Small Ruminant Research 229 (2023) 107144

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Enhancing welfare and reducing stress in surgical embryo collection in sheep: Effects of flunixin and flunixin-dipyrone postoperative protocols in Dorper ewes

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ARTICLE INFO

Keywords:
Analgesic
NSAID
Sheep
Welfare

ABSTRACT

This study aimed to assess the role of dipyrone, in association with flunixin meglumine (FLU), in promoting a more effective modulation of physiological stress indicators during the postoperative period in ewes submitted to surgical embryo collection. Ewes were allocated into either flunixin (G-FLU; $n = 11$) or flunixin and dipyrone (G-FLUDIP; $n = 11$) group after laparotomy: All ewes received 2 mg/kg FLU and the G-FLUDIP received additionally three doses of 50 mg/kg dipyrone intramuscularly immediately, 24 h, and 48 h after the surgery. Embryo recovery by laparotomy had an average duration of 24 ± 0.85 min. The G-FLUDIP-ewes had a shorter interval to eat compared to G-FLU-ewes (9.5 ± 0.3 vs. 12.4 ± 0.4 min; $P = 0.001$). Serum cortisol concentrations presented a tendency ($P = 0.07$) of treatment×time interaction. The interaction effect is immediately after surgery and the G-FLU-ewes had a higher concentration of cortisol compared to G-FLUDIP-ewes ($P = 0.002$). For glycemia, a treatment×time interaction was observed ($P = 0.04$), but overall, concentrations were similar between the treatments ($P = 0.31$). There was a treatment×time interaction in serum globulins ($P = 0.04$), in which G-FLU had greater time to increase those concentrations than G-FLUDIP. The G-FLUDIP-treated ewes showed a lower ($P = 0.01$) overall platelet count compared to G-FLU. A treatment effect was also observed in overall lymphocyte ($P = 0.01$) and monocyte counts ($P = 0.03$). In conclusion, the association of flunixin and dipyrone after surgical embryo collection may promote a more effective modulation of pain and inflammation in ewes since these animals had lesser stress in the initial 24-h period after the procedure. This association was shown to decrease cortisol levels, promote more effective control of thrombocytosis, reduce the acute inflammation recovery time in the ewes, and reduce the interval of time that it took the ewes to eat after the procedure. Thus, the association of both flunixin and dipyrone may be important to increase ewes' welfare after surgical embryo collection.

1. Introduction

Whereas the most used reproductive biotechnology for embryo production in cattle is in vitro embryo production (IVP), multiple ovulation and embryo transfer (MOET) is still the primary assisted reproductive technology used for small ruminant embryos (Souza-Fabjan et al., 2014). The MOET is an important tool in genetic improvement programs because it enables females of high genetic value to produce more offspring when compared to natural breeding (Bruno-Galarraga et al., 2015). In addition, when associated with cryopreservation, MOET enables the conservation and dissemination of genetic material (Pathak et al., 2020). The technique is based on estrus induction or synchronization and superovulation with artificial insemination or natural breeding, followed by embryo collection and the transfer of these embryos to previously synchronized patients.

In small ruminants, embryo collection can be performed by either surgical (laparotomy; Bruno-Galarraga et al., 2015), semi-surgical (laparoscopy; Bari et al., 2001), or non-surgical method (transcervical; Santos et al., 2020; Ribeiro et al., 2023). In sheep, due to the complexity of the cervix, the surgical method still remains the technique of choice

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https://doi.org/10.1016/j.smallrumres.2023.107144
Received 25 July 2023; Received in revised form 29 October 2023; Accepted 31 October 2023
Available online 3 November 2023

worldwide. As an invasive procedure that requires general anesthesia and externalization of the reproductive tract, laparotomy has some disadvantages, such as the risk of adhesions in reproductive organs and stress on animals due to surgical manipulation, as well as its consequent inflammatory response (Ledda and Gonzalez-Bulnes, 2018). Some studies reported an increase in stress indicators, such as acute phase proteins (Oliveira et al., 2018), heart rate, blood glucose, and serum cortisol concentrations (Santos et al., 2020), during the postoperative recovery of sheep submitted to surgical embryo collection. Due to the growing concern for animal welfare, it is necessary to establish protocols to attenuate stress and postoperative discomfort (Windsor et al., 2016). Mitigating stress factors induced by surgical procedures will reflect directly on both inflammation and pain control, corroborating to improve animal welfare (Ahmad and Zakaria, 2015; Bain, 2020).

The use of anti-inflammatory and/or analgesic medications has the potential to alleviate stress and enhance the well-being of farm animals undergoing painful procedures (Windsor et al., 2016). In small ruminants, the postoperative protocols are usually based on the use of non-steroidal anti-inflammatory drugs (NSAIDs; Santos et al., 2020), such as flunixin meglumine (FLU), which is employed in different surgical procedures (Ismail, 2017; Graves et al., 2020), due to their anti-inflammatory and analgesic properties. Additionally, they exhibit the capacity to lower fever and decrease platelet aggregation. These mechanisms are primarily attributed to their ability to inhibit cyclooxygenase, thereby decreasing the production of prostaglandins (Lizarraga and Chambers, 2006; Galatos, 2011). Although FLU induces effective and long-duration visceral analgesia, it may exert a less efficient analgesic effect on skeletal muscle (Anderson and Muir, 2005). In this sense, the association with other analgesic drugs may be a strategy to achieve more effective pain control in small ruminants (Lizarraga and Chambers, 2012). Dipyrone (metamizole) is a non-opioid analgesic that acts by inhibiting COX-3 in the central nervous system with opioidergic and cannabinoid systems activation. Beyond its analgesic effects, dipyrone exerts an antipyretic and spasmolytic effect (Jasiecka et al., 2014) and, in sheep, it offers more extended periods of pain relief compared to other drugs (Lizarraga and Chambers, 2006). However, dipyrone could cause damage to the hematopoietic system, such as agranulocytosis, and although approved for use in sheep in Brazil, this regulation may vary in different countries (Giorgi et al., 2015; Fux et al., 2022). We hypothesize that dipyrone, in association with FLU, may have a role in promoting a more effective modulation of physiological stress indicators during the postoperative period in sheep submitted to laparotomy. Thus, the aim of this study was to compare physiological stress indicators after two different postoperative protocols with FLU administration, given as a singular treatment or in association with dipyrone, in Dorper ewes subjected to surgical embryo collection.

