Received: 22 June 2020 | Accepted: 9 September 2020

DOI: 10.1111/rda.13825

ORIGINAL ARTICLE

Reproduction in Domestic Animals WILEY

Ultrasonographic cervical evaluation: A tool to select ewes for non-surgical embryo recovery

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Funding information
Empresa Brasileira de Pesquisa Agropecuária, Grant/Award Number: 22.13.06.026.00.03 and 22.13.06.026.00.04; Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Grant/Award Number: 00201-17; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: 001

Abstract

This study assessed the cervical ultrasonography mapping as a tool to select donor ewes for non-surgical embryo recovery (NSER). Lacaune ewes had their cervix evaluated by ultrasonography 12 hr after induced oestrus onset (Trial 1, $n = 24$) or 30 min before NSER (Trial 2, $n = 17$). Cervical rings were longitudinally evaluated and classified by their degree of misalignment on ultrasonography (DMUS) into: DMUS-1—cervix rectilinear, DMUS-2—intermediate and DMUS-3—highly asymmetrical. For predicting cervical transposing, only DMUS-1 and DMUS-2 were considered suitable. Similar ranking was attributed to degree of misalignment on the cervical map (DMCM 1–3), established immediately before NSER, which was performed at days 6 to 7 after oestrus. In Trial 1, cervical retraction for NSER was not possible only in three ewes classified as DMUS-3 (3/14, 21.4%). No difference ($p > .05$) was observed in the cervical transposing rates between ewes with different DMUS (ranged from 80% to 100%). In Trial 2, DMUS-1 and DMUS-2 reached 100% of transposing, and the only DMUS-3 ewe has not been transposed. In Trial 1, the prediction performance for successful cervical transposing showed low sensitivity (45%) and no specificity due to a high incidence of false negatives (52%). However, in Trial 2, sensitivity and specificity were both 100%. The DMCM and DMUS were uncorrelated, probably due to cervical stretching required to perform NSER. In conclusion, cervical ultrasound assessment immediately before NSER was more efficient to predict the cervical transposing than at induced oestrus, allowing the classification and selection of ewes eligible for NSER.

KEYWORDS

cervical transposing, cervix, sheep, ultrasound

1 | INTRODUCTION

Cervical anatomy in sheep is the major limitation for the application of reproductive biotechnologies by non-surgical methods (Fonseca et al., 2016; Kershaw et al., 2005) and is highly variable among breeds (Kaabi et al., 2006). The successful cervical transposing is affected by complex anatomy of the cervical lumen and the difficulty of rectal manipulation of the reproductive tract. Furthermore, cervices with higher degree of convolutions and interdigitations are less penetrable by pipette/catheter (Kershaw et al., 2005).

Pharmacological induction of cervical dilation is a crucial point to efficiently perform non-surgical embryo recovery (NSER) in sheep

(Candappa & Bartlewski, 2014; Gusmão et al., 2007, 2009; Masoudi et al., 2012). The hormonal combination of d-cloprostenol, estradiol benzoate and oxytocin was tested in Brazilian sheep breeds (Fonseca, et al., 2019; Fonseca et al., 2019a, 2019b), achieving high rates of cervical transposing, uterine flushing and media recovered. However, the feasibility of cervical access and manipulation (i.e. immobilization, retraction and penetrability) should be tested in individual donor ewes before any embryo collection attempt, aiming better effectiveness in the application of NSER (Fonseca, 2017; Fonseca et al., 2019a, 2019b; Santos et al., 2019).

An attempt at cervical transposing performed between 10 and 12 hr after the oestrus onset (natural, induced or synchronized) with a Hegar dilator allows the creation of a cervical map, recording the geographic-anatomical arrangement of the rings (Fonseca, 2017; Fonseca, et al., 2019). The records of cervical transposing allow for individuals to be ranked by the degree of ease, enabling donor selection and success in the NSER (Fonseca, 2017; Fonseca, et al., 2019; Santos et al., 2019). This evaluation has an accuracy of about 80% due to false positives and negatives (Fonseca et al., 2019a; Santos et al., 2019). Therefore, the degree of cervical dilation during induction of synchronized oestrus may be different from the physiological response to cervical dilation treatment for NSER in superovulated ewes.

