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Effect of Short-Term Glycerin Supplementation on Follicle Dynamics and Pregnancy Rate in Goats

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Abstract: We investigated the effects of short-term glycerin supplementation on follicular dynamics and pregnancy rates. Twenty-five goats with synchronized estrus and follicular waves with three injections of a prostaglandin analog every 7 days were used. Two days after the second injection, 13 goats were randomly chosen to receive an oral drench of 200 mL of glycerin (glycerin group [GG], n = 13) for 6 days, whereas the remaining 12 animals received an oral drench of saline (control group [CG], n = 12). At 24 and 48 h after the third injection, the goats mated. The animals were kept in a collective stall and received the same diet. The GG had higher blood glucose levels during the supplementation period than the CG (76.4 ± 1.9 vs. 50.3 ± 0.7 mg/dL; $p < 0.01$). The glycemic peak was recorded 4 h after the glycerin administration (102.3 ± 5.1 mg/dL) and remained higher than that in the CG 8 and 12 h later. The GG goats had a higher rectal temperature, heart rate, and respiratory rate than the CG goats and showed an increase in these parameters 4, 8, and 12 h after glycerin drenching. The GG animals also exhibited increased stress, urination, and drinking behaviors and reduced rumination. The ultrasonographic analysis showed a higher number of follicles with a diameter >4 mm ($p < 0.05$) and a greater follicular diameter ($p < 0.01$) in the waves before and after ovulation induction. The pregnancy and twinning rates and litter size at parturition were not different between the groups. Short-term supplementation with glycerin positively affects ovarian stimulation but has no effect on the reproductive response after mating.

Keywords: goat; glycerin; follicle; ovary; pregnancy; energy supplementation

1. Introduction

The significant growth in the global goat population observed in recent decades (FAO, 2021) [1] is mainly a product of the ever-increasing interest in the nutritional quality of milk from this species as an alternative to bovine milk [2]. Even today, the main goat farms are located in countries with tropical and subtropical climates, such as Northeast Brazil, where some of the biggest challenges include meeting the energy demands of the animal during lactation and the unfavorable high-temperature environment. As these areas often have a low grain production, alternative food supplements capable of sustaining the nutritional requirements of these animals without compromising the consumption capacity are needed [3].

Among the products that have been most tested in this regard in recent years is glycerin. Several studies have investigated the effects of glycerol on production parameters in dairy cows [4], beef cattle [5], goats [6], and lambs [7]. However, studies on its effects on reproduction, especially in goats, remain scarce. Despite this, the potential of glycerin is high because it can be used in short-term nutritional treatments that focus on supporting numerous reproductive phases. In small ruminants, the effects of glycerin on reproduction have already been demonstrated. For example, in sheep, Gutierrez et al. [8] applied a single dose of glycerol (100 mL) before mating and found an increase in the ovulation rate. However, in goats, the administration of 300 mL of glycerin drench after or during mating induces a reduction in the pregnancy rate [9]. On the other hand, the administration of 200 mL after mating induces an increase in the embryonic quality in superovulated goats [10]. The main challenge for goats, especially in tropical environments, is recommending a glycerin dosing protocol based on the reproductive response. The development of effective protocols requires better knowledge of the impact of the use of glycerin on these animals.

In goats, there is evidence that the diet directly influences follicular development. Kabir et al. [11] stated that diets with a higher energy level promote a greater number of antral follicles, whereas a low energy level promotes follicular degeneration. A hyper-energetic diet can promote an increase in the concentration of leptin in the blood, which induces the secretion of the hormone gonadotropin via hypothalamic and pituitary action in vivo [12]. A reduction in blood glucose levels directly affects oocyte quality and survival because, when they are reduced, oocytes are forced to depend on the use of energy resources derived from body reserves, which leads to changes in their development, owing to a change in mitochondrial β-oxidation [13]. During embryonic development, excess glucose promotes an increase in free radicals, leading to oxidative stress, increased cell proliferation, and the dysregulation of genes involved in mitochondrial function [14]. Cows with an energy deficit may have a higher proportion of morulas and, consequently, a lower proportion of blastocysts, indicating a delay in ovulation or embryonic development during the first few days of gestation [14].

Glycerol, the main component of glycerin, contains a large amount of energy and is considered an important food supplement for ruminants [10]. Part of the ingested glycerol is absorbed directly by the ruminal epithelium, metabolized in the liver, and directed toward gluconeogenesis by glycerol kinase, which converts it into glucose. Therefore, from a glucogenic perspective, the inclusion of crude glycerin increases the glucogenic potential of the diet [15]. Furthermore, another fraction of glycerol can be fermented and converted into volatile fatty acids; mainly propionate, which is the main precursor of gluconeogenesis in the liver and provides energy for cellular metabolism [16].

We hypothesized that glycerin-based supplements may be effective in goats even at short intervals of administration, acting successfully to stimulate ovarian function and reproductive responses after mating. Therefore, the objective of the present study was to verify the effects of short-term oral glycerin drench administration before breeding on the ovarian follicular dynamics, pregnancy rate, and prolificacy in goats and investigate the effects of glycerin on the physiological and behavioral responses of animals.

2. Materials and Methods

2.1. Location, Animal, and Experimental Treatments

This study was conducted on a farm of the School of Veterinary Medicine, Ceará State University, located in the equatorial zone ($4°2'23''$ S and $38°38'14''$ W) of Brazil. This area is characterized by a constant photoperiod regimen and has a warm, tropical, and sub-humid climate.

Twenty-five adult, pluriparous, non-pregnant, crossbred Anglo-Nubian goats of similar ($p > 0.05$) age (3.6 ± 0.6 years; overall mean $\pm$ SD), body weight (36.1 ± 5.3 kg), and body condition (2.7 ± 0.2; from 1 to 5 score) were used. The animals received a diet of chopped elephant grass plus concentrate (ground corn grain, 60%; wheat white, 32%; soybean meal,

3%; and a mineral and vitamin mixture, 5%), which satisfied the nutritional requirements of adult non-dairy maintenance goats (NRC, 2007) [17]. The roughage-to-concentrate ratio of the diets was 60:40. The diet was provided twice a day, early in the morning (07:00) and afternoon (13:00), allowing for 10% of the refused feed. All does had synchronized estrus and follicular waves as described by Viñoles et al. [18] after three injections of 100 μg of the prostaglandin analog (PGF2 alpha) cloprostenol sodium (Prolise-Tecnopec, São Paulo, Brazil) at intervals of 7 days.

