ELSEVIER

Animal Reproduction Science 102 (2007) 66–75

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

Estradiol production by preimplantation blastocysts and increased serum progesterone following estradiol treatment in llamas

Susan A. Powell [a], Bradford B. Smith [b], Karen I. Timm [b], Alfred R. Menino Jr. [a,*]

[a] *Department of Animal Sciences, Oregon State University, Corvallis, OR 97331-6702, USA*
[b] *College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331-6702, USA*

Received 24 August 2006; accepted 3 October 2006
Available online 10 October 2006

Abstract

Estradiol is a potential candidate for the blastocyst signal responsible for maternal recognition of pregnancy in the llama (*Lama glama*). Two experiments were conducted to determine if the llama blastocyst produces estradiol during the presumed period of maternal recognition of pregnancy and if exogenous estradiol can extend the luteal phase. In Experiment 1, llamas were superovulated with eCG and mated 7 days later (Day 0 = day of mating). Blastocysts were collected nonsurgically on Days 7, 9, or 11 or at necropsy on Days 13 and 15 post-mating and cultured for 48 h. Conditioned medium was recovered, replaced with fresh medium at 24-h intervals, and assayed for estradiol-17β. Estradiol production (pg/blastocyst) over the 48-h culture increased ($P < 0.05$) by day of gestation where more estradiol ($P < 0.05$) was produced by Day 11 compared to Day 7 blastocysts, Day 13 compared to Days 7–11 blastocysts, and Day 15 compared to Days 7–13 blastocysts. A dramatic increase was observed between Days 11 and 13 when estradiol production by Day 13 blastocysts increased ($P < 0.05$) more than 50-fold. In Experiment 2, 30 females were induced to ovulate with hCG (Day 0 = day of hCG injection). Starting on Day 7 and continuing through Day 15, animals received daily injections i.m. of 0 ($n = 11$), 5 ($n = 7$), or 10 mg ($n = 12$) estradiol benzoate (EB) dissolved in isopropylmyristate. Sera were collected immediately prior to each injection and on Days 16, 17, 18, 20, and 22 and analyzed for progesterone. Progesterone concentrations were greater ($P < 0.05$) on Days 14, 15, 16, and 17 in llamas treated with 10 mg EB compared to llamas treated with 0 mg EB. These results demonstrate that llama blastocysts produce estradiol and exogenous estradiol can enhance and transiently extend luteal progesterone production. Estradiol produced by the

* Corresponding author. Tel.: +541 737 3011; fax: +541 737 4174.
E-mail address: alfred.r.menino@oregonstate.edu (A.R. Menino Jr.).

0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2006.10.002

preimplantation llama blastocyst may play a role in maternal recognition of pregnancy and early luteal support.

Keywords: Camelids; Lama glama; Corpus luteum; Embryo; Conceptus; Pregnancy

1. Introduction

The blastocyst signal that initiates maternal recognition of pregnancy in the llama (*Lama glama*) is not known. Llamas are induced ovulators with ovulation occurring 1.8–2 days after mating (Bravo and Sumar, 1989; Bravo et al., 1990; Adams et al., 1991). Serum progesterone concentrations ≥1 ng/ml are detectable by Days 4–5 (Day 0 = day of mating) and, in sterile-mated females, the corpus luteum (CL) starts to regress by Days 11–12 (Adam et al., 1989) with receptive behavior returning as early as Days 13–14 (Fernandez-Baca et al., 1970). Successive surges of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ are evident on Days 10–12 (Sumar et al., 1988). In pregnant females, a transient drop below 2 ng/ml is observed from Days 8 to 10 but plasma progesterone concentrations remain ≥2 ng/ml past Day 10 (Adams et al., 1991). Maternal recognition of pregnancy would likely occur from Days 9 to 13 in llamas to prevent destruction of the CL by prostaglandins. Ruminant blastocysts produce interferon-Tau which suppresses estrogen and oxytocin receptor gene expression in the uterine epithelium and prevents uterine release of luteolytic pulses of prostaglandins (Bazer et al., 1996). However, Leaman and Roberts (1992) did not find the interferon-Tau gene in the llama. Another possible candidate for the maternal recognition of pregnancy signal in llamas is estradiol. In pigs, maternal recognition of pregnancy occurs between Days 11 and 12 of gestation when the blastocyst starts producing estrogen and changes from a spherical to filamentous shape (Bazer and Thatcher, 1977; Bazer, 1992). Furthermore, administration of estrogens between Days 11 and 15 of the pig estrous cycle prolongs luteal lifespan significantly (Geisert et al., 1982b).

Estradiol is also involved in the equidistant spacing of blastocysts in the pig uterus. Increased synthesis of estradiol by pig blastocysts occurs concomitantly with blastocyst migration and increased myometrial activity in vitro (Pope et al., 1982b). Horse blastocysts migrate between uterine horns 12–14 times per day during Days 12–18 of pregnancy presumably inhibiting endometrial $PGF_{2\alpha}$ secretion (Ginther, 1984). This period of uterine migration coincides with increased blastocyst estradiol production during Days 8–20 of pregnancy (Ginther, 1984). In llamas, 95–98% of the conceptuses are found in the left uterine horn despite a CL on the right ovary for 50% of these pregnancies (Fernandez-Baca et al., 1970, 1979; Bravo and Varela, 1993). Therefore, a blastocyst conceived in the right oviduct must migrate to the left uterine horn prior to implantation. Skidmore et al. (1994) observed aromatase activity and considerable quantities of estrogen synthesized in the camel blastocyst between Days 10 and 33 after ovulation. The greatest proportion of estrogen produced by camel blastocysts was in the form of estradiol in contrast to the greater estrone:estradiol ratio observed in pig (Perry et al., 1973) and horse blastocysts (Heap et al., 1982). It is not known if llama blastocysts produce estradiol in appreciable quantities during the period of proposed maternal recognition of pregnancy. Therefore, the objectives of this study were two-fold: (1) to determine if the llama blastocyst produces estradiol during the presumed period of maternal recognition of pregnancy and (2) if exogenous estradiol can extend the luteal phase.