A different approach to donor selection would be ultrasonography. In woman, the cervical ultrasound (US) assessment has been used to predict ease of embryo transfer according to the characteristics of the cervical tissue (Stanziano et al., 2017). The use of modern veterinary US equipment can ensure detailed visualization of the cervix in small ruminants, thus allowing new diagnostic and interpretative approaches. We hypothesized that ultrasonography evaluation of the cervical rings misalignment can be an additional tool for predicting cervical transposing and successful NSER in ewes. The present study aimed to assess the ultrasonography as a tool for cervical evaluation and prediction of cervical transposing to select an eligible ewe to undergo NSER.

2 | MATERIALS AND METHODS

2.1 | Experimental conditions

This research was reviewed and approved by the Animal Care Committee of Embrapa Dairy Cattle (Protocol 2512100516/2016) and conducted at the non-breeding season (September–October, Trial 1) and breeding season (April, Trial 2), in Soledade de Minas, Minas Gerais State, Brazil (latitude 22°3′ S, longitude 45°2′ W and altitude of 938 m). The animals were kept indoors in collective pens and fed with corn silage, balanced concentrate, and mineralized salt (DeHeus®) and water ad libitum.

2.2 | Trials

In Trial 1, 28 healthy Lacaune ewes with 15–93 months old, 67.2 ± 2.0 kg of body weight (BW) and 3.4 ± 0.1 of body condition score (BCS) were selected to evaluate the efficiency of ultrasonographic cervical evaluation at oestrus in the prediction of successful cervical transposing for NSER. Ewes received a protocol to induce synchronized oestrus with intravaginal device (60 mg of medroxyprogesterone acetate, Progespon®, Zoetis) for six (*n* = 14) or nine (*n* = 14) days plus 400 IU of eCG (Folligon 5000 IU®, Intervet) and 37.5 μg of d-cloprostenol (Prolise®, Tecnopec) i.m. 24 hr before device removal. The comparison of progestogen exposure for six or nine days was the objective of another study already published (Figueira et al., 2020).

In Trial 2, 24 healthy Lacaune ewes (18–60 months old, BW: 66.4 ± 0.9 kg and BCS: 3.5 ± 0.1) were selected to evaluate the success of cervical transposing using US assessment 30 min before NSER. At this time, the cervix was already under the effects of cervical dilation treatment (described in Section 2.5). The ewes had their oestrus synchronized with a 0.36 g of progesterone intravaginal device (Primer PR®, Tecnopec) for 9 days and received 37.5 μg of d-cloprostenol i.m. (Prolise®, Tecnopec) 24 hr before device removal and 50 μg of gonadorelin (GnRH analogue, Gestran®, Tecnopec) 24 hr after device removal. Superovulatory treatments consisted of 100 mg (*n* = 12) or 200 mg (*n* = 12) of porcine FSH (Folltropin®-V; Bioniche Animal Health), administered i.m. twice a day, for three consecutive days, in decreasing doses (25%, 25%, 15%, 15%, 10% and 10%), starting at 60 hr before device removal. The comparison of pFSH doses was the objective of another study (unpublished data). Therefore, data regarding these treatments are outside the scope of the present study.

2.3 | Oestrous detection

After device removal, oestrous behaviour was monitored by healthy and fertile rams (ratio ram:ewes = 1:6) twice a day (8 a.m. and 6 p.m.). Ewes that exhibited mounting acceptance were considered in oestrus.

2.4 | Ultrasonographic cervical evaluation

Ultrasonographic evaluation was performed at the beginning of experiment to evaluate the genital tract (8.0 MHz, Mindray M5VET®). Cervical assessment was always performed by the same technician using transrectal ultrasonography only in ewes that exhibited oestrous behaviour. Thus, in Trial 1, US was conducted 12 hr after oestrus onset of oestrus in 24 ewes, while in Trial 2 it was done 30 min before NSER in 17 ewes (Figure 1). Four ewes in Trial 1 and seven in Trial 2 did not exhibit oestrus and did not have the cervix evaluated by US. A slow scan was conducted to evaluate the sequential arrangement of cervical rings, which were classified in three degrees of misalignment on ultrasonography (DMUS): DMUS-1—cervix rectilinear, DMUS-2—intermediate and DMUS-3—highly asymmetrical cervix. This nomenclature was proposed due to similarities between the classifications in three degrees of cervical rings misalignment in anatomical samples (Kershaw

