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Effect of prostaglandins E_2 and $F_{2\alpha}$ on in vitro development and hatching of caprine blastocysts

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Abstract

Prostaglandin E_2 has been shown to increase the ovine embryo hatching rate, and $PGF_{2\alpha}$ to reduce the development of rabbit, bovine, and rat embryos. The objective was to determine the effects of PGE_2 and $PGF_{2\alpha}$ on development of caprine embryos. Estrus was synchronized in does ($n = 25$) with medroxyprogesterone acetate (MAP) intravaginal sponges for 12 days, and superovulated with 20 units of FSH. On day 6 following estrus, embryos were flushed ($n = 128$) and incubated individually per well in 25 μl droplets of TCM-199 and BSA (8 mg/ml) for 6 days at 38.5 °C in a 5% CO_2: air with one of the following treatments: (1) control (0.0002% EtOH), (2) PGE_2 (7 ng/ml), (3) $PGF_{2\alpha}$ (7 ng/ml), (4) low PGE_2:high $PGF_{2\alpha}$ (3.5 ng/ml:14 ng/ml), (5) balanced PGE_2:$PGF_{2\alpha}$ (7 ng/ml:7 ng/ml), or (6) high PGE_2:low $PGF_{2\alpha}$ (14 ng/ml:3.5 ng/ml). Treatment with PGE_2 alone reduced ($P < 0.05$) the hatching rate (1/15; 7%). The hatching rate of embryos treated with $PGF_{2\alpha}$ alone (9/18; 50%), low PGE_2:high $PGF_{2\alpha}$ (8/16; 50%), and balanced PGE_2:$PGF_{2\alpha}$ (11/16; 69%) were similar to control (6/18; 33%). In contrast, the hatching rate was non-significantly increased (13/18; 72%) with the high PGE_2:low $PGF_{2\alpha}$ treatment. None of the treatments affected development from the morula to blastocyst stage. From the current data, it can be concluded that PGE_2 alone reduced hatching rate, and $PGF_{2\alpha}$ alone had no effect on the development of caprine embryos. High concentrations of PGE_2 with $PGF_{2\alpha}$ improved the hatching rates. Thus, uterine concentrations of PGE_2 may need to reach a threshold level to improve embryo hatching, as previously reported, while increased uterine concentrations of $PGF_{2\alpha}$ during early pregnancy would not affect development of the embryo.

Keywords: Embryo; Goat; Prostaglandin; Early pregnancy; Blastocyst; Morula

1. Introduction

The ratio of prostaglandins (PG) E_2 and $F_{2\alpha}$ has been implicated in the development and success of early pregnancy in several species (Smith et al., 1991; Rosenkrans et al., 1992; Asselin and Fortier, 2000). Recent studies with Cox-2- and PGE receptor type 2 (EP_2)-deficient mice indicated problems at ovulation, fertilization, embryonic development, and implantation (Lim et al., 1997; Matsumoto et al., 2001). However, no information is available on the role of prostaglandins in the goat during early pregnancy.

Prostaglandin production has been reported from the cumulus–oocyte complex stage (COC; Schuetz et al., 1992; Gurevich et al., 1993; Viggiano et al., 1995), 4-8-cell stage embryo (Sayre and Lewis, 1993), through to the elongated conceptus stage (Lewis and Waterman, 1985). Ovine embryos metabolize arachidonic acid to PGE_2 by day 4 of pregnancy, which continue beyond day 14, while $PGF_{2\alpha}$ production begins at day 10 (Sayre and Lewis, 1993). The cleavage rate of bovine embryos has been shown to be lower in groups with reduced

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concentrations of PGE_2 secreted into the culture media during oocyte maturation (Gurevich et al., 1993), and the hatching rate of ovine embryos increased with treatment of PGE_2 (Sayre and Lewis, 1993). Inhibitors of prostaglandin production reduced the fertilization rate in mice (Viggiano et al., 1995) and hatching rate in various species (Biggers et al., 1978; Baskar et al., 1981; Chida et al., 1986; Sayre and Lewis, 1993).

Endometrial concentrations of $PGF_{2\alpha}$ can be increased by various stressors and when prematurely increased were associated with embryonic deaths in the cow (Schrick et al., 1993). Likewise, $PGF_{2\alpha}$ reduces blastocyst formation and the hatching rate in bovine (Fazio et al., 1997; Scenna et al., 2004), rabbit (Maurer and Beier, 1976), and rat (Buuck et al., 1997) embryos. In contrast, Soto et al. (2003) reported $PGF_{2\alpha}$ to inhibit embryonic development only if treatment was during oocyte maturation or fertilization, but it did not affect the development if treatment was at stages after fertilization.

