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Body condition score and serum metabolites and minerals concentrations as indicators of ovarian activity and pregnancy success in goats on rangeland

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Abstract

Aim of the study: To investigate potential differences in ovarian structures relative to serum metabolite and mineral concentrations at mating. Also, body condition score (BCS), serum metabolites, and mineral profiling at mating were compared between pregnant and non-pregnant goats.

Area of study: Hot zone of northern Mexico (26 °N).

Material and methods: Mixed-breed goats (n= 89) on arid rangeland were exposed to bucks during the non-breeding season. Ovarian structures were recorded at mating and ten days after breeding using ultrasonography. Pregnancy was detected at 30 and 120 days post-mating. BCS, blood metabolites, and minerals were determined at mating.

Main results: Pregnant goats had higher BCS at mating than non-pregnant goats. The mean serum glucose concentration was higher (p<0.05) for pregnant goats than that for non-pregnant ones (87.3 ± 12.1 *vs.* 74.4 ± 11.6 mg/dL). Significantly lower (p<0.01) serum urea nitrogen levels at mating were recorded in non-pregnant (10.7 ± 3.5 mg/dL) than in pregnant goats (12.4 ± 3.7 mg/dL). Lower serum glucose (72.2 ± 6.9 *vs.* 89.4 ± 11.2) and higher non-esterified fatty acids concentrations (NEFA; 0.43 ± 0.23 *vs.* 0.18 ± 0.12) were significantly associated (p<0.05) with pregnancy loss. Higher serum total protein concentrations were associated with a greater number and larger ovulatory follicles. High serum phosphorus was significantly associated with larger ovulatory follicles. Goats with ovulatory follicles ≥7.6 mm were more likely (p<0.05) to get pregnant than goats with smaller ovulatory follicles.

Research highlights: Monitoring BCS, serum glucose, blood urea nitrogen, and NEFA could be used to identify goats at risk for infertility.

Additional keywords: blood urea nitrogen; blood glucose; ovulatory follicle; follicle size; corpus luteum size.

Abbreviations used: BCS (body condition score); BUN (blood urea nitrogen); NEFA (non-esterified fatty acids); TP (total proteins).

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Introduction

Goats play an essential role in developing economies and are a source of subsistence, livelihood, and employment for many rural households. Goats produce meat, milk, skin, and manure under various habitats with scarce vegetation, making them ideal farm animals for resource-poor farmers (Patel *et al*., 2020). In developing countries, goat production systems are characterized by low input and production on degraded rangelands that contribute to inadequate feeding and nutrition (Monau *et al*., 2020; Patrick Baenyi *et al*., 2020), resulting in low productivity (Thomas & Rangnekar, 2004). Browsing and grazing on natural rangelands or shrublands are the primary feed sources in the world's arid and semi-arid pastoral areas, occasionally using crop residues in farming systems where mixed crop-livestock production is practiced (Nair *et al*., 2021).

Seasonality of rainfall distribution, quantity, and quality of forage supply is markedly seasonal in arid rangelands (Larsen *et al*., 2021), with a severe shortage of forage supply and inferior quality during the dry season, which constrains goat production on pasture (Chebli *et al*., 2022). Poor nutrition results in a low growth rate of growing animals, meat and milk production, reproductive performance, loss of body condition, and increased susceptibility to diseases and parasites (Zhou *et al*., 2019; Flores-Najera *et al*., 2020). This is further aggravated by the rearing practice that does not contemplate feed supplementation at any season of the year, due mainly to the high cost of these supplementation feed. Thus, improving nutrition and maximizing the available forage resources should be the primary goal for enhancing goat productivity under marginal rangelands and/or in poor rural households (Mellado *et al*., 2020).

In traditional goat production systems in developing countries, bucks run freely with does; therefore, natural mating occurs most of the year because below 25° north latitude anestrous in goats is almost inexistent (Mellado J *et al*., 2014). However, due to nutritional constraints, goats typically have a great reproductive wastage (Robertson *et al*., 2020; Mellado, 2022). Therefore, proper feeding is key to high fertility in grazing goats. In goats raised in arid ecosystems, reproductive performance is suboptimal, and milk production is low. Even so, these goat production systems are sustainable, meet the dairy and meat demands of low-income communities (Silanikove *et al*., 2010), and provide a significant income source for goat farmers (Mayberry *et al*., 2018; Murali *et al*., 2020).

