

Effects of metabolic changes produced in ewes with subclinical pregnancy toxemia over reproductive parameters

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ABSTRACT - The objective of this work was to determine the repercussion of the metabolic changes that occur in induced subclinical pregnancy toxemia in single- or twin-bearing ewes on the duration of gestation, type and length of lambing, and placental expulsion time. Fifty-one adult Corriedale ewes with a known gestation date and fed in the wild were randomly divided at day 145 of gestation into four groups. Group A included single-bearing ewes, and C twin-bearing ewes, all of which were fed *ad libitum*. Group B was composed of single-bearing ewes, and D of twin-bearing ewes, all subjected to a 75% feed restriction until glycemic values were compatible with subclinical pregnancy toxemia. Metabolic parameters were determined in all ewes from day 145 of gestation to 72 h postpartum. Likewise, the duration of gestation, type of lambing (eutocic/dystocic), labor length, and placental expulsion time were recorded. Glycemia and β -hydroxybutyrate values compatible with subclinical pregnancy toxemia were achieved after 48 h of feed restriction, being 30.67 ± 2.37 mg/dL and 1.87 ± 0.12 mmol/L in single-bearing ewes and 28.40 ± 3.39 mg/dL and 2.21 ± 0.42 mmol/L in twin-bearing ewes, respectively. It is concluded that subclinical pregnancy toxemia induced by feed restriction at the end of gestation, frequent situation that occurs in flock management, produces mild metabolic changes, which return to normal values after the delivery. These metabolic changes registered before lambing do not modify the gestation length, do not increase the percentage of dystocical deliveries, nor influence labor length or placental expulsion time.

Keywords: glycemia, lamb, parturition, peripartum, sheep

1. Introduction

Gestational toxemia is a common metabolic disorder that affects pregnant ewes during the last third of the gestational period and develops consequently to the inability of the organism to maintain energy homeostasis during negative energy balance (Harmeyer and Schlumbohm, 2006; West, 1996). This energy metabolism abnormality can cause a severe form of ketosis, characterized by low circulating levels of blood glucose and high levels of ketone bodies (Sorondo and Cirio, 2011; Rook, 2000). The

fetus's high energy demand exceeds the maternal energy supply during the last pregnancy trimester and constitutes the main cause for the development of this disease (El-Far et al., 2010; Schlumbohm and Harmer, 2008).

In intensively raised flocks, subclinical pregnancy toxemia has a high incidence, reported above 20% (Feijó et al., 2016), and has great economic importance since it is frequently involved in lambs' and sometimes mother's death (Moghaddam and Hassanpour, 2008). Steadily increasing rates of hypoglycemia and hyperketonemia in the absence of clinical signs is characteristic of early subclinical stages of the disease (Barbagianni et al., 2015b; Cal-Pereyra et al., 2015). Subclinical diagnosis is of utmost importance since the detection of the first clinical cases of clinical pregnancy toxemia reflect the existence of a much more important metabolic problem in the rest of the flock (Rook, 2000; Marteniuk and Herdt, 1988). The evaluation of glycemia, serum β -hydroxybutyrate (BHB), and serum cortisol have been shown to define and diagnose subclinical pregnancy toxemia (Cal-Pereyra et al., 2015). The subclinical form of the disease can be diagnosed when blood glucose reaches values of 28.62 ± 4.33 mg/dL and BHB blood values of 2.26 ± 1.03 mmol/L at 48 h past the start of the fasting period (Cal-Pereyra et al., 2015). Ewes suffering from clinical pregnancy toxemia that survive until late gestation tend to develop dystocia and placental retention, leading to metritis and subsequent death (Rook, 2000; Andrews, 1997). However, the effects of the subclinical presentation of this pathology on these reproductive parameters has not yet been described.

The objective of this study was to determine the repercussion of the metabolic changes that occur in induced subclinical pregnancy toxemia in ewes on the duration of gestation, lambing type and length, as well as the placental expulsion time.

2. Material and Methods

The research protocol was carried out under the supervision of the Ethics Commission on the Use of Animals (CEUA/FVET - PI 13/14 - Exp 111130-000636-14), in Libertad, San José, Uruguay ($34^{\circ}38' S$; $56^{\circ}39' W$).

