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Transcervical *vs.* laparotomy embryo collection in ewes: The effectiveness and welfare implications of each technique

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ABSTRACT

This study assessed animal welfare in ewes subjected to transcervical (TC) or laparotomy (LP) embryo collection, and the efficiency of these two techniques. Santa Inês ewes (n = 57) received a protocol for estrus synchronization and superovulation. Cervical dilation protocol was initiated 12 h before embryo collection in all ewes. Depending on the success of cervical passage, the embryos were collected from ewes by either TC or LP. Records were made of physiological (rectal temperature (RT) and heart rate (HR)), endocrine (cortisol concentration), biochemical (glycaemia, total proteins, globulin and albumin concentrations), and behavioral variables. Data were recorded before fasting (BF) and sedation (BS), during (DC) and immediately after embryo collection (IAC), and 1 h (1hAC), 3 h (3hAC), 6 h (6hAC), 12 h (12hAC), 24 h (24hAC), and 48 h (48hAC) after embryo collection. The LP and TC procedures were applied to 22 and 35 ewes (with 100.0% and 94.3% of procedures being successful, respectively). The use of LP took longer than TC ($P = 0.007$) but was less effective in the recovery of uterine fluid and structures ($P = 0.0002$ and $P = 0.0180$, respectively), with no difference in the number of viable embryos recovered per animal. The TC procedure induced a greater RT at DC ($P = 0.002$) and IAC moments ($P < 0.0001$). The heart rate was greater in TC than LP in IAC ($P = 0.036$). On the other hand, HR was greater with LP at 12hAC ($P = 0.033$) and 24hAC ($P = 0.002$). There was no interaction between the procedures and time on total proteins, albumin, or globulin concentrations. The TC procedure induced greater glycaemia than LP in IAC ($P < 0.0001$). LP induced greater serum cortisol concentration than TC at DC, IAC, 1hAC ($P = 0.0004$; $P = 0.0006$; $P = 0.036$, respectively), even though it was greater in the TC than the LP procedure at 3hAC ($P = 0.008$). In conclusion, the TC embryo collection was more effective than the traditional LP procedure. Although both embryo collection procedures affected ewes' welfare, the TC procedure is probably less stressor than the LP.

1. Introduction

The development of assisted reproductive biotechnologies - as *in vivo* production of embryos - has an important impact on the genetic improvement of small ruminant production. In ewes, the anatomy of the cervix makes it difficult to collect embryos without surgery (laparotomy (LP)) [1,2]. Despite the good results obtained in embryo collections by LP [3,4], the costs [5], as well as the risks of post-surgical sequelae [6], require the development of a less invasive transcervical (TC) technique. Moreover, it has also been reported that the TC procedure may be effective in achieving high embryo recovery rates in small ruminants [7].

Even though both procedures - LP and TC - can be effectively used [4,8], both raise welfare concerns. Although the TC procedure is less invasive and faster, the manipulation of the cervix of a

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conscious ewe may be stressful and even painful [9]. Recently, Oliveira et al. [7] reported that the embryo collection procedure did not affect the response of some inflammatory/stress markers (total protein, haptoglobin, fibrinogen, and paraoxonase blood concentrations). However, surgical collection involves procedures such as fasting the animal that affect energy needs at a key moment and can reflect on the quality of the embryo [10]. In addition, the LP procedure includes abdominal cavity opening, anesthesia, and a longer postoperative period [4] and, thereby, may be more stressful and painful for the animals than embryo recovery by the non-surgical TC route.

In summary, despite constant improvements to the TC procedure [11], there is little evidence of its efficiency for obtaining embryos in sheep. Additionally, as both types of embryo collection can be stressful, it is important to determine the key steps, and thus develop alternatives to minimize concerns. In light of this information, our aim was to provide a wide view of the physiological stress responses after collecting embryos with the TC or LP procedures, as well as their effectiveness in embryo collection in ewes.

2. Materials and methods

2.1. Experimental location, animals, and study design

The study was conducted at the Unidade de Pesquisa Experimental em Caprinos e Ovinos at the Universidade Federal Fluminense (22°S, 42°W), Rio de Janeiro State, Brazil. All procedures were performed according to the university's Ethical Committee for Animal Use (protocol 699/2015). The first study to be carried out was then replicated, using different Santa Inês multiparous ewes (4.3 ± 1.0 years old; mean ± SD). All animals were submitted to ultrasonography and clinical exam before the study; only animals without detectable reproductive abnormalities and considered healthy were used. The first study comprised 30 ewes (body weight: 49.0 ± 5.2 kg; mean ± SD), 19 of which were subjected to the TC procedure and 11 to the LP procedure. In this study, only the effectiveness of the procedures was compared. In the replica study, 27 animals (body weight: 47.0 ± 7.4 kg; mean ± SD) were used, comprising 16 for TC and 11 for LP procedures, respectively. In the replica study, the same data were recorded, plus all the samples for welfare analyses. Ewes were maintained in an intensive system, fed with chopped grass (*Pennisetum purpureum*; 2.0 kg DM/day/ewe) and concentrate [12], with free access to mineral salt and water. All the procedures to which the ewes were submitted to obtain embryos are illustrated in Fig. 1.

2.2. Superovulatory treatment and mating

The estrus cycles of all ewes were synchronized according to Pinto et al. [13] in both studies. Briefly, intravaginal sponges impregnated with medroxyprogesterone acetate (60 mg; Progespon; Syntex, Buenos Aires, Argentina) were inserted in all ewes, remaining *in situ* for six days. On the fifth day of progestogen treatment, eCG (300 IU; Novormon; Syntex, Buenos Aires, Argentina) and cloprostenol sodium (0.24 mg; Estron, Agner União, São Paulo, Brazil) were administered IM. Thirty-six hours after sponge withdrawal, all females received gonadorelin acetate IM (0.025 mg; Gestran Plus, Tecnopec, São Paulo, Brazil) (Fig. 2).