There are marked variations in the capacity of goats to graze in harsh environments; therefore, some animals are better able to ingest enough nutrients to reproduce successfully (Mellado M *et al*., 2014). Meeting the nutrient requirements for optimum reproductive performance in grazing/ browsing goats is challenging in the dry season on rangeland (Safari *et al*., 2011). Therefore, assessing the goats' energy status at mating via blood metabolites indicative of body energy reserves is a useful tool to attain an acceptable pregnancy rate of goats on rangeland (Mellado *et al*., 2003; Sarıbay *et al*., 2020). Thus, it would be convenient to find out which goats in a herd can consume a better diet to become pregnant and avoid pregnancy loss.

We hypothesized that grazing goats' fertility would be increased in those animals with higher body condition score (BCS), blood metabolites and minerals indicative of good body energy reserves and that blood metabolites would interact with follicular and corpus luteum development.

Therefore, the present study in mixed-breed goats aimed to investigate potential differences in follicle and corpus luteum number and size and the number of these structures at mating between goats that conceived and those unable to get pregnant during the breeding season. Additionally, this study aimed to investigate the effect of follicle size, BCS, and blood metabolites and minerals concentrations at mating on pregnancy establishment and maintenance.

Material and methods

Study area

The experimental site is located in northeast Mexico (25° 32' N, 103° 40' W) at 1150 m above sea level. Mean annual precipitation is 225 mm, most of which falls as high-intensity thunderstorms from June to October. The mean annual temperature is 22.3° C. The overstory was predominantly *Prosopis* spp., *Larrea tridentata,* and *Atriplex canescens*. Other important shrubs present were *Agave lechuguilla*, and *Opuntia rastrera*. The most abundant forbs are *Sphaeralcea angustifolia*, *Solanum elaeagnifolium*, *Salsola kali,* and *Lepidium virginicum*. Grasses constitute only a small part of the vegetation and grow mainly beneath shrubs. The principal species are *Munroa pulchella*, *Setaria macrostachya,* and *Muhlenbergia porteri*.

Goats and their management

Animal procedures were agreed upon and performed following the Institutional Animal Care and Use Committee of the Agrarian Autonomous University Antonio Narro (Protocol # 03001-2258) and carried out following FASS (2010). The study was conducted from May to October 2021 in a large goat herd in a microphyll desert scrub ecosystem of northern Mexico. A total of 89 mixed-breed (Central Europe dairy breeds × criollo) goats were used in the present study. Goats did not present any physical defects, had not given birth in the previous five months, were not pregnant, and had BCS ranging from poor to good (BCS 1–3 on a scale of 1–5). Goats grazed on open degraded Chihuahuan desert rangeland in plain terrain, yearround, driven by a herdsman for 6 h per day (from 1100 to 1700 h). Goats were confined after returning from grazing

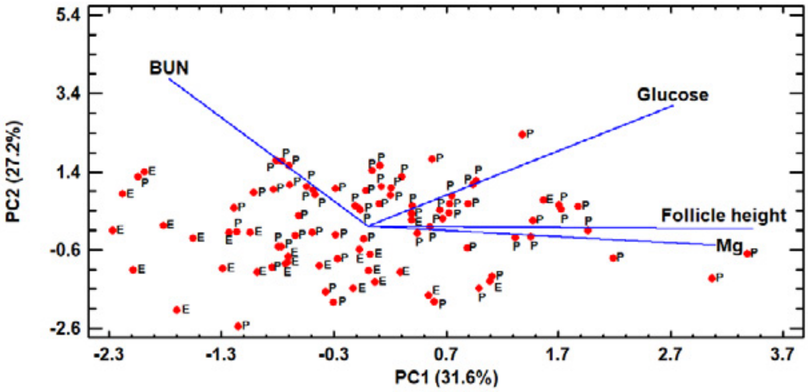

Figure 1. Results of the principal components analysis from some serum metabolites, minerals, and ovarian follicle size. The two principal components with the largest eigenvalues are shown as the x and y axes, respectively. In addition, the loading for each of the input variables concerning these two dimensions is shown. For each point: P= pregnant, and E= "empty".

in an unshaded pen, without access to feed supplementation and water. No salt mineral mix was provided to the goats throughout the year; goats had access to water from a pond only once a day. Goats were not treated against gastrointestinal and external parasites or vaccinated against endemic diseases.

