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Effect of cortisol on bovine oocyte maturation and embryo development *in vitro*

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ABSTRACT

Glucocorticoids (GCs) are important mediators of key cellular events. Herein, we investigated the effect of adding cortisol to the IVM medium on the acquisition of developmental competency in bovine oocytes. Cortisol (0.01, 0.1, or 1 μg/mL) had no effect on cleavage rates or cell numbers of resulting blastocysts; however, supplementation with 0.1 μg/mL during IVM increased blastocyst rates of *in vitro*–fertilized bovine oocytes as compared to untreated controls (41 ± 10% vs. 21 ± 1.2%, $P < 0.05$, respectively). This concentration was chosen to assess changes in the relative expression of potential GC target genes. Oocytes matured in the presence of cortisol and their corresponding cumulus cells did not show changes in expression for genes analyzed as compared to untreated controls. Notably, blastocysts from oocytes matured in cortisol-supplemented medium expressed higher relative levels of glucose transporter 1 (*GLUT1*), fatty acid synthase (*FASN*), and heat shock protein 70 (*HSP70*). This study supports a role for cortisol in the acquisition of bovine oocyte competence. This is evidenced by increased blastocyst development rates and presumably related to elevated embryonic transcripts with roles in glucose and lipid metabolism, as well as the cellular response to stress.

1. Introduction

Oocyte maturation is a long and complex process during which the female gamete acquires the competence to be fertilized as well as to sustain *in vitro* embryo development to the blastocyst stage and, likely, *in vivo* development to term [1–3]. *In vivo*, oocyte maturation occurs within the follicle driven by multiple local and systemic signaling events that require interaction between the oocyte and surrounding somatic cells [4]. Given the inherent challenge involved in trying to replicate these events, *in vivo*–matured oocytes display higher developmental competency than their *in vitro* counterparts [5,6].

Certain messenger RNA and protein expression patterns determine oocyte quality [7,8]. In this regard, it is known that IVC conditions used for oocyte maturation can influence gene expression and thus developmental competency [8,9]. Moreover, although much progress has been made in optimizing maturation media, *in vitro*–matured bovine oocytes rarely yield more than 30% to 40% blastocyst rates as compared to almost two times higher rates obtained with *in vivo*–matured oocytes under the same fertilization

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and embryo culture conditions [6,10]. Therefore, optimization of the oocyte maturation conditions via the addition of hormones and/or growth factors to the culture media is still a research priority [9,11–14].

Glucocorticoid (GC) hormones, in particular cortisol, are important mediators in many cellular events such as apoptosis modulation [15,16], response to stress [17,18], lipid and carbohydrate metabolism [19,20], and mitochondrial activity [21–23]. However, their role in ovarian physiology remains unclear. Studies in pigs and mice showed that supplementation of maturation media with cortisol inhibited or had no effect on oocyte developmental competency, respectively [24,25]. Conversely, a higher cortisol:cortisone ratio in follicular fluid of women undergoing IVF cycles was associated with higher embryo implantation rates [26,27]. Furthermore, in humans, GCs diffused from general circulation into follicular fluid where they were metabolized so that intrafollicular levels of the active metabolite cortisol were higher than those in blood, especially close to the LH peak [28]. On the basis of these findings, low doses of dexamethasone (a synthetic GC) are often administered to women as a cotreatment to increase the ovarian response in assisted reproduction cycles [29]. Altogether, these studies suggest that GCs may have a positive effect on oocyte maturation.

Transcripts for the glucocorticoid receptor (GR) and 11β-hydroxysteroid dehydrogenase type I, the enzyme that catalyzes the conversion of cortisone to its active metabolite cortisol, have been identified in bovine granulosa and theca interna cells from mature ovarian follicles [30]. Moreover, the concentration of cortisol in follicular fluid is highest around the LH peak in cow preovulatory follicles [31]. This suggests that GCs may also play an important role during folliculogenesis and oocyte maturation in the bovine species. Given the importance of improving *in vitro* embryo production methods and the evidence in regards to a potential role of GCs in the acquisition of oocyte developmental competency, the goal of this study was to evaluate the effect of different concentrations of cortisol during *in vitro* oocyte maturation and subsequent bovine embryo development.