Superovulatory treatment and natural mating were also performed as proposed by Pinto et al. [13]. Briefly, 80 h after sponge removal, the ewes received pFSH (133 mg; Folltropin-V, Bioniche Animal Health, Ontario, Canada) in six decreasing doses administered every 12 h IM. A second intravaginal sponge was inserted simultaneously with the first pFSH dose, and removed with the fifth dose. Cloprostenol sodium IM (0.24 mg; Estron, Agner União, São Paulo, Brazil) was also administered simultaneously with the sixth pFSH dose, and gonadorelin acetate IM (0.025 mg; Gestran Plus, Tecnopec, São Paulo, Brazil) 24 h after the sixth dose of pFSH. All ewes were subjected to natural mating every 12 h from the fifth dose of FSH until the end of estrus (Fig. 2).

2.3. Hormonal protocol for cervical dilation

A hormonal protocol for cervical dilation was applied in all animals (n = 57), including the administration of estradiol benzoate IV (100 μg; RIC-BE, Agener Union, São Paulo, Brazil) diluted with 2.5 mL of absolute ethyl alcohol and 2.5 mL of saline solution, and cloprostenol sodium IM (0.12 mg; Estron; Agener União, São Paulo Brazil), 12 h before embryo collection. In addition, 100 IU of oxytocin were administered IV (Ocitocina Forte UCB, Centrovet, Goiânia, Brazil) 15 min before the embryo collection procedure [14].

2.4. Laparoscopy

The *corpora lutea* (CLs) were counted by laparoscopy, since the sensitivity of transrectal ultrasonography for this count is lower in animals with good superovulatory responses [15]. The laparoscopic procedure was performed one day before embryo collection to calculate the recovery rate (number of recovered structures/CL number X 100). All the ewes had no access to food and water for 24 h and 12 h, respectively; this fast was prolonged for a further 12 h, for both food and water, to perform the embryo collection (totaling 36 h and 24 h of food and water fasting). The females were sedated with acepromazine maleate IV (0.1 mg/kg Acepran, Vetnil, Louveira, Brazil) and diazepan IV (0.3 mg/kg Diazepam, Teuto, Anápolis, Brazil), and the laparoscopic procedure was performed as proposed by Bruno-Galarraga et al. [16].

2.5. Transcervical embryo collection

The animals were sedated (0.1 mg/kg; acepromazine maleate IV; and 0.3 mg/kg diazepan IV) and received an epidural anesthesic (ketamine; 2.0 mg/kg; Syntec, São Paulo, Brazil). A cervical transposition procedure [17] was performed to determine whether an ewe would be submitted to a TC (positive result) or LP (negative result) embryo collection procedure. The collection of embryos was performed immediately, as the cervix was already fixed and exteriorized.

Structure recovery was performed with a closed system circuit (Circuito Embrapa for goat/sheep embryo recovery; Embrapa, Brasília, Brazil) according to Fonseca et al. [18]. After the two uterine horns were washed, the uterine lavage was recovered and counted in a beaker after passing through an embryo collection filter.

After this procedure, the average of total structures (number of embryos of all stages and grades of morphological quality, unfertilized oocytes, zona pellucida, and degenerate structures/number of sheep) and viable embryos (number of grade I and II embryos/number of sheep) as well as the percentage of uterine lavage recovered by collection procedure were calculated. Embryos were classified according to the criteria established by the IETS [19]. In addition, the time needed from sedation until the procedure ended, and for the procedure itself, were recorded.

2.6. Surgical embryo collection

The sedation and epidural anesthesic to perform the LP collection was the same used for the procedure of transposing the cervix, so only one preanesthetic medication was performed on the animals. Only those animals which were not successful in the cervical

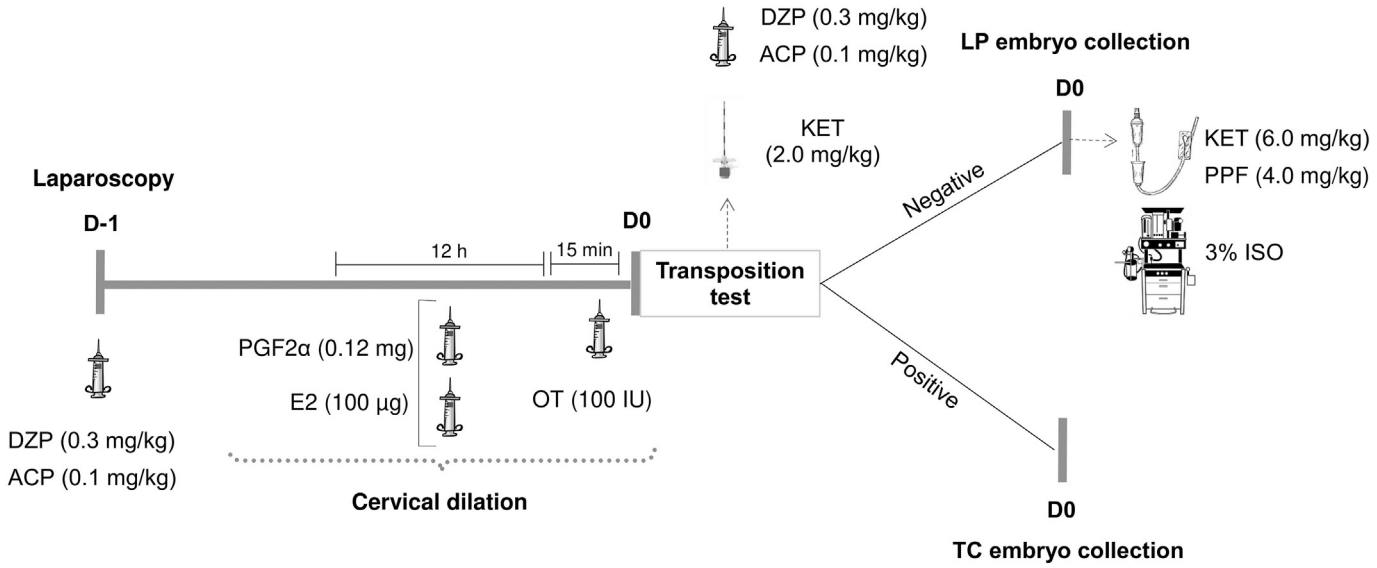

Fig. 1. Graphical scheme of all procedures carried out on Santa Inês ewes to obtain *in vivo* embryos. DZP: diazepam; ACP: acepromazine maleate; KET: ketamine; PPF: propofol; ISO: isofluorane; PGF2α: prostaglandin F2α: cloprostenol sodium; E2: estradiol: estradiol benzoate; OT: oxytocin; laparotomy: LP; transcervical: TC.

passage were subjected to the LP procedure, immediately after the cervical transposition test. The LP collection was performed under general anesthesia, starting with an anesthetic induction using propofol (Provive 1%, União Química, São Paulo, Brazil) at a maximum dose of 4.0 mg/kg (IV) and ketamine (6.0 mg/kg, IV; Syntec, São Paulo, Brazil), maintaining a deep plane of anesthesia with 3% isoflurane (Isoforine, Cristália, São Paulo, Brazil) with the aid of an inhalator anesthesia equipment (HB Hospitalar, São Paulo, Brazil). The uterus was identified through laparoscopy and then a small incision was made to exteriorize and fix the uterus.