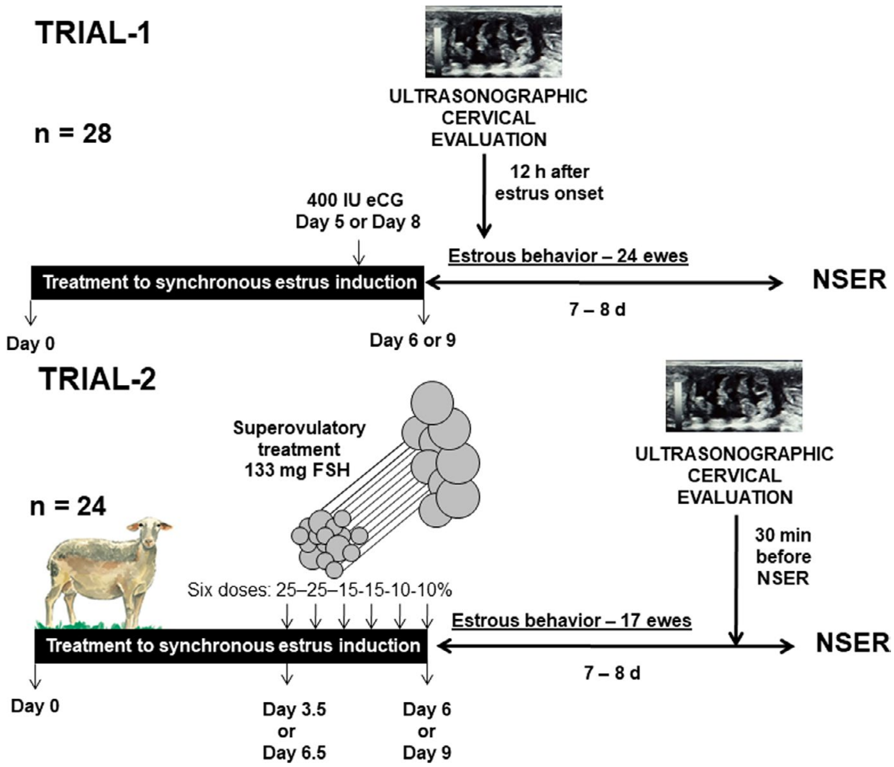

FIGURE 1 Simplified diagram of experimental procedures to evaluate the cervix using B-mode ultrasonography 12 hr after oestrus-induced (Trial 1) or 30 min before collection (Trial 2) in ewes submitted to non-surgical embryo recovery (NSER)

et al., 2005) and the 'cervical maps' performed in vivo (Fonseca, 2017; Fonseca, et al., 2019). The number of rings was counted by US only in Trial 1, due to time constraints in Trial 2. Ultrasonographic images of cervix with different DMUS are shown in Figure 2.

2.5 | Cervical transposing and uterine flushing by NSER technique

The ewes received treatment to induce cervical dilation in both trials. This treatment consisted of a latero-vulvar administration of 37.5 μg of d-cloprostenol and an i.m. injection of 1 mg of estradiol benzoate (Sincrodiol®, OuroFino) 16 hr and 50 IU of oxytocin (Ocitocina forte®, UCB) administered i.v. 20 min before NSER.

The NSER procedure was performed in both trials approximately 6–7 days after oestrus onset by the transcervical method. Immediately after the oxytocin treatment, a combined solution of 40 mg of hyoscine-N-butylbromide and 5 g of sodium dipyrone (Buscofin®, Agener Union) was administered, by both i.v. and i.m. routes, in equal parts. In addition, 0.1 mg/kg of BW of acepromazine maleate (Acepran 1%®, Vetnil) was also administered i.m. 20 min prior to cervical manipulation. The sedation continued for 45 min, enough time for carrying out NSER (Fonseca et al., 2019b). Animals in standing position were restrained in a sheep cart and received 2 ml of 2% lidocaine epidural block (S5-C1) (Lidovet®, Bravet) before cervical manipulation. After insertion of a vaginal speculum, a sterile gauze soaked with 5 ml of 2% lidocaine (without vasoconstrictors) was gently placed ventrally to the cervical opening with the aid of an Allis forceps (26 cm) and left in place for the duration of the procedure. Cervical manipulations (access, immobilization, traction and transposing) and uterine flushing using DPBS (Dulbecco's phosphate-buffered saline, Biodux®) were performed by the same technician, adopting the technique described by Fonseca et al. (2016). The validation of uterine access was done by observing the characteristics of the flushing fluid and ova/embryos recovered.