The goats were randomly divided into two groups: a glycerin group (n = 13) supplemented with 200 mL of bidistilled glycerin (99% glycerol) and a control group (n = 12) that received 200 mL of saline solution. Double-distilled glycerin was administered in the form of a drench (90% glycerol:10% saline solution) provided once a day 1 h after morning feeding for 6 days, starting on the second day after the second application of PGF2α. The estimated energy value for glycerol was 0.38 Mcal of EM/mol [19]. Based on this value, supplementation with glycerin increased EM density by 63% compared with the control diet.

The third PGF2α injection was administered to simultaneously promote estrus and ovulation in all goats. Mating was performed 24 and 48 h after the third application of PGF2α using two Anglo-Nubian bucks of proven fertility.

The experimental animals were kept in open clay communal boxes with free access to mineral supplements and water and divided according to the treatment group. Before the experiments, the goats underwent a 30-day management adaptation after receiving endo- and ecto-parasitic treatments and were vaccinated against clostridiosis. Throughout the pre-experiment, cyclicity and ovarian function were monitored using ultrasound examinations and sexual receptivity in mature fertile males according to Fernandes et al. [20].

2.2. Animal Response Measurements

2.2.1. Thermo-Physiological Measurement

Physiological and environmental parameters were measured daily 0, 4, 8, and 12 h after glycerin drenching. Ambient temperature and relative humidity were recorded using a portable thermohygrometer (AK624, AKSO, São Leopoldo, Brazil). Rectal temperature (RT) was measured using a digital clinical thermometer (G Tech, Hangzhou Sejoy Electronics, Hangzhou, China). Skin surface temperature (ST) was measured on the rump in a previously knitted area (hairless area) using an infrared thermometer (AK32, AKSO, São Leopoldo, Brazil). The heart rate (HR) was assessed with the animal in a standing position using a stethoscope (G Tech/Premium/ProCheck) positioned on the left side of the thorax, close to the heart. The pulse sound was recorded for 15 s, and the resulting value was multiplied by four to obtain the frequency in beats per minute. In sequence, the respiratory rate (FR) was evaluated using a stethoscope. Pulmonary sounds were counted for 15 s, and the resulting value was multiplied by four to obtain the frequency in breaths per minute.

2.2.2. Behavioral Evaluation

To evaluate behavior, the animals were observed every 4 h (0, 4, 8, and 12 h) for 30 min before the administration of glycerin and animal manipulations, totaling 2 h of daily evaluation; a trained observer was positioned in front of the pens to record the activities. According to Bateson and Martin [21], does' behaviors were recorded as activities (water consumption, urination, defecation, rumination, diet consumption, and idleness) and punctual events related to stress indicators (vocalization, isolation, headbutting, sniffing, biting, and moving the head, ears, and thoracic limbs). To allow for an accurate record of the behavior and follicular dynamics, animals from each group were homogeneously segregated for use in two replicates based on Fernandes et al. [22]. A total of 13 and 12 animals were used in the first and second replicates, respectively.

2.2.3. Peripheral Glycemia and Hematological Parameters

Small blood samples were collected by venipuncture using a 1 mL syringe 0, 4, 8, and 12 h after glycerin drenching. Immediately after collection, glycemic measurement was carried out using an automatic device (G Tech Free). To carry out hematological counting of white and red blood cells, a blood sample was used on the 0th and 5th days of glycerin drench via jugular venipuncture using vacuum tubes with 4 mL of EDTA (Becton Dickinson, Franklin Lakes, NJ, USA) and analyzed using an automatic hematological analyzer (Mindray BC 2800 VET, Shenzhen, China).

2.3. Ovarian Follicular Dynamics

Ultrasonography was performed once daily during glycerin supplementation. Ovarian images were captured using a B-mode ultrasound device (Mindray DP 2200 VET, Shenzhen, China) coupled to a linear transducer at a frequency of 7.5 MHz. A video was recorded per ovary for each animal, and images were captured and analyzed using previously calibrated ImageJ. An ovarian follicular wave was defined as the appearance of a cluster of small follicles (3–4 mm) that develop into one or more large follicles ($\geq$5 mm) (Ginther and Kot, 1994) [23]. An intermediate diameter ($\geq$4 mm to <5 mm) was defined as a medium-sized follicle.

2.4. Pregnancy Diagnosis and Post-Partum Measurements

Pregnancy diagnosis was performed 30 and 45 days after breeding by transrectal ultrasound using B-mode ultrasound equipment (Mindray DP 2200 VET, Shenzhen, China) coupled to a linear transducer at a frequency of 7.5 MHz.

Kidding rate and litter size were determined and kids were weighed.

2.5. Statistical Analysis

Statistical analyses were performed using Statistica version 13.4.0.14 (2018; TIBCO Software, Inc., Palo Alto, CA, USA). The data were initially verified for mathematical assumptions using the Kolmogorov–Smirnov and Bartlett's tests. If these conditions were not met, (log10$\times$) transformation was applied.

Data on peripheral glucose concentrations and physiological and behavioral response parameters were analyzed using PROC GLM as factors in the analysis of variance (ANOVA) model for the group (control and glycerin), period of glycerin supplementation (0, 1, 2, 3, 4, and 5 days), time of measurement (0, 4, 8, and 12 h after the glycerin drench), and group vs. day and group vs. hour interactions. Regarding hematological and ovarian response parameters, the ANOVA model included group, time of glycerin supplementation, and the interaction between the factors. All pairwise comparisons were performed using the Newman–Keuls post hoc test. For pregnancy rate, twinning rate, kidding rate, and litter size, the group factor was analyzed using the Kruskal–Wallis ANOVA test.

2.6. Ethical Statement

All procedures in this study were approved by the Ethics Committee on Animal Experimentation of the UECE (No. 3826196/2016, CEUA-UECE).

