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Functional relationships among intrafollicular insulin-like growth factor 1, circulatory gonadotropins, and development of the dominant follicle in mares

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ABSTRACT

The functional relationships among intrafollicular free insulin-like growth factor 1 (IGF1), circulatory gonadotropins, and development of the dominant follicle were studied in 40 mares in two experiments. A GnRH antagonist (Acyline) was given i.m. at the expected beginning of follicular deviation (largest follicle or F1 ≥ 20 mm; Day 0) alone (Acyline group) or in combination with intrafollicular treatment of F1 with rhIGF1 (Acyline/IGF1 group). In Experiment 1, blood samples, follicular-fluid samples, and diameter of F1 were taken on Days 1 and 2. In Experiment 2, daily follicular diameter and blood samples were taken from Day 0 to ovulation. The GnRH antagonist induced a 50% decrease in circulatory FSH concentrations for 1 d and in LH for 2 d. In Experiment 1, control and Acyline/IGF1 groups had greater intrafollicular free IGF1 ($P < 0.05$) and inhibin-A concentrations ($P < 0.08$) than the Acyline group. The intrafollicular concentration of estradiol on Day 2 was greater ($P < 0.05$) in the control group than in the Acyline and the Acyline/IGF1 groups. In Experiment 2, a decrease in diameter of F1 in the Acyline group was followed by a new follicular wave. All IGF-treated follicles grew and ovulated. Results indicated that the increase in intrafollicular free IGF1 observed in F1 in association with deviation is gonadotropin dependent. During the period of lesser gonadotropin concentrations from Acyline treatment, intrafollicular IGF1 stimulated follicular growth and inhibin concentrations, but not intrafollicular estradiol production.

1. Introduction

In monovular farm species (horses, cattle), each major follicular wave, including the wave that originates the ovulatory follicle, is initiated by a surge in FSH (reviewed in Ginther et al., 2003, 2004a; Beg and Ginther, 2006; Adams et al., 2008). Usually, only one of many available follicles develops into the dominant follicle, and this phenomenon is known as follicular selection. The eminent selection event during a follicular wave is a distinctive change in growth rates between the developing dominant follicle and the remaining follicles (subordinates) and is known as follicular deviation. Before deviation, all follicles of the wave grow (common-growth phase) and have the potential for future dominance. The beginning of deviation is characterized by a continuation of the growth rate of the dominant follicle and a reduction in growth rate of the subordinate follicles. Deviation begins when the most advanced follicle reaches a specific developmental stage. In the mare, deviation begins when the two largest follicles are on

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average 22.5 and 19.0 mm. Follicular-fluid concentrations of estradiol, free insulin-like growth factor 1 (IGF1), activin-A, and inhibin-A begin to increase differentially in the future dominant follicle about 1 d before the beginning of diameter deviation. Deviation in the ovulatory wave begins during the progressive decline in circulating FSH concentration and increasing LH concentration.

It has been proposed (Beg and Ginther, 2006) that free IGF1 is the main intrafollicular factor needed for the initiation of deviation in horses as indicated by: (1) free IGF1 begins to increase differentially 1 d before the beginning of spontaneous deviation (Donadeu and Ginther, 2002) and 12 h before a deviation induced by ablation of the largest follicle (Ginther et al., 2002b); (2) injection of recombinant human IGF1 (rhIGF1) into the second-largest follicle at the expected beginning of deviation induces an increased incidence of dominance in the treated follicle (Ginther et al., 2004d, 2008); and (3) injection of IGF-binding protein-3 (IGFBP-3) into the largest follicle at the expected beginning of deviation causes the largest follicle to regress and second-largest follicle to become dominant (Ginther et al., 2004c).

It has been postulated that an FSH-dependent increase in IGFBP protease activity is needed for the increase in free IGF1 in the future dominant follicle (reviewed in Mazerbourg et al., 2003; Spicer, 2004; Beg and Ginther, 2006; Mihm and Evans, 2008). In non-equine species, IGF1 may act as an amplifier of gonadotropin hormonal action (Chun et al., 1994, 1996; Adashi, 1998). *In vitro*, IGF1 stimulated granulosa cell proliferation and synergized with gonadotropins to promote differentiation of follicle cells, enhanced the sensitivity of granulosa cells to FSH, increased estradiol production, and increased secretion of inhibin-A, activin-A, and follistatin from granulosa cells (reviewed in Adashi, 1998; Monget et al., 2002; Mazerbourg et al., 2003; Mihm and Evans, 2008). Some of these factors also act as anti-apoptotic signals or as up-regulators of FSH receptor (Chun et al., 1996; Knight and Glister, 2001; Quirk et al., 2004). However, it is not clear whether these *in vitro* effects of IGF1 occur *in vivo*, whether the effects are modulated by free IGF1 alone or in concert with gonadotropins *in vivo*, and whether these effects apply to the horse.