Eighty adult Corriedale ewes aged four to six years old had their heat synchronized with the use of intravaginal sponges for 12 days, containing 160 mg of progesterone (Cronipres[®] CO, Biogenesis-Bagó) (Da Silva et al., 2016). The animals, identified with numbered caravans, presented homogeneous body weight and body condition score above 2.5 (interval 1-5) (Russel et al., 1969). After removing the sponges, service was performed by natural mounting using three rams of the same breed provided with marker harnesses. The day of mounting was recorded for each ewe as day zero of gestation. At day 35 after mounting, pregnancy diagnosis was performed by transabdominal ultrasound (Moallem et al., 2016). A total of 28 ewes bearing a single fetus and 23 bearing twins were included, while empty ewes and carriers of more than two fetuses were not. During the study period, all 51 ewes included were fed natural pasture (nutritional characteristics described in Table 1). The protocol was designed considering a mean of 147.9 ± 1.9 days as the Corriedale ewe's gestation duration (Benech, 2007). On day 145 of gestation, single-bearing ewes were randomly divided into two groups, and the same treatment was given to twin-bearing ewes, achieving a total of four groups; the former were named

Table 1 - Details of the feed given to ewes during the study

Parameter/Unit	Natural field		Alfalfa bale	
	FM	DM	FM	DM
%DM, 105 °C	13.85	-	90.73	-
%Ash	-	20.08	-	7.76
%CP	-	26.31	-	19.13
%NDF	-	32.45	-	47.41
%ADF	-	22.82	-	27.21
ME (Mcal/kg DM)	-	1.84	-	1.28

DM - dry matter; FM - fresh matter; NDF - neutral detergent fiber; ADF - acid detergent fiber; CP - crude protein; ME - metabolizable energy.

groups A and B, and the latter, groups C and D. Group A (n = 13) and group C (n = 12) were considered control groups and were fed *ad libitum* in the wild until the moment of lambing. On the other hand, to produce a pregnancy toxemia-inducing environment, ewes in groups B (n = 15) and D (n = 11)—treatment groups—were subjected to an acute three-day-maximum feed restriction in confinement in roofed concrete floor pens with free access to water. Group B ewes were fed 0.400 kg/day alfalfa hay ration (equivalent to 25% of the daily needs, 0.89 Mcal metabolizable energy [ME]), and group D ewes were fed 0.525 kg/day alfalfa hay ration (equivalent to 25% of daily needs, 1.22 Mcal ME) (AFRC, 1993). When the glycemia and BHB reached values indicative of subclinical pregnancy toxemia (Cal Pereyra et al., 2015), the animals were withdrawn from the restriction and were fed natural pasture *ad libitum*. After feed restriction was completed, animals in treatment groups and those of control groups were pooled in the same farrowing paddock, and equal nutritional management was resumed.

2.1. Serum parameters

Before lambing, blood samples were obtained by jugular vein puncturing and collected in sodium fluoride and EDTA tubes to determine glycemia and in dry tubes with separating gel to determine BHB. To determine glycemia in ewes included in control groups, animals were bled at day 145 of gestation and at 12, 24, and 48 h thereafter; ewes in treatment groups were bled at day 145 of gestation and at 12, 24, 36, and 48 h after feed restriction began. β -hydroxybutyrate was determined in samples taken at day 145 and at 24 and 48 h thereafter for ewes in treatment and control groups alike. All ewes were bled within the first hour after birth and at 24, 48, and 72 h thereafter to determine glycemia and serum BHB concentration. Glycemia was determined again at 12 h postpartum.

All samples were immediately centrifuged after the extraction, and serum was later frozen at $-20\text{ }^{\circ}\text{C}$ in Eppendorf tubes until processing. Glycemia was determined by an enzymatic colorimetric method, using Glucose Liquicolor[®] Commercial Kit (Human, Germany). Absorbance was measured at 500 nm at $37\text{ }^{\circ}\text{C}$, with the HUMALYSER Junior digital colorimeter. β -hydroxybutyrate was determined by an enzymatic colorimetric method, using Ranbut[®] Commercial Kit (Randox Laboratories Ltd., United Kingdom), and absorbance was determined at 330 nm at $37\text{ }^{\circ}\text{C}$ with the HUMALYSER Junior digital colorimeter.