During the first cervical transposing, the number and anatomical arrangement of rings (according to its 'analog clock' position and its distance from the central axis) were recorded in both trials for creation of a cervical map (CM; as proposed by Fonseca et al., 2019a and applied by Dias et al., 2020) in the ewes that cervix was completely transposed. The idea is to sketch a schematic sequence of 'clocks' (the number of clocks drawn is related to the number of cervical rings). As each ring is transposed, the location of its ostium (that best represents the position and distance from the central axis) is schematically represented in the clock. Depending on the finger manipulation, this position could be changed. But, maintaining the pattern of clamps fixation, the ring position tends to be more constant, and CM can thus be a guide for the next attempts. Once the position on the clocks was drawn, three degrees of misalignment of the CM (DMCM) were assigned, similarly to DMUS: DMCM-1—cervix rectilinear, DMCM-2—intermediate and DMCM-3—highly asymmetrical cervix (Figure 3).

2.6 | Statistical analyses

Data were analysed separately in both Trials, using SAS® software version 9.3 (Statistical Analysis System, SAS Inst.). Cervical retraction and cervical transposing rates were analysed by chi-square or

Fisher's exact test. Time of cervical transposing was tested by generalized linear models using Gamma distribution and log link function. The models included as fixed effect degree of misalignment, number of rings counted during cervical transposing and hormonal treatment (oestrous induction—Trial 1 or superovulation—Trial 2). Post hoc multiple comparison was performed using Tukey's test. The number of rings counted by US or CM was compared by paired *t* test. Associations between variables were evaluated using Pearson's or Spearman's correlation. Results were expressed as percentages or least square means ± standard error of the means (LSMEANS ± *SEM*). Statistical significance was declared at $p < .05$.

The performance (efficacy) of the ultrasonographic cervical evaluation as tool to identify ewes eligible for NSER 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]) and accuracy (Ac = [TP + TN]/n), where TN = true negative, FN = false negative, TP = true positive and FP = false positive. Cohen's kappa index was also used for evaluation of test performance and considered the nomenclature proposed by Landis and Koch (1977): poor ($\kappa < 0.00$), slight ($0.00 < \kappa < 0.20$), fair ($0.21 < \kappa < 0.40$), moderate ($0.41 < \kappa < 0.60$), substantial ($0.61 < \kappa < 0.80$) or almost perfect ($0.81 < \kappa < 1.00$). For these analyses, both DMUS-1 and DMUS-2 were considered to be suitable and DMUS-3 non-suitable for transposing. The cervical transposing was considered successful (value equals to 1) if all cervical rings were transposed, and it failed otherwise (value equals to 0).

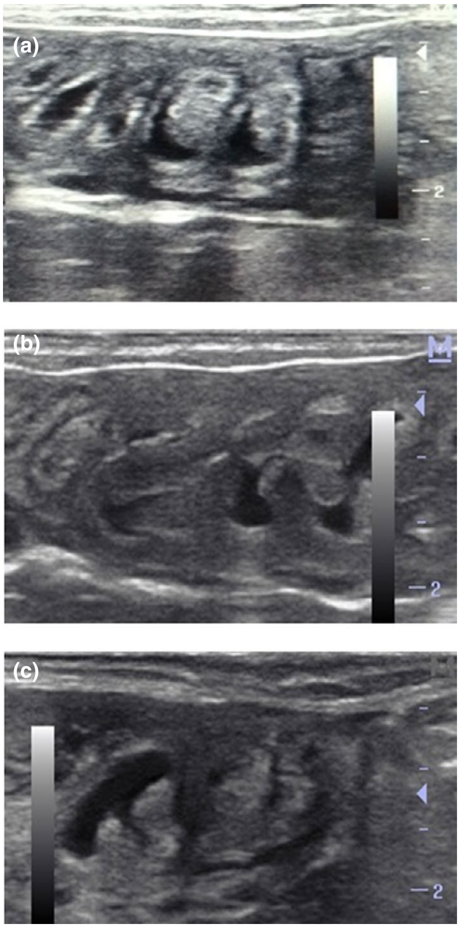

FIGURE 2 Cervical ultrasonography images according to the classification of degree of cervical misalignment on ultrasonography (DMUS) in Lacaune ewes, (a) DMUS-1: cervix rectilinear, (b) DMUS-2: intermediate, and (C) DMUS-3: highly asymmetrical. Images show longitudinal section of the cervix in the caudal cranial direction (left to right)