2. Materials and methods

All chemicals and reagents were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA) unless otherwise stated.

2.1. Experimental design

Two experiments were conducted to understand the potential role of cortisol on bovine oocyte maturation. In experiment 1, embryo development rates were evaluated after IVF of oocytes incubated in IVM medium containing different cortisol concentrations. End point evaluations included cleavage and blastocyst rates, as well as blastocyst cell numbers. The cortisol concentration providing the best outcomes in experiment 1 was chosen for *in vitro* oocyte maturation in experiment 2. Mature oocytes, their corresponding cumulus cells, and resulting Day 8 blastocysts were used to assess the transcript levels for potential GC target genes, which included two genes related to mitochondrial function, cytochrome c oxidase subunit (*COX1*) and nuclear respiratory factor 1 (*NRF1*) [21,32–34]; one gene related to lipid metabolism or fatty acid synthase (*FASN*) [19,35], and one gene related to cellular stress or heat shock protein 70 (*HSP70*) [17,36,37]. In addition, only for embryos, we evaluated the expression of two additional genes with roles in carbohydrate metabolism or glucose transporter 1 (*GLUT1*) [20,38], and cellular respiration or mitochondrial transcription factor (*TFAM*) [22,32].

2.2. In vitro embryo production

2.2.1. Oocyte collection and IVM

Ovaries were obtained from a slaughterhouse and transported in 0.9% sodium chloride solution at room temperature for a maximum of 2 hours. Once in the laboratory, 2- to 8-mm antral follicles were punctured using a syringe and 18-ga needle and follicular fluid was aspirated. Only cumulus–oocyte complexes (COCs) with compact cumulus cells and good morphologic appearance were selected [39].

Groups of 35 to 41 COCs were matured in 400-μL droplets of TCM 199 supplemented with 25-mM sodium bicarbonate, 10% fetal calf serum (Gibco BRL, Grand Island, NY, USA), 11 mg/mL of pyruvate, 50 μg/mL of gentamicin, 0.5 μg/mL of FSH (Folltropin; Bioniche Animal Health, Belleville, Ontario, Canada), and 5 μg/mL of LH (Lutropin; Bioniche Animal Health). A cortisol (Sigma H0135) stock solution was prepared at a concentration of 50 μg/mL in ethanol, and 50-μL aliquots were stored at −20 °C until use. The final ethanol concentration did not exceed 0.1%, which should not affect embryonic development [24]. Thus, COCs were matured for 20 hours in the absence of cortisol (control group) or in the presence of different cortisol concentrations (0.01, 0.1, or 1 μg/mL). Incubations were performed at 38.5 °C in a 5% CO_2, 20% O_2, and 75% N_2 in humidified air atmosphere. Given that GC hormones may bind to plastic, COC maturation was performed in custom-made (1 cm in diameter) glass Petri dishes [40].

2.2.2. IVF and IVC

Frozen semen from one bull (*Bos indicus*) of proven fertility was used for IVF. After thawing, semen was centrifuged at 180 × *g* for 7 minutes through a discontinuous density gradient Percoll column (GE Healthcare Bio-Sciences, Uppsala, Sweden).

In vitro fertilization was performed in 80-μL droplets of TALP-Fert modified medium [41] supplemented with 30 μg/mL of heparin, 1.8-μM of epinephrine, 18-μM penicillamine, 10-μM hypotaurine, and 4 mg/mL of BSA under the same conditions described for IVM. Groups of 20 oocytes per droplet were inseminated with washed sperm at a final concentration of 2×10^6 sperm/mL.

At 26 hours after insemination, presumptive zygotes were denuded from their cumulus cells by repeat pipetting. Groups of 20 were then incubated over a cumulus cell monolayer in 100-μL droplets of synthetic oviductal fluid culture medium supplemented with 3 mg/mL of BSA, 50 μg/mL of gentamicin, and 5% FBS [42]. Incubations