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SHORT COMMUNICATION

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Cervical transposition test using Hegar dilator at oestrus as a tool to select ewes for transcervical embryo collection

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Abstract

This study evaluated the cervical transposition method as a tool to select ewes for embryo collection by transcervical route. Adult Santa Inês ewes (n = 50) received Day 0 protocol for superovulation treatments. The cervix transposition test was performed both at oestrus and at the embryo collection time. The latter was preceded by hormonal cervical dilation. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 85.7%, 66.6%, 85.7%, 66.6% and 80.0%, respectively. The kappa index yielded a moderate score (κ = 0.52). In conclusion, the high sensitivity and accuracy indicate that the cervical transposition test is a screening option to select ewes for embryo collection by transcervical route.

KEYWORDS
cervix, MOET, screening, superovulation

1 | INTRODUCTION

Multiple ovulation and embryo transfer (MOET) significantly increases the reproductive performance of the herd, thereby facilitating rapid genetic improvement. The MOET programme in sheep has two limiting factors, the individual variability in response to superovulation (Bari et al., 2001) and anatomical particularities of the ovine cervix (Kershaw et al., 2005) that impede the nonsurgical (transcervical or TC) embryo collection method. For this reason, both surgical and laparoscopic techniques have been adopted worldwide for embryo recovery in sheep.

On a physiological perspective, at the oestrous time, a natural cervical relaxation occurs due to the effect of oestradiol and oxytocin. In short, the oestradiol increasing throughout the oestrous cycle stimulates oxytocin receptors which promote prostaglandin release (Kershaw et al., 2005). The prostaglandin E_2, in special, is responsible to induce smooth muscle relaxation and glycosaminoglycan production, which acts separating the collagen fibres and increasing the spaces between them, culminating with the cervical relaxation (Kershaw-Young, Khalid, McGowan, Pitsillides, & Scaramuzzi, 2009). In this sense, Leite et al. (2018) have demonstrated advances in hormonal protocols for cervical dilation based on the combination of oestradiol and oxytocin previously to the TC method. Moreover, according to Oliveira et al. (2018), the TC method is less invasive, allows animals to be used successively and has no postsurgical effects such as adherence with consequent fertility impairment. Therefore, this technique could be a viable alternative for embryo collection in sheep MOET programmes.

We hypothesized that there is a positive correlation between the degree of cervical transposition at oestrus and at the embryo collection time if a hormonal dilation protocol based on oestradiol and oxytocin is applied. Thus, this study aimed to evaluate the cervical transposition test using the Hegar dilator at oestrus as a tool to select eligible ewes for submission to nonsurgical (TC) embryo collection.

2 | MATERIAL AND METHODS

2.1 | Animal ethics and management

The study was conducted in the UniPECO/UFF, located at Cachoeiras de Macacu, Rio de Janeiro, Brazil (22°S, 42°W), with 50 adult Santa Ines ewes (47.6 ± 6.8 kg) kept in an intensive system. All procedures were approved in advance by the Ethical Committee for Animal Use of the UFF (protocol 699/2015).

2.2 | Superovulatory treatment, oestrous detection and mating

The Day 0 protocol was used to make all ewes superovulatory and was followed by oestrous detection and natural mating with slight modifications to the protocol proposed by Pinto et al. (2018); this study used 133 mg total dose of pFSH.

2.3 | Cervical transposition test with Hegar dilator

The cervical transposition test was performed using Hegar cervical dilator number three at two moments: at oestrus (32 hr after sponge removal) and at the moment of embryo collection (seven days after oestrus). The latter test determined whether the ewe would be submitted to nonsurgical (TC) or surgical (laparotomy) embryo collection method. Prior to both tests, ewes were sedated with acepromazine maleate IV. (0.1 mg/kg; Vetnil, Louveira, Brazil) and diazepam IV (0.4 mg/kg; Teuto, Anápolis, Brazil). The test at the moment of embryo collection was also preceded by hormonal cervical dilation: Oestradiol benzoate IV (20 μg/ml; Agener União, São Paulo, Brazil) was diluted within 2.5 ml absolute ethyl alcohol and 2.5 ml saline solution and administered 12 hr prior to the moment of embryo collection; cloprostenol IM (0.12 mg; Agener União, São Paulo, Brazil) was administered at the same time; and oxytocin IV (100 IU; Centrovet, Goiânia, Brazil) was administered 15 min prior to embryo collection (Leite et al., 2018). An epidural anaesthetic was performed with ketamine (2.0 mg/kg; Syntec, São Paulo, Brazil).