2. Material and methods

2.1. Ethics, experimental location, and animals

The study was performed in a commercial sheep herd in São Luíz do Paraitinga (23°22'S and 45°26'W), state of São Paulo, Brazil, during the non-reproductive season (October-November). All the procedures were approved by Ethics Committee for the Use of Animals of Universidade de São Paulo (protocol #2717181220). A total of 22 healthy Dorper ewes aged between two and eight years old (3.2 ± 0.1 years), with an average body weight of 64.2 ± 4.0 kg and body condition score (1–5 scale range) of 3.9 ± 0.1 were used in this study. All animals were maintained in an intensive production system and received corn silage and a balanced high-energy diet based on ground corn twice a day, mineral salt, and water ad libitum.

2.2. Experimental design

Ewes were randomly allocated into two groups after surgical embryo collection: flunixin (G-FLU; $n = 11$) or flunixin and dipyrone (G-FLUDIP; $n = 11$). The G-FLU group was submitted to a postoperative protocol with the administration of three doses of 2 mg/kg FLU (Banamine®, MSD Animal Health, São Paulo, Brazil) intramuscularly (IM) immediately after, 24 h and 48 h after the surgical procedure, while the G-FLUDIP group also received three doses of 50 mg/kg sodium dipyrone (D-500®, Zoetis, São Paulo, Brazil; Ribeiro et al., 2023) IM, according to the manufacturer's leaflet for use in sheep, at the same time intervals as FLU administration (Fig. 1). Both groups received three doses of 20 mg/kg antibiotic (Terramycin/LA®, Zoetis) IM immediately after, 24 h, and 48 h after the surgical procedure. The comparison of each treatment regarding stress was assessed by behavioral and physiological indicators measured at 10 different time points (Santos et al., 2020; Fig. 1).

Several indicators were recorded, such as interval from the surgery to get up and to feed; physiological variables [heart rate (HR), respiratory frequency (RF), ruminal movements (RM), and rectal temperature (RT)]; behavioral variables as body posture (standing, arched, sternal or lateral decubitus) and locomotion pattern (normal or limping). In addition, blood samples were collected for hemogram and subsequent measurement of inflammatory markers such as cortisol, glucose, total plasma proteins (TPP), globulin (GLO), albumin (ALB), and fibrinogen (FIB). The embryo collection was performed by the same technician and the time spent in the procedure was registered.

2.3. Superovulation, artificial insemination, and embryo collection

All ewes were submitted to a superovulation protocol and laparoscopic artificial insemination (AI) twice (36 and 42 h after removal of the progesterone device) using commercially cooled semen (Rocha et al., 2022). Before the embryo recovery by laparotomy, all ewes were fasted from food and water for 24 and 12 h, respectively. For the anesthetic induction, animals received 0.1 mg/kg of xylazine hydrochloride 2 % via intravenous (IV). The ewes were placed in a dorsal decubitus position for abdominal trichotomy and anesthesia. The anesthetic plan was achieved by isoflurane (2–4 % 10–15 mL/kg/min de oxygen; Isoforine®, Cristália, São Paulo, Brazil). Embryo recovery was performed by laparotomy according to Bruno-Galarraga et al. (2015).

2.4. Behavioral and physiological parameters

Animal behavior was classified as vigilant or lethargic, and posture variables were measured according to position (standing or recumbency) and locomotion pattern (normal or lameness) in 10 different moments: immediately before anesthetic induction (T1), during the surgical procedure (T2), immediately after the surgical procedure (T3), 1 h (T4), 3 h (T5), 12 h (T6), 24 h (T7), 36 h (T8), 48 h (T9) and 72 h (T10) after the surgical procedure, as shown in Fig. 1. Physiological indicators (HR, RF, MR, and RT) were measured at the same moments. The HR, RF, and MR parameters were measured by auscultation and RT was measured using a digital thermometer. The HR and RF were measured in beats and movements per minute, respectively, whilst the MR was measured in movements per 2 min. Also, ewes were observed immediately after the surgical procedure (T3), and the intervals to stand up and eat were recorded for each animal.

2.5. Hematologic parameters

Blood samples were collected by jugular venipuncture using two 4 mL vacuum collection tubes at the same moments mentioned above (Fig. 1). Samples collected in tubes containing clot activator plus 0.05 mL of glycolysis-inhibiting anticoagulant solution (Glistab®, Labtest, Minas Gerais, Brazil) were centrifuged at 2500×*g* for 15 min for serum separation. Aliquots of serum were frozen at − 20 °C until analysis. Samples collected in tubes containing ethylenediaminetetraacetic acid (EDTA) were used for counting the following blood cells: erythrocytes, total leukocytes, basophils, eosinophils, band neutrophils,