The ratio of PGE_2 to $PGF_{2\alpha}$ during early pregnancy may be involved in the maintenance of pregnancy. Evidence in humans indicate that follicular ratio of PGE_2 to $PGF_{2\alpha}$ was associated with the pregnancy rate after IVF with increased concentrations of PGE_2 to $PGF_{2\alpha}$ being associated with a greater rate of pregnancy (Smith et al., 1991). During the period of signaling to the mother to maintain the pregnancy, uterine concentrations of PGE_2 were increased and the typical peak in concentration of $PGF_{2\alpha}$ for luteolysis was reduced. Endometrial production of PGE_2 in the ewe was increased with embryonic production of interferon-τ (IFNτ), and the enzyme involved in conversion of PGE_2 to $PGF_{2\alpha}$, 9-keto-PGE reductase, was reduced with IFNτ (Asselin and Fortier, 2000). Both of which leads to an overall increase in uterine concentration of PGE_2 and an increased PGE_2:$PGF_{2\alpha}$ ratio. Likewise, estradiol production increased the uterine PGE_2:$PGF_{2\alpha}$ ratio during establishment of pregnancy in the pig (Rosenkrans et al., 1992). The objective of this experiment was to determine the effects of PGE_2 and $PGF_{2\alpha}$ and the PGE_2:$PGF_{2\alpha}$ ratio on blastocoele formation and in vitro hatching of caprine blastocysts.

2. Materials and methods

The Virginia State University (VSU) animal ethics committee approved all animal procedures. Estrus was synchronized in young, cyclic crossbred does ($n = 25$) with intravaginal sponges impregnated with medroxyprogesterone acetate (MPA; 60 mg) (Sigma Chemical, St. Louis, MO) inserted for 12 days and two 7.5 mg doses of $PGF_{2\alpha}$ (Lutalyse; Pfizer Animal Health, Kalamazoo, MI) given 4 h apart on the day of sponge removal (Wade and Lewis, 1996; Wulster-Radcliffe et al., 1999; Cline et al., 2001; Stellflug et al., 2001). Does were superovulated with 20 IU FSH (Sioux Biochemical, Sioux City, IA) administered in a series of five injections; the doses being 5, 5, 5, 2.5, and 2.5 units given i.m. at −36, −24, −12, 0, and 12 h relative to sponge removal, respectively (Rexroad and Powell, 1991). When in estrus, all does were mated to a minimum of two fertile bucks to ensure fertilization. The VSU goat breeding program uses an accelerated program for the production of three kiddings in 2 years. The animals selected from the breeding herd were 7 to 9 months of age. Because of this accelerated system, it was necessary to perform this trial during the early anestrous period (March to June). Data on doe responses to estrous synchronization and superovulation procedures were recorded to determine any effects on ovarian and embryo responses.

On day 6 following the onset of estrus, does were sedated with xylazine (0.2 mg/kg BW; Fort Dodge Animal Health, Fort Dodge, IA). An epidural anesthesia was induced (5 ml of 2% lidocaine; Phoenix, St. Joseph, MO), and does were ovario-hysterectomized via midventral laparotomy. The number of CL were immediately counted, whereafter ovaries and the uteri were transported to the laboratory for the collection of embryos within approximately 1 h. The uteri were trimmed of excess tissue, and embryos were flushed from each horn with 10 ml of TCM-199 media. Of the embryos ($n = 128$) collected, 20 embryos were discarded because of poor quality, and the remainder was incubated with one of the treatments as indicated in Table 1. The embryos discarded were at a stage of development (2-, 4-, or 8-cell) that was not consistent with the day of pregnancy. Embryos were collected from 5 does/week (replicates = 5) with treatments randomized within a replicate to ensure all treatments were represented within each

Table 1
Concentrations of prostaglandins added to culture media for caprine embryo incubation

Treatment	Concentration of PGE_2 (ng/ml)	Concentration of $PGF_{2\alpha}$ (ng/ml)
Control	0.0[a]	0.0[a]
PGE_2	7.0	0.0
$PGF_{2\alpha}$	0.0	7.0
Low PGE_2:$PGF_{2\alpha}$ ratio	3.5	14.0
Balanced PGE_2:$PGF_{2\alpha}$ ratio	7.0	7.0
High PGE_2:$PGF_{2\alpha}$ ratio	14.0	3.5

[a] An equal volume of diluent was added to produce droplets of the same volume.