After the cervix was located with a speculum, the cervical os was clipped with Allis forceps and exteriorized by caudal traction using two Pozzi forceps inserted on each side of cervical os in the fornix region. A maximum of three attempts were made to insert the Hegar dilator through the cervix lumen, with a maximum duration of 5 min per attempt, and intervals between attempts of 10, 20 or 40 min after the preanaesthetic medication (test at oestrus) or after epidural anaesthetic (test at the embryo collection time), respectively. The test was performed by the same operator every time. The test was considered positive if all cervical rings were transposed in any one attempt and otherwise was considered negative.

Results of the test at oestrus were related to the test at embryo collection and classified as follows: true positive (TP, animals with positive results in both tests), true negative (TN, animals with negative results in both tests), false positive (FP, animals with a positive result in the test at oestrus but a negative result in the test at embryo collection) or false negative (FN, animals with a negative result in the test at oestrus but a positive result in the test at embryo collection).

2.4 | Data analysis

The performance of the cervix transposition test as a tool to identify ewes eligible for the nonsurgical (TC) embryo collection method 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).

3 | RESULTS

The numbers of TP, FP, TN and FN results were 30, 5, 10 and 5, respectively. This yielded the following performance measures for the cervical transposition test: SENS of 85.7%, SPEC of 66.6%, PPV of 85.7%, NPV of 66.6% and Ac of 80.0%. There was moderate agreement between both tests (κ = 0.52). At the time of embryo collection, 71.43%, 17.14% and 11.43% of the animals had their cervix transposed in the first, second and third attempt/s, respectively.

4 | DISCUSSION

As occurred previously in bovine species (Castro Neto et al., 2005), many efforts are now being made to eliminate surgical procedures from MOET programmes in small ruminants (Fonseca et al., 2013). In most sheep MOET programmes, the standard embryo collection method is surgical, even when the nonsurgical collection is scheduled, and surgical collection is programmed by default if the TC collection becomes infeasible.

The results in our study propose a screening test to select candidate for TC embryo collection, improving MOET operations in the species. The transposition test at oestrus is a valuable tool, although it presented values, false positives (5/50) and negatives (5/50). With this screening test, sheep MOET programmes could maximize the use of the most promising donors, since in all sheep the surgical procedure would be performed. Additionally, by preplanning the embryo collection method, breeders could decide whether it was worthwhile to proceed with the MOET programme. In other words, the use of the cervical transposition test at oestrus can make sheep MOET programmes less costly, simpler, better planned and without the risks and animal welfare issues associated with surgical procedures.

This study demonstrated the correlation between the degree of penetrability of a sheep's cervix at oestrus and at the moment of TC embryo collection. The Hegar test achieved high SENS (85.7%) and AC (80.0%) when applied at oestrus. The efficiency of diagnostic screening tests is determined primarily by SENS (Grimes & Schulz, 2002), since this percentage determines the number of TP that will emerge from a given population. Based on this measure, the Hegar test was appropriately efficient at detecting ewes that could not be

submitted to TC, allowing the technician to schedule the surgical embryo collection for those animals.

Interestingly, we had a NPV and SPEC of 66.6%. These results highlight the difficulty of standardizing ewe groups due to individual cervix variability. This relatively low percentage can be attributed to the fact that at oestrus, cervical relaxation was not equivalent to the hormonal ambient obtained after cervical dilation treatment at the embryo collection time. Toosi et al. (2010) proposed physiological values for E2 in cyclic ewes throughout an ovulatory wave, describing basal and peak E2 values of 2–3 and 6–7 pg/ml, respectively. In the current study, a higher dose of exogenous E2 (20 μg/ml) was used for cervical dilation, and it is likely that this dose promoted greater cervical relaxation than was observed under physiological conditions at oestrus. Rexroad (1977) used 100 μg of oestradiol, similar to our study, and found a peak plasma concentration of 76.2 pg/ml after 5 hr of application of the hormone in oestrous sheep, which highlights the plasma elevation of oestradiol levels and its possible effect on FN.

The FP animals likely did not respond adequately to the cervical dilation protocol, since relaxation at the time of embryo collection provided the cervical transposition in 70% of the animals (35/50). This finding, together with the FN, may have influenced the kappa score.

In conclusion, this study demonstrated that the cervical transposition test at oestrus has the ability to identify with high sensitivity and accuracy the potential of donor ewes to be submitted to nonsurgical embryo collection in MOET programmes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

JDRS developed the experimental activities, analysed the data and wrote the manuscript; EKNA, MFAB and JMGSF developed the experimental activities, analysed the data and corrected the manuscript; PHNP, CVS and CRL developed the experimental activities; FZB and JFF designed and coordinated the study.

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