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ORIGINAL ARTICLE

Use of meloxicam with or without dipyrone in non-surgical embryo recovery in hair sheep: Effects on animal welfare

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Abstract

The aim of this study was to determine the effectiveness of meloxicam with or without dipyrone on the welfare of ewes subjected to non-surgical embryo recovery (NSER). Two studies were carried out using 51 multiparous Santa Inês ewes. All animals received a standard oestrous synchronization treatment and a superovulatory protocol. In Study 1, 12 ewes received meloxicam (GM) before cervical transposition (1 mg kg^{-1}, i.v.), repeated 24 h after (1 mg kg^{-1}, i.m.), while the other 10 received a saline solution, remaining as a control group (GC1). In Study 2, ewes were allocated into a group of 15 ewes treated as GM of Study 1 associated with dipyrone (GMD; 50 mg kg^{-1}, i.m.) before cervical transposition, 12 h, and 24 h after, or a control group (GC2) of 14 ewes treated with saline solution. In both studies, heart and respiratory rates (RR), cortisol, glucose, total proteins, albumin and globulins blood concentration were recorded before sedation (BS), after sedation (AS), after cervical transposition, immediately after collection (IAC), and 0.5, 1.5, 3, 6, 12, 24 and 48 h after embryo collection (hAC). In Study 1, RR tended to be greater in GC1 ($p = .08$), serum total proteins and globulins values were lower and serum albumin values were greater in this group than GM ($p = .003$, $p < .0001$, and $p < .0001$, respectively). In Study 2, treatment of GMD tended to reduce the glycaemia at AS ($p = .052$) and reduced it at 3hAC ($p < .0001$), and 6hAC ($p = .03$). It also tended to reduce cortisol concentrations ($p = .10$). The other variables varied with NSER without interaction with the experimental treatments. In conclusion, in this study condition, NSER in sheep induced transient changes indicative of stress and possibly pain, therefore, affecting animal welfare. The administration of meloxicam was ineffective to reduce those responses, and the association of dipyrone had only slight effects without modifying the main welfare indicative responses in ewes subjected to NSER.

KEYWORDS
analgesia, metamizole, pain control, transcervical embryo collection, wellness

1 | INTRODUCTION

Assisted reproductive biotechnologies such as multiple ovulation and embryo transfer enhance genetic gains and reproductive efficiency in livestock systems. Embryo collection and transfer are key steps for the success of these programmes and can be performed by surgical or transcervical techniques. Despite the good outcomes and still being the most commercially used method for sheep, surgical procedures for embryo collection involve several animal welfare issues due to the anaesthetic and surgical risks and a limit of successive donor uses as a consequence of post-surgical sequelae in reproductive organs (Fonseca et al., 2016; Gomes et al., 2014; Pinto et al., 2020). However, although there are initial studies on pharmacological protocols for cervical dilation (Dias et al., 2023, 2020; Fonseca, Zambrini, et al., 2019; Leite et al., 2018; Prellwitz et al., 2019), the complex sheep's cervical morphology makes transcervical uterine access difficult (Fonseca et al., 2019a; Kershaw et al., 2005). Although non-surgical embryo recovery (NSER) is less invasive and has good recovery rates of structures, under some drugs preparations/combination and anaesthetic procedures used, it also induces an elevation of inflammatory markers (Oliveira et al., 2018), heart rate, cortisol values and glycaemia (Santos et al., 2020) indicative of stress, and probably pain and discomfort generated by cervical manipulation.

Welfare requirements in farm animals include the improvement and feasibility of less invasive veterinary procedures such as NSER, including the evaluation of specific strategies to reduce the negative responses. In this context, anti-inflammatory and/or analgesic drugs can reduce stress and improve welfare conditions for farm animals subjected to painful procedures (Windsor et al., 2016). To the best of our knowledge, there are no studies with this aim during NSER, but in general, non-steroidal anti-inflammatory drugs (NSAID) are widely used in veterinary medicine because of their easy administration, good half-life and potent anti-inflammatory, and analgesic effects. In short, their therapeutic properties are due to the regulation of cyclooxygenase (COX) enzymes, reducing peripheral inflammatory mediators, and centrally mediated analgesic effects (Adams, 2017; Clark-Price, 2014; Lizarraga & Chambers, 2012). Furthermore, the association of NSAIDs with other analgesics could prevent acute pain and minimize the side effects of each drug (Windsor et al., 2016). In this context, meloxicam, an NSAID, is safe and effective for reducing somatic and visceral pain in ruminants (Colditz et al., 2019; Mauffré et al., 2021). Therefore, meloxicam can be potentially used alone, or associated with dipyrone, a non-opioid analgesic with antipyretic and spasmolytic properties, even though without a strong anti-inflammatory effect (Jasiecka et al., 2014).

The aim of this study was to determine if the administration of meloxicam associated or not with dipyrone reduces the responses indicative of stress and probably pain, improving the welfare of hair ewes submitted to NSER.