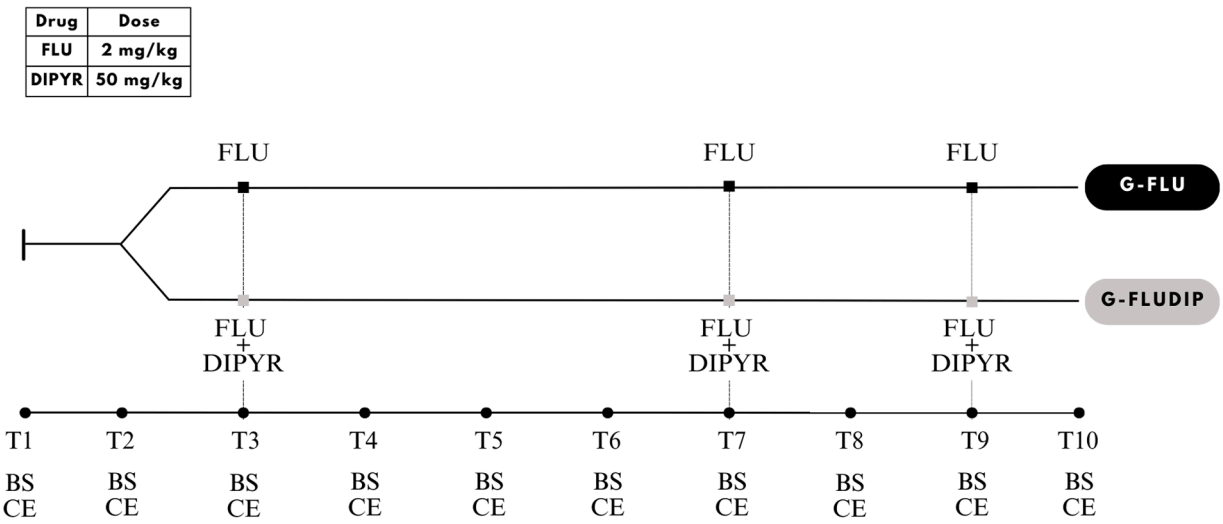

Fig. 1. Experimental design of the study with two postoperative protocols with flunixin in a single (G-FLU; n = 11) or dipyrone association (G-FLUDIP; n = 11) in Dorper ewes after submitted to a surgical embryo collection. BS: blood sample; CE: clinical exam; T1: immediately before anesthetic induction; T2: during the surgical procedure; T3: immediately; T4: 1 h; T5: 3 h; T6: 12 h; T7: 24 h; T8: 36 h; T9: 48 h; and T10: 72 h after the surgical procedure.

segmented neutrophils, lymphocytes, monocytes, and platelets (Harvey, 2012).

Serum cortisol concentration was measured by radioimmunoassay using a commercial kit (MP Diagnostics Division, Orangeburg, NY, USA). The sensitivity and coefficient of variation were 10 ng/mL and 9.8 %, respectively. All data were contained within the minimum and maximum values of the curve. Glucose, TPP, and ALB levels were measured using commercial kits in a semi-automatic biochemical analyzer (BIO 2000®, Labtests, Brazil). The GLO levels were obtained by calculating the difference between TPP and ALB. The FIB levels were obtained using a brix refractometer (Harvey, 2012). Blood cell counts were determined by blood smear examination.

2.6. End points and statistical methods

The behavioral and physiological stress indicators were: interval to stand up, interval to eat, behavior, posture, locomotion pattern, HR, RF, RM, RT, serum cortisol, glucose, TPP, ALB, GLO, FIB, erythrocytes, total leukocytes, basophils, eosinophils, band neutrophils, segmented neutrophils, lymphocytes, monocytes, and platelets. Data analysis was performed in IBM SPSS version 25. Data were compared using a generalized linear mixed model (GLMM), including the treatment, time, and their interaction (treatment × time) as main effects in endpoints with repeated measures. The repetition was included as a random factor. For all endpoints, $P < 0.05$ was considered significant, and $0.05 \leq P < 0.10$ was considered tendency. Data are presented as LSMeans ± SEM, which adjusted values according to random factors to minimize these effects.

3. Results

Embryo recovery had an average duration of 24 ± 0.85 min. There was no difference in the interval to stand up between groups (G-FLUDIP: 8.2 ± 0.3 min vs G-FLU: 8.3 ± 0.5 min; $P > 0.05$), but the G-FLUDIP-ewes had a shorter interval to eat compared to G-FLU-ewes (9.5 ± 0.3 vs. 12.4 ± 0.4 min; $P = 0.001$). Although some effects were observed at different times of evaluation ($P = 0.001$), the treatments did not affect posture ($P = 0.321$), locomotion ($P = 0.944$), and behavior ($P = 0.332$). The effect of treatments, times of evaluation, and their interaction are presented in Table 1.

The physiological indicators obtained after each treatment through times are shown in Fig. 2. Significant differences were observed in RT throughout the evaluation ($P = 0.001$), although it was not affected by the treatment or treatment × time interaction ($P > 0.05$). Both groups showed a minimum temperature at T3 and a peak at T5. The overall HR was significantly higher in G-FLUDIP than in G-FLU ($P = 0.005$). The HR

Table 1
Effect of treatments, time of evaluation, and their interaction on physiological and hematological endpoints in Dorper ewes subjected to surgical embryo collection (LSMeans ± SEM).

