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ScienceDirect
Theriogenology 74 (2010) 912–921
Theriogenology
www.theriojournal.com

Pulsatile LH secretion and ovarian follicular wave emergence and growth in anestrous ewes

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Received 15 September 2009; received in revised form 8 April 2010; accepted 11 April 2010

Abstract

The objective of this study was to determine if pulsatile LH secretion was needed for ovarian follicular wave emergence and growth in the anestrous ewe. In Experiment 1, ewes were either large or small (10 × 0.47 or 5 × 0.47 cm, respectively; n = 5/group) sc implants releasing estradiol-17 beta for 10 d (Day 0 = day of implant insertion), to suppress pulsed LH secretion, but not FSH secretion. Five sham-operated control ewes received no implants. In Experiment 2, 12 ewes received large estradiol-releasing implants for 12 d (Day 0 = day of implant insertion); six were given GnRH (200 ng IV) every 4 h for the last 6 d that the implants were in place (to reinitiate pulsed LH secretion) whereas six Control ewes were given saline. Ovarian ultrasonography and blood sampling were done daily; blood samples were also taken every 12 min for 6 h on Days 5 and 9, and on Days 6 and 12 of the treatment period in Experiments 1 and 2, respectively. Treatment with estradiol blocked pulsatile LH secretion ($P < 0.001$). In Experiment 1, implant treatment halted follicular wave emergence between Days 2 and 10. In Experiment 2, follicular waves were suppressed during treatment with estradiol, but resumed following GnRH treatment. In both experiments, the range of peaks in serum FSH concentrations that preceded and triggered follicular wave emergence was almost the same as control ewes and those given estradiol implants alone or with GnRH; mean concentrations did not differ ($P < 0.05$). We concluded that some level of pulsatile LH secretion was required for the emergence of follicular waves that were triggered by peaks in serum FSH concentrations in the anestrous ewe.

Keywords: Anestrous; Estradiol; FSH; Ovarian follicular waves; Sheep

1. Introduction

With the recent use of transrectal ultrasonography for imaging ovaries in both cyclic and anestrous ewes, it was found that 1–3 ovarian antral follicles emerged or grew from a pool of small follicles, 1–3 mm in diameter, every 4–5 d [1–4]. These follicles grow to ≥5 mm in diameter before regression or ovulation [1–4]. Each wave of follicular growth was preceded by a transient peak of FSH secretion that lasted 3 or 4 d and was characterized by blood samples taken daily [3,5,6].

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0093-691X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2010.04.016

This peak in FSH secretion was the essential trigger for the follicular wave [3,5,6]; however, the maximum concentration that peaks achieved and the nadirs in serum FSH concentrations between peaks, varied considerably among follicular waves [3,4,7].

When cyclic ewes were treated for 10 d with large estradiol-17β releasing implants (10 × 0.47 cm), that created supraphysiological serum concentrations of estradiol-17β (10.4 ± 0.7 pg/mL vs. 3.9 ± 0.7 pg/mL for treated vs. control ewes, respectively), the amplitude of the FSH peaks that preceded follicular waves was reduced and follicular wave emergence was blocked [7]. Injection of ovine FSH (oFSH), to recreate FSH peaks, re-initiated follicular waves [7]. In that study, pulsed LH secretion was not affected. Pulses of LH were released at a frequency of ~1 every 1–6 h in the ewe and lasted no more than ~2 h [8,9]. Although peaks in FSH secretion initiated follicular waves, the role of pulsed LH secretion in the emergence, growth and regression of ovine antral follicles was unclear. Changes in LH pulse frequency during the estrous cycle did not appear to be correlated with or functionally related to specific phases of the growth or regression of follicular waves in the ewe [8,9]. Profiles of FSH in blood samples collected from the jugular vein in ewes were not pulsatile [10,11].

Compared to ewes during the breeding season, seasonally anestrous ewes had lower circulating concentrations of LH, FSH, and estradiol, little or no progesterone, and serum concentrations of estradiol and inhibin which were not correlated with follicular wave development [8,12–15]. The frequency of LH secretory pulses was very low and fluctuated very little across seasonal anestrus [13–17], with no apparent association to stages of follicular wave development and regression. During seasonal anestrus, estradiol exerted a more powerful negative feedback effect on pulsatile LH secretion than during the breeding season, but the effects on FSH secretion appeared to be minimal [18–20].

We hypothesized that although the frequency of pulses of LH secretion were very low in anestrous ewes, they were still essential for emergence and growth of ovarian follicular waves. The objective of the present study was to examine the need for pulsed LH secretion for the emergence and growth of ovarian follicular waves in anestrous ewes. Two experiments were conducted. The purpose of the first experiment was to determine if treatment of anestrous ewes with large estradiol releasing implants (10 × 0.47 cm) that created supraphysiological concentrations of estradiol [7] would suppress the pulsed secretion of LH, but leave FSH peaks with a concentration at their zenith similar to control ewes and in a range that could still initiate a follicular wave. The purpose of the second experiment was to replace LH pulses with frequent GnRH injections, in the experimental model above, to see if restored LH pulsatility would in fact allow restoration of follicular waves. This would confirm that, although peaks in FSH secretion initiated follicular waves in the anestrous ewe, some level of pulsed LH secretion was also required.

2. Materials and methods

2.1. Animals

Care and handling of experimental animals was done according to the Canadian Council on Animal Care's published guidelines. Sexually mature, clinically healthy, seasonally anestrous, Western White Face (WWF) ewes were kept outdoors in sheltered paddocks. Ewes were fed a maintenance diet of hay; cobalt iodized saltlicks and water were freely available. The WWF is a cross between the Columbia and Rambouillet breeds.

2.2. Ultrasound technique

The growth and regression of ovarian antral follicles were monitored in all ewes by transrectal ovarian ultrasonography (scanning), using a 7.5-MHz transducer stiffened with a hollow plastic rod and connected to a B-mode, real-time echo camera (Aloka SSD-900, Overseas Monitor, Richmond, BC, Canada). This technique has been validated for monitoring ovarian follicular dynamics and CL detection in sheep [1,21,22]. All images were viewed at a magnification of × 1.5 with constant gain and focal point settings. Ovarian images were recorded (Panasonic AG 1978, Matsushita Electric, Mississauga, ON, Canada) on high-grade video tapes (Fuji S-VHS, ST-120 N, Fujifilm, Tokyo, Japan) for later examination. The relative position and dimensions of follicles and luteal structures were also sketched on ovarian charts.

2.3. Experimental design

2.3.1. Experiment 1

Fifteen seasonally anestrous (June) WWF ewes (mean body weight of 76.9 ± 2.9 kg) were allocated into three groups of five ewes each. The experimental design is summarized in Figure 1. Five ewes received large subcutaneous silastic rubber implants (10 × 0.47 cm) and five ewes received small subcutaneous silastic rubber implants (5 × 0.47 cm) containing 10% estradiol-17β w/w (Sigma Chemical Company, St. Louis, MO, USA [7]. Five sham operated control ewes received no