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Effect of triiodothyronine on developmental competence of bovine oocytes

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ABSTRACT

Developmental competence of *in vitro*–matured bovine oocytes is a limiting factor in production of embryos *in vitro*. Several studies have suggested a potential positive effect of thyroid hormones on cultured oocytes and/or their supporting cells. Therefore, the aim of the present study was to ascertain whether medium supplementation with triiodothyronine (T3) improved subsequent developmental competence of *in vitro*–matured bovine oocytes. For this purpose, we first documented (using reverse transcription PCR) that whereas bovine cumulus cells expressed both thyroid hormone receptor (TR)-α and TRβ, immature bovine oocytes expressed TRα only. Thereafter, to test the effects of TH on developmental competence, abattoir-derived oocytes were matured *in vitro* in a medium containing 0, 25, 50, or 100 nM T3 and subjected to *in vitro* fertilization. Embryo quality was evaluated by assessing cleavage and blastocyst rates, morphological quality, development kinetics, and total cell number on Day 8 of culture. Notably, addition of 50 or 100 nM T3 to the *in vitro* maturation medium increased ($P < 0.05$) the rate of hatched blastocysts on the eighth day of culture, as compared with other groups (62.4 ± 11.7, 53.1 ± 16.3, and 32.4 ± 5.3, respectively). Next, the relative expression levels of genes related to embryo quality POU-domain transcription factor (*POU5F1*) and glucose transporter-1 (*GLUT 1*) were compared between *in vivo*– and *in vitro*–produced blastocysts. On the basis of the previous experiments, IVP embryos originating from oocytes that were matured *in vitro* in the presence or absence of 50 nM T3 were evaluated. The treatment had no effect ($P > 0.05$) on gene expression. We concluded that supplementation of bovine oocyte *in vitro* maturation medium with T3 may have a beneficial effect on the kinetics of embryo development.

1. Introduction

Oocyte maturation is a long and complex process during which the female gamete acquires the properties required to become fertilized and to sustain development to the blastocyst stage, altogether termed developmental competence [1,2]. *In vivo*, gonadotropins trigger the last stages of oocyte maturation, ultimately resulting in germinal vesicle breakdown (GVBD), arrest at metaphase II (MII), and ovulation in most mammalian species. Moreover, there is evidence that events occurring even before GVBD were also important for acquisition of developmental competence [3]. The improvement of *in vitro* oocyte maturation conditions to ensure optimal development to stages when embryos can be frozen or transferred offers the potential of obtaining more offspring resulting from assisted

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reproductive technologies in valuable livestock species [4]. Notably, *in vitro*–matured bovine oocytes are less developmentally competent than those matured *in vivo* [5,6]. Although improvements in culture conditions during *in vitro* oocyte maturation succeeded in obtaining a reasonable percentage of blastocyst stage embryos, many of these zygotes underwent a cleavage block during the first few cell cycles [7,8]. This is likely related to poor oocyte quality, which mostly depends on the changes during the maturation period, when oocyte competence is acquired [1,9].

Several reports have addressed the effects of supplementing culture media with hormones such as leptin, melatonin, and growth hormone in order to improve the developmental competence of *in vitro*–cultured bovine oocytes [10–12]. In addition, it was recently reported that the thyroid hormone (TH) triiodothyronine (T3) had a critical role in embryonic development in fish [13] and birds [14]. Further supporting these findings, TH receptors (TRs) were identified on pig, human, mouse, and chicken granulosa cells [14–17].