2 | MATERIALS AND METHODS

2.1 | Experimental location and animals

The studies were conducted at the Unidade de Pesquisa Experimental em Caprinos e Ovinos at Universidade Federal Fluminense (UFF), located in Cachoeiras de Macacu (22°S, 42°W), Rio de Janeiro, Brazil. The project was approved by the Ethics Committee on the Use of Animals at Universidade Federal Fluminense (3,155,020,620—ID000931). Two studies were carried out using 51 multiparous Santa Inês ewes. This breed has only slight seasonal changes at this location (Balaro et al., 2014), so all ewes were cycling at the time of both studies. All animals were previously submitted to a clinical and ultrasonographic evaluation, being used only those that were healthy and without reproductive disorders. Ewes were managed under an intensive system, in a suspended pen with a slatted floor, fed with chopped elephant grass (*Pennisetum purpureum* Schum.), 300 g/animal/day of concentrate (16% of crude protein), and free access to water and mineral salt (Ovinofos). Embryo collection and sample processing were performed in temperature-controlled rooms equipped for these procedures.

2.2 | Experimental design

2.2.1 | Study 1

The study was performed during spring, with 22 adult, cyclic, multiparous Santa Inês ewes (42.9 ± 3.7 kg; 2.9 ± 0.3 of body condition score on a scale of 1–5; mean ± SD). The animals were submitted to a Day 0 oestrus synchronization protocol according to Balaro et al. (2016). Briefly, ewes were superovulated with 133 mg of p-FSH i.m. (Folltropin-V, Bioniche Animal Health, Ontario, Canada) in six decreasing doses (25%, 25%, 15%, 15%, 10% and 10% of the total dose) every 12 h, with the first dose given 80 h after intravaginal sponge removal containing medroxyprogesterone acetate (60 mg; Progespon; Syntex, Buenos Aires, Argentina). At this time, a new progesterone intravaginal implant (P4; 0.36 g; Primer PR, Agener União Saúde Animal) was inserted, which remained in situ until the fifth FSH dose. Simultaneously with the last dose of pFSH, sodium cloprostenol (0.24 mg i.m.; Estron, Agner União Saúde Animal, São Paulo, Brazil) was administered, and after 24 h, the ewes received lecirelin (25 μg i.m.; TEC-Relin, Agener União Saúde Animal, Brazil), a GnRH analogue. All females were inseminated thrice using semen from rams with proven fertility through previous andrological evaluation. Inseminations were performed 24, 36 and 48 h after the fifth FSH dose with 0.10 mL of fresh semen into the cervical os (300×10^6 spermatozoa/dose) (Figure 1).

In sequence, ewes were randomly divided into two groups. A treated group (group GM, $n = 12$), in which meloxicam (Maxicam 2%, Ourofino Saúde Animal) was administered before cervical

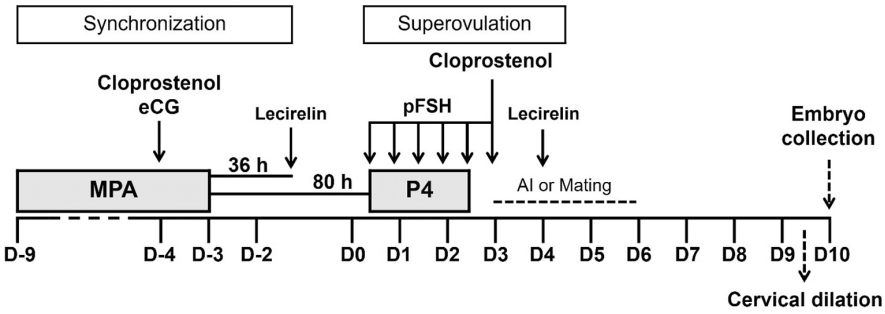

FIGURE 1 Experimental treatment for in vivo embryo production in Santa Inês ewes, including hormonal protocols and embryo collection. Artificial insemination (AI); Equine chorionic gonadotropin (eCG; 300 IU); Lecirelin (25 μg); Medroxyprogesterone acetate (MAP; 24 mg); Porcine follicle stimulating hormone (pFSH; 133 or 200 mg); Progesterone (P4; 0.36 mg); Sodium cloprostenol (0.24 mg).