3. Results

3.1. Peripheral Glucose Levels and Hematological Parameters

Figure 1 shows the plasma glucose concentrations after the administration of the glycerin drench. The measurement of the glucose level at 0 h does not indicate a statistical difference between groups, showing values within the range indicated by the goat species (70 $\pm$ 10 mg/dL) [24]. The glycerin group showed a higher average glucose level for the entire measurement interval than the control group (76.4 $\pm$ 1.9 mg/dL vs. 50.3 $\pm$ 0.7 mg/dL; $p < 0.01$). Peripheral glycemia was influenced by the measurement time ($p < 0.01$), as there was an interaction between the group and time ($p < 0.01$). Unlike the control group, the glycemia levels in the glycerin group showed an increase after 4 h of the drench

(102.3 $\pm$ 5.1 mg/dL) and then partially decreased after 8 and 12 h (Figure 1), but always remaining at significantly higher values ($p < 0.01$) than the group control. There was no effect of the supplementation time or interaction between the group and supplementation time.
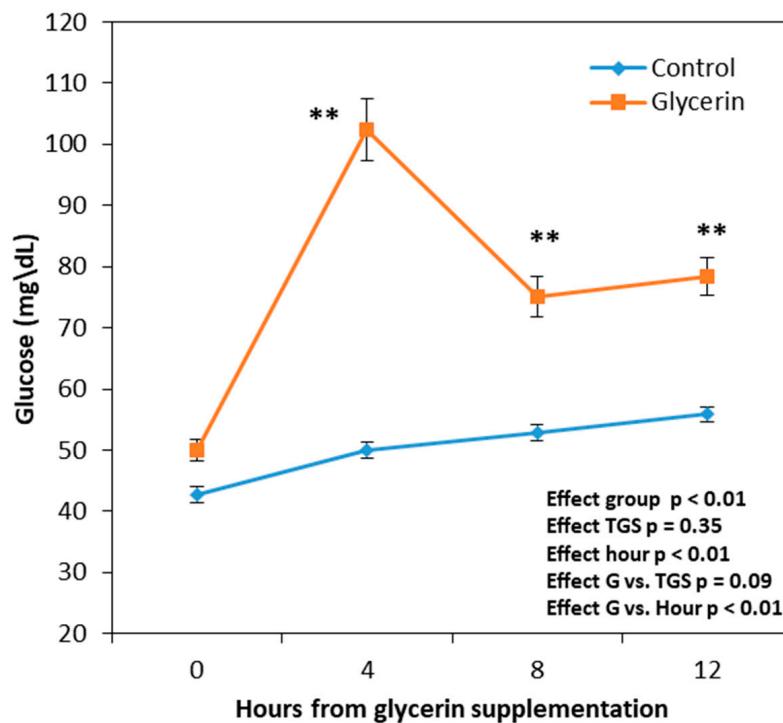

Figure 1. Peripheral glucose levels measured 0, 4, 8, and 12 h after glycerin drench in goats fed a baseline diet or supplemented with glycerin 7 days prior to mating. Time of glycerin supplementation (TGS), group (G). Data are plotted as mean $\pm$ SEM. ANOVA *p*-value for the group, time of glycerin supplementation, hour, and interactions are shown in each figure. ** $p < 0.01$ indicates differences between groups.

No differences were observed between groups with respect to the automatic counting of white and red cells (Table 1). Both parameters showed no changes depending on the supplementation time. With the exception of ST, other physiological parameters increased in the glycerin group (Table 1).

Table 1. Hematological, physiological, and behavioral responses in goats fed a baseline diet or diet supplemented with glycerin drench 7 days prior to mating.

Parameters	Group			*p* Value				
	Control	Glycerin	SEM	Group	TGS	Hour	G $\times$ TGS	G $\times$ Hour
Hematological response *								
WBC, $\times 10^3/\mu$L	11.6	11.8	0.33	0.22	0.94	-	0.88	-
RBC, $\times 10^6/\mu$L	13.4	14.2	0.53	0.84	0.52	-	0.67	-
Physiological response								
HR, beat\min	66.4	90.6	0.85	<0.01	<0.01	<0.01	<0.01	<0.01
RF, breaths\min	32.2	37.2	0.71	<0.01	<0.01	<0.01	0.04	0.74
ST, °C	35.5	35.3	0.07	0.13	<0.01	<0.01	0.20	0.06
RT, °C	38.8	38.9	0.02	<0.01	0.22	<0.01	<0.01	<0.01

Table 1. *Cont.*

Parameters	Group			*p* Value				
	Control	**Glycerin**	**SEM**	**Group**	**TGS**	**Hour**	**G × TGS**	**G × Hour**
	Behavior response							
Rumination, n	1.3	0.4	0.05	<0.01	<0.01	<0.01	0.07	<0.01
Idle, n	1.7	2.6	0.09	<0.01	0.64	<0.01	0.20	0.38
Drinking, n	0.00	0.3	0.02	<0.01	0.05	<0.01	0.05	<0.01
Feeding, n	2.9	2.6	0.11	0.60	<0.01	<0.01	0.57	<0.01
Urination, n	0.1	0.3	0.01	<0.01	0.33	0.61	0.31	0.88
Defecation, n	0.1	0.1	0.01	0.13	0.42	0.04	0.01	0.36
Stress indicators, n	2.8	3.1	0.19	0.02	<0.01	<0.01	0.71	<0.01

* hematological parameters measured at 0th and 5th days of glycerin drench. Abbreviations: WBC, white blood cell; RBC, red blood cell; HR, heart rate; RF, respiratory frequency; ST, superficial temperature; RT, rectal temperature; TGS, ANOVA effect for glycerin supplementation length (0, 1, 2, 3, 4, and 5 days); hour, ANOVA effect for daily reading measures (0, 4, 8, and 12 h after glycerin drench).

3.2. Physiological and Behavior Responses

The minimum and maximum environmental temperatures recorded during the experimental period were 28.7 ± 0.2 °C and 28.81 ± 0.2 °C, respectively. The minimum relative humidity recorded was $67.7 \pm 0.9\%$ and the maximum was $68.6 \pm 0.91\%$. No changes in these parameters were observed between the groups or during the supplementation period. In contrast, there were significant variations ($p < 0.0001$) in the environmental parameters during the daily measurements, with an average increase in temperature (+3.4 °C) at 12:00 a.m. and 16:00 p.m., 4 and 8 h after the glycerin drench. At the same intervals, there was also a decrease in the relative humidity (-10.8%).

