Theriogenology xxx (2015) 1–12

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Characterization of persistent follicles induced by prolonged treatment with progesterone in dairy cows: An experimental model for the study of ovarian follicular cysts

Pablo U. Díaz [a], Matías L. Stangaferro [b], Natalia C. Gareis [a], William J. Silvia [c], Valentina Matiller [a], Natalia R. Salvetti [a], Florencia Rey [a], Fabián Barberis [d], Luciano Cattaneo [d], Hugo H. Ortega [a,*]

[a] Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL), Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Esperanza, Santa Fe, Argentina
[b] Department of Animal Science, Cornell University, Ithaca, New York, USA
[c] Department of Animal and Food Science, University of Kentucky, Lexington, Kentucky, USA
[d] Cátedra de Teriogenología, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina

ARTICLE INFO

Article history:
Received 18 March 2015
Received in revised form 18 June 2015
Accepted 19 June 2015

Keywords:
Follicular dynamics
Endocrine profiles
Follicular persistence
LH
FSH

ABSTRACT

Cystic ovarian disease (COD) is a major factor contributing to poor reproductive efficiency of lactating dairy cows. The objective of the present study was to analyze the endocrine profile, growth dynamics, and histologic characteristics of persistent ovarian follicles-cysts developing in response to long-term administration of intermediate levels of progesterone. To this end, after synchronization of cows, a low dose of progesterone was administered for 5, 10, and 15 days after the expected day of ovulation in treated cows (groups P5, P10, and P15, respectively), using an intravaginal progesterone-releasing device. A significant increase in diameter was detected on Day 11 of progesterone treatment and thereafter ($P < 0.05$), and at Day 15 of persistence, the diameter of the persistent follicle reached a mean of 23 ± 0.6 mm. Microscopically, the persistent follicles had a complete granulosa, an intensely vascularized theca interna, and a collagenous theca externa layer. Temporal changes in the serum concentrations of estradiol, progesterone, and FSH were detected (effects of time, $P < 0.01$). Progesterone treatment completely inhibited the LH preovulatory surge in treated cows and affected the basal concentration of LH. The pulse frequency remained high at 5 and 10 days of persistence and declined ($P < 0.05$) after 15 days of persistence. The LH pulse concentration and pulse amplitude had a significant reduction ($P < 0.05$) during follicular persistence. Changes in the serum levels of estradiol, progesterone, 17-hydroxyprogesterone, and testosterone in serum and follicular fluid were also observed. In serum, estradiol increased gradually from proestrus to Day 10 of follicular persistence ($P < 0.05$), progesterone showed an increase ($P < 0.05$) at Day 5 of follicular persistence, 17-hydroxyprogesterone showed a significant decrease at 5 days of follicular persistence in relation to proestrus, and testosterone showed a significant increase ($P < 0.05$) from proestrus and Day 5 of persistence through Day 15 of follicular persistence. Correlation between serum and follicular fluid steroid concentrations was significant for testosterone ($P < 0.0001$) and not significant for estradiol and progesterone. These findings indicate that ovarian cysts in COD are similar in many ways to the persistent follicles induced by progesterone, with an analogous hormonal and morphologic context, thus

* Corresponding author. Tel.: +54-3496-421037; fax: +54-3496-426304.
E-mail address: hhortega@fcv.unl.edu.ar (H.H. Ortega).

0093-691X/$ – see front matter © 2015 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.theriogenology.2015.06.015

confirming a local role of subluteal levels of progesterone in COD pathogenesis and in the regulatory mechanisms of the ovarian function.

1. Introduction

Cystic ovarian disease (COD) is a major factor contributing to poor reproductive efficiency of lactating dairy cows [1]. However, the pathogenesis of COD has not been clearly established. Cysts develop from preovulatory follicles that fail to ovulate, persist, and then interfere with normal ovarian function [2]. The most widely accepted hypothesis is that COD is the result of a "hormonal imbalance" within the hypothalamic–pituitary–gonadal axis [2]. Silvia et al. [3] have proposed that the primary defect resides in the hypothalamus, which fails to release a surge of GnRH in response to follicular estradiol. This failure leads to a lack of stimulus for the anterior pituitary gland to secrete the preovulatory surge of LH. This hypothalamic insensitivity to estradiol may be due to the intermediate concentrations of progesterone commonly found in cows with COD [4–9].

