Theriogenology 79 (2013) 673–679

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Equine chorionic gonadotropin alters luteal cell morphologic features related to progesterone synthesis

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ARTICLE INFO

Article history:
Received 9 February 2012
Received in revised form 22 November 2012
Accepted 22 November 2012

Keywords:
eCG
Corpus luteum
Bovine
Mitochondria
Progesterone

ABSTRACT

Exogenous eCG for stimulation of a single dominant follicle or for superovulation are common strategies to improve reproductive efficiency by increasing pregnancy rates and embryo production, respectively. Morphofunctional changes in the CL of eCG-treated cattle include increases in CL volume and plasma progesterone concentrations. Therefore, we tested the hypothesis that eCG alters the content of luteal cells and mitochondria related to hormone production. Twelve crossbred beef cows were synchronized and then allocated into three groups (four cows per group) and received no further treatment (control) or were given eCG either before or after follicular deviation (superovulation and stimulation of the dominant follicle, respectively). Six days after ovulation, cows were slaughtered and CL collected for morphohistologic and ultrastructural analysis. Mitochondrial volume per CL was highest in superovulated followed by stimulated and then control cows (18,500 ± 2630, 12,300 ± 2640, and 7670 ± 3400 μm^3; $P < 0.001$), and the density of spherical mitochondria and the total number of large luteal cells were increased ($P < 0.05$) in stimulated cows compared with the other two groups (110.32 ± 14.22, 72.26 ± 8.77, and 70.46 ± 9.58 mitochondria per μm^3 and 678 ± 147, 245 ± 199, and 346 ± 38 × 10^6 cells, respectively. However, the largest diameters of the large luteal cells were increased in superovulated and control cows versus stimulated ones (32.32 ± 0.06, 31.59 ± 0.81, and 29.44 ± 0.77 μm; $P < 0.0001$). In contrast, the total number of small luteal cells was increased in superovulated cows (1456 ± 268, 492 ± 181, and 822 ± 461 × 10^6, $P < 0.05$). In conclusion, there were indications of cellular changes related to increased hormonal production (stimulatory treatment) and increased CL volume (superovulatory treatment).

1. Introduction

The bovine CL undergoes morphologic adaptations, manifested as increased luteal cell number and diameter, or biochemical changes (e.g., augmented expression of enzymes involved in progesterone synthesis [1]). Mitochondria are directly involved in steroidogenesis and their number and shape coincide with metabolic demands during the estrous cycle and various stages of embryo development [2,3].The use of exogenous gonadotropins to control gametogenesis and ovarian endocrine function has increased in the past 10 years [4]. Gonadotropins, which are glycoprotein hormones, include LH and FSH from the anterior pituitary, and hCG and eCG, which are currently widely used in human and veterinary medicine [5]. Exogenous eCG is used to superovulate cattle (dose, approximately 2000 IU) or to improve the quality of the

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0093-691X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2012.11.023

dominant follicle (stimulatory treatment; dose, 400 IU) during fixed time AI. Treatment with eCG affects the follicle and the ensuing CL, including increased CL diameter and function (namely, increased plasma progesterone concentrations [4,6]).

Plasma concentrations of both progesterone and estradiol are increased in cows given eCG [7–9]. The first steroidogenic step stimulated by gonadotropins is the transfer of cholesterol from the outer to the inner mitochondrial membrane, where it is converted to pregnenolone by the enzyme cytochrome P450 through cleavage of the cholesterol side chain [10–12]. This transport is mediated by several factors [13,14], although expression of steroidogenic acute regulatory (STAR) protein appears to be the limiting step [15–17]. Both STAR protein and mRNA expression were upregulated in cows given eCG, as was expression of peroxisome proliferator-activated receptor gamma protein and mRNA, an important regulator of lipid biosynthesis [18]. Although the capacity of small and large luteal cells to produce progesterone in response to gonadotropin has been already established [19], there are apparently no reports regarding the content of luteal cell populations and mitochondria volume and shape in CL after exogenous eCG for superovulatory or stimulatory treatments.

A marked increase in metabolic function might be characterized by changes in mitochondrial shape and number [20], and numbers of large and small luteal cells [3] to increase steroid production. Because progesterone plasma concentration increases in cattle treated with eCG [6,18], we hypothesized that eCG treatment for either single dominant follicle stimulation or for superovulation increases numbers of luteal cells and organelles involved in luteal progesterone synthesis.