For the RT, HR, and RF, the group interaction with the supplementation time was also recorded because of the increase in the values of these parameters in the animals supplemented with glycerin. The interaction between the measurement time and group for the HR and RT was owing to an increase in these parameters in the glycerin group 4, 8, and 12 h after the administration of the supplement.

The glycerin group showed an increase in behaviors (Table 1) related to idleness ($p < 0.01$), urination ($p < 0.01$), water consumption ($p < 0.01$), and stress indicators ($p = 0.02$), whereas the frequency of rumination was decreased ($p < 0.01$), compared with the control group. An interaction between the group and supplementation time ($p = 0.01$) was recorded for defecation frequency, owing to the increase in these behavioral acts in the glycerin group between the third and fifth day of supplementation compared with the control. Interactions were also verified between the group and time of measurement for water consumption owing to the frequency increase in this behavior 4 h after the drench ($p < 0.01$). In contrast, reductions in rumination and feeding were observed in the glycerin group ($p < 0.01$), with more evidence after 4 h of drenching (interaction $p < 0.01$).

On average, the stress markers were higher in the glycerin group ($p = 0.02$), with a pronounced increase 4 and 12 h after supplementation ($p < 0.01$).

3.3. Follicle Traits and Reproductive Response

Table 2 shows the results of the ultrasound analysis of the ovarian follicular population. There was a significant increase in the number of follicles >4 mm in diameter the and follicular diameter before and after ovulation induction in the glycerin group. Regarding the reproductive response (Table 2), there were no differences between the groups in terms of the pregnancy rate, twinning rate, or litter size. The live weight of the kids at birth was also similar between the groups.

Table 2. Follicle traits and reproductive response in goats fed a baseline diet or diet supplemented with glycerin drench 7 days prior to mating.

Parameters	Group			*p* Value		
	Control	Glycerin	SEM	Group	TGS	G × TGS
Follicle traits before ovulation induction *						
Follicles > 4 mm, n\ovary	0.01	0.06	0.01	0.04	0.38	0.04
Total follicles, n\ovary	3.4	3.4	0.06	0.53	0.51	0.63
Follicle diameter, mm	3.4	3.7	0.05	<0.01	0.03	0.78
Follicle traits after ovulation induction **						
Follicles > 4 mm, n\ovary	0.08	0.2	0.01	0.01	0.04	0.61
Total follicles, n\ovary	3.4	3.5	0.05	0.31	0.69	0.79
Follicle diameter, mm	3.9	4.2	0.04	<0.01	<0.01	0.92
Reproductive response						
Pregnancy rate, % (n/n)	66.7 (8/12)	76.9 (10/13)	-	0.57	-	-
Twinning rate, % (n/n)	25.0 (2/8)	30.0 (3/10)	-	0.74	-	-
Kidding rate, % (n/n)	62.5 (5/8)	80.0 (8/10)	-	0.27	-	-
Litter size	1.5	1.4	0.15	0.84	-	-
Kid weight at partum/doe, kg	4.9	5.2	0.56	0.86	-	-

* Follicle ultrasonography traits measured after the second PG2α administration on the 0th day to the 4th day of glycerin supplementation. ** Follicle ultrasonography traits measured after the third PG2α administration on the 5th day to the 6th day of glycerin supplementation. TGS, ANOVA for glycerin supplementation time.

4. Discussion

The results obtained in this study partially confirmed the initial hypothesis of the study. The application of glycerin-based supplements for a brief interval before mating was successful in stimulating ovarian function, increasing the number of large follicles and follicular size, but had no effect on the pregnancy rate or multiple pregnancies.

Regarding the impact of the supplementation on the animal response, no clinical signs of hyperglycemia were observed during the experimental period; however, a transient elevation in the glycemic level was recorded 4 h after the administration of the supplement. Although the blood glucose values returned to normal in successive measurements, the glycemic peak observed at that specific interval showed an inadequate homeostatic response in the animal. The increase in the plasma glucose concentration observed in the present study was probably owing to the high rate of glycerol absorption when the glycerin was administered. After a meal, pancreatic β-cells secrete insulin to coordinate systemic glucose homeostasis in response to elevated blood glucose and other metabolite levels. This homeostasis is driven by tissue insulin sensitivity, which generally describes the efficiency of a given insulin concentration to normalize blood glucose levels [25]. The administration of glycerol to ruminants promotes metabolic changes by increasing plasma glucose and insulin concentrations in a dose-dependent manner. Although the latter mechanism is well-established, animal responses are largely dependent on the dose and form of administration. In sheep treated with intravenous glycerol infusion (170 mL), blood glucose and insulin levels peak 1 and 2 h, respectively, after administration [26]. In sheep, Ferraro et al. [27] observed an increase in the peak glucose and plasma insulin levels from 3 to 12 h after the application of glycerin in the form of an oral drench (270 mL). In goats, Rodrigues et al. [9] applied a 300 mL oral glycerin dose and found an increase in glycemia and insulinemia 6 and 12 h after administration, respectively.

Very high blood glucose levels can lead to hyperglycemia, in which the high peak in a short time of glycemic flow is difficult to administer by the tissues and can create a cascade effect on tissue receptors. This well-regulated homeostatic mechanism typically involves multiple processes in multiple organs, including the attenuation of glucose release from the liver, increased glucose uptake in the muscle and adipose tissue, the suppression of free fatty acid release from adipocytes (lipolysis), and increased lipid accumulation in the liver and adipocytes, regulated by insulin-dependent signaling [28].