Reproductive management

Four adult (64.5 ± 7.3 kg BW) mixed-breed bucks with adequate BCS (3.5; scale 1-5) with previous mating experience and an account of satisfactory kidding percentages were joined to dry goats for 30 days in May 2021. Approximately thirty days after the end of the mating season, pregnancy was detected using ultrasonography. Pregnancy detection was again assessed 120 days after mating. At kidding, kidding rate and litter size were recorded.

Ultrasound examination

At mating, trans-rectal ovarian ultrasonographic evaluations were performed using an ultrasound scanner (Aloka 500V, Corometrics Med. Syst. Inc., Wallingford, CT, USA) equipped with a linear array transrectal probe (7.5 MHz transducer) by a single experienced operator. Follicles were counted, and their height was recorded on a frozen image. Follicles were classified according to follicular height as small (<3 mm in size), growing mid-sized follicles (3 to 5 mm), and large follicles (>5 mm) (Rateb *et al*., 2019). The ovulation rate was determined by identifying the corpus luteum and the disappearance of the previously observed mature follicle(s) 10 days after mating.

Blood metabolites and minerals determination

Blood was sampled from the selected goats by jugular venipuncture in non-anticoagulation gel separator vacuum tubes (Vacuntainer®). Immediately after collection, the samples were centrifuged, and the serum was placed in Eppendorf tubes and stored at −20 °C until it was assayed. Serum glucose, total protein (TP), cholesterol, blood urea nitrogen (BUN), creatinine, and phosphorus (P) concentrations were determined using colorimetric methods following protocols supplied by the kits´ manufacturers (Sigma Diagnostics Inc., Livonia, MI, USA). Non-esterified fatty acids (NEFA) were determined using a commercial kit (WAKO; Mountain View, CA, USA). In addition, serum minerals were determined by atomic absorption spectrophotometry.

Statistical analyses

Continuous variables were tested for normality and group homogeneity using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC, USA, vers. 9.4); continuous data were normally distributed. The variables were compared between groups (pregnant *vs.* non-pregnant; pregnancy loss *vs.* non-pregnancy loss, single *vs.* twin pregnancy) using the GLM procedure of SAS. Variables were described as mean values ± standard deviation, differences between groups with 95% confidence intervals were computed (TTEST procedure of SAS), and significance was declared when α was 5%.

These variables were dichotomized for the effect of serum metabolites and minerals on ovarian structures, using their mean as a cutting point for classifying concentrations below or above the mean. Then, the GLM procedure of SAS was used to detect differences between levels of metabolites and minerals on the number of total follicles and ovulatory follicles, mean ovulatory follicles height, number of corpus luteum, and average corpus luteum height. Parity was included in the model as a covariate.

Canonical correlations for exploring the relationships between two multivariate sets of variables were applied

Table 1. Between-group comparison of body condition score (BCS), serum metabolites, and minerals of non-pregnant and pregnant goats at about 30 days post-service on a desert rangeland. Values for groups are means ± standard deviations.

