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Animal Reproduction Science 99 (2007) 342–353

ANIMAL REPRODUCTION SCIENCE

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In vitro production of llama (*Lama glama*) embryos by intracytoplasmic sperm injection: Effect of chemical activation treatments and culture conditions

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Received 25 February 2005; accepted 29 May 2006

Available online 18 July 2006

Abstract

Assisted reproductive technologies in the llama (*Lama glama*) are needed to provide alternative methods for the propagation, selection and genetic improvement; however, recovery of adequate quantity and quality of spermatozoa for conventional IVF is problematic. Therefore, an effort was made to adapt the intracytoplasmic sperm injection (ICSI) procedure for the *in vitro* production of llama embryos. The specific objectives of this study were: (1) to determine *in vitro* maturation rates of oocytes recovered by transvaginal ultrasound-guided oocyte aspiration (TUGA) or flank laparotomy; (2) to evaluate the effects of activation treatments following ICSI; (3) to evaluate the development of llama ICSI embryos in CR1aa medium or in an oviduct cell co-culture system. Llamas were superstimulated by double dominant follicle reduction followed by oFSH administered in daily descending doses over a 3-day interval. Oocytes were harvested by flank laparotomy or TUGA and matured *in vitro* for 30 h. Mature oocytes were subjected to ICSI followed by no chemical activation (Treatment A), ionomycin only (Treatment B) or ionomycin/DMAP activation (Treatment C). More oocytes were recovered by flank laparotomy procedure compared with TUGA (94% versus 61%, $P < 0.05$) and a greater number of oocytes harvested by flank laparotomy reached the metaphase-II stage (77% versus 44%, $P < 0.05$). After ICSI, the proportion of cleaved and 4–8-cell stages embryos was significantly greater when injected oocytes were activated with ionomycin/DMAP combination (63% and 38%, respectively, $P < 0.05$).

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0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2006.05.020

The co-culture of ICSI embryos with llama oviduct epithelial cells resulted in progression to morula (25%) and blastocyst (12%) stages; whereas, all embryos cultured in CR1aa medium arrested at the 8–16-cell developmental stage.

Keywords: Camelid; Llama; Oocyte; ICSI; Activation; Embryo; Co-culture

1. Introduction

The importance of South American camelids in the United States and South America has previously been reported (Bravo et al., 2000; Chaves et al., 2002). Llama (*Lama glama*) has been the subject of intensive selection for meat and fiber quality in South American countries. In the United States, these animals have had a steady demand mainly as exotic pets and companion animals; however, current regulations and importation restrictions (i.e., foot and mouth disease) limit the introduction of new genetic lines to the existing populations. Assisted reproductive technologies could provide alternative methods to the propagation, selection and genetic improvement in this species.

Intracytoplasmic sperm injection (ICSI) is an important assisted reproduction technology, particularly in the treatment of male factor infertility in humans. Since the first report of ICSI in hamsters (Uehara and Yanagimachi, 1976), live births in mice (Kimura and Yanagimachi, 1995), rabbits (Hosoi et al., 1998), sheep (Catt et al., 1996), cattle (Hamano et al., 1999), horses (Cochran et al., 1998), domestic cats (Gomez et al., 2000), pigs (Martin, 2000; Kolbe and Holtz, 2000; Lai et al., 2001) and humans (Palermo et al., 1992; Tesarik, 1996) have been produced by the application of this micromanipulation technique.

Intracytoplasmic sperm injection has also provided a valuable tool in the study of cell cycle control, the mechanisms underlying sperm-induced oocyte activation and the need for further chemical activation after ICSI. In hamsters, mice and humans, the mechanical disruption of ICSI alone seems to be sufficient to trigger the cascade of events of oocyte activation (Kimura and Yanagimachi, 1995; Kuretake et al., 1996; Perreault et al., 1988; Tesarik and Sousa, 1995). In contrast, other species such as the cow (Rho et al., 1998) and pig (Lee et al., 2003) require additional activation of the oocyte following sperm injection. To date, it is unclear whether the activation of llama oocytes will be required following the ICSI procedure.

The general the objective of the present study was to produce llama embryos *in vitro* using the ICSI procedure. Specific objectives were: (1) to determine the *in vitro* maturation rates of cumulus–oocytes complexes (COCs) recovered by transvaginal ultrasound-guided oocyte aspiration (TUGA) or flank laparotomy; (2) to evaluate the effects of additional activation treatments of llama oocytes following ICSI on *in vitro* embryonic development; and (3) to evaluate the *in vitro* embryonic development of llama ICSI embryos in CR1aa medium or oviduct cell co-culture system.

2. Materials and methods

2.1. Ovarian superstimulation, oocyte recovery and in vitro maturation

The ovarian superstimulation of donor animals was conducted in Bozeman, MT during the month of August. A total of 11 adult female llamas with good body condition (scores 6–8,