3 | RESULTS

3.1 | Trial 1

The percentage of ewes with corpus luteum (CL) at trial onset was 57.1% (14/28) in Trial 1. Cervical retraction and transposing rates, as well as the time for cervical transposing, did not differ ($p > .05$) among the degrees of misalignment or number of rings on US (Table 1). The time for cervical transposing was lower ($p < .05$) in DMCM-1 compared to DMCM-2 and DMCM-3, but no difference ($p > .05$) was found between DMCM-2 and DMCM-3. Cervical transposing time

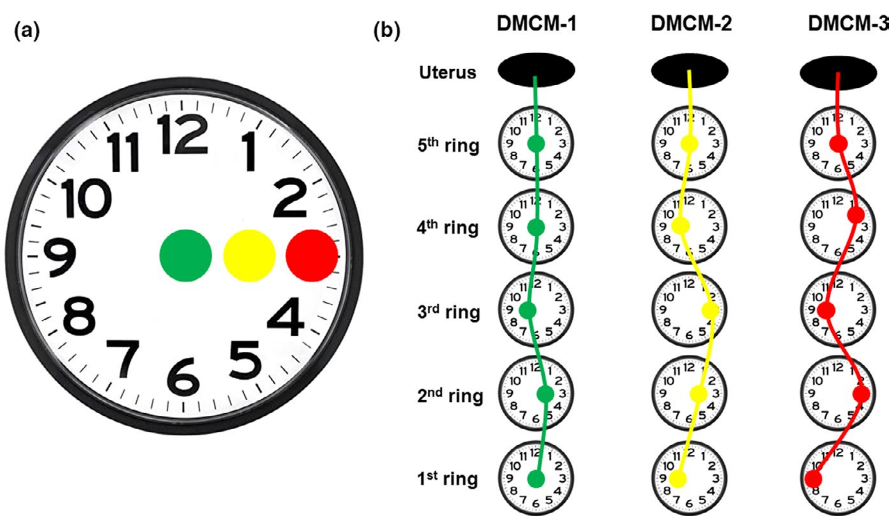

FIGURE 3 Assessment of the degree of misalignment on 'cervical map'. (a) The ostium of each cervical ring is recorded in a central position (green ball) or according to its intermediate (yellow ball) or distal (red ball) position on the circle representing an analog clock. (b) The route from the first cervical ring to the uterus is evaluated according to the degree of misalignment: cervix rectilinear (DMCM-1, green line), intermediate (DMCM-2, yellow line), and highly asymmetrical (DMUS-3, red line)

was positively correlated with DMCM ($r = .67$, $p < .01$) and was not correlated with DMUS ($r = .01$, $p > .05$), as well as with the number of rings counted by US ($r = .01$, $p > .05$) or CM ($r = -.19$, $p > .05$). The two methods of assessing cervical misalignment, DMUS and DMCM, were not correlated ($r = -.15$, $p > .05$).

The number of rings was higher ($p < .05$) on CM (6.5 ± 0.2, range 6–9) than in US counting (6.0 ± 0.1, range 5–8), without correlation ($r = .37$, $p > .05$) between methods due to over- (5%, 1/20) or underestimation (45%, 9/20). The number of rings coincided in both techniques in 50% of cases, and considering one ring's difference to be an acceptable estimate of error (slight variation), the US count allowed estimate the number of rings in 85% of cases.

Evaluating the performance of US for prediction of cervical transposing in Trial 1, high number of false negatives was observed, which dramatically reduced specificity and negative predictive value, resulting in low accuracy and poor Cohen's kappa index (Table 2).