2.2. Reproductive parameters and lamb body weight

Gestation length for each ewe was determined in days, considering the time elapsed between day of mounting and day of lambing. Labor length was recorded in minutes, considering the time elapsed between the recorded hour of the beginning of labor (allantochorionic sac appearance through the vulva, followed by the amnion together with part of the fetus) (Fernández Abella, 1993) and the hour of lamb expulsion. In cases of twin-bearing ewes, labor total duration was considered from the beginning of the first lamb labor until the second lamb was expelled.

Types of lambing were classified as eutocic, when no operator intervention was needed, or dystocic, if the ewes needed to be attended during parturition. Placental expulsion time was recorded in minutes and was established as the time elapsed between lambing and total placenta expulsion (Saelzer et al., 1999). After mother-lamb link was established, all lambs born of mothers included in the studied groups were weighed with a digital scale and weight was recorded.

2.3. Statistical analysis

Normality in the distribution of the different variables was determined using Shapiro-Wilk test. Glycemia and BHB serum levels, gestation length, and lamb body weight were normally distributed, and the presence of significant difference among groups was analyzed with one-way ANOVA, followed by Scheffe's test. The assessment of differences in glycemia and BHB concentration between samples from the same group was carried out with Student's test for paired samples. The remaining reproductive parameters evaluated (type of lambing and placental expulsion time) were not distributed normally; therefore, Kruskal Wallis test was used. To determine differences in type of lambing between groups,

χ^2 and Fisher's exact tests were performed. Differences were considered significant at values of $P < 0.05$, and statistical analysis was performed using the program STATA 15.2 (Statistics/Data Analysis. StataCorp LLC).

3. Results

3.1. Metabolic parameters prior to lambing

Glycemia and BHB values compatible with subclinical pregnancy toxemia were achieved after 48 h of feed restriction, 30.67 ± 2.37 mg/dL and 1.87 ± 0.12 mmol/L in single-bearing ewes and 28.40 ± 3.39 mg/dL and 2.21 ± 0.42 mmol/L in twin-bearing ewes, respectively.

Prior to the start of feed restriction, on day 145 of gestation, the glycemic concentration of all experimental groups did not show significant differences (Table 2). In group B, 48 h past the feed restriction started, a lower blood glucose value was found ($P < 0.001$) compared with that in group A (Table 2). In group D, blood glucose drop after fasting was more pronounced than in group C. Twelve hours after starting feed restriction, a decrease in glycemia concentration was observed ($P = 0.004$) when compared with group C. In addition, blood glucose concentration observed 48 h past the start of the trial was different from the concentration found at the beginning of the trial, in single-bearing ewes with feed restriction and in the two groups of twin-bearing ewes ($P = 0.0001$).

On day 145 of gestation, BHB concentration did not show significant differences between treatment and control groups (Table 2). Twenty-four hours after the feed restriction began, a difference in ketone body was observed among groups ($P = 0.0001$). Differences were observed between the groups of feed restricted ewes when compared with their respective controls. Moreover, a statistically significant difference was observed in the value of this analyte between the feed restricted groups, being the mean higher in the group of twin-bearing ewes. After 48 h of feed restriction, a difference was found between the single-bearing and twin-bearing ewes ($P < 0.0001$). When analyzing the behavior of the ketone body during time, a difference was found between day 145 of gestation and 48 h past feed restriction started in group A ($P = 0.002$), B ($P < 0.0001$), and D ($P = 0.0344$).

Subclinical pregnancy toxemia was diagnosed in all cases included in group B, 69.23% (9/13) reached glycemia and BHB values compatible with this disease 48 h after the feed restriction started, 23.08% (3/13) of the cases developed subclinical pregnancy toxemia after a 72-h feed restriction period, and 7.69% (1/13) within 24 h past the referred moment. In group D, 54.55% (6/11) of the cases were diagnosed with subclinical pregnancy toxemia after a 24-h period of feed restriction and 45.45% (5/11) after a 48-h period. In none of the studied cases, lambing occurred during feed restriction.