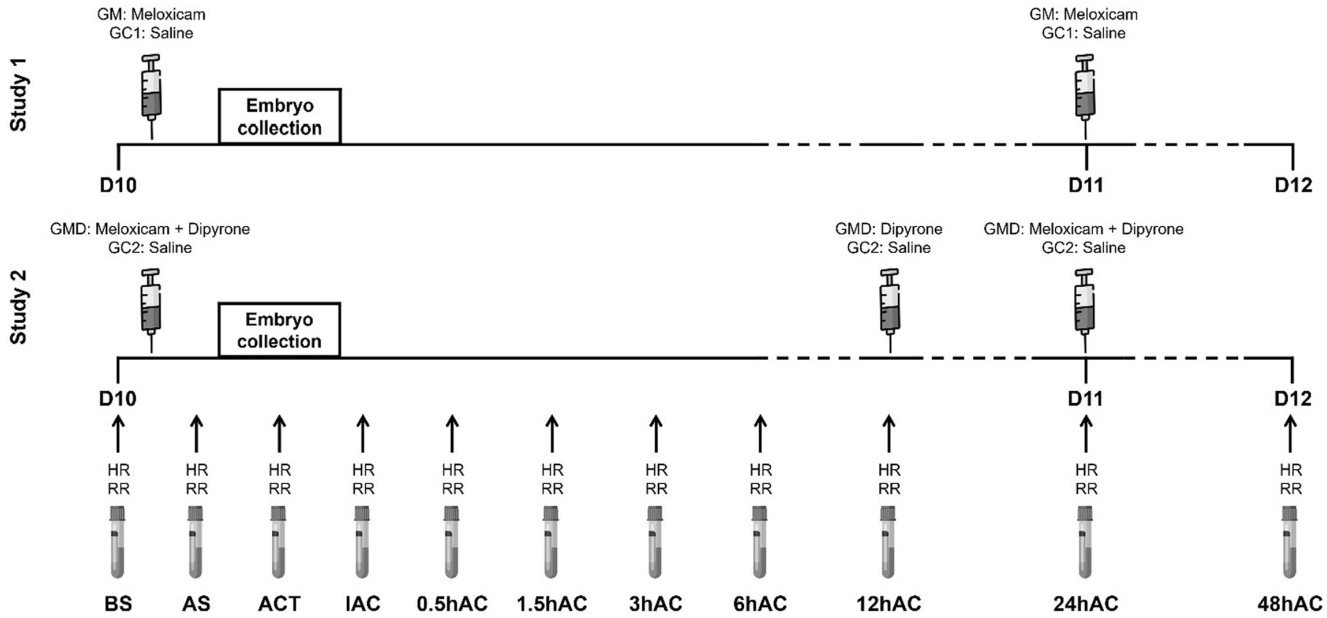

FIGURE 2 Pain control protocols and animal welfare responses in Santa Inês ewes submitted to non-surgical embryo recovery. 0.5hAC, 0.5 h after collection; 1.5hAC, 1.5 h after collection; 12hAC, 12 h after collection; 24hAC, 24 h after collection; 3hAC, 3 h after collection; 48hAC, 48 h after collectio; 6hAC, 6 h after collection; ACT, after cervical transposition; AS, after sedation; BS, before sedation; GC1 and GC2, control groups; GM, meloxicam group; GMD, meloxicam and dipyrone group; HR, heart rate; IAC, immediately after collection; RR, respiratory rate.

transposition (1 mg kg^{-1}, i.v.) and 24 h after cervical transposition (1 mg kg^{-1}, i.m). A control group (group GC1, $n = 10$) received saline solution (volume calculated according to each animal weight and drug dose) following the same routes and administered at the same moments (Figure 2).

2.2.2 | Study 2

The second study was performed during the spring, with 29 adult, cyclic, multiparous Santa Inês ewes (52.2 ± 10.3 kg; 3.0 ± 0.3 of BCS). All animals underwent the same oestrus synchronization protocol described in Study 1 and were superovulated with 133 ($n = 14$) or 200 mg of pFSH ($n = 15$), subsequently following the same hormonal protocol previously described. All ewes were subjected to natural mating every 12 h from the sixth FSH dose to the estrus ending (Figure 1).

In sequence, ewes were randomly allocated into two homogeneous groups, regarding the superovulatory treatments, that is, the effect of the FSH dose was blocked. Fifteen ewes received meloxicam before (1 mg kg^{-1}, i.v.) and 24 h after cervical transposition (1 mg kg^{-1}, i.m.), associated with dipyrone (50 mg kg^{-1}, i.m.; D-500, Zoetis) before, 12 and 24 h after cervical transposition (group GMD). The other 14 ewes remained as a control group (group GC2), receiving saline solution (volume calculated according to each animal weight and drug dose) following the same routes and time (Figure 2).

2.3 | Cervical dilation and non-surgical embryo recovery

In both studies, ewes underwent a hormonal protocol for cervical dilation (Leite et al., 2018), that included the administration of estradiol benzoate (100 μg i.v.; RIC-BE, Agener Union, São Paulo, Brazil)

diluted with 2.5 mL of absolute ethyl alcohol and 2.5 mL of saline and sodium cloprostenol (0.12 mg i.m.; Estron; Agener União, São Paulo, Brazil) 12 h before embryo collection, and oxytocin (100 IU i.v.; Oxytocin Forte UCB, Centrovet, Goiânia, Brazil), 15 min before collection. Ewes were sedated with acepromazine maleate (0.1 mg kg^{-1} i.v.; Acepran, Vetnil, Louveira, Brazil) and diazepam (0.2 mg kg^{-1} i.v.; Diazepam, Teuto, Anápolis, Brazil) and then submitted to epidural anaesthesia with ketamine hydrochloride (2.0 mg kg^{-1}; Cetamin, Syntec, Barueri, Brazil). For NSER, cervical fixation and traction were performed followed by both uterine horn washing and recovery of structures through a closed circuit (Circuito Embrapa for recovery of goat/sheep embryos, Embrapa, Brasília, Brazil) (Fonseca et al., 2013).

2.4 | Welfare responses

In both studies, heart (HR) and respiratory rates (RR) were recorded by auscultation before sedation (BS), after sedation (AS), after cervical transposition (ACT), immediately after collection (IAC), and 0.5, 1.5, 3, 6, 12, 24 and 48 h after embryo collection (hAC). Immediately after recording HR and RR, blood samples were collected by jugular venipuncture in sterile vacuum tubes containing sodium fluoride/potassium oxalate and without anticoagulant (Becton, Dickinson and Company) to obtain plasma and serum, respectively (Figure 2). All samples were divided into aliquots and stored in a freezer at −20° for further laboratory processing.

