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Gene expression and embryo quality in superovulated goats supplemented with crude glycerin after mating

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ABSTRACT

This study aimed to assess the effects of dietary supplementation with crude glycerin on embryo quality and expression profile of genes from superovulated goats. Twenty-four hours (1 day) and 72 h (3 days) after mating animals received an oral drench of 200 mL saline solution (Control group), 100 mL glycerol (100 mL group) or 200 mL glycerol (200 mL group). Both insulin and glucose levels were measured after glycerol drenching. The relative expression of genes (Glut-1, IGF-1, Hsp70, Sod and Bax) in grade I and II embryos from groups was analyzed by sensitive TaqMan qPCR. Administration of crude glycerin raised insulin and glucose ($p < 0.05$) plasma concentrations. Similar rates of superovulatory response (94.12%; 16/17) and recovery of transferable embryos (N embryo grades 1, 2 and 3/N ovulation), ($44.69 \pm 6.83\%$; 100/224) were obtained. The 200 mL group showed a greater proportion of transferable embryos compared to the degenerated embryos (8.83 ± 2.30 vs. 1.17 ± 0.83, $p < 0.05$), that achieved values 2-fold higher than control group (4.80 ± 2.30 vs. 0.20 ± 0.20, $p < 0.05$) and 100 mL group (3.83 ± 1.25 vs. 1.17 ± 0.48, $p > 0.05$). A higher relative abundance of IGF-1 transcripts was observed in 200 mL group. Also none differences were observed for transcripts of apoptotic (Bax) and stress (Hsp70) genes. In conclusion, enhancement of circulating glucose and insulin generated from drenching crude glycerin after mating was associated with higher IGF-I expression and increased proportion of transferable embryos.

1. Introduction

Glycerin is a co-product of biodiesel from the reaction of vegetable or animal fat with methanol (Shin et al., 2012). It is estimated that each liter of biodiesel produced generates about 100 mL crude glycerin (Dasari et al., 2005). As glycerin production increases, glycerin supply may exceed the demand for food and personal care products, such that it may become an increasingly important substitute for concentrate feedstuffs for livestock (Shin et al., 2012). Previous studies have shown that crude (DeFrain et al., 2004) and pure glycerin (Carvalho et al., 2011) have been consumed successfully at 5 to 15% of dietary dry matter by lactating dairy cows. In addition, studies have shown glycerin to be a potential glucogenic feed additive, increasing the proportion of propionic acid in the rumen of dairy cows (Wilbert

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et al., 2013). Elevation of glucose concentration alone (Goff and Horst, 2001), or together with insulin concentration (Linke et al., 2004), have been reported after drenching large amounts of glycerol via an esophageal pump (Goff and Horst, 2001) or orally (Linke et al., 2004). However, to our knowledge, there is no study regarding the effects of the dietary supplementation with crude glycerin on in vivo embryo production, embryo quality and gene expression in goats.

Currently, is been established that energy uptake has a significant impact on early embryonic development (Boland et al., 2001) and that relative abundance of several mRNA transcripts can predict the developmental potential of embryos in vivo (Balasubramanian et al., 2007). This implies that specific dietary components should change relative abundance of specific genes involved with metabolism of glucose (glucose transporter – 1, Glut-1), development (insulin-like growth factor-1, IGF-1), stress response (heat shock proteins, Hsp70 and superoxide dismutase manganese, MnSod) and apoptosis (Bax), during early developmental embryo. Thus, the aims of this work were to study the effects of supplementation with crude glycerin on embryo production and quality, and expression profile of genes related to embryonic metabolism, development, oxidative stress and apoptosis in embryos from superovulated goats.

2. Materials and methods

2.1. 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), carried out in October and November, during the dry season. The study was approved by the Ethics Committee for experimental animal use of the State University of Ceará, under protocol no. 10724486-1/08.

