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Stimulation of the largest subordinate follicle by intrafollicular treatment with insulin-like growth factor 1 is associated with inhibition of the dominant follicle in heifers

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Abstract

The effect of intrafollicular treatment of the second-largest follicle (F2) with insulin-like growth factor (IGF) 1 on the largest follicle (F1) and F2 was studied in heifers. Treatment of F2 was done when F1 reached ≥8.2 mm (expected beginning of follicle deviation; Day 0 or Hour 0). In each of two experiments, three groups (n = 6 or 7 heifers/group) were used: controls, F2 treated with vehicle and F2 treated with IGF1. The IGF1 treatment consisted of 200 μg of recombinant human IGF1 (pharmacological dose) in 20 μL of vehicle. In Experiment 1, the hypothesis that treatment of F2 with IGF1 has a stimulatory effect on F2 was supported by a greater ($P < 0.05$) incidence of F2 dominance (≥10 mm) in the IGF1 group (71%) than in the other two groups (8%), and a greater ($P < 0.02$) growth rate of F2 on Days 0–2. Unexpectedly, treatment of F2 with IGF1 had an inhibitory effect on F1, as indicated by a reduced ($P < 0.03$) growth rate of F1 during Days 0–1 and Days 0–4 and a lesser ($P < 0.05$) maximum diameter of F1 in the IGF1 group. In Experiment 2, the hypothesis of an inhibitory effect on F1 when F2 was treated with IGF1 was supported by a lesser ($P < 0.04$) increase in diameter of F1 and a lesser ($P < 0.04$) percentage of follicle wall with power-Doppler signals of blood flow between Hours 0 and 14 in the IGF1 group. Circulating concentrations of FSH and LH were not altered significantly in either experiment. In conclusion, treatment of F2 with IGF1 at the expected beginning of deviation had a stimulatory effect on F2, but an inhibitory effect on F1.

Keywords: Follicle deviation; IGF1; Ovarian follicles; Ovarian follicular development; Blood flow

1. Introduction

In monovular species (cattle, horses, humans), the follicular wave that originates the ovulatory follicle is initiated by a surge in FSH [1–4]. The future dominant follicle emerges approximately 10 h before the future largest subordinate follicle, using 1 mm [5] or 3 mm [6] to define emergence. Therefore, the largest follicle of the wave has a size advantage for becoming the dominant follicle. All follicles of the wave grow for several days in a common-growth phase, and all have the capability for future dominance. However, only one follicle continues to grow and develop into the dominant follicle, whereas the remaining follicles are se-

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lected against and become subordinates. The eminent event during selection against the future subordinates is a distinctive reduction in growth rate in the future subordinate follicles, but not in the developing dominant follicle beginning at the end of the common-growth phase, and is known as follicle deviation [6].

In cattle, deviation begins when the most advanced follicle reaches a specific development stage and the two largest follicles are an average of 8.5 and 7.2 mm [2,7]. Deviation begins during a progressive decrease in concentrations of the wave-stimulating FSH surge and greater concentrations of LH. A two-way functional relationship between FSH and follicles is close temporally, so that a change in either event causes a detectable change in the other in <8 h in heifers [8,9]. The close functional two-way FSH/follicle relationship is considered an integral component of follicle deviation [10].

In cattle, a differential response among follicles to LH is also involved in deviation [2]. The involvement of LH has been demonstrated by a smaller postdeviation diameter of the largest follicle when LH was experimentally reduced [11,12]. In addition, concentration of LH was transiently greater during deviation [9,12,13], and the difference between the two largest follicles in granulosa LH receptor mRNA [14] increased 8 h before the beginning of deviation [15].

The largest follicle develops greater responsiveness to FSH and LH at the time of deviation, enabled by follicular-fluid factors, and therefore it alone is able to respond to the low gonadotropin concentrations (reviewed in Beg and Ginther, 2006 [16]). The intrafollicular factors that cause the apparently greater response of the future dominant follicle in heifers include estradiol and insulin-like growth factor 1 (IGF1). Follicular-fluid concentrations of estradiol increase more rapidly in the future dominant follicle (F1) than in the largest future subordinate follicle (F2), beginning approximately 1 d before the beginning of diameter deviation [17]. Free IGF1 in F1 remains at an elevated concentration encompassing the beginning of deviation [15,17,18], whereas the concentration begins to decrease in F2 before the beginning of deviation. Studies on experimental deviation by ablation of F1 at the expected beginning of deviation have indicated that F2 changes its course within 12 h to become the dominant follicle, as indicated by an increased diameter and greater concentrations of estradiol and free IGF1 [17,19].

Intrafollicular injection of recombinant human (rh) IGF1 into F2 or an IGF binding protein (IGFBP) into F1 at the expected beginning of deviation has been used to study the role of free IGF1 in follicle deviation in mares. Based on these experiments, free IGF1 was the main intrafollicular factor needed for the initiation of deviation [20–22]. Similar studies have not been done in cattle.

The purpose of the present two experiments was to test the hypothesis that IGF1 stimulates follicle growth in heifers, as determined by intrafollicular IGF1 treatment of F2 at the expected beginning of deviation. An unexpected finding in Experiment 1 was that the growth rate of F1 was reduced when F2 was treated with IGF1. Experiment 2 tested the hypothesis that stimulation of F2 with IGF1 decreases the growth and vascularization of F1 without an effect on circulating gonadotropin concentrations.

2. Materials and methods

2.1. Animals and ultrasonography

Holstein heifers aged 17–21 mo and weighing 480–660 kg were used. All heifers had a single ovulation during the experimental estrous cycle with at least two previous cycles of 16–24 d. Only heifers in which the two largest follicles were increasing in diameter on the day of treatment were used. The heifers did not have apparent abnormalities of the reproductive tract, based on transrectal ultrasonic examinations [23], and were acclimated to the handling procedures for at least 2 wk prior to the experiment. The feeding program consisted of *ad libitum* access to a mixture of grass and alfalfa hay, water, and minerals with grain supplementation. Heifers remained healthy and in good body condition throughout the experiment. Animals were handled in accordance with the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Research.

A duplex B-mode (grey scale) and pulsed-wave color-Doppler ultrasonic instrument (Aloka SSD 3500; Aloka American, Wallingford, CT, USA) equipped with a linear array 7.5-MHz transducer was used. Transrectal scanning of the ovaries for measuring follicles and detecting ovulation was done as described [23]. Heifers were checked once daily for ovulation beginning when a preovulatory follicle (≥12 mm) was present. Follicle ablations were done by ultrasonic-guided transvaginal aspiration of the contents of all follicles ≥5 mm 4 d after ovulation, using an 18-ga needle connected to a vacuum pump as described [24]. Follicle ablations were done to induce an FSH surge with development of a new follicular wave. This was done as an aid in sequential identity of individual follicles by eliminating the mixing of follicles from a previous wave with those of the experimen-