The structure recovery was carried out as proposed by Lima et al. [20]. The animals received an anti-inflammatory (0.5 mg/kg IM; Maxican, Ourofino, São Paulo, Brazil) for three consecutive days, and three applications of antibiotics (20 mg/kg IM; Terramicina/LA, Zoetis, São Paulo, Brazil) every 48 h. As in the TC procedure, the mean total and viable structures recovered were also recorded,

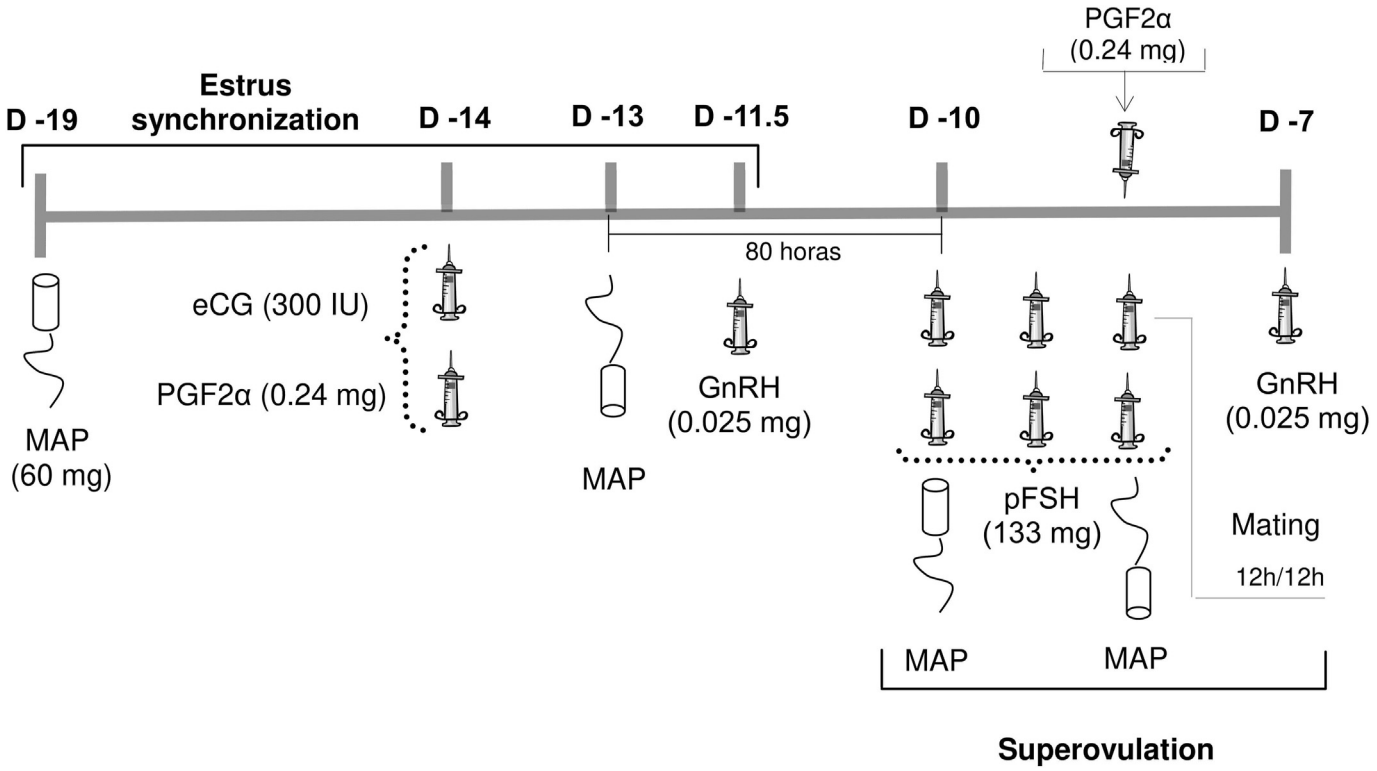

Fig. 2. Hormonal protocol for estrus synchronization and superovulation in Santa Inês ewes for *in vivo* embryo production. MAP: medroxyprogesterone acetate; eCG: equine chorionic gonadotropin; PGF2α: prostaglandin F2α: cloprostenol sodium; GnRH: gonadotropin-releasing hormone: gonadorelin acetate.

as well as the time required from sedation until the collection ended and the time-restricted procedure itself.

2.7. Welfare responses

All animals were observed after the collection procedures, and the time needed by each animal to stand up and eat was recorded.

The rectal temperature (RT) was measured with a digital thermometer and the heart rate (HR) was recorded by auscultation. Both parameters were measured at different moments: before fasting (BF), before sedation (BS), during embryo collection (DC), immediately after embryo collection ended (IAC), and 1 (1hAC), 3 (3hAC), 6 (6hAC), 12 (12hAC), 24 (24hAC), and 48 h (48hAC) after the end of the procedures (Fig. 3).

Blood samples were also collected from all animals by jugular venipuncture at the same moments mentioned above, after HR measurement to avoid the blood collection influencing the HR. Blood was collected in tubes without anticoagulant, allowed to clot for 20 min at room temperature, and centrifuged for 20 min at 1500 X g. Serum was separated and maintained in triplicate aliquots at −20 °C until being analysed.

Total protein, albumin, and glucose concentrations were measured with commercial kits (Labtest, Labtest Diagnóstica AS, Minas Gerais, Brazil). Serum globulin values were calculated by subtracting the serum albumin values from the serum total protein values.

