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Effect of oral drenching of glycerin as a source of pre-mating energetic supplementation on reproductive response in goats

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Abstract

The availability of glycerol has increased because of the biofuels industry, and glycerol can have a significant effect on reproductive efficiency when used as an alternative energy source in animal feeds. The aim of this study was to investigate the effects of pre-mating oral drenching of glycerin on ovarian and fertility responses in goats. Sixty Anglonubian mixed-breed goats were submitted to estrus synchronization by CIDR-prostaglandin PGF2α treatment and mated. At CIDR removal, onset of estrus, and 24 h after estrus behavior, the animals received 150 ml of saline solution (control group, n = 20), 150 ml of glycerol (150 ml group, n = 20), or 300 ml of glycerol (300 ml group, n = 20). The administration of glycerin increased plasma glucose in the 300 ml group ($P < 0.05$) and the insulin concentration at 12 h after glycerin drenching in both treated groups. Goats from the 300 ml group showed a lower ovulation rate when compared to the control group (1.15 ± 0.08 *vs*. 0.89 ± 0.14; $P < 0.05$) but exhibited larger follicles at 48, 24, and 12 h prior to ovulation ($P < 0.05$). Administration of 300 ml of glycerol was also associated with a significant reduction in the pregnancy rate (80.00% *vs*. 38.89%; $P < 0.05$) and in pregnant animals it was associated with lower growth of embryonic vesicles (1.78 ± 0.07 mm/day *vs*. 1.31 ± 0.07 mm/day; $P < 0.05$) compared to the control treatment. Gestational losses in the 300 ml group occurred between mating and the 45th day of pregnancy. No differences were found for the reproductive parameters analyzed in the study between the 150 ml and control groups. In conclusion, the supplementation with glycerol before the mating did not appear to be a viable alternative to increase reproductive efficiency of adult does.

Keywords: glycerin, goats, ovulation rate, pregnancy rate.

Introduction

Energy balance is an essential condition in assisted reproduction technologies. In tropical arid areas in particular, the main drawback of goat husbandry continues to be how to sustain the nutritional status during the prolonged seasonal food shortage. Thus, the use of alternative nutrients is important in the composition of feeds because they help decrease production costs (Donkin, 2008). Recently, the expansion of the biofuels industry has increased the availability of glycerol, which may become an increasingly important substitute for concentrate feedstuffs for livestock. According to the Food and Drug Administration - FDA (2006), glycerol is recognized as a safe ingredient for use in animal feeds.

Several studies have shown glycerol to be a potential glucogenic feed additive that increases the proportion of propionic acid in the rumen of dairy cows (DeFrain *et al*., 2004; Linke *et al*., 2004). Recent studies indicate that glycerol can be used to replace up to 30% of corn in the dry matter of the diet without any negative effects on lamb performance or carcass characteristics (Gomes *et al*., 2011). Carvalho *et al*. (2011) indicated that glycerol is a suitable replacement for corn grain in diets for transition dairy cows. In addition, earlier studies also indicated an elevation in glucose concentration alone or together with insulin concentration (Linke *et al*., 2004) after drenching with glycerol. Insulinemia is commonly improved by the administration of propylene glycol in cows (Miyoshi *et al*., 2001) and sheep (Chiofalo *et al*., 2005) during the post-partum period.

In the ovary, insulin acts as an important mediator of follicular development, steroidogenesis, oocyte maturation, and subsequent development of the embryo (Yaseen *et al*., 2001). In bovine, it was shown that receptors are widely distributed throughout all ovarian tissues, including the granulosa and theca cells and stromal tissue (Shimizu *et al*., 2008). In ruminants, insulin is a recognized signal for mediating changes in nutrient intake and follicular dynamics. Selvaraju *et al*. (2003), after a superovulation treatment in goats observed that the ovarian response was enhanced by insulin injections. Another study reported that an enhancement of circulating insulin levels immediately before or after mating was associated with a high proportion of viable embryos in donors (Souza *et al*., 2008). Hidalgo *et al*. (2004) utilized propylene glycol to increase pregnancy rates in recipient heifers after embryo transfer. However, limited data are available in the literature regarding supplementation with glycerol prior mating, particularly in goats. Thus, the aim of this study was to evaluate the effects of orally administered glycerol on estrous, ovarian, and fertility responses in hormonally treated goats.

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Material and Methods

Animals

The study was conducted at the "Campo da Semente" experimental farm, located in Guaiuba, CE, Brazil (4°S, 38°E),under a continuous photoperiod regimen (equatorial zone) from December to August. The study was approved by the Ethics Commission for the Use of Animals of the State University of Ceará (CEUA-UECE), under Protocol nº. 10724486-1/08.