Endpoints	Treatment		P-value		
	G-FLU*	G-FLUDIP*	Treat	Time	T × T
Physiological parameters					
Rectal temperature (°C)	38.7 ± 0.1	38.8 ± 0.1	0.616	0.001	0.850
Heart rate (bpm)	100.1 ± 2.5	106.2 ± 2.2	0.005	0.001	0.267
Respiratory frequency	37.3 ± 1.2	37.5 ± 1.4	0.830	0.001	0.598
Rumen movement	1.8 ± 0.1	1.7 ± 0.1	0.856	0.001	0.806
Hematologic parameters					
Cortisol (ng/mL)	24.1 ± 2.3	25.6 ± 2.7	0.717	0.001	0.070
Glycaemia (mg/dL)	84.7 ± 4.4	81.8 ± 4.3	0.309	0.001	0.043
Total protein (g/dL)	6.4 ± 0.1	6.4 ± 0.1	0.835	0.227	0.118
Serum albumin (g/dL)	2.4 ± 0.1	2.4 ± 0.1	0.337	0.017	0.488
Serum globulin (g/dL)	4.0 ± 0.1	4.0 ± 0.1	0.969	0.635	0.044
Fibrinogen (mg/dL)	501.9 ± 26.5	554.5 ± 29.3	0.186	0.263	0.779
Erythrocyte ($\times 10^6$/μL)	3.9 ± 0.2	4.0 ± 0.2	0.632	0.001	0.984
Total leukocyte (/μL)	5744.5 ± 262.3	5341.9 ± 261.0	0.278	0.001	0.595
Platelets ($\times 10^3$/μL)	638.9 ± 18.9	572.7 ± 16.3	0.010	0.915	0.912
Basophils (/μL)	14.8 ± 3.2	20.4 ± 3.6	0.231	0.001	0.296
Eosinophils (/μL)	274.8 ± 31.1	214.2 ± 26.6	0.115	0.127	0.986
Band neutrophils (/μL)	14.6 ± 4.3	10.4 ± 3.7	0.467	0.137	0.873
Segmented neutrophils (/μL)	2793.7 ± 179.0	2896.7 ± 178.1	0.684	0.001	0.763
Lymphocytes (/μL)	2403.1 ± 108.1	2008.1 ± 107.6	0.010	0.001	0.770
Monocytes (/μL)	245.6 ± 22.9	192.9 ± 17.6	0.038	0.001	0.907

* Average values of the ten different moments of evaluation: immediately before anesthetic induction, during the surgical procedure, immediately after the surgical procedure, 1 h, 3 h, 12 h, 24 h, 36 h, 48 h, and 72 h after the surgical procedure. Treat: treatment; T × T: Treatment and time interaction.

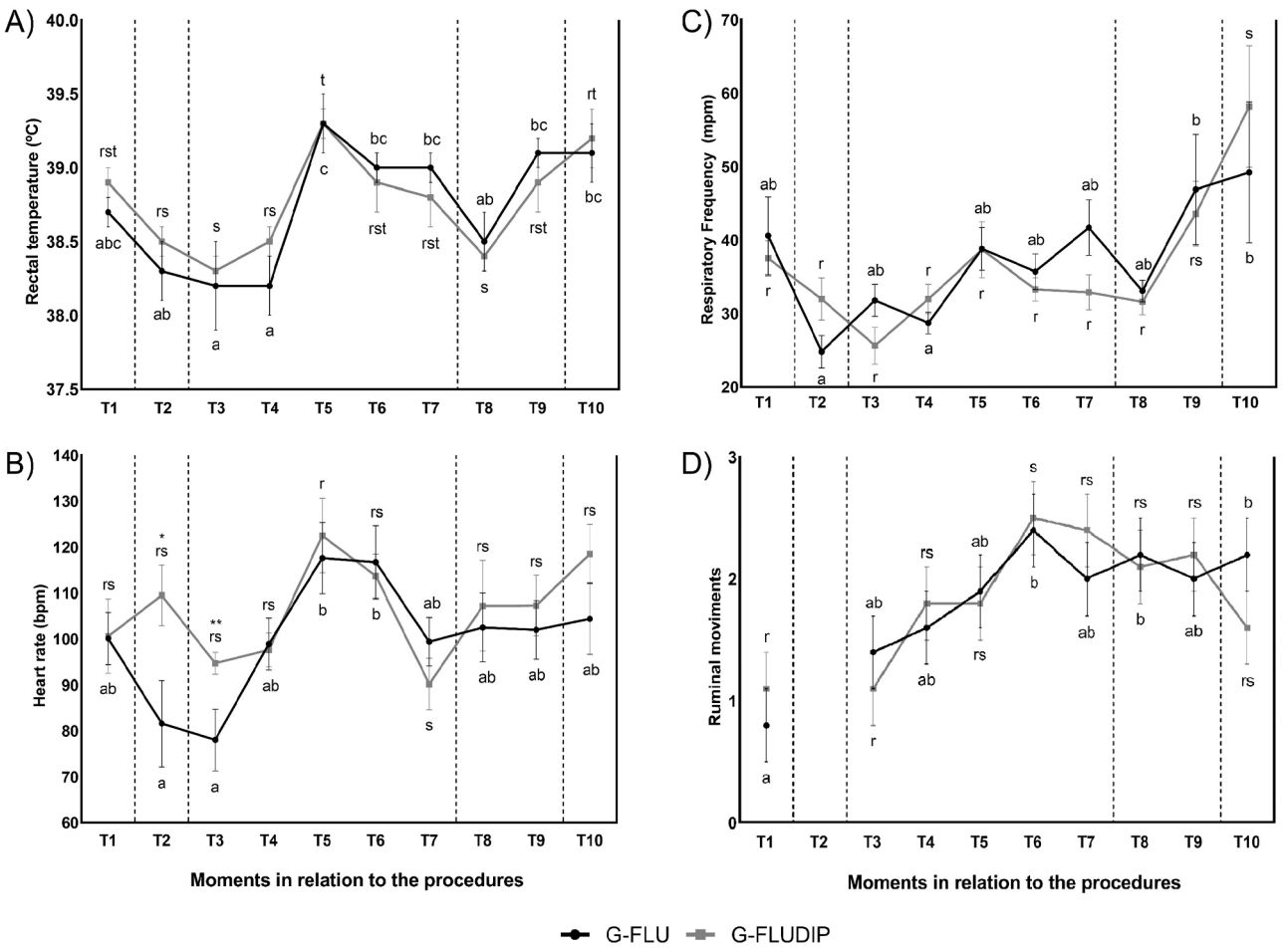

Fig. 2. Averages of (A) Rectal temperature (°C), (B) heart rate (bpm), (C) respiratory frequency (mpm), (D) ruminal movements in Dorper ewes submitted to surgical embryo collection. T1: immediately before anesthetic induction; T2: during the surgical procedure; T3: immediately after the surgical procedure; T4: 1 h; T5: 3 h; T6: 12 h; T7: 24 h; T8: 36 h; T9: 48 h; and T10: 72 h after the surgical procedure. *Indicates a difference between treatments ($P < 0.05$). Differences throughout the evaluations within each treatment are indicated by different letters (G-FLU: a, b, c; G-FLUDIP: r, s, t; $p < 0.05$).

was also affected by the time of evaluation ($P = 0.001$), although no interaction effect was observed ($P > 0.05$). The G-FLUDIP-treated ewes showed higher HR in comparison with the G-FLU group at T2 and T3 ($P < 0.05$). No treatment effect was observed on RR or RM ($P > 0.05$); these parameters only differed through times ($P = 0.001$). The fluctuation pattern was similar between groups over time, with both groups showing a peak of RR at T10 and a peak of RM at T6.