Serum values of total proteins and albumin, in addition to plasma glucose, were measured using commercial kits (Labtest Diagnóstica S.A., Minas Gerais, Brazil) in a semi-automatic biochemical analyser (BIO 2000, Bioplus Produtos Para Laboratórios Ltda., São Paulo, Brazil). Serum globulins were estimated by subtracting albumin concentration from total protein values. Serum cortisol concentrations were measured by radioimmunoassay using a commercial kit (MP Diagnostics Division). The sensitivity and intra-assay coefficients of variation were 0.05 ng/mL and 7.8%, respectively. All data were within the minimum and maximum values of the curve.

2.5 | Statistical analysis

Data analysis was performed using the SAS statistical software (SAS on Demand for Academics). Data were compared using a mixed model, including treatment, time and their interaction as main effects in variables with repeated measurements, and the pdiff procedure to compare specific points. Data are presented as LSmean ± SEM. For all tests, $p \leq .05$ was considered significant, and $p = .05 <$, $p \leq .1$ were considered as tendencies.

3 | RESULTS

Treatment, time and their interaction effects on welfare responses in Santa Inês ewes are presented in Tables 1 and 2 (Study 1 and Study 2).

3.1 | Study 1

In Study 1, HR, glycaemia and cortisol did not vary according to treatments nor to interactions between treatments and time in any variable analysed. RR tended to be greater in GC1 than GM ewes ($p = .08$). The HR, RR, glycaemia and cortisol concentration varied in relation to NSER (time effect). In general, HR increased from AS to ACT ($p < .0001$), from ACT to IAC ($p < .0001$), and decreased progressively thereafter until 12hAC ($p < .05$). The RR increased at AS ($p = .002$), decreasing from ACT to IAC ($p = .04$) (Figure 3a,b). Serum cortisol values increased at AS ($p < .05$), with a peak at ACT and IAC returning to baseline values at 12hAC, and glycaemia increased from AS to 3hAC ($p < .05$; Figure 4a,b).

Serum total proteins and globulins values were greater and serum albumin values were lower in GM than GC1 ewes ($p = .003$, $p < .0001$, and $p < .0001$, respectively). Despite treatments, total protein concentration decreased at ACT, IAC, 0.5hAC, 24hAC and 48hAC; albumin at ACT, 24hAC, and 48hAC; and globulins at IAC ($p < .05$ for all comparisons; Figure 5a–c).

TABLE 1 Treatment, time, and their interaction effects on welfare responses in Santa Inês ewes submitted to non-surgical embryo recovery and treated or not with meloxicam (Study 1).

	Treatment				*p*		
Parameter	GC1	SEM	GM	SEM	Treatment	Time	Treatment × time
HR (bpm)	108.7	2.7	111.4	2.2	ns	<.0001	ns
RR (rpm)	32.7	1.0	30.8	0.7	.08	<.0001	ns
Glucose (mg/dL)	84.4	2.6	80.3	1.9	ns	<.0001	ns
Cortisol (nmoL/L)	97.7	10.6	79.0	7.7	ns	<.0001	ns
Total proteins (g/dL)	7.06	0.04	7.26	0.05	.003	<.0001	ns
Albumin (g/dL)	2.62	0.02	2.49	0.02	<.0001	.001	ns
Globulins (g/dL)	4.44	0.04	4.77	0.05	<.0001	.0001	ns

Note: Data are presented as LS mean.

Abbreviations: GC1, Study 1 control group; GM, group treated with meloxicam (1 mg kg^{-1}: before cervical transposition, and 24 h after); HR, heart rate; RR, respiratory rate; SEM, standard error of the mean.

TABLE 2 Treatment, time, and their interaction effects on welfare responses in Santa Inês ewes submitted to non-surgical embryo recovery and treated or not with meloxicam and dipyrone (Study 2).

	Treatment				*P*		
Parameter	GC2	SEM	GMD	SEM	Treatment	Time	Treatment × time
HR (bpm)	98.4	1.8	97.5	1.8	ns	<.0001	ns
RR (rpm)	30.4	0.7	30.7	0.6	ns	.0003	ns
Glucose (mg/dL)	74.2	1.8	69.2	1.8	ns	<.0001	<.0001
Cortisol (nmoL/L)	121.1	7.9	104.4	7.5	.10	<.0001	ns
Total proteins (g/dL)	7.50	0.05	6.64	0.06	<.0001	<.0001	ns
Albumin (g/dL)	2.31	0.02	2.34	0.03	ns	<.0001	ns
Globulins (g/dL)	5.19	0.05	4.30	0.05	<.0001	.003	ns

Note: Data are presented as LSmean.

Abbreviations: GC2, Study 2 control group; GMD, group treated with meloxicam (1 mg kg^{-1}: before cervical transposition, and 24 h after) and dipyrone (50 mg kg^{-1}: before cervical transposition, 12 h, and 24 h after); HR, heart rate; RR, respiratory rate; SEM, standard error of the mean.