2. Materials and methods

Twelve, nonlactating, crossbred (Nelore × Angus) cows aged between 2 and 5 years were used. These cows were kept on pasture and supplemented with ground corn (17.03%), soybean meal (4.65%), urea (1.13%), ammonium sulfate (0.11%), minerals (1.69%), salt (0.53%), and corn silage (74.86%). All procedures were approved by the Committee in Ethics for the Use of Experimental Animals of the Faculty of Veterinary Medicine and Animal Sciences, University of São Paulo, SP, Brazil (protocol number1637/2009). Before the experiment began, all cows were evaluated for body condition score on a scale of one to five [21] and for ovarian status by transrectal palpation, as described [22]. Only cows with a body condition score between 2.0 and 3.0 were selected.

2.1. Hormonal treatments

Cows were randomly distributed into three groups: control (N = 4), superovulated (N = 4), and stimulated (N = 4, Fig. 1). Briefly, on Day 0 (random day of the estrous cycle), all animals received an intravaginal device containing 1 g of progesterone (bovine intravaginal device; progesterone: 1 g; Primer, Technopec, São Paulo, SP, Brazil) and 2 mg estradiol benzoate im (Estrogin, Farmavet, São Paulo, SP, Brazil). On Day 8, intravaginal devices were removed from control and stimulated cows, and 0.15 mg of d-cloprostenol ($PGF_{2\alpha}$; Prolise; Arsa, Buenos Aires, Argentina) was given. Control cows did not receive eCG (Novormon; Syntex, Buenos Aires, Argentina), and those in the stimulated group received 400 IU on Day 8. At 48 hours after device removal, control and stimulated cows received 0.025 mg of lecirelin (GnRH; Gestran Plus, Arsa). Superovulated cows received 2000 IU of eCG on Day 4 and 0.15 mg of $PGF_{2\alpha}$ on Day 6. On Day 7, devices were removed, and another dose of $PGF_{2\alpha}$ was given, with 0.025 mg GnRH given 12 hours later. Six days after ovulation, cows were slaughtered, ovaries collected, and corpora lutea dissected for subsequent processing.

2.2. Light microscopy

After dissection, CL were submerged in 2.5% paraformaldehyde for tissue fixation and conventional embedding procedures. Paraffin blocks were cut into 3 μm sections using a microtome (Leica RM 2125 RT; Wetzlar, Germany), and sections were stained with hematoxylin-eosin for observation under a light microscope (Olympus BX 60, Olympus, Tokyo, Japan) at magnification ×400. Large and small luteal cells (LLC and SLC) were counted, and the largest diameter of these cells was measured with AxionVision 4.8 software (Cameras Zeiss AxioCam, Oberkochen, Germany). Micrographs were taken along the vertical and horizontal diameters, forming an imaginary cross where one field did not overlap with the next one, thereby preventing cells from being counted twice. Then, micrographs were randomly selected from three cows per group (total of 30 micrographs counted for each group). To determine numerical density of LLCs and SLCs, the following formulas (Eq. 1 and 2) were used:

$$A_T = AM \times NM/Mag^2 \quad \text{(Eq. 1)}$$

$$N_A = \sum Q/AT \quad \text{(Eq. 2)}$$

where (A_T) is the total area; A_M is the area of the micrograph; N_M is the total number of micrographs; Mag is the magnification; N_A is the numerical density; and $\sum Q$ is the total number of cells counted in 10 micrographs divided by the total area A_T.

The total number of SLCS and LLCs was estimated by multiplying the numerical density with CL volume (1495.18 ± 137.01, 1177.37 ± 167.07, and 830.33 ± 234.99 for superovulated, stimulated, and control cows, respectively).

Measurements of the largest diameter of the LLCs and SLCs were made for 200 cells per cow using the same micrographs. The mean of these values was calculated and used for further analysis.

2.3. Transmission electron microscopy

The CL were cut into four fragments from the periphery and four fragments from the center, each measuring 3 mm^3. All fragments were fixed in modified Karnovsky solution containing 2.5% glutaraldehyde (Merck, Darmstadt, Germany) and 2% paraformaldehyde (F1021-01 BL;