We have shown that a single dose of 3 mg Acyline (a synthetic GnRH antagonist) induced a temporary (24 h) decrease in FSH concentrations in mares (Checura et al., 2009). All follicles ≥6 mm were ablated at 10 d after ovulation and mares were treated with Acyline after the peak of the resulting FSH surge and near the beginning of expected deviation. Acyline induced an immediate decrease in FSH and retardation of follicle growth.

In the present two experiments, the following hypotheses were examined in mares: (1) The increase in free IGF1 in the follicular fluid of the largest follicle after the beginning of deviation is dependent on gonadotropins, as determined by comparison of intrafollicular concentrations of free IGF1 in control versus Acyline-treated mares (Experiment 1). In addition, the relationships between gonadotropins and free IGF1 with other intrafollicular factors were considered. (2) The increase in intrafollicular free IGF1 at the expected time of deviation directly stimulates continued development of the follicle, as determined by administration of intrafollicular rhIGF1 during Acyline-induced gonadotropin suppression (Experiment 2).

2. Materials and methods

2.1. Animals, ultrasonography, and treatment hormones

The mares were nonlactating mixed breeds of large ponies and apparent pony-horse crosses aged 6–17 yr and weighing 250–400 kg. Mares with docile temperament and no apparent abnormalities of the reproductive tract, as determined by ultrasound examinations (Ginther, 1995), were used. The mares were kept under natural light in an open shelter and outdoor paddock with free access to alfalfa/grass hay, water, and trace-mineralized salt. Mares were handled in accordance with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching.

The follicles were examined by transrectal ultrasonography, using B-mode of an ultrasonic scanner (Aloka SSD-3500; Aloka America, Wallingford, CT, USA) equipped with a finger-mounted 7.5-MHz convex transducer as described (Ginther, 1995; Acosta et al., 2004b). The day of ovulation was determined by disappearance of the preovulatory follicle with subsequent development of a corpus luteum. Diameter of the follicles was measured with the electronic calipers at the apparent maximal area on the two-dimensional image, using the average of height and width from two frozen images. A new follicular wave was induced by transvaginal ultrasonic-guided follicle ablation by aspiration of contents of all follicles ≥6 mm 10 d after ovulation as described (Gastal et al., 1997). When the largest follicle of the new follicular wave reached a diameter ≥14 mm, all but the three largest follicles were ablated to simplify follicle identification as described (Acosta et al., 2004b). The first-, second-, and third-largest follicles of the wave were designated F1, F2, and F3, respectively, when F1 reached ≥20.0 mm (expected beginning of deviation = Day 0).

The procedure for ultrasonic-guided transvaginal treatment of follicles or sampling of 200 μl follicular fluid has been described (Gastal et al., 1999). Briefly, a double-needle system was used. A 20-gauge outer needle was positioned against the follicular wall and a 25-gauge inner needle was advanced to puncture the follicle for treatment or sampling. A diameter decrease in F1 during the 24 h after intrafollicular injection or sampling that exceeded 7 mm was considered as excessive fluid loss, as reported (Acosta et al., 2004a; Ginther et al., 2004d). For the collection of all follicular contents, a single 17-gauge needle attached to a 10 ml syringe was used to puncture the follicle and aspirate its contents.

The GnRH antagonist, Acyline (CBD 3883H, NICHHD/NIH), was dissolved in 5% aqueous solution of D-mannitol (M-9546, Sigma Chemical Co., St. Louis, MO, USA) to a concentration of 1 mg/ml and was given as a single intramuscular injection of 3 ml. The dose of Acyline was based on a reported titration study (Checura et al., 2009). For intrafollicular injection of rhIGF1 (Genentech, San Francisco, CA, USA), a dose that has been shown (Ginther