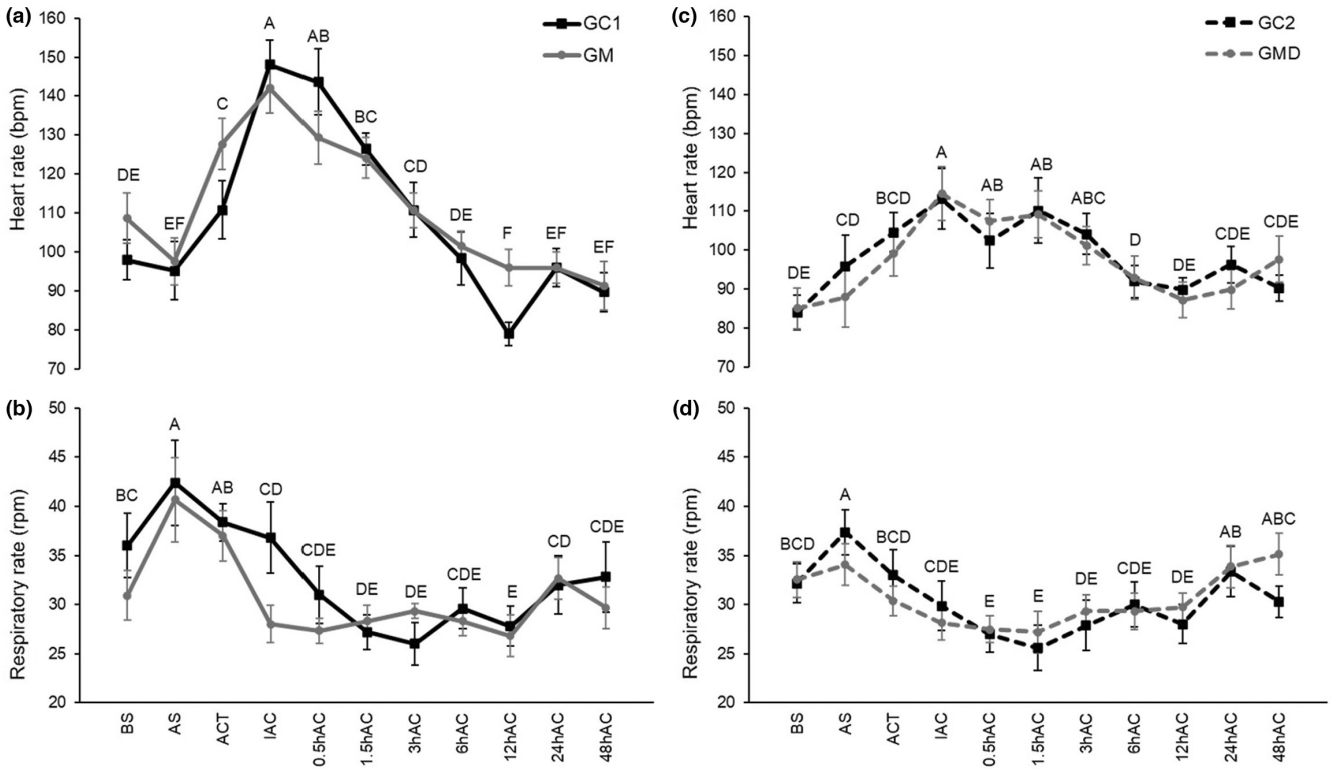

FIGURE 3 Heart rate (bpm) and respiratory rate (rpm) in ewes submitted to non-surgical embryo recovery and treated with meloxicam (Study 1 – a and b) or meloxicam and dipyrone (Study 2 – c and d). 0.5hAC, 0.5 h after collection; 1.5hAC, 1.5 h after collection; 12hAC, 12 h after collection; 24hAC, 24 h after collection; 3hAC, 3 h after collection; 48hAC, 48 h after collection; 6hAC, 6 h after collection; ACT, after cervical transposition; AS, after sedation; BS, before sedation; GC1 and GC2, control groups; GM, meloxicam group; GMD, meloxicam and dipyrone group; IAC, immediately after collection. Different capital letters indicate differences over time ($p < .05$). Data are presented as LSmean ± SEM.

3.2 | Study 2

In Study 2, HR and RR were not affected by treatments and there was no interaction between treatments and time, varying only throughout time. Heart rate increased from ACT to IAC ($p = .02$), decreasing from 3hAC to 6hAC ($p = .045$). The RR increased at AS ($p = .04$) and decreased at 0.5hAC and 1.5hAC ($p < .05$; Figure 4c,d).

Glycaemia was not affected by treatments, but there was a significant interaction between treatments and time ($p < .0001$), tending to be greater in GC2 than GMD at AS ($p = .052$), and being greater at 3hAC ($p < .0001$), and 6hAC ($p = .03$). The glycaemia in GMD ewes increased from BS to AS ($p = .02$), and from AS to ACT ($p < .0001$) returning to baseline values at 6hAC ($p < .05$). The GC2 ewes increased blood glucose from BS to AS ($p < .0001$), peaking at 1.5hAC