Serum cortisol concentrations presented a tendency ($P = 0.07$) of treatment × time interaction. The interaction effect is shown in T3, presenting a higher concentration of cortisol for ewes in G-FLU compared to G-FLUDIP ($P = 0.002$). Serum cortisol was also affected by the time of evaluation ($P = 0.001$), showing an increase in both groups at T2, followed by a decrease throughout time. Overall serum cortisol concentrations were similar between the treatments ($P = 0.717$). For glycemia, a treatment × time interaction was observed ($P = 0.043$). Glycemia was significantly higher at T3 in G-FLUDIP-ewes ($P = 0.038$), and at T4, in G-FLU-ewes ($P = 0.001$). The time effect was also observed ($P = 0.001$). The glycemia varied throughout time with a similar pattern between the groups, with an increase at T2 in relation to T1, and a decrease at T5. Overall, glycemia concentrations were similar between the treatments ($P = 0.309$). Both serum cortisol and glycemia concentrations are shown in Fig. 3. No significant main effects were observed in TPP, FIB, and ALB concentrations ($P > 0.05$). There was a treatment × time interaction in serum GLO ($P = 0.044$). This interaction tended to increase serum GLO in G-FLUDIP-treated ewes at T7 ($P = 0.06$) and was significantly higher in ewes at G-FLU at T9 ($P = 0.019$). These parameters are shown in Fig. 4.

The erythrocyte counts were not affected by the treatment × time interaction ($P > 0.05$). However, these counts were significantly affected by times of evaluation ($P = 0.001$), and both groups varied similarly (Fig. 5). Total leukocyte was affected throughout the time of evaluation ($P = 0.001$), but no differences were observed between the treatments ($P > 0.05$). The G-FLUDIP-treated ewes showed a significantly lower overall platelet count compared to G-FLU ($P = 0.01$), which was not affected by the time of evaluation ($P > 0.05$). The overview of blood counts over each treatment and through times are shown in Fig. 5. When each leukocyte population was analyzed, the time of evaluation exerted a significant effect on basophil, segmented neutrophil, lymphocyte, and monocyte counts ($P = 0.001$), while no effect on eosinophil and band neutrophil counts was seen ($P > 0.05$). A treatment effect was also observed in overall lymphocyte ($P = 0.01$) and monocyte counts ($P = 0.038$). The leukocyte populations over each treatment and through times are shown in Fig. S1.

4. Discussion

To the best of the authors' knowledge, this is the first study comparing physiological, behavioral, and biochemical responses after the use of an analgesic drug, associated with FLU, after a laparotomy procedure in sheep. We hypothesized that the association of dipyrone with FLU could promote a more effective modulation of stress and inflammation and our results support, at least moderately, this idea.

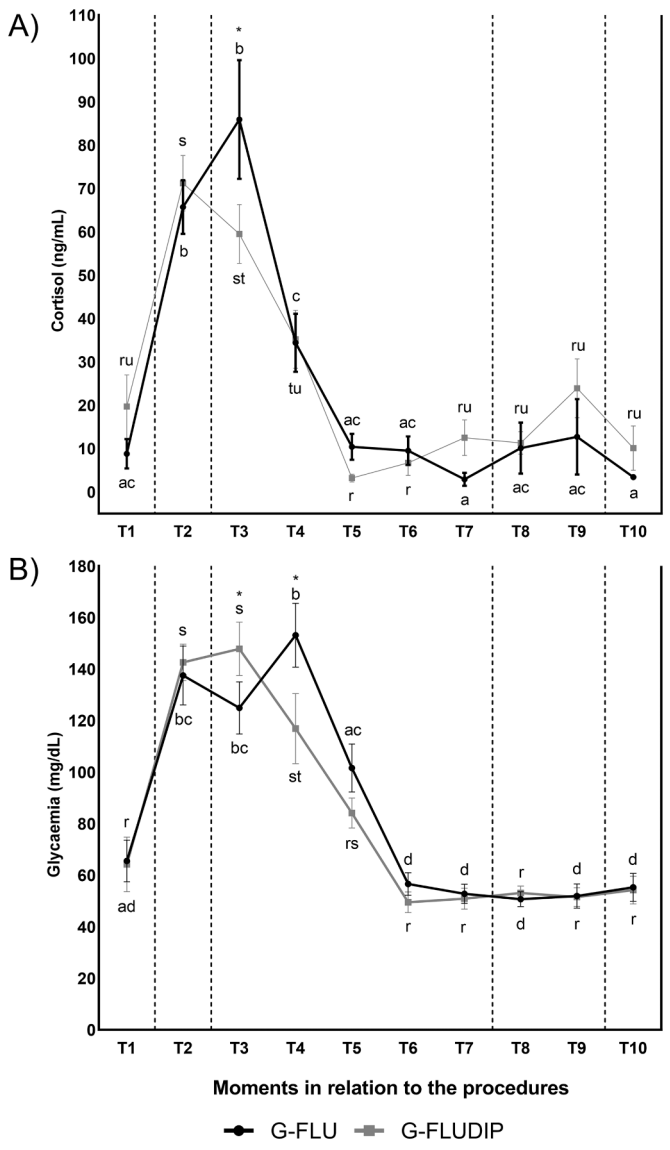

Fig. 3. Averages of (A) serum cortisol concentration (ng/mL) and (B) Glycaemia (mg/dL) in Dorper ewes submitted to surgical embryo collection. T1: immediately before anesthetic induction; T2: during the surgical procedure; T3: immediately after the surgical procedure; T4: 1 h; T5: 3 h; T6: 12 h; T7: 24 h; T8: 36 h; T9: 48 h; and T10: 72 h after the surgical procedure. *Indicates interaction between treatments (G-FLU and G-FLUDIP) × time ($P < 0.05$). Differences throughout the evaluations in each treatment are indicated by different letters (G-FLU: a, b, c, d; G-FLUDIP: r, s, t, u; $p < 0.05$).