Variables	Non-pregnant (NP; n= 25)	Pregnant (P; n= 64)	NP-P difference 95% CI	*p*-value
BCS (units)	2.1 ± 0.56	2.2 ± 0.45	-0.1 (-0.33 – 0.11)	0.035
Age (years)	3.5 ± 1.1	3.2 ± 1.4	0.3 (-0.27 – 0.96)	0.662
Glucose (mg/dL)	74.4 ± 11.6	87.3 ± 12.1	-12.9 (-18.5 – 7.2)	0.019
BUN (mg/dL)	10.7 ± 3.5	12.4 ± 3.7	-1.7 (-3.5 – -0.02)	0.008
Creatinine (mg/dL)	2.5 ± 0.4	2.5 ± 0.5	0.09 (-0.12 – 0.30)	0.471
Cholesterol (mg/dL)	133.3 ± 37.5	131.5 ± 30.4	1.8 (-13.5 – 17.0)	0.702
Total protein (mg/dL)	5.8 ± 1.9	5.8 ± 2.0	0.006 (-0.9 – 0.9)	0.381
NEFA (mmol/L)	0.41 ± 0.28	0.40 ± 0.23	0.02 (-0.09 – 0.13)	0.441
Copper (mg/L)	0.38 ± 0.11	0.43 ± 0.18	-0.04 (-0.12 – 0.03)	0.075
Zinc (mg/L)	1.13 ± 0.28	1.10 ± 0.31	0.01 (-0.14 – 0.16)	0.639
Magnesium (mg/dL)	1.89 ± 0.69	2.07 ± 0.76	-0.24 (-0.62 – 0.13)	0.131
Phosphorus (mg/dL)	4.17 ± 1.43	4.37 ± 1.38	-0.09 (-0.81 – 0.62)	0.257

BUN: blood urea nitrogen. NEFA: non-esterified fatty acids. CI: confidence intervals.

to select follicular traits to identify groups of metabolites with a related biological role in ovarian structures using Statgraphics Centurion 19 (Statgraphics Technol. Inc., The Plains, VA, USA). Also, principal component analyses were carried out to understand the sources of variation of data for pregnancy of goats and see distances between important serum variables affecting pregnancy rate. Significance was declared at $p<0.05$.

Results

In the present study, of the 89 experimental goats, 64 were confirmed as pregnant (72% pregnancy rate) on day 30 post-service using ultrasonography. Upon reexamination on day 120 of mating, ultrasonography indicated eight pregnancy losses, and consequently kidding rate was 63%, with a mean litter size of 1.5 ± 0.59 (± SD). Principal components derived from serum metabolites, ovulatory follicle height, minerals, and ovarian variables showed a clear separation between pregnant and non-pregnant goats (Fig. 1). The first two principal components explained 58.8% of the variation in the data.

Data for BCS, serum metabolites, and minerals for pregnant and non-pregnant goats are shown in Table 1. The univariate general linear model analyses showed that BCS was significantly higher ($p<0.05$) in pregnant than non-pregnant goats. No significant effect of the confounding variable age on pregnancy outcome was observed. Mean serum glucose concentration was 13 mg/kg higher ($p<0.05$) for goats that became pregnant compared with non-pregnant goats. There was a significant ($p<0.01$) effect of pregnancy on serum BUN concentrations, which indicated that high blood BUN levels were associated with pregnancy outcomes. All other serum metabolites indicative of body energy reserves and minerals were not different for non-pregnant compared with pregnant goats. No effect was observed for BCS, serum metabolites, and minerals concentration on litter size of goats ($p>0.05$).

Mean serum glucose concentrations at mating were much higher ($p<0.01$) in goats that did not lose their pregnancy than in goats that experienced a pregnancy loss before 120 days post-mating (Table 2). In addition, serum NEFA concentration at mating was 2.4 times higher ($p<0.05$) in goats that did not lose their pregnancy than in goats that lost their pregnancy. All other serum metabolites and minerals were not different at mating for goats with pregnancy loss and animals with no pregnancy loss. Regarding ovarian structure characteristics at mating, the mean ovulatory follicular height was significantly higher ($p<0.01$) in goats that became pregnant than in non-fecundated goats (Table 3).