In the present study, two distinct mechanisms were identified in relation to the glycemic response of the animals. The first was related to the peak after glycerin administration, as previously described, and the second was an interaction that may have occurred with the environment. In our study, an increase in the environmental temperature was recorded when the blood glucose levels remained high, which was probably the cause of the physiological responses of the animals. The increase in the physiological parameters was important, especially in relation to the heart rate, which demonstrates the discomfort of the animals, a fact that was associated with the behavioral changes recorded in the supplemented animals. Despite the positive results described previously, some aspects limit the use of glycerin in folliculogenesis stimulation protocols for small ruminants. In large herds or in the case of limited access to animals, as in extensive systems, the best form of administration and dose for the application of this energy supplement are still debatable. On the one hand, the use of glycerin in the form of an oral drench provides ease of application and allows for the control of the administered amount [29]. On the other hand, it involves greater risks in terms of individual reactions to high doses [27], and the number of applications is limited because frequent animal restraint is required. Owing to its easy handling, the administration of glycerin in feed favors the application of continuous treatments for long periods [30]. Nonetheless, it does not allow for the efficient control of the effective ingested dose, especially when animals feed collectively.

According to Sharma et al. [31], blood glucose levels can increase in response to stress owing to the release of hormones such as glucocorticoids and catecholamines. Stress can lead to the increased activity of the sympathoadrenal system, which reduces glucose tolerance and increases the risk of acute cardiovascular events such as an increased heart rate [32]. Increased catecholamine levels lead to increased glycolysis, glycogenolysis, and gluconeogenesis, and can cause the insulin-mediated suppression of glycogenesis under physiological circumstances, leading to hyperglycemia [33]. Activating β adrenergic receptors (AR) via epinephrine and norepinephrine results in insulin resistance [31].

As anticipated, the supplementation strategy successfully stimulated follicular dynamics. The chosen dosage and application period positively affected the size of the follicles and number of large follicles before and after ovulation induction. Considering the literature, these results were partially expected. For example, Andrade et al. [34] and Oliveira et al. [35] supplemented sheep with glycerin and found a significant increase in the number of large follicles. Alves et al. [36] found that glycerol supplementation significantly increases the number of small follicles. The probable cause of ovarian stimulation was the glycemic surplus that occurred during the supplementation period. According to Viñoles et al. [18], greater nutritional intake in sheep can lead to higher circulating concentrations of FSH and ovarian steroids and increased ovulation rates. Furthermore, Guo et al. [37] stated that increasing dietary energy intake 4–10 days before ovulation results in an increase in the production of glucose, insulin, and IGF-1 in the ovary, which stimulates the synthesis of FSH receptors, leading to an increase in the ovulation rate. Thus, the energy content of diets can affect follicular development via the direct action of nutrients in the ovarian microenvironment and the development of gonadotropin receptors [37]. Dietary glycerol levels promote increases in glucose concentrations, which are directly related to an increase in the circulating concentrations of IGF-1, which could modify the sensitivity of the ovary to gonadotropins and promote greater follicular development [38]. Furthermore, in sheep fed high-energy diets for a short period of time, there is an increase in the number of follicles and amount of double ovulation, possibly promoted by the high levels of estradiol (E2) in plasma [39]. Steroid hormones (E2 and progesterone) are involved in regulating the recruitment, selection, and development of ovulatory follicles, and E2 signaling modulates the coupling of reproductive functions with energy metabolism [40].

Despite the evidence of follicular growth, the supplementation with an oral glycerin drench did not produce any advantages in terms of pregnancy rates or multiple pregnancies. These results were unexpected but can be justified if framed in what happened with the glycemic values. Excessive glycemic stimulation in the case of a glycerol overdose can lead

to hyperglycemia in the herd. Oocyte and embryo quality can also be reduced by excessive energy intake, especially from diets rich in glycemic precursors [41]. This is because an increase in insulin levels induces epigenetic changes, a decrease in the glutathione enzyme, possibly increasing oxidative stress, and sorbitol accumulation in the cells, resulting in the accumulation of reactive oxygen species (ROS) [42]. Hyperinsulinemia can overstimulate the IGF system and negatively affect oocyte quality and pregnancy rates [9]. At supraphysiological concentrations, insulin has a stimulatory effect on oocyte cleavage and maturation in vitro, but prolonged hyperinsulinemia in vivo negatively affects oocyte developmental competence [43]. According to Adamiak et al. [44], changes in the circulating levels of insulin and IGF-1 induced by dietary modifications promote an increase in follicular recruitment but also have negative effects on oocyte quality before and after fertilization. Regarding the initial development of the embryo, a greater availability of insulin promotes greater metabolic activity, leading to an increase in oxidative stress, which could be a possible explanation for its decreased viability. According to Laskowski et al. [45], embryos from oocytes with high insulin levels have greater mitochondrial activity, which is indicative of greater ATP metabolism for mitosis and the proliferation of embryonic cells.

Increasing glycemic indices in the ovary can lead to changes in the activity of antioxidant enzymes, such as glutathione peroxidase. Glucose is an essential energy source for cells as it is used to generate ATP molecules. However, some of the generated energy produces ROS, including superoxide (O^{2-}) and nonreactive H_2O_2 [46]. A previous study demonstrated the involvement of ROS in the follicular fluid environment, folliculogenesis, and steroidogenesis, providing strong evidence that ROS are involved in the onset of apoptosis in antral follicles caused by various chemical and physical agents in the follicular fluid [47]. The increase in glucose availability is one of the "immediate nutrients" effects on the ovulation rate, as the glucose entry rate explained 63% of the variation in the ovulation rate of sheep that receive glucose infusion; thus, energy intake is one of the most important factors influencing the reproductive performance of sheep [48]. This relationship may involve effects mediated by the glucose–insulin intrafollicular system, as well as energy detection mechanisms [49].

The increase in intra-follicular glucose levels can compromise development, resulting in the absence of large follicles, as in the 3-day oral group, which may be related to hyperinsulinemic stimulation, which promotes the inadequate differentiation of granulosa cells, leading to the early exposure of LH receptors controlling follicular growth [50]. According to Viñoles et al. [51], supplementation increases leptin concentrations from the second to the fifth day of supplementation, with higher values recorded on the third day after the beginning of feeding. This suggests that the increase in glucose, insulin, and leptin concentrations from the first to the third day of feeding prolongs the useful life of the last non-ovulatory follicle and, therefore, delays the occurrence of atresia, slowing down the renewal of the follicle and resulting in the occurrence of fewer follicular waves in sheep [51]. Previous studies have shown that supplementation with lupine grains for 4 days can increase the ovulation rate in sheep [52], which is associated with an increase in energy-producing nutrients [51].