Sixty Anglonubian mixed-breed adult cyclical and pluriparous goats, with homogeneous weight (mean ± SD) and body condition (35.42 ± 5.94 kg and 2.3 ± 0.4, respectively) were chosen from a flock on the farm. All animals were maintained under similar feeding and management conditions. The goats were kept in one-pen shelters measuring 8 × 8 m, where they received mineral salt and water *ad libitum*. The pen was clayed with concrete and faced an east-west direction. The goats were submitted to 30 days of housing adaptation. During this period, internal and external parasite treatment and control of ovary function by ultrasonography were performed.

The does received a common ration composed of a mixture of chopped Bermudagrass hay and a commercial ration (14% crude protein and 74% total digestible nutrients on a dry matter basis). The diet formulation was prepared according to the requirements for maintenance and breeding (National Research Council - NRC, 2007) for adult non-dairy does. The diets were provided twice a day (07:00 and 15:00 h), from 20 days before the mating to the weaning, 60 days after parturition. After parturition the kids remained together with the does and were weaned at 60 days of age. The kidding period was 31 days long.

Estrus synchronization and mating

Estrus was induced using a vaginal pressary impregnated with 0.33 g of progesterone (Eazi-Breed CIDR®, InterAg, Hamilton, New Zealand), which was left in the cranial portion of the vagina for 5 days. Upon removal of the device (time zero), the goats received 50 µg of prostaglandin PGF2α (Lutalyse®, Upjohn, Kalamazoo, USA), and 24 h after removal of the device, they were exposed to two Anglo-Nubian bucks of proven fertility, equipped with marker pigtails, which remained with the females for 72 consecutive hours. After the does were mated, they were separated from the bucks. The onset of estrus was considered as the first observation of marker dye in the rump region of the females.

Glycerol and experimental design

At CIDR removal, onset of estrus, and 24 h after estrus behavior, the animals received 150 ml of saline solution (control group, n = 20), 150 ml of glycerol (150 ml group, n = 20), or 300 ml of glycerol (300 ml group, n = 20). Glycerol in the form of refined glycerin (99% glycerol) was used as an energetic supplementation and administrated as a drench of glycogenic solution (90% glycerol: 10% saline solution) provided 1 h after feeding. Each oral dose of glycerol was equivalent to 0.77 Mcal (150 ml group) and 1.54 Mcal (300 ml group) of metabolizable energy (Mach *et al*., 2009).

Ultrasonography

In all animals, the growth pattern of the ovarian follicles was monitored by ultrasonography from 48 h after CIDR removal for three consecutive days, twice daily. Transrectal ultrasonography was also used to determine the ovulation rate by counting the number of corpora lutea (CL) 24 h after mating. The diameter of the CL per ovary was measured. Ultrasonography analysis was conducted by real-time ultrasonography (Shenzhen Mindray Bio-medical Eletronics Co., LTDA, China, model DP-2200Vet), using a linear transrectal transducer of 5.0–10.0 MHz. The ultrasonographic images were recorded and transferred to a computer hard disk for further detailed evaluation.

Diagnosis of pregnancy by ultrasonography was performed on the 30th and 45th days of pregnancy, and embryonic/fetal structures were evaluated according to the parameters proposed by Santos *et al*. (2004): vesicle diameter (VD) and cranial-caudal length (CCL). On the 45th day of pregnancy, the biparietal diameter and abdominal diameter were determined. The thoracic diameter was determined on the 45th day of pregnancy, according to the method of Lee *et al*. (2005). For measurement of structures of interest, ultrasonographic examinations were recorded in the form of videos, followed by the capture and measurement of at least three images for each structure using the Image J program (Image J, National Institutes of Health, Millersville, USA), which had been previously calibrated. In twin pregnancy, the mean value for two embryos/fetuses was considered, following the method described by Bulnes *et al.* (1998). The measurements were used to calculate the daily rates of embryonic/fetal growth (mm/day).

According to Silva *et al*. (2011), pregnancy failure (0-20 days) was classified in the negative goats at the time of pregnancy diagnosis by ultrasound when the progesterone level at 20 days after CIDR removal was below 2 ng/ml, whereas early mortality (21-45 days) was noted when the progesterone level at 21 days after CIDR removal was higher than 2 ng/ml.