3.2 | Trial 2

The percentage of ewes with CL at trial onset was 66.6% (16/24) in Trial 2. All females had the cervix successfully retracted in Trial 2. Cervical transposing was not possible in only one ewe classified as DMUS-3. Cervical transposing time did not differ ($p > .05$) between DMUS-1 and DMUS-2 as well as between DMCM-1 and DMCM-2 (Table 3). There was moderate positive correlation between DMUS and DMCM ($r = .59$, $p = .02$). The DMUS and DMCM were not correlated to cervical transposing time ($r = .04$, $p = .87$ and $r = .15$, $p = .56$, respectively). Number of rings counted by the technician also was not correlated with the cervical transposing time ($r = .01$, $p = .95$).

The US performance for cervical transposing prediction was enhanced in Trial 2. There were no false positives or false negatives, resulting in 100% positive and negative predictive values, excellent accuracy and almost perfect Cohen's kappa index (Table 2).

TABLE 1 Cervical retraction and cervical transposing rates (%) and time of cervical transposing (LSMEANS ± *SEM*) according to the degree of misalignment and number of rings determined by ultrasonography (DMUS) or cervical map (DMCM) evaluation in synchronous oestrus-induced Lacaune ewes submitted to cervical dilation treatment (Trial 1).†

End points	Percentage of animals, % (*n*/*N*)	Cervical retraction rate, % (*n*/*N*)‡	Cervical transposing rate, % (*n*/*N*)‡	Cervical transposing time, min (*n*)
Ultrasonography				
DMUS-1	20.8 (5/24)	100.0 (5/5)	100.0 (5/5)	4.8 ± 1.5 (5)
DMUS-2	20.8 (5/24)	100.0 (5/5)	80.0 (4/5)	4.6 ± 1.4 (4)
DMUS-3	58.3 (14/24)	78.6 (11/14)	100.0 (11/11)	5.9 ± 1.4 (11)
5 rings	16.7 (4/24)	100.0 (4/4)	100.0 (4/4)	5.4 ± 1.7 (4)
6 rings	58.3 (14/24)	92.9 (13/14)	92.3 (12/13)	4.2 ± 0.9 (12)
7 rings	25.0 (6/24)	66.7 (4/6)	100.0 (4/4)	5.7 ± 1.8 (4)
Overall	100.0 (24/24)	87.5 (21/24)	95.2 (20/21)	5.6 ± 0.8 (20)
Cervical map				
DMCM-1	55.0 (11/20)	–	–	3.3 ± 0.6^b (11)
DMCM-2	20.0 (4/20)	–	–	7.5 ± 2.0^a (4)
DMCM-3	25.0 (5/20)	–	–	8.7 ± 2.2^a (5)
5 rings	10.0 (2/20)	–	–	7.6 ± 2.7 (2)
6 rings	50.0 (10/20)	–	–	6.2 ± 0.9 (10)
7 rings	25.0 (5/20)	–	–	5.1 ± 1.4 (5)
8 rings	15.0 (3/20)	–	–	5.5 ± 1.6 (3)
Overall	100.0 (20/20)	–	–	5.6 ± 0.8 (20)

Note: ^{a,b}Means with different superscript letters within columns of the same category (Tukey's test; $p < .05$).

†Cervical dilation treatment: 37.5 μg of d-cloprostenol latero-vulvar and 1 mg of estradiol benzoate intramuscular at 16 hr, and 50 IU of oxytocin intravenous at 20 min before non-surgical embryo recovery.

‡There is no such answer for the cervical map, since it was only done when the cervix was transposed.

TABLE 2 Prediction performance of cervical transposing by ultrasonography of cervix performed at 12 hr after onset of induced oestrus (Trial 1, $n = 24$ ewes) or 16 hr after cervical dilation treatment (Trial 2, $n = 17$ ewes) in Lacaune ewes

Trials	TP	FP	TN	FN	SENS	SPEC	PPV	NPV	AC	κ
# 1	9	1	0	11	45%	0%	90%	0%	43%	−0.09
# 2	16	0	1	0	100%	100%	100%	100%	100%	1.00

Note: Abbreviations: AC, accuracy; FN, false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value; SENS, sensitivity; SPEC, specificity; TN, true negative; TP, True positive; κ, Cohen's kappa index.