Table 2 - Mean \pm SEM blood β -hydroxybutyrate (BHB) and glucose concentrations in ewes under feed restriction and ewes fed in natural field, from day 145 of pregnancy and at 12, 24, 36, and 48 h after the treatment started

	Glycemia (mg/dL)				BHB (mmol/L)			
	A	B	C	D	A	B	C	D
Day 145	50.53 \pm 1.68	55.13 \pm 2.14A	57.17 \pm 2.67A	51.27 \pm 2.73A	0.69 \pm 0.05A	0.47 \pm 0.07A	0.72 \pm 0.14	0.85 \pm 0.20A
12 h	53.00 \pm 3.84	46.93 \pm 2.31	56.33 \pm 1.83a	41.72 \pm 2.26b	-	-	-	-
24 h	49.83 \pm 1.19	40.93 \pm 2.08	56.00 \pm 3.05a	37.40 \pm 4.30b	0.55 \pm 0.08a	1.52 \pm 0.14b	0.67 \pm 0.09a	2.17 \pm 0.24c
36 h	-	33.69 \pm 2.18	-	29.60 \pm 4.20	-	-	-	-
48 h	44.69 \pm 2.75a	30.67 \pm 2.37Bb	40.83 \pm 1.60B	28.40 \pm 3.39B	0.34 \pm 0.04Ba	1.87 \pm 0.12Bb	0.63 \pm 0.05a	2.21 \pm 0.42Bb

SEM - standard error of the mean.

Group A: single-bearing ewes fed *ad libitum*; group B: single-bearing ewes under feed restriction; group C: twin-bearing ewes fed *ad libitum*; and group D: twin-bearing ewes under feed restriction.

A,B - Statistical differences between times within the same study group found with Student's test ($P < 0.05$), in the column, are represented by different uppercase letters following mean values for each time.

a,b,c - Statistical differences among study groups for each time, found with ANOVA and Sheffe's test ($P < 0.05$), are represented by different lowercase letters following mean values.

The absence of uppercase or lowercase letters implies the lack of significant differences among times or groups.

3.2. Metabolic parameters after lambing

In all groups, the mean glycemia concentration observed within the hour of lambing was above the reference interval, and 12 h later, a decrease was found (Table 3). No statistically significant differences were observed regarding glycemia at 12, 24, 48, and 72 h after lambing in ewes within the same group nor among the four groups. Within the first hour after lambing (Table 3), serum BHB concentration was significantly higher in single-bearing ewes subjected to feed restriction and in both groups of twin-bearing ewes than in single-bearing ewes without feed restriction ($P = 0.0002$). In twin-bearing ewes, there were no differences found between groups at any of the moments studied.

Table 3 - Mean±SEM blood β -hydroxybutyrate (BHB) and glucose concentrations in ewes under feed restriction and ewes fed in natural field at 1, 12, 24, 48, and 72 h postpartum

Time (h)	Glycemia (mg/dL)				BHB (mmol/L)			
	A	B	C	D	A	B	C	D
1	107.00±7.53A	110.86±5.39A	88.09±8.48A	88.73±6.84A	0.53±0.10a	1.3±0.10b	1.50±0.25b	1.55±0.21b
12	53.00±3.84B	60.21±3.86B	62.44±2.29B	65.5±4.92B	-	-	-	-
24	48.67±3.89B	59.93±3.16B	59.00±4.47B	62.91±3.83B	1.07±0.11	1.33±0.14	1.23±0.31	1.22±0.17
48	50.77±3.30B	52.14±4.20B	49.43±5.11B	52.78±5.71B	0.99±0.14	1.22±0.10	0.93±0.30	1.06±0.18
72	51.55±2.72B	53.00±4.14B	51.38±4.18B	49.80±3.42B	0.92±0.12	1.21±0.18	1.40±0.25	1.30±0.18

SEM - standard error of the mean.

Group A: single-bearing ewes fed *ad libitum*; group B: single-bearing ewes under feed restriction; group C: twin-bearing ewes fed *ad libitum*; and group D: twin-bearing ewes under feed restriction.

A,B - Statistical differences between times within the same study group found with Student's test ($P < 0.05$), in the column, are represented by different uppercase letters following mean values for each time.

a,b - Statistical differences among study groups for each time, found with ANOVA and Sheffe's test ($P < 0.05$), are represented by different lowercase letters following mean values.

The absence of uppercase or lowercase letters implies the lack of significant differences among times or groups.