The study of the processes that lead to ovulatory failure and persistence of the dominant follicle is the key to understand the pathogenesis of COD. The major difficulty in investigating cysts is that their formation can only be retrospectively recognized after the follicle has undergone extensive pathologic changes. Therefore, prediction of the time of cystic structure formation through follicular development in experimental models is a formidable opportunity to understand their pathogenesis. In this sense, numerous experimental models have been developed to induce the formation of follicular cysts [10–23]. However, the possible role of intermediate levels of progesterone in promoting the formation of ovarian follicular cysts has been investigated only in short-term models without reaching the time of follicular persistence necessary to define ovarian structures as follicular cysts. Thus, the objective of the present study was to analyze the endocrine profile, growth dynamics, and histologic characteristics of persistent ovarian follicles and cysts developing in response to long-term administration of intermediate levels of progesterone.

2. Materials and methods

2.1. Animals

All the procedures were approved by the Institutional Ethics and Security Committee of the Faculty of Veterinary Sciences of the Universidad Nacional del Litoral, Argentina (protocol no. 131-12) and are consistent with the "Guide for the Care and Use of Agricultural Animals in Research and Teaching, Third Edition" (Federation of Animal Science Societies, 2010). This study was performed in nonlactating Holstein cows (N = 25) with regular estrous cycles, all of which had calved at least once. Cows were obtained at the end of lactation from local commercial farms and housed outside in an open lot, except during blood collection or ultrasound examinations (when they were moved to a stanchion barn). The cows were fed a diet based on alfalfa pasture, oat, or rye grass grazing supplemented with corn silage, alfalfa silage, corn grain, soybean expeller, and hay, following the recommendations of the Nutrient Requirements of Dairy Cattle (2001).

2.2. Experimental model

2.2.1. Synchronization

Ovarian activity was synchronized starting with the procedure commonly referred to as G6G [24] with some modifications (see Fig. 1). Holstein cows with one or more corpora lutea (CL) identified by transrectal ovarian ultrasonography were enrolled to start the experiment. The synchronization protocol consisted of two doses of $PGF_{2\alpha}$ (150-μg D-Cloprostenol, Enzaprost D-C; Biogénesis-Bagó, Argentina) administered 12 hours apart on Day 0 to induce luteolysis, followed by a dose of GnRH (20-μg buserelin acetate, Gonaxal; Biogénesis-Bagó) 2 days later to stimulate ovulation of the preovulatory follicles present. Six days after the first GnRH dose, the cows started Ovsynch with an injection of GnRH. Seven days later, cows received two doses of $PGF_{2\alpha}$, 12 hours apart, to ensure luteolysis (completion of the modified synchronization protocol).

2.2.2. Groups and treatments

After synchronization, the cows were divided into five groups: C1 (control 1; n = 5), C2 (control 2; n = 5), P5 (5 days of follicular persistence; n = 5), P10 (10 days of follicular persistence; n = 5), and P15 (15 days of follicular persistence; n = 5).

Control cows (groups C1 and C2) received no additional hormonal treatment. Cows from group C2 were used to determine the time of ovulation, defined as Day 0 through a sequence of ovarian ultrasonography and blood samples (described in the following). On average, ovulation occurred around 4 days after administration of the first $PGF_{2\alpha}$ dose (range, 101–106 hours).

Treated cows (groups P5, P10, and P15) were administered a low dose of progesterone until 5, 10, and 15 days after the expected day of ovulation (Day 0). Progesterone was administered using an intravaginal progesterone-releasing device (750 mg of micronized progesterone; Pro-Ciclar P4-Zoovet) inserted 1 day after the first $PGF_{2\alpha}$ injection of Ovsynch. This device was kept in the cows for 5 days after the expected day of ovulation in group P5 and for 8 days in groups P10 and P15. In the latter two groups, a new intravaginal progesterone-releasing device was introduced 1 day before the removal of the first one, to maintain a more consistent concentration of progesterone throughout the treatment period. In group P15, a third intravaginal progesterone-releasing device was introduced on Day 11 of persistence, again 1 day before removal of the second one.