TABLE 3 Cervical retraction and cervical transposing rates (%) and time of cervical transposing (LSMEANS ± *SEM*) according to the degree of cervical misalignment determined by ultrasonography (DMUS) or cervical map (DMCM) evaluation in superovulated Lacaune ewes submitted to cervical dilation treatment (Trial 2).†

End points	Percentage of animals, % (*n*/*N*)	Cervical retraction rate, % (*n*/*N*)‡	Cervical transposing rate, % (*n*/*N*)‡	Cervical transposing time, min (*n*)
Ultrasonography				
DMUS-1	29.4 (5/17)	100.0 (5/5)	100.0 (5/5)	3.8 ± 0.9 (5)
DMUS-2	64.7 (11/17)	100.0 (11/11)	100.0 (11/11)	4.0 ± 0.6 (11)
DMUS-3	5.9 (1/17)	100.0 (1/1)	0.0 (0/1)	–
Overall	100.0 (17/17)	100.0 (17/17)	94.1 (16/17)	3.9 ± 0.6 (16)
Cervical map				
DMCM-1	37.5 (6/16)	–	–	4.5 ± 1.1 (6)
DMCM-2	62.5 (10/16)	–	–	4.2 ± 0.8 (16)
DMCM-3	0.0 (0/16)	–	–	–
6 rings	12.5 (2/16)	–	–	3.4 ± 1.1 (2)
7 rings	37.5 (6/16)	–	–	4.8 ± 1.0 (6)
8 rings	43.8 (7/16)	–	–	3.0 ± 0.5 (7)
9 rings	6.2 (1/16)	–	–	7.3 ± 3.5 (1)
Overall	100.0 (16/16)	–	–	3.9 ± 0.6 (16)

†Cervical dilation treatment: 37.5 μg of d-cloprostenol latero-vulvar and 1 mg of estradiol benzoate intramuscular at 16 hr, and 50 IU of oxytocin intravenous at 20 min before non-surgical embryo recovery.

‡There is no such answer for the cervical map, since it was only done when the cervix was transposed.

4 | DISCUSSION

This is the first study performed in small ruminants that assessed the use of ultrasonography as a tool for cervical evaluation and prediction of cervical transposing to select an eligible ewe to undergo NSER. Cervical ultrasonography was able to identify nuances of the lumen and cervical rings, allowing estimation of the misalignment degree. The presence of mucus in the vaginal lumen helped to locate the cervical external *os* and to start ring counting but does not allow detailing external cervical *os* type. The acoustic enhancement below the cervical rings provides a basis for the ring count. It is important to highlight that the B-mode image quality seems essential for obtaining detailed cervical images. It is expected that continuous improvements in US equipment will allow acquisition of better images, further facilitating diagnostic approaches (Medan & Abd El-Aty, 2010).

The high cervical transposing rate did not allow obtaining statistical differences between DMUS or DMCM. According to Fonseca, et al. (2019), cervical transposing will be easier in ewes with grade 1 and 2 on the cervical map, and it would be more difficult or even impossible in ewes with grade 3. In anatomic samples, Kershaw et al. (2005) observed lower penetrability in grade 3 cervices. In the present study, the penetrability in DMUS-3 ewes probably was improved by stretching after retraction and manipulation of the cervix with the thumb and index finger.

The high cervical transposing rate in DMUS-1 (100% in both trials) and the high positive predictive value observed in both Trials (90% and 100%) show the importance of US for choosing animals eligible for NSER. However, 0% of specificity and 0% of negative predictive value in Trial 1 suggest that ewes should not be discarded by US alone. An interesting finding was that restriction to cervical retraction occurred only in ewes classified as grade 3 in Trial 1. In Trial 2, the only ewe classified as DMUS-3 was not transposed. Therefore, the DMUS-3 or DMCM-3 ewes when used as a donor could be preventively submitted to fasting for surgical collection in case of non-penetration.

The time required to complete cervical transposing is another indicator of the difficulty of the NSER procedure (Fonseca, et al., 2019). Only DMCM in Trial 1 presented a high positive correlation with time of cervical transposing. This method seems more predictive of difficulty observed by the technician in successive attempts. Therefore, the cervical transposing attempt with a Hegar dilator at oestrus (Fonseca et al., 2019a; Santos et al., 2019) could be performed in association with a US diagnostic mainly in DMUS-3 ewes. The number of rings does not seem to be as limiting of cervical retraction and cervical transposing as the degree of misalignment. In a study, cervices with flap-like folds were penetrated easier than those with ring-like folds (Kaabi et al., 2006), but neither US nor CM methods allow this kind of distinction. In this study, errors associated with the count of number of rings by US and CM methods may have resulted in over- or underestimation. The fact of counts were equal in 50% of cases, and in 85% cervical ring count divergence did not exceed one ring suggests that it is possible to at least estimate with a minor margin of error the number of ring and encouraged validation possibly with subsequent dissection of anatomical samples either.