High BCS, serum glucose, and BUN concentrations did not significantly affect the number or size of ovulatory follicles and corpus luteum. The total number of ovulatory follicles at mating was significantly higher ($p<0.01$) in goats with high serum TP than in goats with lower blood TP levels (Table 4). Likewise, higher serum TP concentrations led to more prominent follicles ($p<0.05$). Goats with higher serum P concentration presented significantly bigger ($p<0.05$) ovulatory follicles than goats with lower serum P levels. None of the variables studied affected the number of corpus luteum. The average height of the corpus luteum was larger ($p<0.05$) in goats with lower serum creatinine levels than in goats with high serum creatinine concentrations. Lower serum magnesium concentration

Table 2. Between-group comparison of body condition score (BCS), serum metabolites, and minerals of goats that maintained their gestation to term or goats that suffered pregnancy loss on a desert rangeland.

Variable	Pregnancy to term (PT; n= 56)	Pregnancy loss (PL; n= 8)	PT-PL difference 95% CI	*p*-value
BCS (units)	2.19 ± 0.51	1.92 ± 0.34	0.27 (-0.13 – 0.65)	0.628
Age (years)	3.4 ± 1.3	2.7 ± 1.6	0.73 (-0.34 – 1.81)	0.393
Glucose (mg/dL)	89.4 ± 11.2	72.2 ± 6.9	17.2 (9.1 – 25.4)	0.004
BUN (mg/dL)	12.6 ± 3.4	11.3 ± 5.9	1.26 (-1.75 – 4.27)	0.592
Creatinine (mg/dL)	2.46 ± 0.47	2.38 ± 0.14	0.08 (-0.28 – 0.44)	0.759
Cholesterol (mg/dL)	130.1 ± 30.7	143.6 ± 27.5	-13.5 (-37.8 – 10.8)	0.593
Total protein (mg/dL)	5.8 ± 1.9	5.8 ± 2.5	0.06 (-1.5 – 1.6)	0.581
NEFA (mmol/L)	0.18 ± 0.12	0.43 ± 0.23	-0.25 (-0.38 – 0.41)	0.018
Copper (mg/L)	0.43 ± 0.18	0.43 ± 0.17	-00.2 (-0.15 – 0.14)	0.795
Zinc (mg/L)	1.12 ± 0.29	0.91 ± 0.36	0.21 (-0.02 – 0.44	0.110
Magnesium (mg/dL)	2.30 ± 0.68	2.06 ± 0.46	0.25 (-.28 – 0.78)	0.906
Phosphorus (mg/dL)	4.41 ± 1.31	4.29 ± 1.01	0.12 (-0.97 – 1.20)	0.447

BUN: blood urea nitrogen. NEFA: non-esterified fatty acids. CI: confidence intervals.

was associated with a shorter corpus luteum ($p<0.05$). Additionally, the canonical correlation showed that follicle traits, BCS, and some blood metabolites were moderately correlated (r = 0.45; $p<0.05$), which indicates that serum glucose, creatinine, and NEFA had a significant but moderate association with the number of follicles and size.

Discussion

Optimization of reproductive efficiency of goat herds on arid rangelands is a continuous challenge to goat producers. In the present study kidding rate of unsupplemented goats bred during the non-breeding season was 63%, a figure close to 72% reported in the same environment and nutritional conditions (De Santiago-Miramontes *et al*., 2011; Mellado *et al*., 2020). The suboptimal reproductive performance observed in this study is attributable to the arid conditions under which the goats are reared.

The present study evaluated the variations in BCS, serum metabolites, and mineral concentrations in pregnant and non-pregnant goats on rangeland fecundated during the non-breeding season (May). The results indicated that BCS at mating was higher in pregnant than in non-pregnant goats. These findings align with previous works showing that adequate body energy reserves, mainly represented by body fat and muscle content in goats, are required for maximum estrus response (Rivas-Muñoz *et al*., 2010) and pregnancy rate (Serin *et al*., 2010). In addition, BCS in goats is associated with blood glucose concentration (Milosevic-Stankovic *et al*., 2020; Sitaresmi *et al*., 2020), BUN (Sitaresmi *et al*., 2020) and NEFA (Lunesu *et al*., 2021), which means that BCS vary with the change of energy balance. In goats, reduced BCS leads to ovarian dysfunction (inactive or acyclic; Widiyono *et al*., 2020). Thus, goats with higher body energy reserves had more energy for reproductive function, which was reflected in higher odds of getting pregnant.