3.3. Gestation and labor length, type of lambing, and placental expulsion time

The mean of gestation duration in the four experimental groups was of 148.06 ± 0.54 days (Table 4). No statistically significant difference was found among all experimental groups. Considering type of lambing, 92.31% (12/13) of the ewes included in group A and 66.67% (10/15) in group B had an eutocic parturition. Group C presented 91.67% (11/12) of eutocic parturition and group D, 90.91% (10/11) (Table 4). No statistically significant differences were found between the experimental groups regarding this variable ($P = 0.272$). No statistically significant differences were found among the four experimental groups, regarding lambing duration, type of lambing (eutocic or dystotic), and placental expulsion time (Table 4).

Table 4 - Reproductive parameters¹ measured in ewes

Group	Gestation length	Eutocia			Dystocia			Total no. of ewes
		No. of ewes	Labor length	Placental expulsion time	No. of ewes	Labor length	Placental expulsion time	
A	148.32±0.66	12	26.0 (8.0-65.0)	183.0 (30.0-233.0)	1	32.0	301.0	13
B	148.07±0.28	10	19.5 (7.0-55.0)	174.5 (107.0-411.0)	5	124.0 (55.5-160.0)	140.0 (123.0-202.0)	15
C	147.75±0.3	11	27.0 (12.0-97.0)	204.0 (47.0-359.0)	1	65.0	151.0	12
D	148.09±0.30	10	44.0 (11.0-75.0)	199.0 (150.0-283.0)	1	155.0	175.0	11
Total	148.06±0.54	43	27.0 (8.0-65.0)	188.0 (107.0-289.0)	8	101.0 (32.0-160.0)	162.0 (123.0-301.0)	51

SEM - standard error of the mean.

Group A: single-bearing ewes fed *ad libitum*; group B: single-bearing ewes under feed restriction; group C: twin-bearing ewes fed *ad libitum*; and group D: twin-bearing ewes under feed restriction.

¹ Gestation length expressed in days (mean±SEM); labor length and placental expulsion time expressed in minutes [median (p5-p95)].

The absence of letters implies the lack of significant differences among times or groups.

Regarding the weight of lambs after parturition, statistically significant differences were found when comparing the weights of single-born lambs (group A and B) against the weights of twin-born lambs (groups C and D), without considering the mother's treatment ($P = 0.0001$). However, no statistically significant differences were observed when comparing within groups with the same type of lambing (Table 5).

Table 5 - Lamb weight (mean \pm SEM; g) after birth

A (n = 13)	B (n = 14)	C (n = 19)	D (n = 19)
4969.23 \pm 171.11a	5093.33 \pm 184.51a	3884.78 \pm 120.91b	4154.54 \pm 137.07b

SEM - standard error of the mean.

Group A: lambs born from ewes fed *ad libitum*; group B: lambs born from ewes under feed restriction; group C: twin lambs born from ewes fed *ad libitum*; and group D: twin lambs born from ewes under feed restriction.

a,b - Statistical differences among groups, found with ANOVA and Sheffe's test ($P < 0.05$), are represented by different letters following mean values.

4. Discussion

Glycemic values recorded in this study at day 145 of gestation were consistent with that reported by other authors as physiological in single or twin-bearing ewes at term (Cal-Pereyra et al., 2015; Raoofi et al., 2015; El-Far et al., 2010). In this trial, the animals received only 25% of the energy requirements needed, situation that decreases glucose production rate. Some authors suggest that the most important factor for the development of metabolic diseases involves a decrease in maternal nutritional levels (Constable et al., 2017; Barbagianni et al., 2015a). Moreover, the decrease in glycemia concentration evidenced in treatment groups once feed restriction begun has been explained by several authors. It is suggested that pregnant ewes subjected to fasting or feed restriction quickly develop hypoglycemia, associated not only with a decrease in glucose production rate due to a lack of precursors, but also to an excessive fetal glucose consumption, causing a dysregulation in maternal homeostasis, which can lower blood glucose levels to 20 mg/dL (Cal-Pereyra et al., 2015; Schlumbohm and Harmer, 2008).