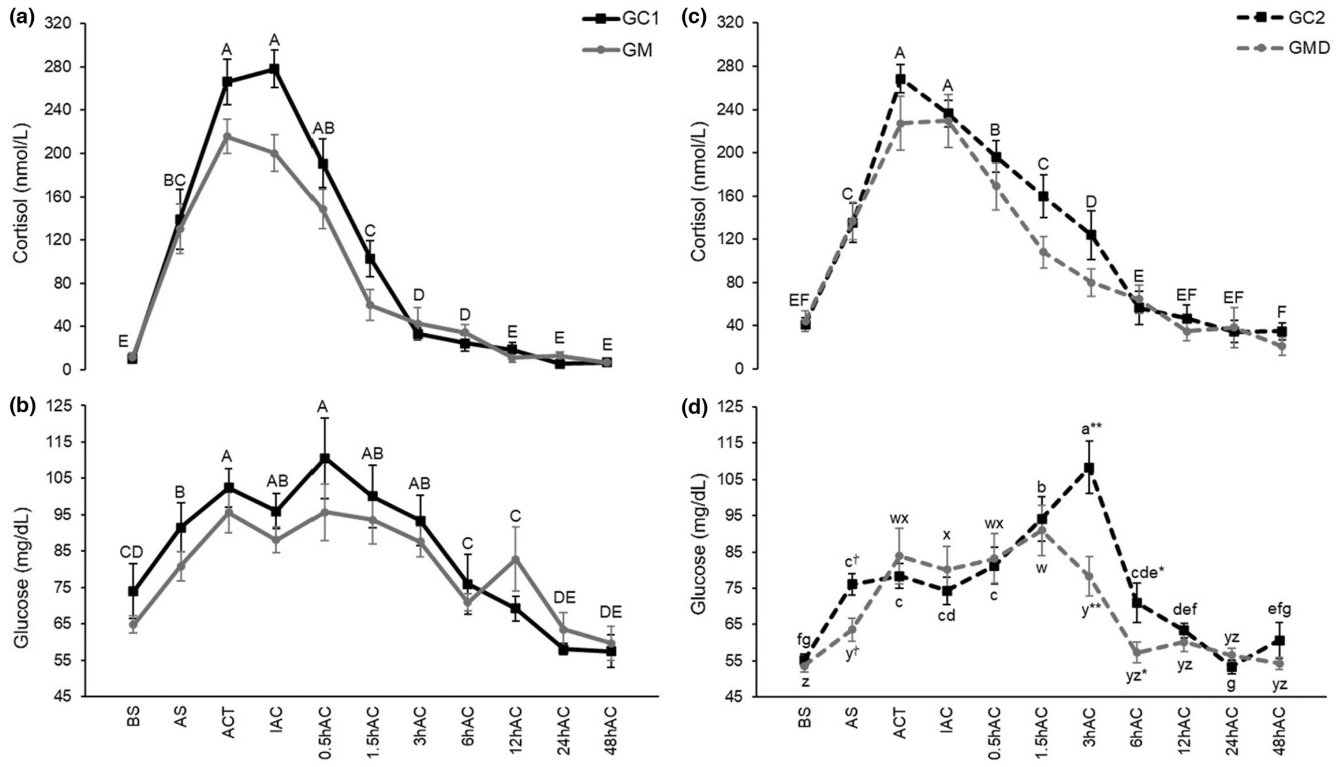

FIGURE 4 Plasma glucose and serum cortisol in ewes submitted to non-surgical embryo recovery and treated with meloxicam (Study 1 – a and b) or meloxicam and dipyrone (Study 2 – c and d). 0.5hAC, 0.5 h after collection; 1.5hAC, 1.5 h after collection; 12hAC, 12 h after collection; 24hAC, 24 h after collection; 3hAC, 3 h after collection; 48hAC, 48 h after collection; 6hAC, 6 h after collection; ACT, after cervical transposition; AS, after sedation; BS, before sedation; GC1 and GC2, control groups; GM, meloxicam group; GMD, meloxicam and dipyrone group; IAC, immediately after collection. $^{*}p < .05$; $^{**}p < .001$; †$p = .0521$ between treatments in that time point. Different capital letters indicate differences over time ($p < .05$). Different lowercase letters indicate differences throughout the time in each treatment (GC: a, b, c, d, e, f, g; GMD: w, x, y, z; $p < .05$). Data are presented as LSmean ± SEM.

and 3hAC returning to baseline values at 12hAC ($p < .05$). Cortisol values tended to be greater in GC2 than in GMD ($p = .10$), but there was no interaction between treatments and time. Considering both groups, cortisol increased at AS ($p < .05$), with a peak at ACT and IAC, and decreased progressively until reaching baseline values at 6hAC ($p < .05$ for all comparisons) (Figure 5c,d).

The serum total proteins and globulins values were greater in the GC2 than the GMD ewes ($p < .0001$), but there were no effects of treatments on serum albumin values. These variables had no interaction between treatments and time, varying only throughout time. In general, there was a reduction in total proteins and globulins concentrations from AS to ACT ($p < .0001$ and $p = .001$ respectively), returning to baseline values at 3hAC ($p < .05$ in both). Albumin concentration decreased from AS to ACT ($p < .0001$), returning to baseline values at 1.5hAC ($p < .05$; Figure 5d–f).