Table 3. Between-group comparison of ovarian structures of non-pregnant and pregnant goats on a desert rangeland.

Variables	Non-pregnant (NP; n= 25)	Pregnant (P; n= 64)	NP-P difference 95% CI	*p*-value
Total follicles	5.20 ± 2.10	4.67 ± 1.67	0.53 (-0.34 – 1.40)	0.663
Ovulatory follicles	2.36 ± 1.63	2.26 ± 0.97	0.09 (-0.46 – 0.65)	0.838
Average ovulatory follicle size (mm)	6.70 ± 1.74	7.63 ± 1.57	-0.93 (-1.69 – -0.17)	0.016
Number of corpus luteum	1.48 ± 0.91	1.60 ± 0.63	-0.13 (-0.46 – 0.21)	0.450
Average corpus luteum size (mm)	13.00 ± 2.10	12.30 ± 3.64	0.69 (-0.85 – 2.23)	0.375

CI: confidence intervals.

Table 4. Mean (± SD) number and height of ovulatory follicles and corpus luteum relative to classes of body condition score (BCS) and serum metabolites and minerals goats on a desert rangeland.

Variables	Ovulatory follicles		Corpus luteum	
	Number	Height	Number	Height
BCS (units)				
≥ 2.5 (n=42)	2.36 ± 1.32	7.40 ± 1.53	1.69 ± 0.78	12.98 ± 2.81
< 2.5 (n=47)	2.23 ± 1.06	7.36 ± 1.78	1.46 ± 0.65	12.07 ± 3.63
Glucose (mg/dL)				
≥ 85 (n=40)	2.43 ± 1.17	7.62 ± 1.71	1.53 ± 0.64	12.52 ± 3.72
< 85 (n=49)	2.13 ± 1.20	7.07 ± 1.57	1.62 ± 0.80	12.48 ± 2.70
BUN (mg/dL)				
≥ 11.5 (n=49)	2.31 ± 1.06	7.20 ± 1.38	1.53 ± 0.70	12.72 ± 3.09
< 11.5 (n= 40)	2.28 ± 1.33	7.59 ± 1.96	1.62 ± 0.70	12.24 ± 3.52
Creatinine (mg/dL)				
> 2.4 (n= 40)	2.18 ± 1.15	7.30 ± 1.84	1.55 ± 0.81	11.67 ± 4.25[a]
< 2.4 (n= 49)	2.28 ± 1.02	7.44 ± 1.52	1.59 ± 0.64	13.18 ± 2.01[b]
Cholesterol (mg/dL)				
> 132 (n= 43)	2.26 ± 1.05	7.35 ± 1.81	1.53 ± 0.82	12.34 ± 3.87
< 132 (n= 46)	2.22 ± 1.11	7.40 ± 1.52	1.61 ± 0.61)	12.65 ± 2.66
Total protein (mg/dL)				
> 5.8 (n= 47)	2.53 ± 1.06[A]	7.74 ± 1.85[a]	1.53 ± 0.65	12.85 ± 2.63
< 5.8 (n= 42)	1.90 ± 1.01[B]	7.06 ± 1.41[b]	1.62 ± 0.79	12.11 ± 3.88
NEFA (mmol/L)				
> 0.4 (n= 51)	1.97 ± 0.97[a]	7.71 ± 1.84	1.53 ± 0.60	12.18 ± 3.96
< 0.4 (n= 38)	2.43 ± 1.12[b]	7.13 ± 1.84	1.61 ± 0.80	12.74 ± 2.69
Copper (mg/L)				
> 0.41 (n= 32)	2.25 ± 1.08	7.38 ± 1.41	1.59 ± 0.66	12.38 ± 3.04
< 0.41 (n= 57)	2.22 ± 1.09	7.38 ± 1.80	1.56 ± 0.75	12.57 ± 3.43
Zinc (mg/L)				
> 1.1 (n= 45)	2.24 ± 1.13	7.38 ± 1.88	1.58 ± 0.75	12.79 ± 1.90
< 1.1 (n= 44)	2.23 ± 1.03	7.38 ± 1.43	1.57 ± 0.69	12.21 ± 4.26
Magnesium (mg/dL)				
> 2.2 (n= 45)	2.20 ± 0.99	7.61 ± 1.79	1.60 ± 0.68	11.83 ± 3.67[a]
< 2.2 (n= 44)	2.27 ± 1.16	7.13 ± 1.49	1.55 ± 0.76	13.19 ± 2.71[b]
Phosphorus (mg/dL)				
> 4.3 (n= 38)	2.29 ± 1.04	7.83 ± 1.64[a]	1.52 ± 0.82	12.32 ± 3.39
< 4.3 (n= 51)	2.20 ± 1.11	7.03 ± 1.60[b]	1.61 ± 0.63	12.64 ± 3.2

