate and oxytocin; OX, cervical dilation protocol using oxytocin; D0, day of oestrus after an oestrous synchronization protocol; D7 to D13 sequence after D0 (measured every 24 hr). [a,b]Different letters between moments of evaluation differ significantly ($p < .05$)

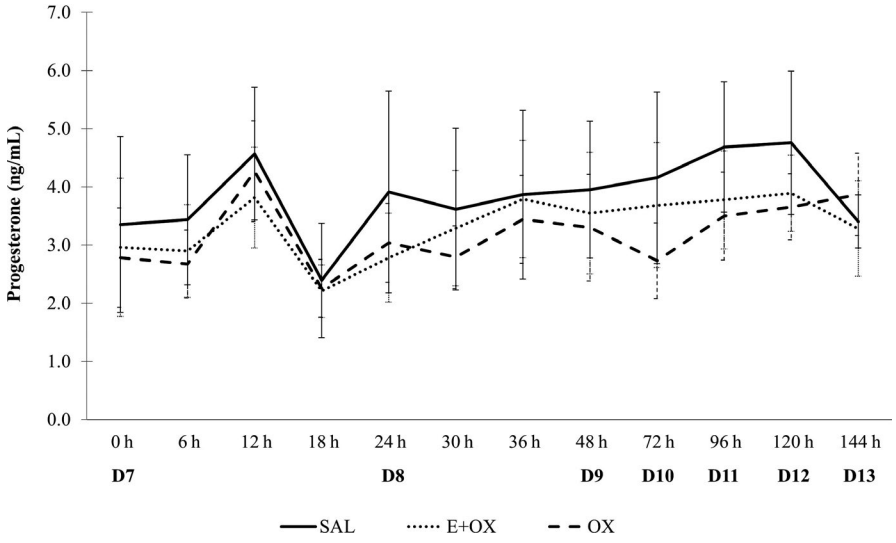

FIGURE 4 Serum progesterone values in Santa Inês ewes submitted to the following treatments at dioestrus: SAL, administration of saline solution; E + OX, cervical dilation protocol associating oestradiol benzoate and oxytocin; OX, cervical dilation protocol using oxytocin ($p > .05$; among treatments on the same time and throughout time). D0 was considered as the onset of oestrus after an oestrous synchronization protocol

modulates changes in cervical collagen fibres and smooth muscles and induces hydration through hyaluronic acid, leading to lower tension and easier distension of the cervix (Cabrol et al., 1987; Feltovich et al., 2005; Kershaw-Young, Khalib, McGowan, Pitsillides, & Scaramuzzi, 2009). As expected, the control group (SAL) had lower rates of CT than the treatment groups (E + OX, OX). However, Khalifa et al. (1992) and Gusmão et al. (2007, 2009) described a CT rate of 0% in animals that did not receive drugs for cervical dilation (control group). This discrepancy in CT between control groups suggests that other variables such as breed, CT technique and operator experience may have an effect on CT rates in sheep. Dias et al. (2020) also suggested that there may be a breed effect in response to the same CDP. Another observation is that our transposing rates improved around 20% in the treated groups; however, a considerable amount of animals could have had achieved CT without the influence of hormones. We believe that even within breeds, it is possible to find animals with distinct cervical conformation, and thus, there are subgroups of animals for which CT can be easier, harder or even impossible.

Progesterone plays an important role in early embryonic development (Lonergan, 2011). Therefore, CDP for NSET must be developed to ensure CL viability. All groups showed a drop in P4 values at 18 hr (after D7); however, this oscillation can be seen in initial luteogenesis in sheep bearing functional CLs (Figueira et al., 2015; Quirke, Hanrahan, & Gosling, 1979; Rhind, Chesworth, & Robinson, 1978). The most relevant factor is that serum P4 values be maintained at greater than 2.0 ng/ml at all times, regardless of the CDP protocol. Such values can be considered physiological and expected in ewes with functional CL (Boscos, Samartzi, Lymberopoulos, Stefanakis, & Belibasaki, 2003). For natural luteolysis, binding between oxytocin and its specific receptors in the endometrium is necessary to increase PGF2α pulsatility and subsequent CL regression (Trevisol, Ferreira, Ackermann, Destro, & Amaral, 2013). However, the expression of oxytocin receptors in the uterus is at its minimum during the luteal phase of the oestrous cycle (Robinson, Mann, Lamming, & Wathes, 2001). In addition, in vitro studies have shown that P4 can suppress the endometrial production of PGF2α (Duras, Mlynarczuk, & Kotwica, 2005). In this way, the low number of oxytocin receptors associated with the suppressive effect of P4 might be responsible for the prevention of luteolysis when oxytocin is administered at D8. Moreover, Wulster-Radcliffe, Costine, and Lewis (1999) and Lewis (2010) verified that administration of oxytocin, oestradiol or a combination of both does not impair embryo development, pregnancy rates or luteal function. In addition, no effect on CL morphology (area and diameter) or blood flow was detected on any of the CDP tested.