4 | DISCUSSION

Overall, ewes subjected to NSER with or without analgesic/anti-inflammatory treatments responded similarly in terms of the variables studies as indicators of stress responses, reaffirming that NSER requires the development of strategies to reduce welfare concerns (Oliveira et al., 2018; Santos et al., 2020). The development of NSER provided a practical tool, that allows collecting embryos without the requirements of surgeries, but as confirmed in both studies, is not completely innocuous in terms of animal welfare, inducing changes in most of the clinical and blood markers studied, indicating stress and probably pain responses. In general, although the treatments applied reduced some of these responses, apparently the main effect was limited to glycaemia when meloxicam and dipyrone were simultaneously applied, and to blood protein responses. On the other hand, cortisol secretion was not significantly reduced, suggesting that the treatments did not affect the hypothalamus–pituitary–adrenal response, or it was secreted at its maximum possible concentrations despite the administration of analgesic-anti-inflammatory treatments, considering the high concentrations compared with those observed in similar studies (Santos et al., 2020) or in relation to increases produced by electroejaculation (Abril-Sánchez et al., 2018). Overall, considering the slight positive effects, it should be required testing drugs with stronger effects or consider modifying the moment of the administration of the anti-inflammatory and/or analgesics drugs in relation to cervical manipulation to reduce the stress responses. It should also be important to study other responses, specifically.

In Study 1, regardless of treatments, the increase in HR may be related to cervical manipulation (press and caudal traction) for

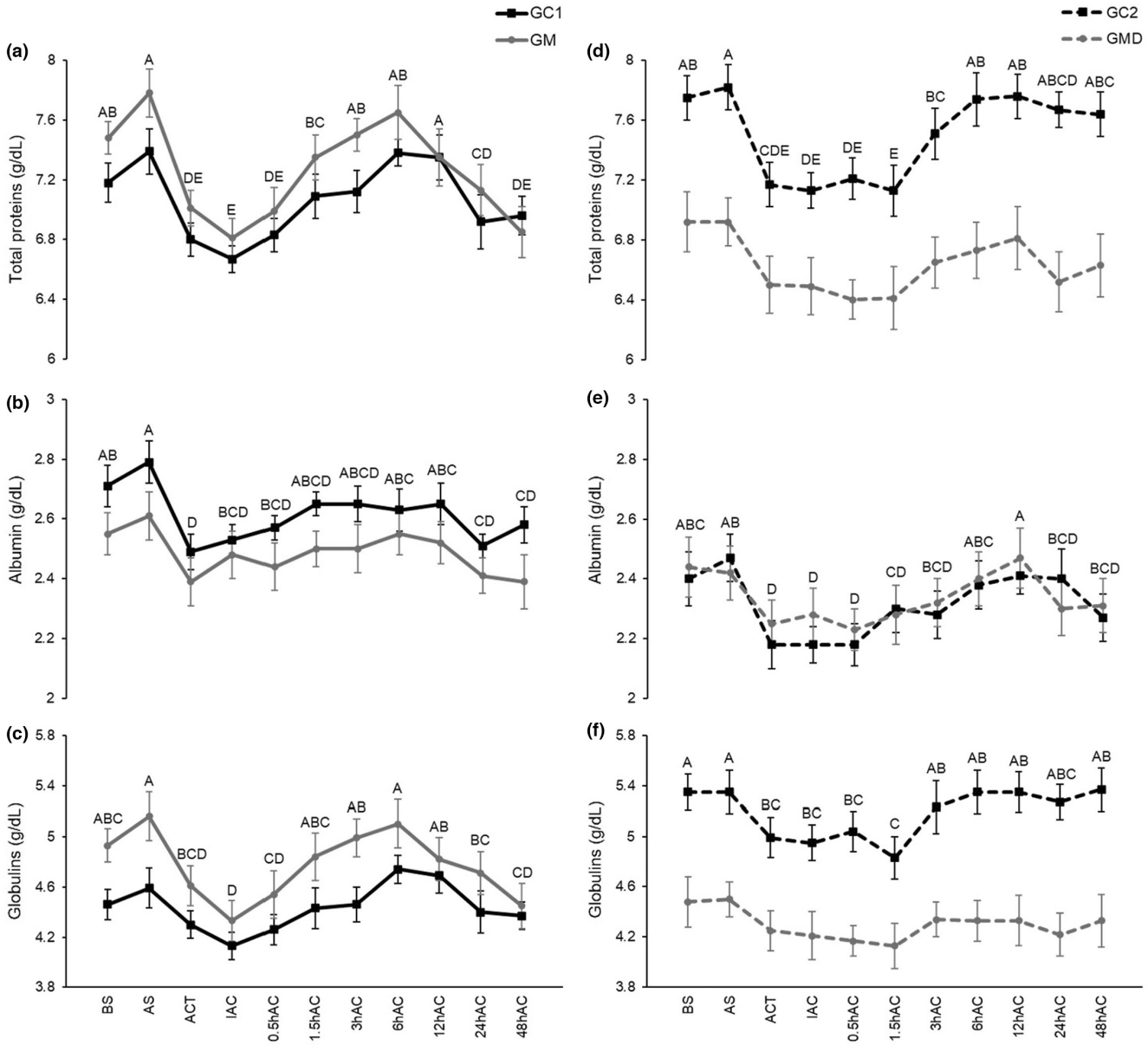

FIGURE 5 Serum total proteins, albumin and globulins in ewes submitted to non-surgical embryo recovery and treated with meloxicam (Study 1 – a–c) or meloxicam and dipyrone (Study 2 – d–f). 0.5hAC, 0.5 h after collection; 1.5hAC, 1.5 h after collection; 12hAC, 12 h after collection; 24hAC, 24 h after collection; 3hAC, 3 h after collection; 48hAC, 48 h after collection; 6hAC, 6 h after collection; ACT, after cervical transposition; AS, after sedation; BS, before sedation; GC1 and GC2, control groups; GM, meloxicam group; GMD, meloxicam and dipyrone group; IAC, immediately after collection. Different capital letters indicate differences over time ($p < .05$). Data are presented as LSmean ± SEM.