The anatomy of the ovine cervix is variable between breeds, and this affects the degree of ease in cervical transposing. The Lacaune seems to be one of the breeds in which cervical anatomy offers less limitation to catheter penetration, as it presented high pregnancy rates after cervical insemination compared to other breeds (Kaabi et al., 2006). In fact, cervical transposing rates reached 95% in non-superovulated Lacaune ewes submitted to NSER (Figueira et al., 2020). Therefore, it is possible that ultrasonographic cervical evaluation should be more effective in breeds with lower effectiveness in the cervical transposing.

Although the experiments have been conducted in different seasons, the percentage of ewes with CL was similar between trials, suggesting similar hormonal background. Possibly, there are differences in the cervical dilation intensity observed at oestrus (Trial 1) and after treatment for cervical dilation (Trial 2), as in the first trial DMUS-3 was more frequent, while in the second trial DMUS-3 was less frequent. In a study, cervical transposing screening test with a Hegar dilator at oestrus fails in 20% of cases, producing a false positive or false negative when a second attempt at NSER was performed (Santos et al., 2019). Fonseca et al. (2019a) also observed similar loss of accuracy. Therefore, US evaluation after treatment for cervical dilation seems more representative of conditions for transposing at NSER than oestrous time.

Finally, from a practical point of view, the synchronized oestrous induction plus eCG single-shot allows to evaluate by US the donor potential to promote multiple ovulations (Bruno-Galarraga et al., 2015), as well as simulate all stages of NSER following cervical dilation treatment in synchronized ewes (Figueira et al., 2020). The US is also a powerful tool to measure the efficiency in multiple ovulation and embryo transfer (MOET) programmes based on NSER by CL counting (Oliveira et al., 2018; Pinto et al., 2018). Likewise, ultrasonographic cervical evaluation can also become a valuable tool to classify and select eligible animals for NSER. The combination of DMUS-3 and/or DMCM-3 before NSER with high BW, vaginal narrowing and/or insufficient ovulatory response would be the primary criteria for disqualifying a donor candidate in a MOET programme based on NSER. Furthermore, ranking of animals by score could also help to determine the order of collections, taking out or leaving to the end the animals supposedly more difficult, if necessary, to perform the surgical procedure.

5 | CONCLUSIONS

Ultrasonographic cervical evaluation 30 min before NSER is more predictive of cervical transposing than at oestrus. The ultrasonographic cervical evaluation can be considered an additional tool to select ewes eligible for NSER.

ACKNOWLEDGEMENTS

The authors also thank Embrapa Goats and Sheep (Project Superov 22.13.06.026.00.04) and the Minas Gerais Research Foundation (FAPEMIG; Project CVZ-PPM 00201–17) for financial support. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brazil (CAPES)—Finance Code 001. M.E.F. Oliveira, J.F. Fonseca and J.M.G. Souza-Fabjan are CNPq (National Council for Scientific and Technological Development) fellows. J.M.G. Souza-Fabjan is FAPERJ (Carlos Chagas Filho Research Support Foundation) fellow.

CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTIONS

LM Figueira elaborated the hypothesis, implemented the study, analysed the data and wrote the first version of the manuscript; GB Vergani implemented the study and corrected the manuscript; NG Alves designed the study, analysed the data and corrected the manuscript; JMG Souza-Fabjan and MEFO implemented the study and corrected the manuscript; RR Lima analysed the data and corrected the manuscript; JF Fonseca elaborated the hypothesis, designed and coordinated the study.

DATA AVAILABILITY

The data on which the findings of this study were drawn are available from the corresponding author upon request.

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How to cite this article: Figueira LM, Alves NG, Souza-Fabjan JMG, et al. Ultrasonographic cervical evaluation: A tool to select ewes for non-surgical embryo recovery. *Reprod. Dom. Anim.* 2020;55:1638–1645. https://doi.org/10.1111/rda.13825