Animal Reproduction Science 128 (2011) 52–59

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Treatment with recombinant equine follicle stimulating hormone (reFSH) followed by recombinant equine luteinizing hormone (reLH) increases embryo recovery in superovulated mares

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ARTICLE INFO

Article history:
Received 8 December 2010
Received in revised form 26 August 2011
Accepted 9 September 2011
Available online 17 September 2011

Keywords:
Recombinant equine FSH and LH
Mares
Superovulation
Embryo recovery
Anovulatory follicles
Hormones

ABSTRACT

The dynamics of ovarian follicular development depend on a timely interaction of gonadotropins and gonadal feedback in the mare. The development and efficacy of genetically cloned recombinant equine gonadotropins (reFSH and reLH) increase follicular activity and induce ovulation, respectively, but an optimum embryo recovery regimen in superovulated mares has not been established. The objective of this study was to determine if treatment with reFSH followed by reLH would increase the embryo per ovulation ratio and the number of embryos recovered after superovulation in mares. Sixteen estrous cycling mares of light horse breeds (4–12 years) were randomly assigned to one of two groups: Group 1; reFSH (0.65 mg)/PBS ($n=8$) and Group 2; reFSH (0.65 mg)/reLH (1.5 mg) ($n=8$). On the day of a 22–25 mm follicle post-ovulation mares were injected IV twice daily with reFSH for 3 days ($PGF_{2\alpha}$ given IM on the second day of treatment) and once per day thereafter until a follicle or cohort of follicles reached 29 mm after which either PBS or reLH was added and both groups injected IV twice daily until the presence of a 32 mm follicles, when reFSH was discontinued. Thereafter, mares were injected three times daily IV with only PBS or reLH until a majority of follicles reached 35–38 mm when treatment was discontinued. Mares were given hCG IV (2500 IU) to induce ovulation and bred. Embryo recovery was performed on day 8 day post-treatment ovulation. Daily jugular blood samples were collected from the time of first ovulation until 8 days post-treatment ovulation. Blood samples were analyzed for LH, FSH, estradiol, progesterone and inhibin by validated RIA. Duration of treatment to a $\geq$35 mm follicle(s) and number of ovulatory size follicles were similar between reFSH/reLH and reFSH/PBS treated mares. The number of ovulations was greater ($P<0.01$) in the reFSH/reLH group, while the number of anovulatory follicles was less ($P<0.05$) compared to the reFSH/PBS group. Number of total embryos recovered were greater in reFSH/reLH mares than in the reFSH/PBS mares ($P\leq0.01$). The embryo per ovulation ratio tended to be greater ($P=0.07$) in the reFSH/reLH mares. Circulating concentrations of estradiol, inhibin, LH and progesterone were not statistically different between groups. Plasma concentrations of FSH were less ($P<0.01$) in the reFSH/reLH treated mares on days 0, 1, 4, 6, 7 and 8 post-treatment ovulation. In summary, reFSH with the addition of reLH, which is critical for final follicular and oocyte maturation, was effective in increasing the number of ovulations and embryos recovered, as well as reduce the number of anovulatory follicles, making this a more viable option than treatment with reFSH alone. Further evaluation is needed to determine the dose and regimen of reFSH/reLH to significantly increase the embryo per ovulation ratio.

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doi:10.1016/j.anireprosci.2011.09.002

1. Introduction

Embryo recovery rates per ovulation in some superovulation studies have been less than spontaneously ovulating mares, whereas other studies have reported no differences (Squires, 2006; McCue et al., 2008). Approximately 25% of uterine flushes conducted on superovulated mares resulted in no embryos at all (Squires, 2006). Previous studies noted that with increased number of ovulations, embryo recovery per ovulation remained the same or decreased, following repeated injections of equine pituitary extracts (EPE) or a partially purified pituitary equine follicle stimulating hormone (eFSH; Bioniche Animal Health, Bogart, GA) (Rosas et al., 1998; Alvarenga et al., 2001; Scoggin et al., 2002; Machado et al., 2005; Squires, 2006; Welch et al., 2006; Logan et al., 2007; Araujo et al., 2009; Raz et al., 2009). Several factors can affect the embryo per ovulation ratio such as oocyte quality (Geary et al., 1989; Woods, 1989; Carnevale and Ginther, 1995; Carnevale et al., 1999) and the capability of the oocyte to enter into the oviduct via the ovulation fossa (Carmo et al., 2006). According to Carmo et al. (2006), there was no significant difference in the number of oocytes that appeared in the oviduct from superovulated mares compared to control mares supporting the concept that oocyte development and maturation may be a critical factor affecting the embryo per ovulation ratio in superovulated mares. The findings that an increased number of anovulatory follicles are present after superovulation regimens (Alvarenga et al., 2001; Squires, 2006; Welch et al., 2006; Raz et al., 2009) also supports the concept that a less than optimum embryo per ovulation ratio may be a function of oocyte dysfunction.