From the data obtained in this study, we were able to obtain five main conclusions: firstly, G-FLUDIP declined cortisol faster and can promote more effective control of thrombocytosis than G-FLU; G-FLUDIP-ewes had a shorter interval to eat compared to G-FLU-ewes; the latter has a delayed time to recover from the acute inflammation; and finally, both G-FLUDIP and G-FLU can control glycemia. Like the majority of the studies conducted in the field, this one has a few limitations that should be highlighted. A relatively small number of animals was assessed per group, which may have affected the possibility of finding more differences between the groups. This number was unfortunately limited by the logistics, due to the need for surgery and several parameters collected in the animals in a short time and contemporaneously. Another limitation is the absence of a control group either without any drug or only treated with dipyrone. However, those approaches would not be approved by the Brazilian Ethical Committee, since animals underwent a relatively invasive surgery. Finally, the third limitation is that regulations vary in different countries, and in some of them, dipyrone is not allowed to be used in sheep.

Variations in the RT were similar in both groups, suggesting that both strategies are efficient in fever control, even though a febrile response may occur as a consequence of acute inflammation and/or invasive procedures (Anderson and Muir, 2005). This result may be explained by the antipyretic effect of NSAIDs (Botting, 2004; Stillman and Whittaker, 2019). As expected, a decrease in the RT was observed while the ewes were anesthetized, followed by a return to standard values a few hours after the surgical procedure (Hall et al., 2001). Although the overall HR was higher in G-FLUDIP, the most significant differences between the groups were observed before the animals received any drug. Therefore, these punctual differences could be related to the pharmacological treatment but also allied to individual animals' response to manipulation. In contrast, the significant post-operative increase of this parameter occurred similarly throughout the evaluation in ewes subjected to both treatments. Similarly to our findings, Santos et al. (2020) observed that the time had an influence on HR in ewes subjected to surgical embryo recovery, with its most accentuated increase 1 h after the procedure.

Serum cortisol and glycemia are important biomarkers of stress. In the present study, both parameters increased during embryo collection, suggesting an acute inflammatory response to surgical manipulation. Under stressful stimuli, the hypothalamic-pituitary-adrenal axis is activated resulting in cortisol release from the adrenal cortex and an elevation in blood glucose (Miller and O'Callaghan (2002)). Serum cortisol levels started to decline after the surgery in both groups (immediately after and 3 h after in G-FLUDIP and G-FLU, respectively), in agreement with a previous report (Santos et al., 2020). The serum cortisol decrease was followed by a glycemia decrease in both experimental groups. Despite that, serum cortisol levels were significantly lower in G-FLUDIP at 3 h after the surgery. It has been described that in sheep the active metabolites of dipyrone per IM route may achieve their maximum plasma concentration rapidly and could be detected during approximately 10 h (Giorgi et al., 2015). These results may indicate that dipyrone inhibited the increase of serum cortisol levels immediately after surgery due to its analgesic effects.

Hematological indicators are important points to the understanding of the course of treatments. Acute phase proteins are produced by hepatocytes in response to proinflammatory cytokines (Germolec et al., 2010), often resulting in an increase of TPP levels after injuries, either rapidly or belatedly (Oliveira et al., 2018). Although early TPP alterations were expected after the surgical procedure, we found no difference in the present study. In the current study, with the exception of minimal isolated differences, ALB was not affected by the treatments. Similarly, no differences were observed in the FIB count between the treatments, time, or treatment × time, corroborating a previous study (Santos et al., 2020). The ALB is a negative acute-phase protein and the main determinant of colloid osmotic pressure, being associated with edema formation in acute inflammation (Fischer et al., 1999). The overall FIB count detected in this study was slightly higher than the established standard for sheep, which ranges from 100 to 500 mg/dL (Ceciliani et al., 2012; Kramer, 2000). The FIB is a positive acute-phase protein and is considered one classical biomarker of acute inflammation (Germolec et al., 2010). The complete absence of any effect of ALB and FIB suggests that, in the current study, they were not sensitive markers of inflammation or stress. The GLO is a positive acute-phase protein as well as FIB. A treatment × time interaction was observed in GLO, showing that G-FLU had greater time to increase GLO than G-FLUDIP, i.e., the FLU alone delays the recovery from the effect of acute inflammation.

The erythrocyte counts were affected throughout the time, and both groups varied similarly throughout the moments. However, G-FLU at T4 and T6 showed a greater number of erythrocytes compared to G-FLUDIP at that same time frame. The increase observed may be due to an attempt of the body to return to homeostasis by controlling the blood volume