Glucose, insulin and progesterone assay

Blood samples were collected on CIDR removal (day 0) and every 6 h in each 24h period after

day 0 in heparinized tubes by jugular venipuncture for insulin determination. The blood collections were always performed in the morning, before the administration of food and glycerol. The blood was centrifuged at 3000 rpm for 15 min, and the plasma obtained was frozen at -20ºC until analysis. Concomitantly, small blood samples were collected in the syringe, and glucose levels were immediately measured using a glucose metering apparatus (Accu-Chek Active, Roche Diagnostics GmbH, Mannheim, Germany), with a measurement range of 10-600 mg/dl. Insulin determination was performed by microparticle enzyme immunoassay (MEIA; Abbott Diagnostics AxSYM® SYSTEM), using a commercial kit (Axsym Insulin; Abbott Japan Co., Ltd, Tokyo, Japan). The sensitivity of the test was1.0 µU/ml, and the intra- and interassay coefficients of variation were 3.2 and 2.3%, respectively. In addition, upon removal of the CIDR (day 0) and on days 4, 8 12, 16, and 20 after removal of the device, blood samples were collected for progesterone (P4) determination by microparticle enzyme immunoassay (MEIA; Abbott Diagnostics AxSYM® SYSTEM), using a commercial kit (Axsym P_4; Abbott Japan Co., Ltd, Tokyo, Japan). The sensitivity of the test was 0.2 ng/ml, and the intra- and interassay coefficients of variation were 7.9 and 3.3%, respectively.

Statistical analysis

All data were analyzed using the statistical program Statistica (StatSoft Inc., Tulsa, OK, USA). The effects tested by ANOVA in plasmatic concentrations of glucose, insulin, and progesterone were group (control, 150 ml, and 300 ml), interval of assessment considered (time), and the group vs. time interaction. For parameters of estrus and ovarian responses, body weight, litter size, pregnancy length, kids weaned/doe and weaned kid weight/doe, the effect tested was the group. In both models, ANOVA were performed using GLM procedures. Data for follicular size growth and embryonic and fetal measurements were analyzed using the GLM procedures for repeated measures analysis of variance (ANOVA). The effects tested were group and interval of assessment considered. The images of the structures (1, 2, and 3) were the repeated measures. For the number of marked goats, pregnancy rate, twinning rate, kidding rate, and mortality variables, the effect of group was analyzed by the Kruskal-Wallis ANOVA test. Comparisons between means were determined by the Duncan test. Comparisons between numbers were performed using the chi-squared test.

Results

The plasma levels of glucose and insulin monitored for 24 h after the first application of glycerin are shown in Fig. 1 and 2, respectively. The results showed a statistically significant increase in plasma glucose ($P < 0.05$) at 6, 12, 18, and 24 h after administration of the energy supplement in animals treated with 300 ml of glycerin (Fig. 1). From 12 h, plasma insulin values significantly increased ($P < 0.05$) in both groups treated with glycerin (Fig. 2).

The main results regarding estrus and ovarian responses after estrus synchronization treatment are shown in Table 1. No effect of treatment ($P > 0.05$) in the response to estrus was observed after hormonal synchronization treatment. The average fraction of marked animals was 96.6% (58/60). Regarding the ovarian response, the ovulation rate tended to decrease ($P < 0.05$) with increased doses of administered glycerin. The follicular growth rate and corpus luteum size were positive and similar among treatments (Table 2). The mean follicular diameter was significantly higher ($P < 0.05$) in animals that received 300 ml of glycerin 48, 24, and 12 h prior to ovulation when compared to the control group (Table 1).

The pregnancy rate was significantly ($P <0.05$) modified by the treatment, showing a marked reduction in the 300 mlgroup, in which less than 40% of the animals (7/18) were positive in the ultrasonographic assessment when compared to control (16/20) and 150 ml (14/20) groups respectively (Table 3).

In pregnant animals, treatment with 300 ml of glycerol reduced the rate of embryonic vesicle growth and the average diameter of the structure measured at 45 days ($P < 0.05$), but increased the duration of gestation when compared to the other treatments (Table 3).

Prolificacy was statistically similar among groups ($P > 0.05$), with an overall average of 1.13 ± 0.35. For the kidding rate, twinning rate, body weight of kids at birth, and weaning, no differences were observed among groups (Table 3). No differences in the incidence of multiple pregnancies and in the other parameters of ultrasonographic fetal measurements were observed.

Gestational losses resulting from failures in pregnancy from mating to 20 days after CIDR removal and from 21 days after CIDR removal to pregnancy diagnosis were distributed with a similar frequency ($P > 0.05$) in all three groups (Table 3). In Fig. 4, the respective values of plasma progesterone are shown.