The decrease in glycemia was greater in ewes pregnant with twins, a widely reported finding that suggests that the condition of gestating multiple fetuses increases the risk for developing pregnancy toxemia (Barbagianni et al., 2015b; Cal-Pereyra et al., 2015; Brozos et al., 2011; Moghaddam and Hassanpour, 2008). It is also suggested that the stress generated by feed restriction produces a greater demand in energy homeostasis of mothers carrying more than one fetus (El-Far et al., 2010; Schlumbohm and Harmer, 2008), metabolic situation that is more controllable in those ewes pregnant with only one lamb, because the first have a lower glucose exchange rate than production rate (Raoofi et al., 2015). In this study, single-bearing ewes that continued to be fed natural pasture did not show a significant difference in glycemic values between the beginning of the study and the subsequent 48 h, showing that the amount offered to these animals was sufficient to maintain energy homeostasis. On the other hand, twin-bearing ewes with equal feeding treatment had a significant decrease in blood glucose level 48 h after the start of the trial. This situation confirms what has been reported by several authors, who affirm that there is a competition between the pregnant uterus against the rumen capacity at the end of gestation, favoring a decline in the intake capacity that leads to a lower glucose production. Therefore, ewes in this condition can be able to enter in subclinical pregnancy toxemia despite not being subjected to feed restriction, as frequently happens in twin-bearing ewes in extensive pastoral systems (Ratanapob et al., 2018; Raoofi et al., 2015; Cal-Pereyra et al., 2012).

In our experimental conditions, BHB concentrations were not significantly different among groups prior to starting the feed restriction. The increase in BHB blood concentration observed in ewes subjected to dietary restriction can be explained by the lack of food-derived precursors for glyconeogenesis, producing a negative energy balance, which leads to mobilization of body reserves. The increase in this ketone body has been widely reported to reflect the magnitude of lipomobilizations (Ratanapob et al., 2018; Sakha, 2016; Cal-Pereyra et al., 2015). The increase in the concentration of this

metabolite was greater in ewes pregnant with twins, when compared with single-bearing ewes. This event can be associated to the important adipose reserve mobilization frequently observed in twin-bearing ewes due to the high energy requirements that characterize this situation (Cal-Pereyra et al., 2012; Rook, 2000). Moreover, this finding concurs with other authors' reports, who affirm that gestating multiple fetuses is a risk factor for developing the disease (Ratanapob et al., 2018; Barbagianni et al., 2015a; Raoofi et al., 2015).

In this study, 48 h after the feed restriction started, glycemia and BHB concentrations in treatment groups was found to be compatible with subclinical pregnancy toxemia diagnostic values according to Cal-Pereyra et al. (2015). Considering glycemia and BHB values and the fact that no animals included presented associated clinical signs to the disease, we can assume that these animals were developing subclinical pregnancy toxemia (Duffield, 2000).

Blood glucose concentrations recorded at lambing in single- and twin-bearing ewes were high and matched those reported by Araujo et al. (2014) for well-nourished ewes during pregnancy. These authors reported that hyperglycemia occurs immediately after lambing, in either single- or twin-bearing ewes, possibly due to an increase in glucagon reserves and in glucocorticoids concentration, promoting liver glycogen depletion (González et al., 2000). Nevertheless, glycemia concentration tended to progressively decrease in the postpartum period, accordingly with Campos et al. (2010). Twelve hours after lambing in all experimental groups, the blood glucose concentration was reduced to values considered by many authors as physiological in non-pregnant ewes (50-80 mg/dL) (Feijó et al., 2016; Santos et al., 2011). After 24 h of lambing, ewes of all groups presented glycemic values considered normal for the first two weeks of lactation in dairy ewes (Raoofi et al., 2015; Araujo et al., 2014; Caldeira, 2005).

One hour after lambing, BHB concentration in the single-bearing ewes fed *ad libitum* was significantly lower than that in the feed restricted group bearing the same number of fetuses. This situation was not observed in groups of ewes bearing twins. This scenario agrees with Raoofi et al. (2015), who demonstrated that at the end of gestation and during early lactation, ewes bearing two lambs have significantly higher BHB concentration than those ewes carrying a single lamb. These authors suggested that the regulatory and metabolic stress present in late-gestation twin-bearing ewes is disproportionately higher than in single-bearing ewes (Raoofi et al., 2015; Moallem et al., 2012). Moreover, this ketone body concentration was similar in all experimental groups after 24 h of lambing, in line with Caldeira (2005) for dairy sheep during the first two weeks of lactation. Additionally, the production rate of hepatic ketone bodies generally increases four to five times in ewes during late gestation and lactation, as these animals continue to have a negative energy balance (Raoofi et al., 2015; González et al., 2000). Glycemia and BHB values obtained from ewes under feed restriction that presented subclinical pregnancy toxemia before lambing demonstrated that this pathological condition had no repercussion on mothers after birth occurred.