Serum cortisol concentrations were measured by radioimmunoassay using commercial kits (MP Diagnostics Division, Orangeburg, NY, USA). Sensitivity and intra-assay coefficient of variation were 10 ng/mL and 9.8%, respectively. All data were within the minimum and maximum values of the curve.

2.8. Statistical analysis

Data analysis was performed with SAS statistical software (SAS University Edition). Data were compared using a mixed model, including the procedure, time, and their interaction as main effects in variables with repeated measurements, and the pdiff procedure to compare specific points. The repetition was included as a random factor in the model. For all tests, $P < 0.05$ was considered significant. Data are presented as LSmeans (±SEM), which are adjusted values according to the random factors to minimize these effects.

3. Results

3.1. Procedures and recovery rate

The overall estrus response was 77.2% (44/57). Twenty-eight of these animals were submitted to TC (28/44; 63.6%) while 16 ewes were subjected to the LP procedure (16/44; 36.4%). Embryo collection was performed in all animals, regardless of superovulatory response. The TC was faster than the LP procedure, and the uterine flushing recovery and recovery rate were greater with the TC than with the LP procedure. In this sense, the percentage of grade I and II embryos was also greater in the TC collection than the LP. However, the averages of total structures and viable embryos recovered per animal (sheep that did not respond to the superovulation protocol were removed from the calculus) were similar between the procedures (Table 1).

3.2. Welfare responses

Ewes subjected to the TC tended to stand up and eat again earlier than those subjected to the LP (TC: 25.6 ± 5.1 min vs. LP: 40.1 ± 6.2 min and TC: 26.3 ± 5.1 min vs. LP: 41.6 ± 6.1 min, $P = 0.08$, $P = 0.06$, respectively).

The effect on welfare of the collection procedure, time of evaluation, and interaction between them are presented in Table 2. The TC procedure induced a greater RT ($P = 0.039$), varied with time ($P < 0.0001$), and there was an interaction between the procedure and time: RT was greater in the TC than the LP procedure during ($P = 0.0021$) and immediately after the procedures ended ($P < 0.0001$) (Fig. 4A). In relation at the time of evaluation, in the TC procedure there was a decrease of temperature in the times DC, IAC, 1hAC, with an elevation in 3hAC in relation to the other moments evaluated ($P < 0.05$). A similar pattern was observed in the LP procedure, with a more evident drop at IAC moment (Fig. 4A). The HR was greater in the LP than in the TC procedure ($P = 0.010$), and there was a significant interaction between the procedures and time ($P = 0.037$). The HR was greater immediately after the TC than

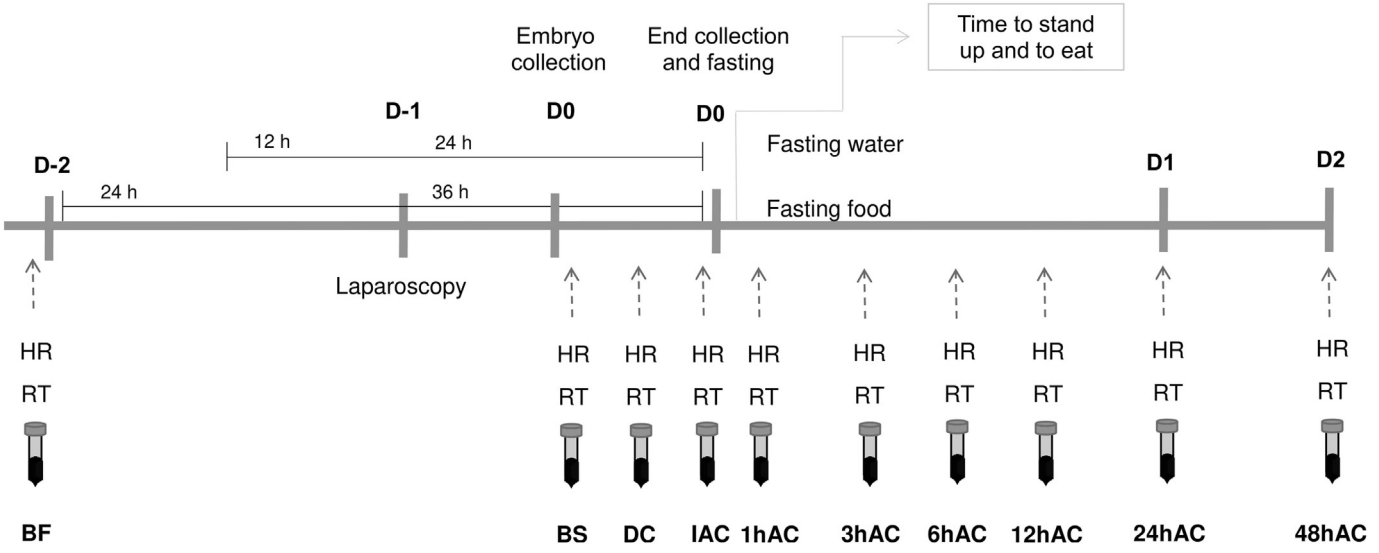

Fig. 3. Animal welfare analyses in Santa Inês ewes submitted to embryo collection by either laparotomy (LP) or transcervical route (TC). RT: rectal temperature; HR: heart rate; BF: before fasting; BS: before sedation; DC: during collection; 1hAC: 1 h after collection; 3hAC: 3 h after collection; 6hAC: 6 h after collection; 12hAC: 12 h after collection; 24hAC: 24 h after collection; 48hAC: 48 h after collection.

Table 1
Results (mean ± standard error of mean or %) of embryo collection procedures (Transcervical or Laparotomy) in Santa Inês ewes submitted to a short-term protocol for estrus synchronization and superovulation and hormonal protocol for cervical dilation.