BUN: blood urea nitrogen. NEFA: non-esterified fatty acids. [a,b]Means with different superscripts in the same column and within variable differ ($p<0.05$). [A,B]Means with different superscripts in the same column and within variable differ ($p<0.01$).

A comparison of the mean serum glucose concentrations between pregnant and non-pregnant goats also indicated that the higher circulating glucose concentrations occurred in goats that become pregnant. Glucose is the primary source of energy for the body's cells (Milosevic-Stankovic *et al*., 2020) and the primary indicator of energy status in goats (Khan & Ludri, 2002). Improved nutrition directly affects animal metabolism by providing substrates for metabolism and cellular processes (Scaramuzzi *et al*., 2006). Well-fed goats show higher serum glucose concentration than underfed animals (Mellado *et al*., 2020).

Glucose availability for ovarian follicles can be used for energy production (Sutton-McDowall *et al*., 2010), and circulating glucose, insulin, and glucagon levels affect folliculogenesis and the intrafollicular environment (Ying *et al*., 2011; Al-Hamedawi *et al*., 2017). Additionally, glycemia is critical in regulating ovarian follicle responsiveness to gonadotropins (Selvaraju *et al*., 2003). In this context, the present study confirmed that high serum glucose availability at mating in grazing goats increases the odds of pregnancy. Furthermore, short-term nutritional supplementation rises the number of ovulatory follicles, and the ovulation rate is associated with blood glucose levels in goats (Zabuli *et al*., 2010). This explains the higher proportion of pregnant goats with higher serum glucose concentration when joined to bucks.

These results showed that low serum BUN in goats resulted in a reduction in pregnancy rate. BUN reflects a higher nitrogen intake, as a positive correlation exists between protein intake and BUN concentration in goats (Rondina *et al*., 2005; Senosy *et al*., 2017). Pregnancy was more likely in goats that had a mean BUN value of 12.6 mg/dL, suggesting that high BUN in goats grazing arid rangeland is favorable for conception as the increased serum BUN in goats on rangeland indicates a positive effect of diet on rumen ammonia-nitrogen concentration (Zhu *et al*., 2020).

Serum cholesterol concentrations were not significantly different among pregnant, non-pregnant, and goats that experienced pregnancy loss or those whose pregnancy was carried to term. Also, this metabolite did not affect the number or size of ovarian structures. This response could be explained by the fact that serum cholesterol concentrations do not differ among BCS grades (Moeini *et al*., 2014; Sitaresmi *et al*., 2020) and, therefore, under the current nutritional conditions, may not have influenced the reproductive outcome.

Blood metabolites and minerals were not different at mating between single and twin-bearing goats. These results align with observations of Cepeda-Palacios *et al.* (2018), who documented that the number of developing fetuses did not affect any measured hematochemical parameters. However, other studies have found differences between goats carrying singles or twins (Cappai *et al*., 2019; Sarıbay *et al*., 2020). However, in these previous studies, blood metabolite concentrations were not determined at the time of fecundation.