Nogueira et al. [38] found that the supplementation of goats with corn for a short period (9 days) reduces the number of large follicles, increases the number of small follicles, and stimulates the ovulatory rate of these animals, although without changes in the metabolic profile. In sheep, short-term (6 days) energy supplementation results in an increase in the serum levels of glucose, cholesterol, and insulin, as well as an increase in the rate of double ovulation [39]. This is explained by Muñoz-Gutiérrez et al. [53] because, according to the authors, dietary energy can directly stimulate folliculogenesis in recruited and selected follicles, and this effect can be mediated by changes in systemic leptin concentrations and the hexosamine pathway in the follicle. Viñoles et al. [51] applied nutritional supplementation for 6 days, administered from the 9th to 14th day of the estrous cycle, and showed no effect on the ovulation rate. However, Viñoles [54], who supplemented sheep for 7 days, from days 8 to 14 of the estrous cycle, observed an increase in the ovulation

rate by 14%. The inconsistent effect of the short-term supplementation on the ovulation rate suggests that an increase in the ovulation rate depends on the follicular state at the beginning of the nutritional treatment [51].

5. Conclusions

Under the present experimental conditions, the use of 200 mL of glycerin during the 6 days of application successfully increased the glycemic rate in the goats but also markedly altered the physiological and behavioral responses. Furthermore, the oral glycerol supplementation did not affect the reproductive performance after mating, but provided better follicular growth. Therefore, we hope to conduct further studies using new dosages and administration times to provide adequate nutritional support for the entire embryo formation process.

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References

1. FAO. Agricultural Production Statistics 2000–2021.FAO. Available online: https://www.fao.org/3/cc3751en/cc3751en.pdf (accessed on 20 July 2023).
2. Verruck, S.; Dantas, A.; Prudencio, E.S. Functionality of the components from goat's milk, recent advances for functional dairy products development and its implications on human health. *J. Funct. Foods* **2019**, *52*, 243–257. [CrossRef]
3. Silva, M.R.L.; Alves, J.P.M.; Fernandes, C.C.L.; Cavalcanti, C.M.; Conde, A.J.H.; Bezerra, A.F.; Soares, A.C.S.; Tetaping, G.M.; De Sá, N.A.R.; Teixeira, D.Í.A.; et al. Use of green microalgae Chlorella as a nutritional supplement to support oocyte and embryo production in goats. *Anim. Reprod. Sci.* **2023**, *256*, 107296. [CrossRef] [PubMed]
4. Syahniar, T.M.; Andriani, M.; Ridla, M.; Laconi, E.B.; Nahrowi, N.; Jayanegara, A. Glycerine as a feed supplement for beef and dairy cattle: A meta-analysis on performance, rumen fermentation, blood metabolites and product characteristics. *J. Anim. Physiol. Anim. Nutr.* **2020**, *105*, 419–430. [CrossRef] [PubMed]
5. Almeida, V.V.S.; Oliveira, A.C.; Silva, R.R.; Ribeiro, J.S.; Oliveira, H.C.; Santos, L.S.; Lima, D.M., Jr. Crude glycerin in the supplementation of crossbred heifers in tropical pastures. *Animals* **2021**, *15*, 100088. [CrossRef] [PubMed]
6. Freire, L.F.D.S.; Cruz, G.R.B.; Costa, R.G.; Ribeiro, N.L.; Guerra, R.R.; Sousa, S.; Nascimento, G.V.D.; Lima, A.M.; Silva, G.F.; Sant'ana, A.M.S.; et al. Glycerin diet affects the size of the fat globule and the fatty acid profile of goat's milk. *Food Sci. Technol.* **2022**, *42*, e85821. [CrossRef]
7. Merlim, F.A.; Silva Sobrinho, A.G.; Borghi, T.H.; Cirne, L.G.A.; Valença, R.L.; Almeida, F.A.; Viviane Endo, V.; Viegas, C.R.; Zeola, N.M.B.L. Crude glycerin is an efficient alternative to corn in the diet of feedlot lambs. *Arch. Anim. Breed.* **2021**, *64*, 387–393. [CrossRef]
8. Gutierrez, C.G.; Ferraro, S.; Martinez, V.; Saharrea, A.; Cortez, C.; Lassala, A.; Basurto, H.; Hernandez, J. Increasing ovulation quota: More than a matter of energy. *Acta Sci. Vet.* **2011**, *39*, s305–s316.
9. Rodrigues, F.V.; Silva, C.M.G.; Lima, I.M.T.; Silva, A.M.; Fernandes, C.C.L.; Rondina, D. Effect of oral drenching of glycerin as a source of pre-mating energetic supplementation on reproductive response in goats. *Anim. Reprod.* **2015**, *12*, 890–898.
10. Silva, L.M.; Oliveira, C.H.A.; Silva, A.M.; Fernandes, C.C.L.; Duarte, S.S.; Lima, I.M.T.; Silva, C.M.G.; Martins, L.T.; Calderón, C.E.M.; Tavares, K.C.S.; et al. Gene expression and embryo quality in superovulated goats supplemented with crude glycerin after mating. *Small Rumin. Res.* **2014**, *120*, 71–77. [CrossRef]

11. Kabir, M.E.; Miraz, F.H.; Alam, M.H.; Sarker, M.B.; Hashem, M.A.; Khandoker, M.Y.; Moniruzzaman, M. Dietary energy influences ovarian morphology and in vitro maturation of oocytes in goats. *J. Appl. Anim. Res.* **2022**, *50*, 47–53. [CrossRef]

12. Childs, G.V.; Odle, A.K.; MacNicol, M.C.; MacNicol, A.M. The importance of leptin to reproduction. *Endocrinology* **2021**, *162*, bqaa204. [CrossRef] [PubMed]

13. Bach, À. Effects of nutrition and genetics on fertility in dairy cows. *Reprod. Fertil. Dev.* **2019**, *31*, 40–54. [CrossRef] [PubMed]

14. Chaput, C.; Sirard, M.A. Embryonic response to high beta-hydroxybutyrate (BHB) levels in postpartum dairy cows. *Domest. Anim. Endocrinol.* **2020**, *72*, 106431. [CrossRef] [PubMed]