End-points	Collection procedures		*P*-value	Overall
	Transcervical	Laparotomy		
Number of ewes	35	22	–	–
Estrus response (%)	63.6 (28/44)	36.4 (16/44)	–	77.2 (44/57)
Ewes from which embryos were successfully collected (%)	94.3 (33/35)	100 (22/22)	–	–
Cervical penetration (%)	61.4 (35/57)	–	–	–
Time from sedation until embryo collection ended (min)	50.1 ± 2.6[b]	79.7 ± 3.2[a]	$P < 0.0001$	61.5 ± 2.8
Duration of embryo collection procedure (min)	24.5 ± 1.1[b]	31.6 ± 3.0[a]	$P = 0.0077$	27.2 ± 1.4
Fluid recovery efficiency (%)	99.2 ± 1.1[a]	92.0 ± 1.4[b]	$P = 0.0002$	96.5 ± 1.0
CL number per animal	6.1 ± 1.1	6.6 ± 1.3	$P = 0.6463$	6.3 ± 0.8
Recovery of structures (%)[a]	60.5 ± 6.1[a]	37.1 ± 7.4[b]	$P = 0.0180$	51.0 ± 4.9
Number of structures recovered per animal	4.2 ± 0.7	3.0 ± 0.7	$P = 0.2592$	3.7 ± 0.5
Number of viable embryos per animal	2.7 ± 0.6	1.4 ± 0.5	$P = 0.1493$	3.2 ± 0.5
Grade I and II embryos (%)	63.8 (83/130)	46.0 (29/63)	$P = 0.0205$	–
Grade III embryos (%)	11.5 (15/130)	4.8 (3/63)	$P = 0.1868$	–

[a,b] Different letters, on the same line, are significantly different.
[a] Recovery of structures (%): Structures recovered/CL X 100.

the LP procedure (P = 0.036), but lower at 12hAC and 24hAC (P = 0.034 and P = 0.003, respectively) (Fig. 4B). Throughout the evaluations, there was a peak of HR at 1hAC in the LP procedure in relation to the previous moments, followed by an unstable pattern of falls and elevations of the HR. In general, the analysis of the different moments in the TC procedure showed a similar pattern to LP procedure, where a general peak occurred at 1hAC who differed from all other times, with exception of IAC (Fig. 4B).

Total serum protein concentration and globulin concentration were not affected by the procedures or by the interaction between the procedures and time (Table 2), varying only with time (Fig. 5A and 5C, respectively). Serum albumin concentrations were greater with the TC than with the LP procedure (P = 0.014). The interaction between the procedures and time tended to be significant (P = 0.064) (Fig. 5B).

The serum cortisol values were not affected by the procedures, but there was a significant interaction between the procedures and time (P < 0.0001). This interaction was greater with the LP than with the TC procedure at moments DC (P = 0.0004), IAC (P = 0.0006), 1hAC (P = 0.0364). However, serum cortisol values were greater in the TC than the LP procedure at 3hAC (P = 0.0079) (Fig. 6A). In TC procedure, there was a marked increase in DC, IAC, 1hAC, however there was an abrupt decrease between 1hAC and 3hAC. The LP procedure also presented similar pattern (Fig. 6A). Similarly, the glycemia was not affected by the procedures, but there was a significant interaction between the procedures and time (P < 0.0001), this being greater with the TC than with the LP procedure in IAC (P < 0.0001) (Fig. 6B). In general, in the TC procedure there was a peak at the moments DC, IAC, 1hAC and 3hAC in relation to the other moments. In the LP procedure, there was an increase in glucose concentrations at the same moments (DC, IAC, 1hAC and 3hAC), however there was an oscillation characterized by a reduction in IAC followed by a peak at 1hAC (Fig. 6B).

4. Discussion

To the best of our knowledge, this is the first study comparing the welfare problems involved in physiological, behavioural, and biochemical responses raised by TC and LP procedures used for embryo collection in sheep. The results of our study showed that embryo collection is stressful for the animals, as described by Oliveira et al. [7] and Fonseca et al. [21]; therefore, there is a requirement to develop complementary aids to prevent or at least decrease these problems, whatever the procedure used. In this sense, both procedures triggered typical stress responses such as increases in HR, cortisol, and glycemia, since these variables increase in stressful situations [22,23].

As a less invasive method, the TC procedure was faster than the LP procedure, mainly because the latter involves general anesthesia and other surgical inputs (laparoscopy to fix the uterus, sutures, etc). In this sense, it should be considered that TC simplifies the procedure and avoids the use of general anesthesia, whose application puts the life of the animals at risk [24]. Moreover, the post-anesthetic recovery is also risky, affects animals' welfare, and requires intensive monitoring of the animals. Therefore, avoiding the use of general anesthesia is beneficial per se, in addition to the simplification of the procedure. In contrast with these results, Oliveira et al. [7] reported that both procedures required a similar time; however, these authors used different anesthetic protocols and, as was mentioned, the protocol itself should be carefully

Table 2
Effect of embryo collection procedures, evaluation time, and their interactions on welfare parameters in Santa Inês sheep submitted to the cervical dilation hormonal protocol.

Parameter	Embryo collection procedures				*P*-value or significance		
	LP	SEM	TC	SEM	ECP	Time	ECPxTime
Rectal temperature (°C)	38.09	0.10	38.28	0.09	0.039	<0.0001	<0.0001
Heart rate (bpm)	98.04	2.69	91.64	2.48	0.010	<0.0001	0.037
Total protein (g/dL)	5.81	0.22	6.04	0.22	0.060	<0.0001	NS
Serum albumin (g/dL)	2.10	0.23	2.31	0.23	0.014	<0.0001	0.064
Serum globulin (g/dL)	3.72	0.07	3.74	0.06	NS	<0.0001	NS
Glycaemia (mg/dL)	70.51	3.34	72.74	3.06	NS	<0.0001	<0.0001
Cortisol (ng/mL)	32.34	2.69	29.52	2.23	NS	<0.0001	<0.0001

All data are presented as LSmeans. LP, laparotomy; TC, transcervical procedure; ECP, embryo collection procedure; ECPxTime, embryo collection procedure by time interaction; SEM, standard error; NS, non-significant.