Regarding pregnancy losses, low serum glucose concentration at mating led to higher spontaneous loss of a pregnancy. Of the several possible mechanisms bringing non-infectious gestational failure in goats, hypoglycemia in the mother and subsequently in the fetus trigger the premature eviction of fetuses (Mellado *et al*., 2004, 2020). Energy-deficient diets during gestation, especially in young goats or bearing double fetuses, are important factors triggering fetal losses (Waideland & Loken, 1991; Cronjé, 1998). The few aborted fetuses observed in these grazing goats did not show signs of decomposed fetuses or placentitis, which suggests that malnutrition at mating and subsequent weeks of pregnancy caused pregnancy loss. Goats that maintain their gestation to term in this ecosystem select diets higher in nutrients than goats suffering pregnancy loss (Mellado M *et al*., 2014); therefore, goats' genotypes unable to ingest nutrient-rich diets would present low serum blood glucose, which eventually leads to fetal expulsion.

Another blood metabolite at mating linked to pregnancy loss was NEFA. Goats with high serum NEFA concentrations were more likely to lose their pregnancy than goats with low circulating NEFA. This finding is in line with Hussain *et al.* (1996) who observed higher blood NEFA concentrations in goats with nonviable pregnancy than in goats with no pregnancy loss. This suggests increased lipolysis in goats with pregnancy loss, which presented elevated blood NEFA concentrations, indicative of acute energy restriction and mobilization of body reserves when the glucose level decreased (Veerkamp *et al*., 2003). Perhaps, the higher serum NEFA concentration was related to deficient nutrition for non-adapted goats to lower forage quality or a high fiber diet on rangeland, and they had lower energy status to sustain pregnancy than their better-adapted counterparts (Mellado M *et al*., 2014).

Ovulatory follicle size affected fertility when ovulation occurred after the buck stimulus. Contreras-Solís *et al.* (2021) observed that large follicles from prepubertal ewes had higher estradiol and progesterone concentrations, more competent oocytes, and blastocyst produced in vitro than less developed follicles. Also, it has been reported that GnRH-induced ovulation of follicles ≤11 mm results in lower pregnancy rates and augmented late embryonic/fetal mortality, associated with lower circulating estradiol concentrations and decreased circulating progesterone concentrations (Perry *et al*., 2005). Therefore, ovulatory follicle size is a robust indicator of fertility (Perry *et al*., 2007) and follicular growth stimulation with equine chorionic gonadotropin leads to greater pregnancy rate in goats (Hameed *et al.,* 2020).

Lower serum creatinine concentrations were associated with larger corpus luteum. High blood creatinine concentrations are a reliable biomarker of body protein breakdown and muscle mass change (Patel *et al*., 2013), and therefore, creatinine concentrations change in response to body protein mobilization. In goats, creatinine increased linearly with the decreasing crude protein concentration in the diet (Zhu *et al*., 2020). Therefore, researchers have used this metabolite to monitor nutrient status and muscle mass (Turner *et al*., 2005).

Higher serum TP concentrations were associated with a greater number of ovulatory follicles and their size. This metabolite accommodated both the variations in the albumin and globulins, showing a clear separation for the undernourished and well-fed sheep (Caldeira *et al*., 2007). Thus, this study reaffirms the effects of energy level during the antral phase and subsequent follicular development on follicle recruitment and size. This response has been observed in goats, where dietary energy levels positively influence oocyte follicular development and meiotic competence (Kabir *et al*., 2022).

The fewer ovulatory follicles in goats with elevated serum NEFA concentrations clearly show the effect of the well-known decrease in basal metabolic rate in animals in a state of undernutrition. Energy supplementation in sheep has increased the number of follicles and the amount of double ovulation (Habibizad *et al*., 2015), which are connected to a rise in the number and size of preovulatory follicles (Cuadro *et al*., 2018). Also, greater serum P concentration resulted in larger preovulatory follicles, a singularity of this mineral that improves reproductive performance of anestrus sheep restoring their ovarian activity, increasing the number and size of ovarian follicles and size of corpora lutea (Senosy *et al.*, 2018).

In summary, this study showed that high serum BUN and glucose, greater follicular development, and better BCS at mating are sensitive biochemical and physical markers to detect grazing goats capable to become pregnant. Further, high serum glucose and low NEFA concentration at mating are predictive of goats maintaining pregnancy on rangeland.

Authors' contributions

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