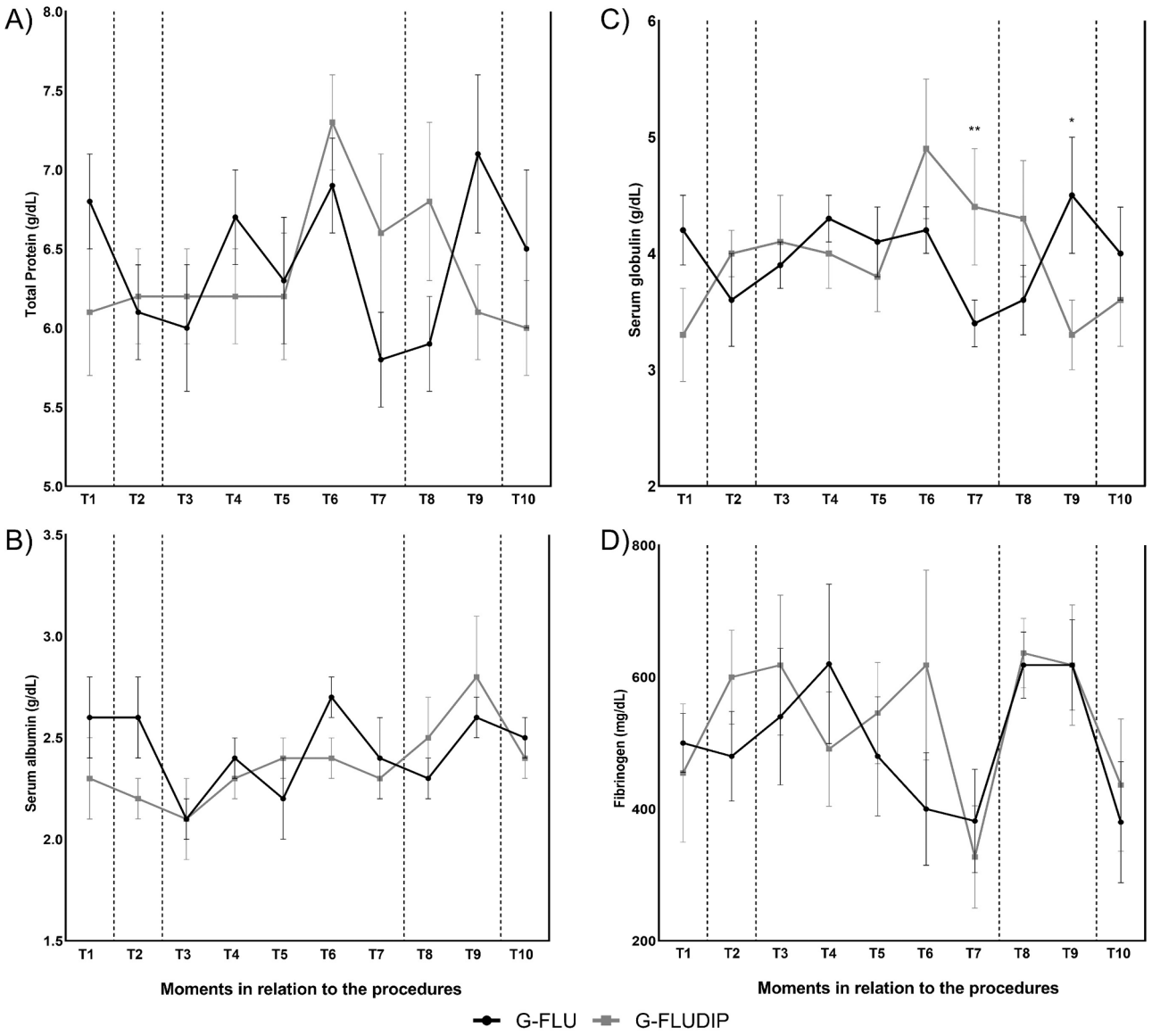

Fig. 4. Averages of (A) serum total protein concentration (g/dL), (B) serum albumin concentration (g/dL), (C) serum globulin concentration (g/dL), and (D) fibrinogen concentration (mg/dL) in Dorper ewes submitted to surgical embryo collection. T1: immediately before anesthetic induction; T2: during the surgical procedure; T3: immediately after the surgical procedure; T4: 1 h; T5: 3 h; T6: 12 h; T7: 24 h; T8: 36 h; T9: 48 h; and T10: 72 h after the surgical procedure. *Indicates interaction between treatments (G-FLU and G-FLUDIP) × time ($P < 0.05$). **Indicates interaction tendency between treatments (G-FLU and G-FLUDIP) × time ($P < 0.10$).

(Pugh et al., 2015). The G-FLU-ewes had a higher overall platelet count than G-FLUDIP-ewes. Although the platelet's primary function is on hemostasis, thrombocytosis may also be expected as a response to acute inflammatory processes (Germolec et al., 2010). A significant difference was observed 72 h after the surgery when the dipyrone treatment appeared to promote more effective control of the platelet count. These results suggest a beneficial additional effect of the non-opioid analgesic on inflammation control.

Leukocyte levels were similarly affected by the time in both groups. The mobilization of leukocytes is an important response to inflammatory stimuli, as an attempt of the organism to combat any pathological disorder (Stockham and Scott, 2002). In this sense, modifications in the blood leukocyte population in response to inflammatory processes caused by surgical procedures could be useful in the assessment of animal welfare (Cockram et al., 1993). Moreover, physiological stress may also increase segmented neutrophils in response to corticosteroid release (Germolec et al., 2010). In the present study, all ewes showed an increase in band neutrophil counts. This result, in addition to the significant variation of segmented neutrophils throughout time in both groups, indicates that surgical procedure induces acute inflammation despite the treatment. Basophil, monocyte, and lymphocyte levels were also significantly affected by the time and showed a similar fluctuation pattern between the groups. As a key role in the removal of dead or injured cells, monocytes are usually recruited during inflammatory processes (Germolec et al., 2010). In addition, the increase in the lymphocyte counts may be indicative of an acute stressful response, as a result of the possible release of catecholamines (Pascual-Alonso et al., 2017), an event that appeared to be more effectively controlled in ewes treated with dipyrone. The stress caused by the surgical procedure can promote leukocytosis (Fig. 5). In this study, the count of leukocytes was higher in G-FLU until T5. Moreover, there is an increase in lymphocytes and monocytes in the G-FLU. These results may be related to the benefits of associating dipyrone with anti-inflammatory effects in the first hour after surgery reducing the inflammatory response.