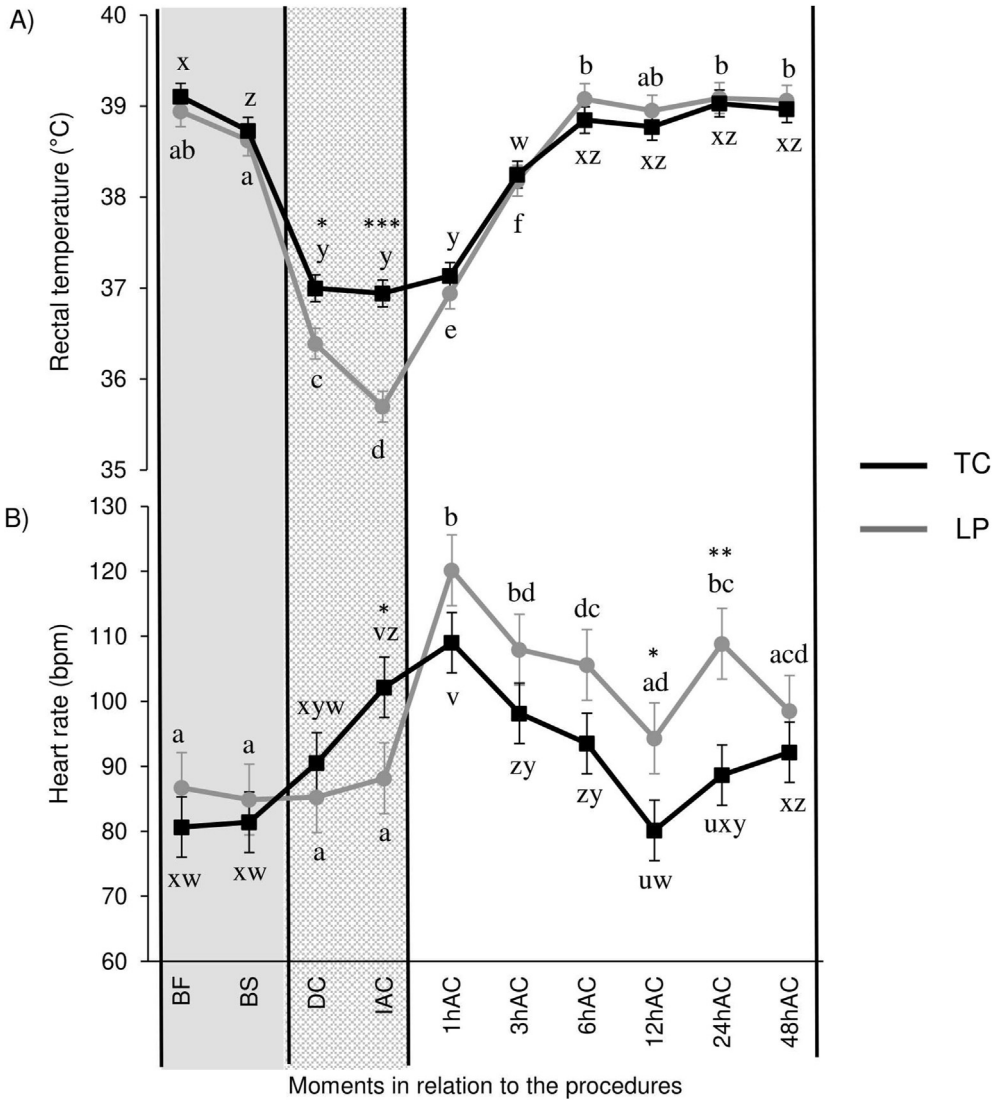

Fig. 4. A) Rectal temperature (°C); and B) heart rate (bpm) in ewes submitted to embryo collection by either laparotomy (LP) or the transcervical route (TC). BF: before fasting; BS: before sedation; DC: during collection; 1hAC: 1 h after collection; 3hAC: 3 h after collection; 6hAC: 6 h after collection; 12hAC: 12 h after collection; 24hAC: 24 h after collection; 48hAC: 48 h after collection. * Indicates interaction between embryo collection procedures (LP and TC) x Time ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$). Differences throughout the evaluations in each embryo collection procedure are indicated by different letters (TC: u, v, x, z, y, w; LP: a, b, c, d, e, f; $P < 0.05$).

considered to avoid other negative effects on animal welfare and/or deleterious effects on embryo quality. On the other hand, the shorter time needed by TC coincides with previous reports on ewes [8,25] and goats, in which Andrioli et al. [26] highlighted the practical advantages of TC. However, it should be considered that bypassing the cervix is more difficult in sheep than in goats and, moreover, there is a wide variation among different sheep breeds [27]. Thus, more studies of different breeds and conditions should be performed before definitive conclusions can be drawn.

As expected, RT decreased more while the LP animals were anesthetized, and typical stress responses - such as HR, glycemia, and cortisol concentrations - increased more during the period in which they were recovering from the anesthesia. Therefore, although the anesthesia partially controlled sensitivity to the procedure, most acute responses were observed immediately after the LP procedure ended, even considering that the time required by the animals to stand up and eat again was similar in both procedures. In this sense, it is possible that although the anesthesia exerted a positive effect while it was acting, there was a rebound effect related with the post-anesthesia recovery of the animals, indicating that although the anesthesia may be temporarily beneficial, the whole procedure may be even more stressful and painful for the animals.

Both HR and RT increased after the LP procedure ended, probably due to the longer period needed for anesthetic recovery and the pain caused by pneumoperitoneum and manipulation of the viscera during the laparoscopy [28]. Two interesting findings are worth mentioning: the increase of HR in the TC procedure shortly after the collection ended, probably related to the prompt return from sedation which generates a slight agitation; and a decrease in RT during the intraoperative LP collection, probably due to the use of anesthetics such as propofol and isoflurane [29,30]. Therefore, although some stress responses were greater with the LP procedure (HR and cortisol), avoiding the use of general and/or epidural anesthesia would require the use of more intensive analgesia protocols to decrease the discomfort of embryo collection.