Sixteen mixed-breed cyclical and pluriparous does with homogeneous (mean ± SD) body weights, body condition scores and ages (40.2 ± 6.1 kg, 2.8 ± 0.3 and 32.6 ± 4.7 months of age, respectively) were selected from a herd of 40 females. All animals were maintained under similar feeding and management conditions. Goats were submitted to 30 days of housing adaptation. At the beginning of this period, ecto and endoparasite treatments were performed. The control of ovary function and cyclicity was done by regular ultrasonographic examinations during the adaptation period.

The goats received a common diet composed of mixture of chopped Bermudagrass hay and pelleted commercial ration (15% Crude Protein and 67% Total Digestible Nutrients on Dry Matter basis). The diet formulation was based according to 1.5 times the energy requirements for maintenance of live weight (NRC, 2007) for adult non-dairy does and presented the same concentrate:roughage ratio (60:40). The diets were provided twice a day (07:00 and 15:00 h).

2.2. Superovulation protocol

Goats were synchronized using a 60-mg Medroxyprogesterone Acetate (MPA, Progespon®, Syntex, Argentina) vaginal sponge for 11 days, with 50 μg $PGF_{2\alpha}$ (Prolise®, Tecnopec, Argentina) given 48 h prior to sponge removal. For superovulation, a total of 200 mg pFSH (Folltropin®, Vetrepharm, Canada) was administered i.m. in six doses, given twice daily at 12-h intervals (40/40, 40/40 and 20/20 mg), from Day 9 to Day 11, when the sponges were removed. Thirty-six hours before sponge removal, 100 mg GnRH (Gestran-plus®, Licirelin, Argentina) was administered i.m. Estrous detection was conducted by teasing with a male every 4 h for 1 h, from 4 up to 72 h after sponge removal. Females in estrus were subjected to controlled mating using Anglo-Nubian fertile mature bucks.

2.3. Crude glycerin and experimental design

Crude glycerin used in this study was obtained from a biodiesel facility (Petrobras Biocombustível S.A., Quixadá, Ceará, Brazil), which contained 80.0% glycerol, 13.0% water, 7.0% salt, and 0.1% methanol. Glycerol, in the form of crude glycerin was used as an energetic supplementation. Animals received a drench of 200 mL saline solution (Control group, $n = 5$), or 100 mL (100 mL group, $n = 6$) or 200 mL (200 mL group, $n = 6$) glycerol, administrated as a drench of crude glycerin in saline solution (9:1), 1 h after feeding, on Day 1 (24 h) and on Day 3 (72 h) after mating. Each oral dose of crude glycerin was equivalent to 0.51 Mcal (100 mL group) e 1.03 Mcal (200 mL group) of metabolizable energy (Mach et al., 2009).

2.4. Surgical embryo recovery

Embryos were collected surgically on Day 7 after mating in accordance to Baril et al. (1995), with some modifications. All females were deprived of food and water for 24 h before embryo recovery. Goats were sedated by an i.v. combination of 0.1 mg/kg atropine sulfate at 1% (UCB, Jaboticabal, Brazil) and 2% xylazine hydrochloride (Coopazine; Coopers, São Paulo, Brazil). In addition, immediately prior to surgery, all animals were anesthetized with an injection of 4 mL of 2% lidocaine solution (Pearson, São Paulo, Brazil) into the epidural space at the lumbosacral junction. A medio-ventral incision was made, and the reproductive tract was exposed. The number of corpora lutea was verified, and embryo recovery was performed by flushing each uterine horn with a catheter inserted in the infundibulum and connected to a syringe containing 20 mL of flushing medium (DMPBS Flush; Nutricell, São Paulo, Brazil). Embryos were recovered by flushing each uterine horn with 20 mL flushing medium. Recovered structures were morphologically classified according to the developmental stage and four quality grades: grades I (excellent), II (fair), III (poor), and IV (dead/degenerated), or unfertilized (Ova with no signs of cleavage), based on Leibfried-Rutledge and Firts (1979), as adapted to the goat. Only grades I and II embryos at different developmental stages (compact morula, early blastocyst and expanded blastocyst) were snap-frozen and stored at −80 °C, pending