15. Bezerra, H.; Santos, E.; Oliveira, J.; Carvalho, G.; Silva, F.; Cassuce, M.; Perazzo, A.; Zanini, A.; Pinho, R. Performance and ruminal parameters of boer crossbred goats fed diets that contain crude glycerin. *Animals* **2019**, *9*, 967. [CrossRef] [PubMed]

16. Lima, A.M.; Cruz, G.R.B.; Costa, R.G.; Ribeiro, N.L.; Beltrão Filho, E.M.; Sousa, S.; Justino, E.S.; Santos, D.G. Physical-chemical and microbiological quality of milk and cheese of goats fed with bidestilated glycerin. *Food Sci. Technol.* **2021**, *41*, 25–33. [CrossRef]

17. NRC. *Nutrient Requirements of Small Ruminants*; National Academy Press: Washington, DC, USA, 2007.

18. Viñoles, C.; Paganoni, B.; Glover, K.M.M.; Milton, J.T.B.; Blache, D.; Blackberry, M.A.; Martin, G.B. The use of a'first-wave'model to study the effect of nutrition on ovarian follicular dynamics and ovulation rate in the sheep. *Reproduction* **2010**, *140*, 865. [CrossRef] [PubMed]

19. Mach, N.; Bach, A.; Devant, M. Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. *J. Anim. Sci.* **2009**, *87*, 632–638. [CrossRef] [PubMed]

20. Fernandes, C.C.L.; Aguiar, L.H.; Calder'on, C.E.M.; Silva, A.M.; Alves, J.P.M.; Rossetto, R.; Bertolini, L.R.; Bertolini, M.; Rondina, D. Nutritional impact on gene expression and competence of oocytes used to support embryo development and livebirth by cloning procedures in goats. *Anim. Reprod. Sci.* **2018**, *188*, 1–12. [CrossRef]

21. Bateson, M.; Martin, P. *Measuring Behaviour: An Introductory Guide*, 3rd ed.; Cambridge University Press: Cambridge, UK, 2007.

22. Fernandes, C.C.L.; Feltrin, C.; Martins, L.T.; Gaudêncio Neto, S.; Aguiar, L.H.; Silva, A.M.; Oliveira, C.H.A.; Silva, L.M.; Silva, C.M.G.; Bertolini, M.; et al. Goat oocyte quality and competence to undergo IVM and embryo development after parthenogenetic activation from goats fed with different levels of cashew nut bran as source of dietary lipids. *Theriogenology* **2014**, *82*, 332–337. [CrossRef]

23. Ginther, O.J.; Kot, K. Follicular dynamics during the ovulatory season in goats. *Theriogenology* **1994**, *42*, 987–1001. [CrossRef]

24. Quandt, J.E.; Barletta, M.; Cornell, K.K.; Giguère, S.; Hofmeister, E.H. Evaluation of a point-of-care blood glucose monitor in healthy goats. *J. Vet. Emerg. Crit. Care* **2018**, *28*, 45–53. [CrossRef]

25. Chadt, A.; Al-Hasani, H. Glucose transporters in adipose tissue, liver, and skeletal muscle in metabolic health and disease. *Pflugers Arch. Eur. J. Physiol.* **2020**, *472*, 1273–1298. [CrossRef]

26. Kalyesubula, M.; Rosov, A.; Alon, T.; Moallem, U.; Dvir, H. Intravenous infusions of glycerol versus propylene glycol for the regulation of negative energy balance in sheep: A randomized trial. *Animals* **2019**, *9*, 731. [CrossRef]

27. Ferraro, S.M.; Mendoza, G.D.; Miranda, L.A.; Gutiérrez, C.G. In vitro ruminal fermentation of glycerol, propylene glycol and molasses combined with forages and their effect on glucose and insulin blood plasma concentrations after an oral drench in sheep. *Anim. Feed. Sci. Technol.* **2016**, *213*, 74–80. [CrossRef]

28. James, D.E.; Stöckli, J.; Birnbaum, M.J. The aetiology and molecular landscape of insulin resistance. *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 751–771. [CrossRef]

29. Porcu, C.; Manca, C.; Cabiddu, A.; Dattena, M.; Gallus, M.; Pasciu, V.; Succu, S.; Naitana, S.; Berlinguer, F.; Molle, G. Effects of short-term administration of a glucogenic mixture at mating on feed intake, metabolism, milk yield and reproductive performance of lactating dairy ewes. *Anim. Feed. Sci. Technol.* **2018**, *243*, 10–21. [CrossRef]

30. van Cleef, E.H.C.B.; Almeida, M.T.C.; Perez, H.L.; Paschoaloto, J.R.; Castro Filho, E.S.; Ezequiel, J.M.B. Effects of partial or total replacement of corn cracked grain with high concentrations of crude glycerin on rumen metabolism of crossbred sheep. *Small Rumin. Res.* **2018**, *159*, 45–51. [CrossRef]

31. Sharma, K.; Akre, S.; Chakole, S.; Wanjari, M.B. Stress-Induced Diabetes: A Review. *Cureus* **2022**, *14*, e29142. [CrossRef]

32. Hillis, G.S.; Woodward, M.; Rodgers, A.; Chow, C.K.; Li, Q.; Zoungas, S.; Patel, A.; Webster, R.; Batty, G.D.; Ninomiya, T.; et al. Resting heart rate and the risk of death and cardiovascular complications in patients with type 2 diabetes mellitus. *Diabetologia* **2012**, *55*, 1283–1290. [CrossRef]

33. Shah, A.; Wondisford, F.E. Gluconeogenesis Flux in Metabolic Disease. *Annu. Rev. Nutr.* **2023**, *43*, 153–177. [CrossRef]

34. Andrade, M.A.M.M.; Alves, J.P.M.; Galvão, I.T.O.M.; Cavalcanti, C.M.; Silva, M.R.L.; Conde, A.J.H.; Bezerra, A.F.; Fernandes, C.C.L.; Teixeira, D.I.A.; Rondina, D. Glycerin supplementation strategies for three or seven days affects oxidative stress, follicle dynamics and ovulatory response in Morada Nova sheep. *Anim. Reprod.* **2022**, *19*, e20200025. [CrossRef]