Although TC induced lower increases of serum cortisol, the reduction of these concentrations was slower than after LP. Probably,

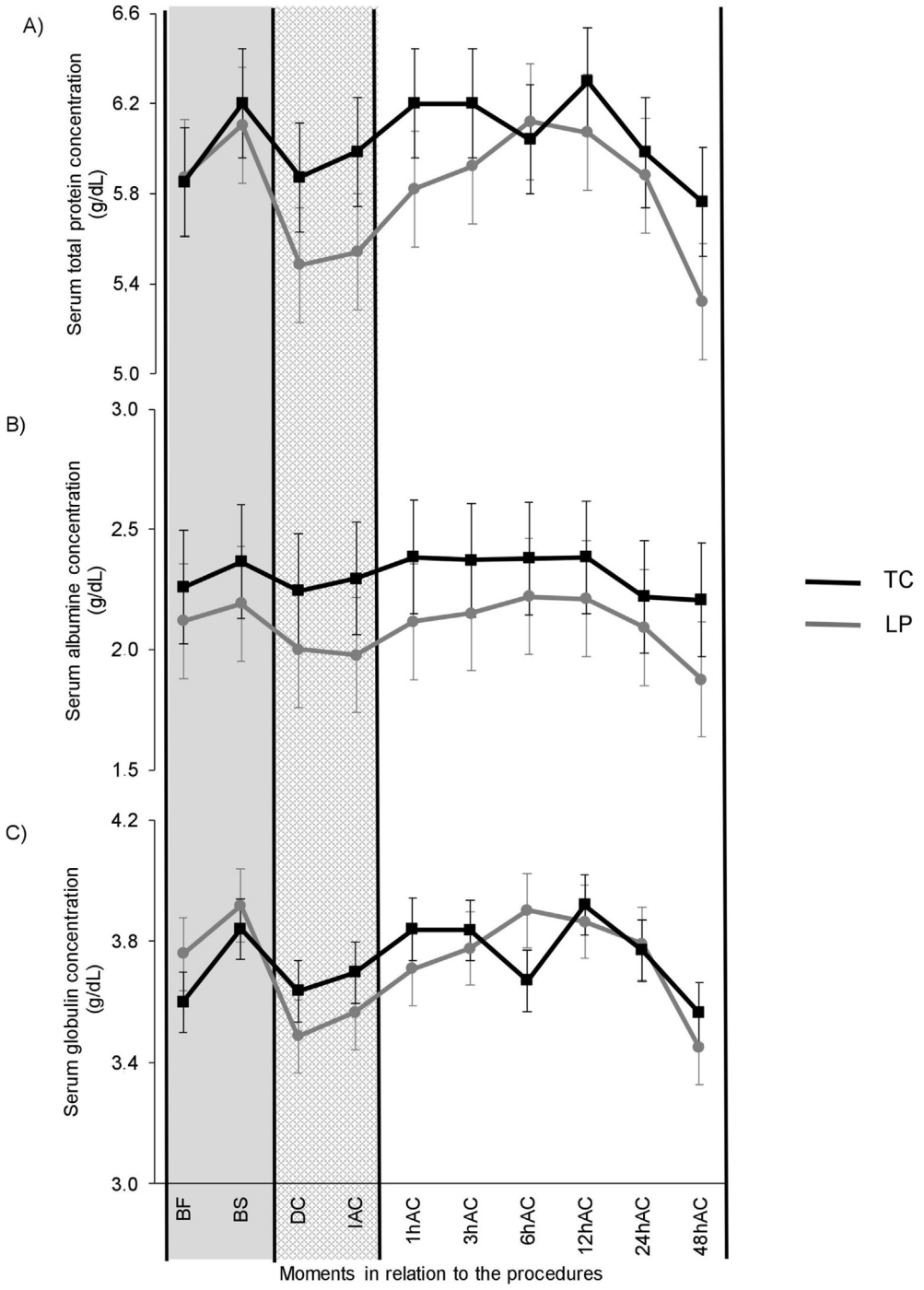

Fig. 5. A) Serum total protein (g/dL); B) albumin (g/dL); and C) globulin (g/dL) values in ewes submitted to embryo collection by either laparotomy (LP) or the transcervical route (TC). BF: before fasting; BS: before sedation; DC: during collection; 1hAC: 1 h after collection; 3hAC: 3 h after collection; 6hAC: 6 h after collection; 12hAC: 12 h after collection; 24hAC: 24 h after collection; 48hAC: 48 h after collection.

the discomfort and pain caused by manipulation of the cervix (clipping and caudal traction) without the analgesia induced by the anesthesia explains the maintenance of elevated cortisol concentrations, as the analgesia provided by epidural anesthesia with ketamine lasts approximately 50 min in small ruminants [31]. Although the TC procedure is easier to carry out and seems to be less invasive, it can also induce an inflammatory response due to the damage to the cervical epithelium caused by the manipulation of the cervix, which also activates the uterine immune response [32]. This may explain the high glycemia induced, and the lack of differences in total protein, albumin, and globulin concentrations in the two procedures (ECPxTime: NS, 0.064, NS; respectively). Therefore, the inclusion of a pain control protocol with the administration of an anti-inflammatory agent and/or analgesia associated with the TC collection may be necessary to decrease welfare concerns.

In contrast to previous reports [3,4,33], the rate of structure recovery under the LP procedure was notably lower in this study (50–80% in the cited studies *vs* 37.1% in this study). However, this