Pregnant animals from all treatments showed increased progesterone levels ($P < 0.05$; Fig. 3). From the eighth day after CIDR removal, progesterone levels were higher than 2 ng/ml of plasma, indicating the presence of a gestational corpus luteum. In the 300 ml group, at day 16 after CIDR removal, the progesterone level was higher than that in other treatments (Fig. 3). In the same group, the overall mean progesterone level was higher ($P < 0.05$) than that in the 150mL group and control group (5.56 ± 0.67 ng/ml *vs*. 4.99 ± 0.36 ng/ml *vs*. 4.44 ± 0.31 ng/ml).

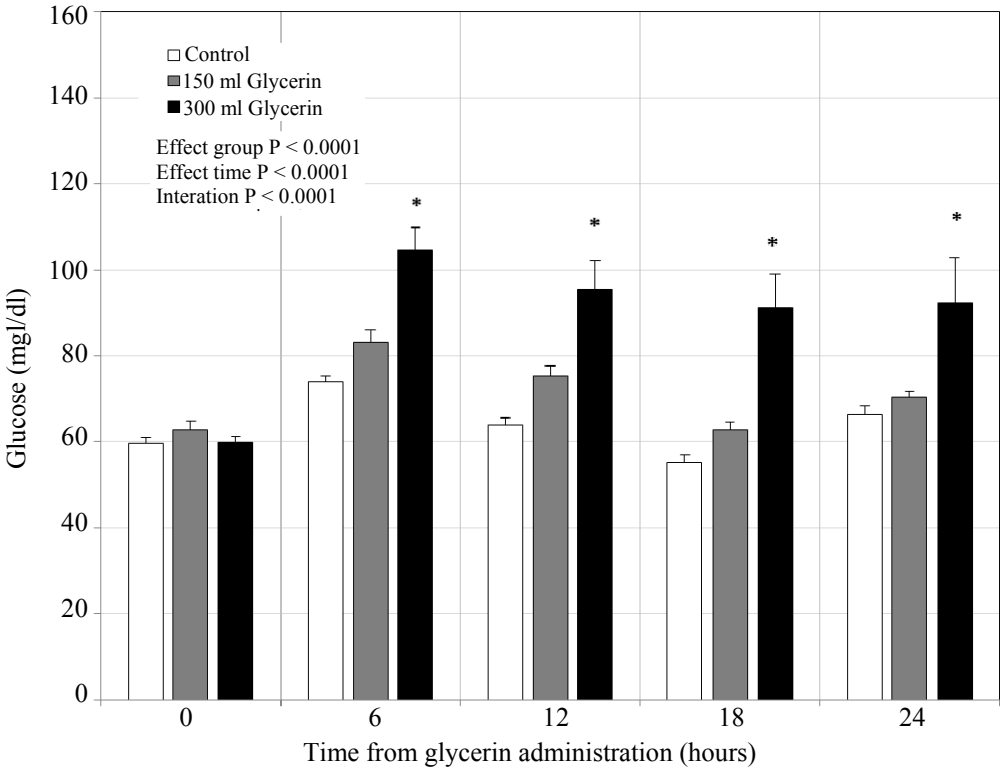

Figure 1. Glucose level measured in goats during 24 h after glycerin administration. Values are given in means ± SEM. *P < 0.05 differences from control group in each period. Statistically significant effect of Group, Time treatments and Interaction are given in the figure.