35. Oliveira, F.B.B.; da Silva, C.P.; Fernandes, C.C.L.; de Sousa, F.C.; Silva, A.M.; Pinheiro, D.C.N.; Rodrigues, L.F.S.; Rondina, D. Ovarian follicular growth in Morada Nova sheep supplemented with crude glycerin before mating. *Rev. Acad. Ciênc. Anim.* **2016**, *14*, 235. [CrossRef]

36. Alves, J.P.M.; Fernandes, C.C.L.; Rossetto, R.; da Silva, C.P.; Galvão, I.T.O.M.; Bertolini, M.; Rondina, D. Impact of short nutrient stimuli with different energy source on follicle dynamics and quality of oocyte from hormonally stimulated goats. *Reprod. Domest. Anim.* **2019**, *54*, 1206–1216. [CrossRef]

37. Guo, Y.X.; Duan, C.H.; Hao, Q.H.; Liu, Y.Q.; Li, T.; Zhang, Y.J. Effect of short-term nutritional supplementation on hormone concentrations in ovarian follicular fluid and steroid regulating gene mRNA abundances in granulosa cells of ewes. *Anim. Reprod. Sci.* **2019**, *211*, 106208. [CrossRef]

38. Nogueira, D.M.; Eshtaeba, A.; Cavalieri, J.; Fitzpatrick, L.A.; Gummow, B.; Blache, D.; Parker, A.J. Short-term supplementation with maize increases ovulation rate in goats when dietary metabolizable energy provides requirements for both maintenance and 1.5 times maintenance. *Theriogenology* **2017**, *89*, 97–105. [CrossRef] [PubMed]

39. Habibizad, J.; Riasi, A.; Kohram, H.; Rahmani, H.R. Effect of long-term or short-term supplementation of high energy or high energy-protein diets on ovarian follicles and blood metabolites and hormones in ewes. *Small Rumin. Res.* **2015**, *132*, 37–43. [CrossRef]

40. Fernandez, J.; Bruno-Galarraga, M.M.; Soto, A.T.; de la Sota, R.L.; Cueto, M.I.; Lacau, I.M.; Gibbons, A.E. Hormonal therapeutic strategy on the induction of accessory corpora lutea in relation to follicle size and on the increase of progesterone in sheep. *Theriogenology* **2018**, *105*, 184–188. [CrossRef]

41. Van Eetvelde, M.; Heras, S.; Leroy, J.L.M.R.; Van Soom, A.; Opsomer, G. The importance of the periconception period: Immediate effects in cattle breeding and in assisted reproduction such as artificial insemination and embryo transfer. *Periconception Physiol. Med.* **2017**, *1014*, 41–68.

42. Uhde, K.; van Tol, H.T.; Stout, T.A.; Roelen, B.A. Exposure to elevated glucose concentrations alters the metabolomic profile of bovine blastocysts. *PLoS ONE* **2018**, *13*, e0199310. [CrossRef]

43. Dupont, J.; Scaramuzzi, R.J. Insulin signalling and glucose transport in the ovary and ovarian function during the ovarian cycle. *Biochem. J.* **2016**, *473*, 1483–1501. [CrossRef]

44. Adamiak, S.J.; Powell, K.; Rooke, J.A.; Webb, R.; Sinclair, K.D. Body composition, dietary carbohydrates and fatty acids determine post-fertilisation development of bovine oocytes in vitro. *Reproduction* **2006**, *131*, 247–258. [CrossRef] [PubMed]

45. Laskowski, D.; Båge, R.; Humblot, P.; Andersson, G.; Sirard, M.A.; Sjunnesson, Y. Insulin during in vitro oocyte maturation has an impact on development, mitochondria, and cytoskeleton in bovine day 8 blastocysts. *Theriogenology* **2017**, *101*, 15–25. [CrossRef] [PubMed]

46. Turrens, J.F. Mitochondrial formation of reactive oxygen species. *J. Physiol.* **2003**, *552*, 335–344. [CrossRef] [PubMed]

47. Ciani, F.; Cocchia, N.; d'Angelo, D.; Tafuri, S. Influence of ROS on ovarian functions. *New Discov. Embryol.* **2015**, *21*, 41–73. [CrossRef]

48. Senosy, W.; Mahmoud, G.B.; Abdel-Raheem, S.M. Influence of short-term energy supplementation on estrus, ovarian activity, and blood biochemistry in Ossimi ewes synchronized with fluorogestone acetate in the subtropics. *Theriogenology* **2017**, *88*, 152–157. [CrossRef] [PubMed]

49. Scaramuzzi, R.J.; Zouaïdi, N.; Menassol, J.B.; Dupont, J. The effects of intravenous, glucose versus saline on ovarian follicles and their levels of some mediators of insulin signalling. *Reprod. Biol. Endocrinol.* **2015**, *13*, 6. [CrossRef]

50. Sánchez, F.; Romero, S.; Smitz, J. Oocyte and cumulus cell transcripts from cultured mouse follicles are induced to deviate from normal in vivo conditions by combinations of insulin, follicle-stimulating hormone, and human chorionic gonadotropin. *Biol. Reprod.* **2011**, *85*, 565–574. [CrossRef]

51. Viñoles, C.; Forsberg, M.; Martin, G.B.; Cajarville, C.; Repetto, J.; Meikle, A. Short-term nutritional supplementation of ewes in low body condition affects follicle development due to an increase in glucose and metabolic hormones. *Reproduction* **2005**, *129*, 299–309. [CrossRef]

52. Stewart, R.; Oldham, C.M. Feeding lupins for 4 days during the luteal phase can increase ovulation rate. *Proc. Aust. Soc. Anim. Prod.* **1986**, *16*, 367.

53. Muñoz-Gutiérrez, M.; Blache, D.; Martin, G.B.; Scaramuzzi, R.J. Folliculogenesis and ovarian expression of mRNA encoding aromatase in anoestrous sheep after 5 days of glucose or glucosamine infusion or supplementary lupin feeding. *Reproduction* **2002**, *124*, 721–731. [CrossRef]

54. Viñoles, G.C. Effect of Nutrition of Follicle Development and Ovulation Rate in the Ewe. Ph.D. Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, 2003.