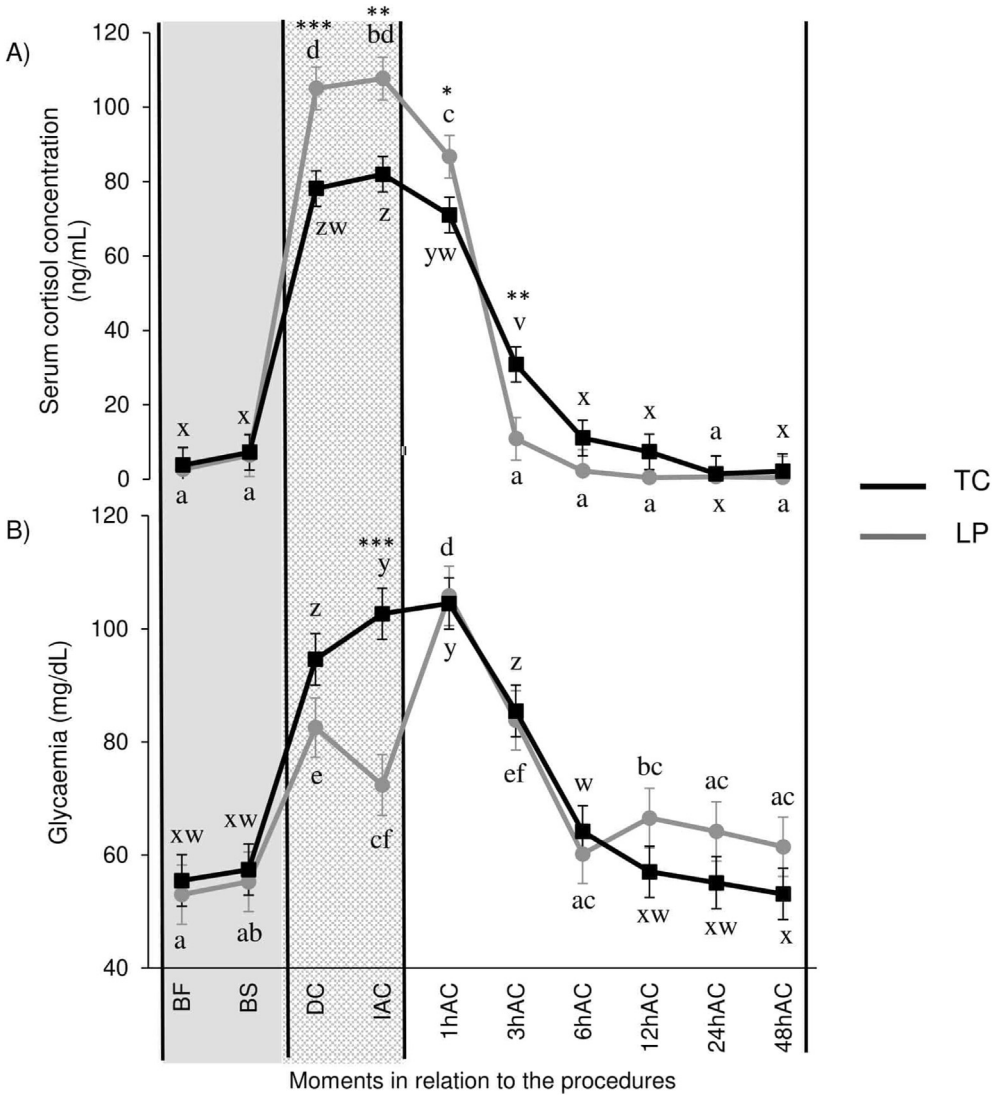

Fig. 6. A) Cortisol (ng/mL); and B) glycaemia (mg/dL) values in ewes submitted to embryo collection by laparotomy (LP) or the transcervical route (TC). BF: before fasting; BS: before sedation; DC: during collection; 1hAC: 1 h after collection; 3hAC: 3 h after collection; 6hAC: 6 h after collection; 12hAC: 12 h after collection; 24hAC: 24 h after collection; 48hAC: 48 h after collection. * Indicates interaction between embryo collection procedures (LP and TC) x Time ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$). Differences throughout the evaluations in each embryo collection procedure are indicated by different letters (TC: v, x, z, y, w; LP: a, b, c, d, e, f; $P < 0.05$).

was unrelated to the fluid recovery, which was very high in both procedures (even lower with LP, which is conceivable since the amount of liquid injected in the TC procedure is four times greater than that in LP). It is possible that the administration of oxytocin before the procedure had a negative influence on the recovery of structures, as it promotes contractility of the uterine horns [34], probably inducing uterine contractions during the collection and thus reducing the recovery rate. Although the effect of the administration of this hormone was visible during collection (myometrial tetany), the consequence of this action on embryo progression and embryo quality is still unclear [35]. Considering both recovery rates, it seems that the structure recovery depletion in the LP procedure is not a consequence of the fluid recovery, so the recovery of the structures may be more affected by changes in uterine tonus than with the TC procedure.

The number of viable embryos recovered per animal with the TC procedure is similar to that stated in previous reports [2,36,37]. It is important to note that in the cited studies, different cervical dilation protocols were used, among which the best results refer to the use of misoprostol, a hormone which is difficult to obtain due to its abortifacient effect in humans. Similarly, the LP procedure showed good quality embryos, as previously described [3,4,20]. Interestingly, the TC procedure obtained the highest percentage of grade I and II embryos. It is possible that the anesthetics used in the LP procedure also influenced this parameter, since it has been found that increasing the concentration of isofluorane in mice inhibits the initial development of *in vitro* produced embryos [38]. Thus, it is plausible that the use of volatile anesthetics influenced the quality and embryonic development in this procedure.

Although the objective of the study was to compare the two procedures, the transposition test (performed only to decide which procedure to apply) indicated failure in 38.6% (since 61.4% of the animals had their cervix transposed) of the animals used. Although this is a high percentage, it may be related to breed characteristics. In effect, these ewes display wide variation in the shape and length of the cervix [25]. Anatomical features such as vaginal-vestibule stenosis, elongated/tortuous cervices, and insufficient cervical distension are also cited by the authors as a frequently found

obstacle in this breed. Although not considered in this study, some of these aspects were observed. This low percentage can also be attributed to the use of different cervix relaxation protocols. In this regard, Fonseca et al. [11] demonstrated that the same hormone (in different compositions: estradiol benzoate vs. estradiol cypionate) administered at different intervals led to a 40% reduction in the passage of the cervix.

5. Conclusion

The TC embryo collection was more effective than the traditional LP procedure. Although both embryo collection procedures affected ewes' welfare, the TC procedure is probably less stressor than the LP. In this sense, it is essential to study possible treatments associated with analgesia or other complementary pharmacological strategies.

Declaration of competing interest

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

CRediT authorship contribution statement

Juliana Dantas Rodrigues Santos: Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Rodolfo Ungerfeld:** Conceptualization, Methodology, Validation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Mário Felipe Alvarez Balaro:** Methodology, Investigation, Writing - review & editing. **Joanna Maria Gonçalves Souza-Fabjan:** Methodology, Investigation, Writing - review & editing. **Isabel Oliveira Cosentino:** Methodology, Investigation. **Viviane Lopes Brair:** Methodology, Investigation. **Clara Vieira de Souza:** Methodology, Investigation. **Pedro Henrique Nicolau Pinto:** Methodology, Investigation. **Ana Luiza Cunha Bade:** Methodology, Investigation. **Jeferson Ferreira da Fonseca:** Methodology, Investigation, Writing - review & editing. **Felipe Zandonadi Brandão:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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