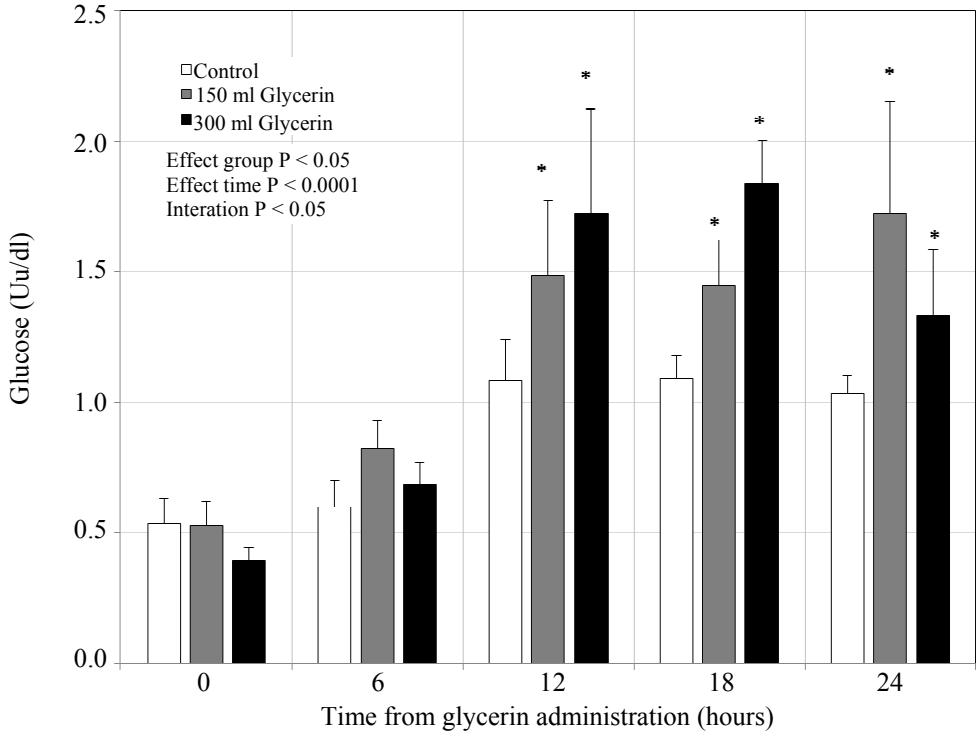

Figure 2. Insulin level measured in goats during 24 h after glycerin administration. Values are given in means ± SEM. *P < 0.05 differences from control group in each period. Statistically significant effect of Group, Time treatments and Interaction are given in the figure.

Table 1. Estrus and ovarian responses in goats treated with glycerin before mating. Values are means ± SEM.

Attributes	Groups		
	Control	150 ml	300 ml
N. of does exposed	20	20	20
Body weight (kg)	35.48 ± 1.37	35.77 ± 1.33	35.02 ± 1.34
Body condition	2.37 ± 0.09	2.31 ± 0.10	2.34 ± 0.09
Estrus response			
N. of does in estrus, % (n/n)	100.00 (20/20)	95.00 (19/20)	95.00 (19/20)
CIDR removal - onsetestrus, h	48.00 ± 2.43	48.00 ± 2.43	54.31 ± 2.81
CIDR removal – ovulation, h	77.37 ± 2.15	78.67 ± 2.27	75.43 ± 2.57
Onset estrus – ovulation, h	29.37 ± 1.94	26.67 ± 1.62	24.00 ± 1.66
Ovarian response			
N. of does ovulated, % (n/n)	100.00 (20/20)	90.00 (18/20)	77.78 (14/18)*
Ovulation rate	1.15 ± 0.08^{a}	1.00 ± 0.10^{ab}	0.89 ± 0.14^{b}
Follicular growth rate*, mm/h	0.03 ± 0.002	0.03 ± 0.001	0.03 ± 0.002
Area of corpus luteum**, mm^2	1.29 ± 0.003	1.29 ± 0.01	1.29 ± 0.002

^{a,b}comparison among groups (P < 0.05). *In this group two animals were ruled out due to a hyperglycemic crisis. *performed during 48 h before ovulation. **performed 24 h after ovulation.

Table 2. Follicular diameter (mm) in goats treated with glycerin before mating. Values are mean ± SEM.

Groups	N	Follicular diameter (number of animals / number of follicles analyzed)			
		48 h*	36 h*	24 h*	12 h*
Control	20	3.20 ± 0.06 (1/1)^{a,A}	3.70 ± 0.03 (3/4)^{B}	4.04 ± 0.01 (15/18)^{a,C}	4.38 ± 0.01 (19/22)^{a,D}
150 ml	18	-	3.80 ± 0.20 (6/6)^{A}	4.09 ± 0.01 (13/13)^{a,b,B}	4.40 ± 0.01 (18/20)^{a,b,C}
300 ml	14	3.45 ± 0.06 (1/1)^{b,A}	3.89 ± 0.06 (1/1)^{B}	4.12 ± 0.02 (9/10)^{b,C}	4.47 ± 0.01 (14/16)^{b,D}

*Hours before ovulation. ^{a,b}Different letters in the same column indicate significant differences (P < 0.05). ^{A,B,C,D}Different letters between columns indicate significant differences (P < 0.05).