In this study, feed restriction occurred from day 145 of gestation, which was within the probable interval of date of lambing for Corriedale ewes (Benech, 2007; Fernández Abella, 1993; Durán del Campo, 1993). Gestation length was not shortened in groups subjected to feed restriction, evidencing the same duration of pregnancy in all experimental groups as reported by Benech (2007) for the Corriedale breed.

In this research, lambing assistance was within the percentages valued as normal in ovine species (Dwyer, 2003). Although there was no difference in lambing ease, single-bearing ewes subjected to feed restriction presented a higher percentage of assistance, compared with the other groups analyzed. In addition, lambs born from this experimental group were the ones with the highest body weight recorded, in accordance with what the literature suggests (Benech, 2007; Dwyer, 2003; Owens et al., 1985). According to Fernández Abella (2015), dystocic births generally occur due to excessive size of the fetus and frequently occur in ewes suffering from clinical pregnancy toxemia that survive until the end of gestation, associated with poor uterine and abdominal musculature activity and with poor cervical dilation. In many of these cases, placental retention also occurs, leading to metritis and later

death (Rook, 2000; Andrews, 1997; Marteniuk and Herdt, 1988). Lambing duration was not affected by feed restriction in this study, since no statistically significant differences among the studied groups were found. The maximum lambing duration in this trial was 75 min, which was documented as physiological for the species (Senger, 2003).

In ruminants, placental retention is more frequent than in other species due to the type of anchorage presented (Benech, 2007), and ewes arriving at parturition with clinical pregnancy toxemia have been reported to develop fetal membrane retention, associated with weak abdominal and uterine activity (Ioannidi et al., 2020; Cal Pereyra, 2012; Brozos et al., 2011). Furthermore, Ioannidi et al. (2020) suggested that placental retention is also associated with energy deficiency in pregnant ewes, since it affects protein metabolism, possibly negatively impacting the enzymatic pathways that lead to cotyledon proteolysis. In this trial, no significant difference was found among groups involving placental expulsion time, and the maximum placental expulsion time in single and twin-bearing ewes was within the limits reported by various authors (Benech, 2007; Saelzer et al., 1999; Owens et al., 1985), possibly associated to the fact that none of the cases studied developed clinical pregnancy toxemia.

5. Conclusions

Subclinical pregnancy toxemia induced by feed restriction at the end of gestation, frequent situation that occurs in flock management, produces mild metabolic changes, which can return to normal values after the delivery. These metabolic changes registered before lambing do not modify the gestation length, do not increase the percentage of dystocical deliveries, nor do they influence labor length or placental expulsion time.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M.C. Abreu-Palermo and L. Cal-Pereyra. Data curation: M.C. Abreu-Palermo, P. Rodríguez-Gamarra and L. Cal-Pereyra. Formal analysis: M.C. Abreu-Palermo and L. Cal-Pereyra. Funding acquisition: L. Cal-Pereyra. Investigation: M.C. Abreu-Palermo, A. Benech-Gulla, J.R. González-Montaña and L. Cal-Pereyra. Methodology: M.C. Abreu-Palermo, P. Rodríguez-Gamarra, A. Benech-Gulla and L. Cal-Pereyra. Project administration: M.C. Abreu-Palermo and L. Cal-Pereyra. Resources: L. Cal-Pereyra. Supervision: J.R. González-Montaña and L. Cal-Pereyra. Visualization: P. Rodríguez-Gamarra, S. Perini-Perera and J. Acosta-Dibarrat. Writing-original draft: M.C. Abreu-Palermo, S. Perini-Perera, J. Acosta-Dibarrat, A. Benech-Gulla, J.R. González-Montaña and L. Cal-Pereyra. Writing-review & editing: M.C. Abreu-Palermo, P. Rodríguez-Gamarra, S. Perini-Perera, J. Acosta-Dibarrat, A. Benech-Gulla, J.R. González-Montaña and L. Cal-Pereyra.

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