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ScienceDirect
Theriogenology 75 (2011) 1482–1488
Theriogenology
www.theriojournal.com

In vitro fertilization and development of cumulus oocytes complexes collected by ultrasound-guided follicle aspiration in superstimulated llamas

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Received 30 July 2010; received in revised form 30 November 2010; accepted 30 November 2010

Abstract

The objective was to evaluate the developmental competence of cumulus-oocyte complexes (COC) collected by follicular aspiration in llamas treated with FSH or eCG. Llamas were assigned randomly to two groups (n = 16 per group) and treated, at the time of ovarian follicular wave emergence, with either: 1) 25 mg of FSH im, twice daily for 4 d; or 2) 1000 IU of eCG as a single i.m. dose. The start of gonadotropin treatment was considered Day 0. Both groups were given 5 mg of Armour Standard LH im on Day 6, and COC were collected by follicle aspiration on Day 7. Expanded COC collected from FSH- (n = 157) and eCG-treated llamas (n = 151) were fertilized *in vitro* using epididymal sperm, and presumptive zygotes were *in vitro* cultured in SOF medium for 8 d. The FSH and eCG treatment groups did not differ with respect to: the number of follicles ≥7 mm (16.0 ± 2.7 vs 14.0 ± 1.9, respectively; P = 0.5); the number of COC collected (11.5 ± 1.9 vs 9.7 ± 1.2; P = 0.4); the number of expanded COC (9.8 ± 1.4 vs 9.4 ± 1.2; P = 0.8); or the percentage of presumptive zygotes which developed into 2 to 8 cell stage embryos (65.3 vs 63.1), morulas (46.2 vs 42.5), or blastocysts (23.1 vs 20.5; P > 0.05). In conclusion, FSH and eCG treatments were equally effective for recovery of a high number of expanded COC which were used directly for *in vitro* fertilization. Furthermore, rate of embryo development was not significantly affected by the gonadotropin treatment used.

Keywords: Oocyte competence; Superstimulation; in vitro fertilization; Camelids; Gonadotropins

1. Introduction

Since the report of the first llama cria by nonsurgical procedures [1], embryo transfer technology has been applied with limited success in South American camelids. *In vivo* production of llama/alpaca embryos can be achieved by nonsurgical uterine flushing after gonadotrophin superstimulatory treatment [2], or by the recovery of a single embryo in non-superstimulated females [3,4]. Several limiting steps have been associated with the slow development of the embryo transfer

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0093-691X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2010.11.047

technique in camelids, including extremely variable follicular responses after superstimulatory treatment [2], and inconsistent results regarding semen preservation and AI [5]. Furthermore, recovery of embryos after uterine flushing in hatched blastocyst stage made it difficult to apply other technologies, e.g., cryopreservation techniques, thereby limiting the potential for international trade of camelid [6,7]. Development of an *in vitro* embryo production system in camelids may overcome some of the problems associated with embryo transfer. However, improvements in oocyte maturation, fertilization, and embryo culture are necessary to achieve success in development of any *in vitro* fertilization program.

The first llama embryos were produced *in vitro* using Cumulus Oocyte Complexes (COC) collected by follicular puncture from abbatoir-derived ovaries [8]; this was followed by the first *in vitro* production of alpaca and llama embryos from COC collected by surgical follicular aspiration from females superstimulated with gonadotrophin [9,10]. Regarding other camelid species, e.g. dromedary, blastocysts have been produced *in vitro* using COC collected by follicle puncture form abattoir-derived ovaries [11,12]. Although the first dromedary offspring was obtained by transfer of *in vitro* hatched blastocysts to synchronized recipients [13], there are not apparently reports documenting the births of llamas or alpacas produced with this technique. However, development and application of transvaginal ultrasound-guided follicular aspiration in llamas and dromedary during the last 10 y [14–17] to collect COC from i*n vivo* superstimulated and non-superstimulated females will facilitate the use of this technique as a valuable tool to maximize the genetic potential of these species.

Ovarian superstimulation for oocyte collection in alpacas [18], llamas [10,16,19,20] and vicuñas [21], with either FSH or a single dose of eCG, followed by GnRH or LH, have resulted in a high recovery rates of expanded COC. As a result of the long biological half-life of eCG, ovarian superstimulatory protocols based on a single dose of eCG have the advantage of minimizing animal handling and stress compared to multiple-dose protocols based on FSH. The long half-life of eCG, however, has been associated with premature maturation of bovine oocytes during superstimulatory treatment, resulting in deleterious effects on oocyte quality and subsequent embryo development [22–24]. In a more recent bovine study [25], eCG treatment resulted in the recovery of a fewer good quality COC than FSH treatment.

In a previous study [16] no differences were detected between FSH- and eCG-treated llamas in the number of expanded COC collected (8.3 ± 2.1 vs 10.6 ± 2.2) or the number of COC at the MII stage (6.9 ± 1.8 vs 8.9 ± 1.9). In a similar study in alpacas [18], FSH- and eCG-treated animals did not differ with respect to the number of follicles > 6 mm at the time of COC collection (20.0 ± 7.5 vs 27.0 ± 3.3), the number of COC collected (26.2 ± 8.4 vs 23.3 ± 3.7), number of expanded COC collected (11.5 ± 2.9 vs 8.8 ± 2.8), or the number of expanded COC in MII (8.5 ± 1.9 vs 6.0 ± 2.1). However, a direct comparison between FSH and eCG treatment on oocyte competence in llamas after *in vitro* fertilization has apparently not been reported.

The objective of the present study was to compare developmental competence of COC collected by ultrasound-guided follicular aspiration in llamas treated with FSH or eCG.

2. Materials and methods

2.1. Animals and treatments

Mature, non-pregnant female llamas (n = 32), ≥ 3 y of age and weighing an average of 120 kg, were used during the breeding season (January to March) at the Llama del Sur Ranch, in the Department of Temuco, Chile (38° S, 72° W, at sea level). To synchronize follicular wave emergence, females were given caudal epidural anesthesia (2.5 mL of 2 % of lidocaine; Laboratorio Chile, Santiago, Chile), restrained in dorsal recumbency, and all ovarian follicles ≥ 5 mm were ablated by transvaginal ultrasound-guided follicle aspiration, using a 5.0 MHz convex-array ultrasound transducer (Aloka SSD-500, Tokyo, Japan) and a 19-gauge needle [14–16]. At 48 h after follicle ablation [i.e., expected time of follicular wave emergence; (15)], llamas were assigned randomly to two groups (n = 16 per group) and given either: 1) 25 mg of FSH (Folltropin, Bioniche Animal Health Canada Inc., Belleville, ON, Canada) i.m., twice daily for 4 d; or 2) 1000 IU of eCG (Novormon, Bioniche Animal Health Canada Inc.) as a single im dose. The start of gonadotropin treatment was considered Day 0. Llamas in both groups were given 5 mg Armour Standard LH (Lutropin, Bioniche Animal Health Canada Inc.) i.m. on Day 6. The ovarian response was assessed by transrectal ultrasonography, using a 7.5 MHz linear-array transducer (Aloka SSD-500) immediately before COC collection on Day 7 (24 to 26 h after LH treatment).