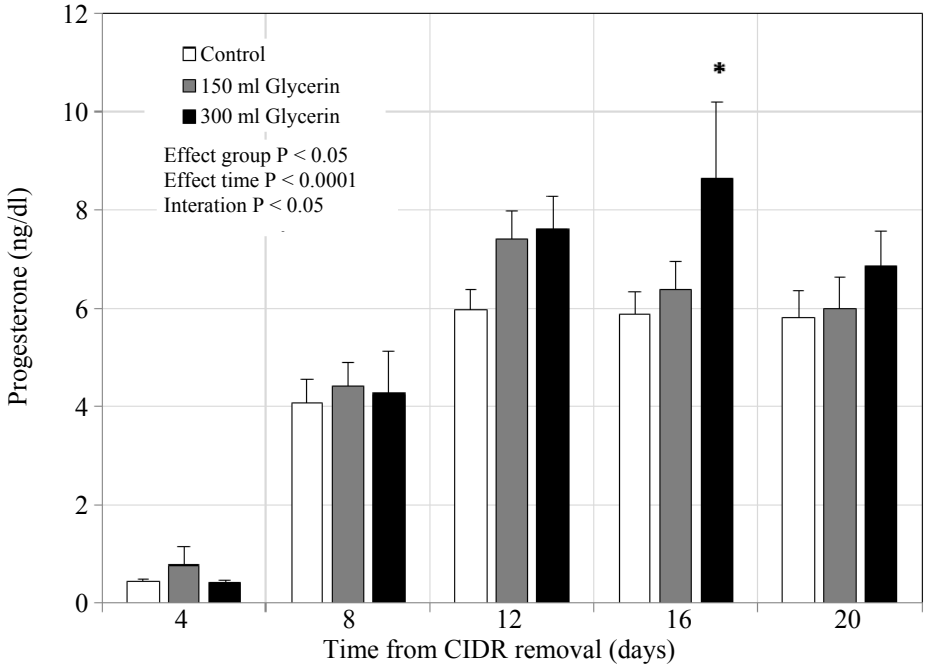

Figure 3. Plasma P4 concentrations in pregnant goats up to day 20 after CIDR removal. Values are given in means ± SEM. *P < 0.05 differences from control group in each period. Statistically significant effect of Group, Time treatments and Interaction are given in the figure.

Table 3. Reproductive response in goats treated with glycerin before mating. Values are means ± SEM.

Attributes	Groups		
	Control	150 mll	300 ml
Pregnancy rate, % (n/n)**	80.00 (16/20)[a]	70.00 (14/20)[a]	38.89 (7/18)[b,*]
Twinning rate, % (n/n)	25.00 (4/16)	7.14 (1/14)	14.28 (1/7)
Ultrasonographic fetometry			
Diameter of embryonic vesicle, mm			
30 days of gestation	27.45 ± 0.67	29.44 ± 0.80	29.47 ± 0.71
45 days of gestation	54.11 ± 0.99[a]	52.96 ± 0.73[a]	49.15 ± 0.56[b]
Growth rate, mm/d	1.78 ± 0.07[a]	1.57 ± 0.07[a]	1.31 ± 0.07[b]
Crown-rump length, mm			
30 days of gestation	9.78 ± 0.20	10.16 ± 0.27	9.97 ± 0.35
45 days of gestation	33.65 ± 0.70	32.79 ± 0.41	34.35 ± 0.77
Growth rate, mm/d	1.59 ± 0.04	1.51 ± 0.03	1.62 ± 0.04
Biparietal diameter 45 days, mm	10.13 ± 0.13	10.82 ±0.13	10.39 ± 0.18
Diameter of thorax 45 days, mm	8.14 ± 0.13	8.09 ± 0.14	7.54 ± 2.57
Diameter of abdomen 45 days, mm	11.19 ± 0.18	10.72 ± 0.11	10.95 ± 0.26
Pregnancy length, days	147.17 ± 0.66[a]	147.82 ± 0.44[a]	149.71 ± 0.68[b]
Doe body weight			
Parturition, kg	36.37 ± 1.61	34.54 ± 1.67	37.60 ± 2.91
Weaning, kg	36.60 ± 1.86	33.04 ± 1.54	36.17 ± 2.59
Kidding rate, % (n/n)	75.00 (12/16)	78.57 (11/14)	100.00 (7/7)
Litter size	1.33 ± 0.14	1.09 ± 0.09	1.14 ± 0.14
Twinning rate, % (n/n)	33.33% (4/12)	9.1% (1/11)	14.28% (1/7)
Kids body weight			
Birth, kg	3.01 ± 0.04	2.99 ± 0.09	3.06 ± 0.09
Weaning, kg	8.72 ± 0.48	8.90 ± 0.67	9.46 ± 0.93
Kids weaned/doe	0.92 ± 0.19	1.09 ± 0.09	1.14 ± 0.14
Kids weight weaned/doe, kg	10.66 ± 1.55	8.90 ± 0.67	10.81 ± 1.93
Pregnancy failure***, % (n/n)	12.50 (1/8)	22.22 (2/9)	45.45 (5/11)
Early mortality****, % (n/n)	37.50 (3/8)	44.44 (4/9)	54.54 (6/11)

[a,b]comparison among groups (P < 0.05). * In this group two animals were ruled out due to a hyperglycemic crisis. **Performed at 45 days of gestation. ***Gestation failure from mating to days 20 after CIDR removal. ****Mortality from days 21 after CIDR removal to pregnancy diagnosis.