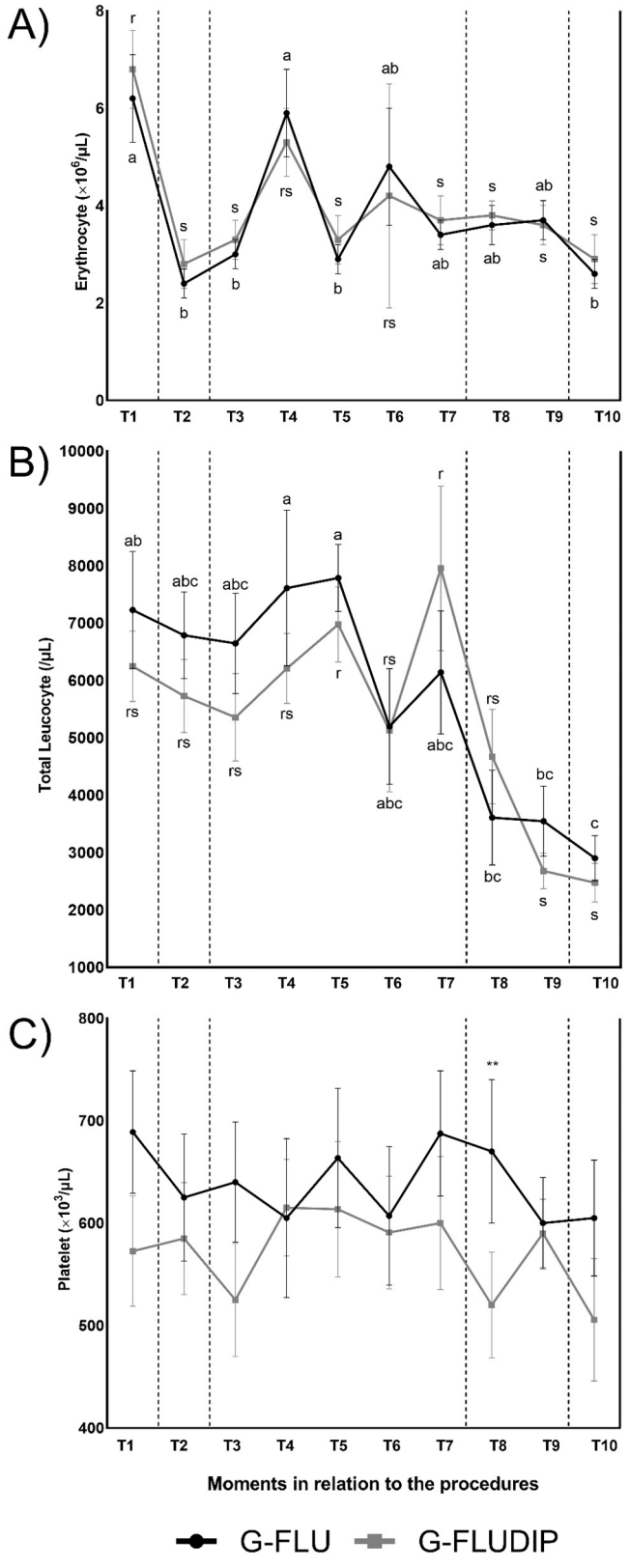

Fig. 5. Averages of (A) erythrocyte (x106/µL), (B) total leukocyte (/µL), and platelets (x103/µL) in Dorper ewes submitted to surgical embryo collection. T1: immediately before anesthetic induction; T2: during the surgical procedure; T3: immediately after the surgical procedure; T4: 1 h; T5: 3 h; T6: 12 h; T7: 24 h; T8: 36 h; T9: 48 h; and T10: 72 h after the surgical procedure. **Indicates interaction tendency between treatments (G-FLU and G-FLUDIP) × time ($P < 0.10$). Differences throughout the evaluations in each treatment are indicated by different letters (G-FLU: a, b, c; G-FLUDIP: r, s; $P < 0.05$).

5. Conclusions

In conclusion, the association of FLU and dipyrone after surgical embryo collection promotes a more effective modulation of pain and inflammation since ewes had lesser stress in the initial 24 h after the procedure. Reasons to support this affirmation involved the effect of this protocol in declining cortisol, promoting a more effective control of thrombocytosis, reducing the acute inflammation recovery time in the ewes, and reducing the interval of time that it took the ewes to eat after the procedure. In this sense, more studies are necessary to explore animal behavior aiming at animal welfare. Thus, the association of FLU and dipyrone may be important to increase ewes' welfare after surgical embryo collection.

CRediT authorship contribution statement

Mirela Balistrieri: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Marcela Sene Rocha:** Data curation, Investigation, Validation. **Paula Renata Cortat:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Maria Clara Cruz Morais:** Data curation, Investigation. Writing – original draft. **Ana Clara Sarzedas Ribeiro:** Methodology, Writing – review & editing. **Lucas Francisco Leodido Correia:** Conceptualization, Formal analysis, Methodology, Software, Visualization, Writing – review & editing. **Felipe Zandonadi Brandão:** Resources, Writing – review & editing, Validation. **Claudio Alvarenga de Oliveira:** Supervision, Writing – review & editing, and Validation. **Joanna Maria Gonçalves Souza-Fabjan:** Conceptualization, Formal analysis, Investigation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

On behalf of all the authors, there are no conflicts of interest to declare.

Acknowledgments

This work was supported by the Rio de Janeiro State Research Funding Agency (FAPERJ), the Brazilian National Scientific and Technological Development Council (CNPq), and the Coordination of Higher Education Personnel (CAPES, Finance Code 001), all from Brazil. FZB and JMGS-F are CNPq and FAPERJ fellows. The authors also thank both Post-Graduation Programs from Fluminense Federal University: Science and Biotechnology (PPBI) and Veterinary Medicine (PPGMedVet).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.smallrumres.2023.107144.

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