The development and efficacy of genetically cloned recombinant equine gonadotropins (reFSH and reLH) have recently been reported (Jablonka-Shariff et al., 2007; Yoon et al., 2007; Jennings et al., 2009; Meyers-Brown et al., 2010). A recent study indicated that reFSH could initiate follicular development in ovaries of normal breeding mares whose follicular activity had been suppressed with progesterone and estradiol (Jennings et al., 2009). Mares treated with repeated injections reFSH had greater numbers of pre-ovulatory follicles. However, they also had a greater number of anovulatory follicles, similar ovulation rates and a suppressed LH surge (Jennings et al., 2009). In a second study using normal estrous cycling mares treated with repeated injections of reFSH to stimulate follicular development and increase embryo numbers, there was an increase in the number of ovulations, a greater number of anovulatory follicles, a suppressed LH surge and no difference in the embryo per ovulation ratio compared to controls (Meyers-Brown et al., 2010). Similar results were also noted in another superovulation study in the mare that evaluated repeated injections of eFSH (Bioniche) and decreasing doses of EPE (Machado et al., 2008). The studies above suggest that the disruption in the hormone milieu may have an effect on oocyte maturation associated with the number of anovulatory follicles present (Machado et al., 2008; Jennings et al., 2009; Meyers-Brown et al., 2010).

An optimum hormone milieu is important for normal oocyte maturations. Oocytes require the presence of LH to stimulate granulosa and cumulus cells to secrete factors important for oocyte maturation and competence for fertilization (Goudet et al., 1999; Hinrichs and Schmidt, 2000; Hillier, 2001; Accardo et al., 2004; Webb et al., 2004; Mihm et al., 2006; Silvestre et al., 2007; Lindbloom et al., 2008; Rosen et al., 2009). An increase in LH receptors on granulosa and cumulus cells has been correlated with an increase in follicular diameter and oocyte competence in the mare (Goudet et al., 1999). Lindbloom et al. (2008) reported that reLH was effective in increasing epidermal-like growth factors in granulosa cells and an isoform of phosphodiesterase in equine oocytes within 9 h after incubation which are essential for oocyte maturation. The studies using reFSH, eFSH and decreasing doses of EPE had greater systemic concentrations of estrogen and inhibin (Machado et al., 2008; Jennings et al., 2009; Meyers-Brown et al., 2010). Greater concentrations of estradiol and inhibin acting as paracrine/autocrine factors have been shown to be detrimental to oocyte maturation (Silva et al., 1999; Bing et al., 2001; Rosen et al., 2009) which may in turn eventually affect the embryo per ovulation ratio.

Due to the fact that, EPE or partially purified pituitary eFSH contain both FSH and LH, treating with either material from the beginning of follicular emergence and deviation to the time right before ovulation does not simulate the physiological events that occur in the estrous cycling mare as described by Ginther et al. (2005a,b). In addition treating with just reFSH does not result in an increase in the embryo per ovulation ratio (Roser et al., 2008; Jennings et al., 2009). Both FSH and LH are critical for follicular and oocyte development but must be available in the proper quantities at the correct time for optimum fertilization (Ginther et al., 2005a,b).

The present research was designed to test the hypothesis that a superovulation regimen involving repeated injections of reFSH followed by multiple injections of reLH would establish a hormone environment conducive to maximum embryo recovery efficiency. The objectives of the present study were to determine the efficacy of treatment with reFSH followed by reLH: (1) to increase the number of multiple ovulating follicles and reduce the number of anovulatory follicles, (2) to increase the embryo recovery per flush and embryo per ovulation ratio and (3) to determine changes in the hormone profiles of mares treated with reFSH/reLH compared to those treated with reFSH/PBS.

2. Materials and methods

2.1. Animals

Sixteen normal estrous cycling mares of light horse breeds between the ages of 4 and 12 years were housed in paddocks at the UC Davis Animal Science Horse Barn, from June to September 2008. Mares were fed a diet of alfalfa and oat hay and had access to fresh water at all times. Experiments were approved by the Animal Care and Use Committee at the University of California, Davis.