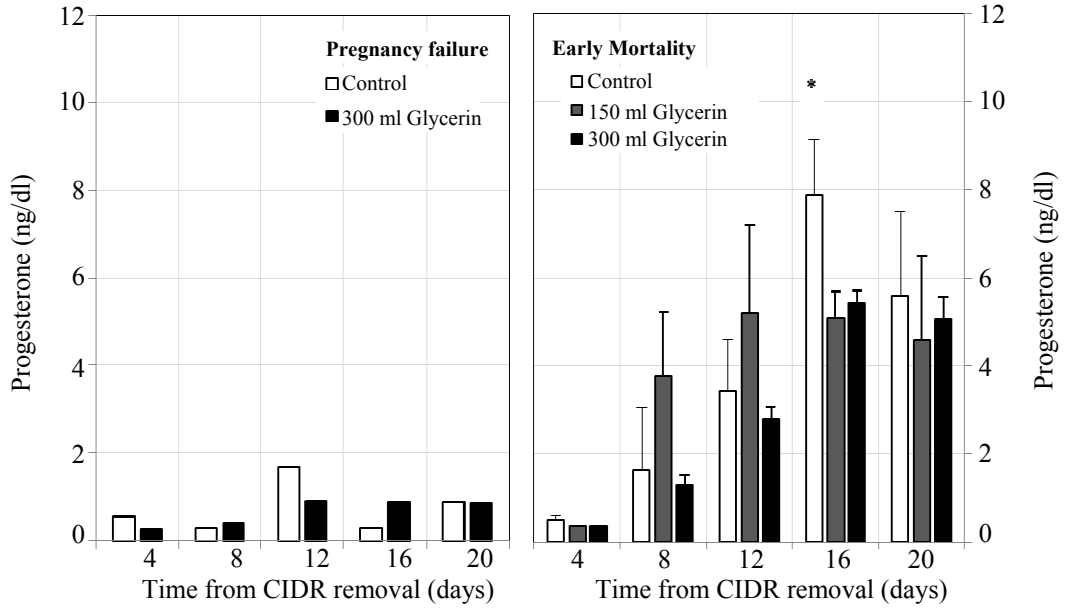

Figure 4. Plasma P4 concentrations in goats negative for diagnosis of pregnancy up to day 20 after CIDR removal. Values are given in means ± SEM. *P < 0.05 differences from control group in each period.

Discussion

In the present study, administration of glycerol represented an extra energetic supply in the nutritional plane of breeding goats (NRC, 2007) and was used to promote a short nutritional stimulus during follicular development. This nutritional stimulus resulted in an increase in insulin levels but did not enhance the ovulation rate. The effect of the 150 ml dosage was similar to that observed in the control group, and the 300 ml dosage was associated with a decrease in pregnancy rate. Thus, supplementation with 150 ml glycerol did not stimulate follicle growth in this short period of time, whereas supplementation with 300 ml glycerol seemed to have been excessive, suggesting that neither concentration tested was adequate for improving the reproductive parameters in goats.

Based on our findings, supplementation with 150 ml glycerol promotes a significant increase in insulin levels, but does not stimulate an increase in plasma glucose. We believe that the explanation for these results involves several molecular mechanisms. However, the signal transduction pathway between the activation of insulin receptors and glucose transport has not been elucidated to date at the molecular level in ruminants. However, supplementation with 300 ml glycerol promotes a marked increase in the plasma levels of glucose and insulin. Similar results were reported by Gomes *et al.* (2011), who also observed an increase in blood levels of glucose and insulin in sheep after supplementation with 15% or 30% glycerin. These authors believe that this effect was the result of increased ruminal production of propionic acid from fermentation of glycerol in the rumen. In another study, Pethick *et al*. (2000) reported that a mixture of 3.5% glycerol and 1.5% propylene glycol caused hyperglycemia in sheep, with a consequent increase in plasma insulin concentrations. According to Nielsen and Ingvartsen (2004), glycerol can be absorbed in the rumen and small intestine, and is an excellent precursor of glucose in gluconeogenic pathways in the liver. In addition, some studies have indicated that the administration of glycerol as a drench seems to be more effective than administration in the diet (Goff and Horst, 2001; Linke *et al*., 2004) or drinking water (Osborne *et al*., 2009) for stimulating glucose levels in the blood. However, to date, the molecular events of the mechanism of insulin action and the characteristics of glucose transport system in ruminants have not been well established.