The CT Hegar test showed good sensitivity, indicating its satisfactory ability to identify ewes in which transcervical embryo transfer is more likely to be achieved. In addition, the low number of FNs indicates that an animal whose cervix could not be transposed at oestrus is unlikely to have the cervix transposed at dioestrus (moment of embryo transfer). Particularly, in the oxytocin group (OX), a fair accuracy, PPV, and NPV and moderate agreement (kappa index) were observed. Likewise, using the same screening test but for selecting ewe donors, Santos et al. (2019) reported a similar sensitivity (85.7%) and FN rate (10%). However, the high proportion of FPs found in this study contributed to the low test specificity, indicating that CT at oestrus does not guarantee CT at embryo transfer time, which is a limitation. However, this limitation may be partially overcome by subjecting animals that are CT negative at NSET to semi-laparoscopic embryo transfer (Figueira et al., 2019). Otherwise, the results (sensitivity, accuracy, PPV, NPV, FN, kappa) support the suggestion that ewes negative for the CT Hegar test at oestrus should be excluded from non-surgical MOET programmes. This information allows for decisions to be made such as the adjustment of the number of recipients needed for NSET or even inseminating/mating ewes that are CT negative at oestrus. An inconvenient aspect of the CT Hegar test is the required extra handling of the animals (contention and manipulation at oestrus), which can be unfeasible in scenarios such as a large-scale embryo transfer programme. Factors such as age, previous lambing, breed, individual anatomical features and time since last delivery can also interfere with successful CT rates

(Fonseca, Oliveira, Brandão, Batista, et al., 2019; Kaabi et al., 2006; Naqvi et al., 2005; Prellwitz et al., 2019), and these factors must be taken into account when selecting sheep for NSET. One hypothesis that rises is that a strict selection of the sheep donor, focusing on NSER and NSET, could lead to a scenario similar to that seen in goats, where it is possible to perform CT without the use of CDP (Fonseca, Oliveira, Brandão, Batista, et al., 2019).

In cattle, one of the major factors that resulted in widespread use of embryo production and transfer was the establishment of transcervical methods, which replaced surgical procedures (Betteridge, 2003). In addition, these methods increased animal welfare, as invasive procedures (surgical) were no longer needed for in vivo embryo production. In most sheep MOET programmes, standard methods for evaluating the superovulatory response, collecting and transferring embryos are still performed by surgical methods. However, efforts have been made to replace these procedures and allow MOET to become totally non-surgical (Fonseca, Oliveira, Brandão, Batista, et al., 2019; Pinto et al., 2018; Wulster-Radcliffe et al., 1999). In short, this study presents the relevant findings that a CDP based only on oxytocin is efficient and safe for the CL and that a screening test for NSET is useful. Furthermore, the findings of this study can contribute to improving NSET by offering an alternative technique that is in balance with current demands for greater animal welfare standards (Coleman, 2018; Grandin, 2014).

5 | CONCLUSIONS

CDP based on oxytocin only resulted in satisfactory CT rates in embryo-recipient Santa Inês sheep. Likewise, the administration of oxytocin (100 IU) at dioestrus does not interfere with luteal function. Finally, a previous CT Hegar test at oestrus can be used as a screening method to remove ewes with difficult CT from NSET programmes.

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CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTIONS

VMBS contributed with data collection and first draft production; PHNP participated at study design, data interpretation and first draft production; MFAB contributed with data collection, statistics, data interpretation and first draft production; JDRS, ART, CGES and FMG worked in data collection. Finally, JFF and FZB participated in the conception of the study, developed the hypothesis, study design, data collection and first draft production. In addition, all authors revised and approved the final version of the manuscript.

DATA AVAILABILITY

The data that support the findings of this experiment are available from the corresponding author upon reasonable request.

ORCID

Pedro Henrique N. Pinto https://orcid.org/0000-0001-5137-0106
Juliana D. R. Santos https://orcid.org/0000-0001-8324-1309
Caroline G. do Espirito Santo https://orcid.org/0000-0002-0157-6226
Jeferson F. da Fonseca https://orcid.org/0000-0001-8028-3984

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