accessing the uterus, as this probably generates pain and tissue damage (Andrioli et al., 1999; Campbell et al., 1996). Increases in HR and RR may be associated with acute and chronic pain, related to sympathetic nervous system activation, and secretion of catecholamines, that trigger increases in blood pressure, cardiac output and systemic vascular resistance (DeMarco & Pascoe, 2008; Karas et al., 2008; Stewart et al., 2010). The animal handling immediately after recovering from sedation may have contributed to the maintenance of this state in the first evaluation after the end of embryo collection, as described by Santos et al. (2020), who also found an increase in this variable more accentuated at the first hour after NSER. The RR was similarly elevated at the time after sedation, in both studies, similar to what was previously observed by Leite et al. (2021) using the same sedation and epidural protocol in sheep. These authors also observed a reduction in RR from 45 to 60 min after the epidural procedure in agreement with our findings. Moreover, it was stated that although safe and effective for cervical manipulation, the anaesthetic protocol can induce mild cardiorespiratory changes (Leite et al., 2021). Celly et al. (1997) and Nishimura et al. (2017) also reported a transient reduction in RR in ewes sedated with diazepam or acepromazine, respectively. The punctual elevation of the RR at the AS moment may be related only to the stress provoked by the animal

handling or transporting to the veterinary room since the baseline assessments (BS) were carried out in the pens when animals were allocated, a more friendly environment for them. Similarly, in agreement with those results, cortisol concentration increased in similar magnitude in both treatments, mainly after sedation, reaching very high peaks after cervical manipulation. Glycaemia variations tend to follow those of cortisol, as expected due to its gluconeogenic effects (Goff, 2015; Lee et al., 2015). Overall, these findings demonstrate that even under sedation and epidural anaesthesia, receiving or no analgesia, NSER was stressful and probably painful partially explained by the inflammatory response associated with cervical manipulation and trauma. Therefore, overall, at least considering those physiological responses, it appears that the treatment was not effective in reducing the negative effects. Although it cannot be discarded that these increases be potentiated by the general handling management of the flock, movement of the animals, capturing and restraining the animals before anaesthetising them, the staff participating in both studies was the same, experienced technicians. Analgesic treatments were effective to reduce some physiological responses, including a temporary reduction in the increase of glycaemia. In particular, it appears that dipyrone was required to reduce the increase of glycaemia (Study 2), as meloxicam per se was not effective (Study 2). This response was consistent with the tendency of the same treatment to reduce cortisol secretion. Although treated and untreated ewes' blood protein concentrations differed, these were the main effects, and therefore, probably unrelated to the drug administration. Oliveira et al. (2018) observed an acute inflammatory response in sheep undergoing surgical embryo collection or NSER, with an elevation of total protein and haptoglobin values, but, on the other hand, Santos et al. (2020) did not record any difference between collection methods (surgical or transcervical) on total protein, albumin, and globulins values, although varied after embryo recovery. As with the other physiological responses, analgesic treatments were ineffective to control the main responses, or NSER per se triggered the maximum responses despite ewes received analgesia or not.

It is worth mentioning that even with time variation, HR and RR values in Study 2 remained within the normal range for species, remaining unclear why the response in Study 1 was apparently greater than that in Study 2, even in control ewes. In agreement, cortisol concentrations returned to baseline earlier in Study 2 than in Study 1, although in both studies reached high concentrations. As pointed out, although the cause remains unknown, the great differences observed between GC1 and GC2 ewes, as well as the differences in the magnitude of the responses in these studies and Santos et al. (2020) demonstrate that other factors not considered have a great influence on how ewes respond to NSER, reinforcing the concept that there is room to study deeply the factors causing greater or slighter responses.

5 | CONCLUSION

Both studies reinforced that the procedures required for NSER, including the movement of the animals, sedation, restraint and embryo collection were stressful, and NSER per se was also probably painful, affecting animal welfare. The administration of meloxicam was ineffective to reduce those responses, and the association of dipyrone had only slight effects without modifying the main welfare indicative responses in ewes subjected to NSER. Therefore, other analgesic options should be considered to improve welfare during NSER in sheep.

AUTHOR CONTRIBUTIONS

ACS Ribeiro, R Ungerfeld and FZ Brandão: conceptualization, methodology, investigation, data curation, formal analysis, writing – original draft, writing – review & editing. AR Taira, VC Santos, VL Brair and MPF Lopes: investigation, data curation. MFA Balaro, JMG Souza-Fabjan and JF Fonseca: conceptualization, methodology, investigation, writing – review & editing. FZ Brandão: project administration.

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CONFLICT OF INTEREST STATEMENT

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

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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