After treatment with progestin, at least 95% of the animals came into estrus, demonstrating that supplementation with glycerol did not affect this parameter. These results are in agreement with those of Motlomelo *et al*. (2002), and higher than the levels reported by Lehloenya and Greyling (2010) in Boer goats (71.4%). We consider that this high response rate to estrous synchronization protocol is related to the homogeneous and adequate body condition of the experimental animals. According to Rondina and Galeati (2010), body condition in goats is a major factor that influences the response to hormonal treatment. However, the mechanisms by which energy reserves control the reproductive function of goats are still unclear.

In this work, we observed that the elevation of insulin derived from the glycerol drench stimulated follicular development but was not sufficient to promote an increase in ovulation rate. These results are in agreement with the findings of Haruna *et al*. (2009), who after evaluating the effect of nutritional supplementation for a short period in goats (7 days) did not observe an effect on ovulation rate. In contrast, Gutierrez *et al*. (2011) reported that nutritional supplementation for short periods can stimulate follicular development, which is known as an "acute effect of nutrition." In addition, an increase in energy levels results in an increase in serum levels of insulin, which in turn stimulates the functionality of the ovary (Miyoshi *et al*., 2001).

In the present study, supplementation with 300 ml of glycerol was associated with a reduction in the pregnancy rate of goats. This result may reflect subnormal development of the tertiary follicles that resulted in poor quality oocytes. This hypothesis is supported by other studies suggesting the supply of an inadequate feed plane as the main cause of deficient oocyte quality (Borowczyk *et al*., 2006). For example, Lozano *et al*. (2003) demonstrated that the supply of high concentrations of dietary energy (*ad libitum*) reduced the efficiency of a superovulation treatment, obtaining a low rate of ovulation and poor embryo development. In the same study, a lower number of follicles and a higher number of unfertilized oocytes were also observed in sheep that received a low-energy diet (0.5 ×energy requirements for maintenance). These results indicate that the response to acute changes in nutrition during the pre-mating period can change the pattern of follicular and oocyte development, with a consequent effect on embryonic development and pregnancy rate.

Plasma levels of progesterone measured during early embryonic development were similar among groups, demonstrating that the glycerol drench did not affect the quality of the corpus luteum. However, in pregnant animals supplemented with 300 ml of glycerol, there was an increase in plasma progesterone levels compared to other treatments that was associated with a reduction in the growth of embryonic vesicles. These results demonstrate that supplementation with 300 ml of glycerol resulted in high insulin levels, which may have promoted an overstimulation of the insulin/IGF1 axis, resulting in a micro-environment unfavorable to embryo development and subsequent pregnancy in goats. Similar results have been reported in previous studies in which high concentrations of insulin resulted in

detrimental effects on oocyte developmental competence, reflected as a lower rate of blastocyst generation (Adamiak *et al*., 2005). According to Garnsworthy *et al.* (2009), exacerbated insulinemia can overstimulate the IGF system and may adversely affect the pregnancy rate. In another study, Adamiak *et al*. (2006) observed in cattle that changes in circulating levels of insulin and IGF-1 induced by dietary modifications promoted an increase in follicular recruitment but also had negative effects on oocyte quality before and after fertilization.

The use of glycerol as a drench did not cause any increase in parameters related to economic viability, such as prolificacy or birth and weaning weights. These results are inconsistent with those of other studies that used supplementation with glycerol as an alternative method to alleviate the symptoms of negative energy balance and to improve the fertility of dairy cows (Lomander *et al*., 2012) and sheep (Gutierrez *et al*., 2011). This inconsistency among different studies may be related to the use of different sources of gluconeogenic substances, durations of supplementation, or species studied.

In conclusion, the use of increased doses of glycerol as an energy supplement before the mating period showed no positive effects on the reproductive performance of goats. Furthermore, supplementation with 300 ml of glycerol significantly altered the ovarian response and was associated with a decrease in pregnancy rate. These findings are contradictory to the idea that increasing insulin levels during the phase of follicular growth improves the ovulation rate. Possibly, these discrepancies occurred due to the dosage of glycerol used in this study, which promoted an excessive increase in the insulin levels (hyperinsulinaemia), which influenced negatively the quality of oocytes and future embryonic development. Thus, on the basis of the conditions of our experiments, the supplementation with glycerol before mating did not appear to be a viable alternative to increase reproductive efficiency of adult does. However, further studies are needed to test the use of glycerol supplementation in distinct dosages, schedules and for brief or prolonged supplementation periods.

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