In mammals, *in vitro* studies on the action of T3 on the ovary have yielded conflicting results, perhaps due to species-specific differences or inconsistencies in TH doses used [18–20]. In one study, T3 acted on cumulus oophorus cells isolated from immature and adult mice by decreasing the FSH-driven aromatase activity in these cells and thereby reducing estradiol production [19]. In another study, murine preantral follicles co-cultured with increasing doses of T3 lost the ability to form an antrum, and the corresponding oocytes had a decreased ability to progress through meiosis beyond GVBD [18]. Conversely, when T3 and thyroxine (T4) were added to the *in vitro* culture (IVC) medium for bovine granulosa and theca cells, they exerted some positive effects on steroid production. For instance, both T3 and T4 stimulated mildly FSH- or LH-induced progesterone production by granulosa or theca cells, respectively. In the presence of insulin and LH, both THs increased androstenedione production in theca cells [21]. Similarly, T4 increased FSH-induced aromatase activity in porcine granulosa cells in culture, increasing production of progesterone, 17 beta-estradiol, and estrone [22]. Regarding the effects of TH supplementation on developmental competence of bovine oocytes, supplementing the embryo culture but not the *in vitro* oocyte maturation medium with T3 and T4 increased blastocyst and hatching rates, and increased viability of post-thaw embryos [20]. Nonetheless, this study did not address the individual effects of each TH, because both T3 and T4 were always combined in the culture medium.

The disparity in findings regarding the role of TH on ovarian function, paired with the fact that T3 is the active TH on cell metabolism and that its action on oocyte growth occurs presumably through its supporting cells, prompted us to study the effects of T3 on the acquisition of bovine oocyte developmental competence. For this purpose, we first ascertained whether both bovine oocytes and cumulus cells expressed TR. Then, various concentrations of T3 were tested for their ability to support oocyte maturation and subsequent developmental competence. Finally, relative expression levels of two genes important for normal mammalian embryonic development, glucose transporter-1 (*GLUT 1*) and POU-domain transcription factor (*POU5F1*), were measured in embryos derived from oocytes matured *in vitro* with or without T3-supplemented medium, and compared with those of *in vivo*–produced embryos.

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

Three experiments were conducted to evaluate the role of T3 on *in vitro* maturation (IVM) of bovine oocytes. Experiment 1 consisted of a qualitative analysis using real-time PCR to identify whether TRα and TRβ were present in immature oocytes and cumulus cells. In experiment 2, blastocyst rate, morphology, development kinetics, and the total number of cells were evaluated in embryos resulting from oocytes matured in media supplemented with various concentrations of T3. The T3 concentration, providing the best results in the experiment 2, was used in experiment 3, in which gene expression levels were analyzed to assess the effects of T3 supplementation during oocyte maturation on subsequent embryo quality. *In vivo*–produced embryos were used as positive controls for IVP embryos to compare the expression levels of genes related to embryo quality (*POU5F1* and *GLUT 1*).

2.2. In vitro embryo production

2.2.1. Oocyte collection and IVM

Bovine ovaries were obtained from a local abbatoir and transported to the laboratory in 0.9% saline solution at room temperature for ~2 hours. Cumulus-oocyte complexes (COCs) were recovered from small antral follicles (2–8 mm) by follicular aspiration. Only compact COCs with good morphological characteristics [5] were selected. Groups of 36 to 41 COCs were cultured in a 400-μL droplet of TCM-199 supplemented with 2.2 g/L sodium bicarbonate, 10% FBS (vol/vol; Gibco BRL, Grand Island, NY, USA), 11 mg/mL pyruvate, 50 μg/mL gentamicin, 50 μM beta-mercaptoethanol, 0.5 μg/mL FSH (Folltropin, Bioniche Animal Health, Belleville, ON, Canada), and 5.0 μg/mL LH (Lutropin, Bioniche Animal Health). In addition, a stock solution of T3 was dissolved in DMSO, stored at 22 °C, and then further diluted in TCM-199 medium and finally in IVM medium. Final concentrations of DMSO never exceeded 0.1%. The COCs were matured for 18 hours in the absence of T3 (control group) or in the presence of various concentrations of T3 (25, 50, or 100 nM) at 38.5 °C in a 5% CO_2 atmosphere in humidified air. Because T3 is liposoluble, COCs were cultured in four-well dishes without the addition of mineral oil. In addition, COCs were also matured in a 35-mm petri dish within 50-μL droplets covered with mineral oil in order to obtain a monolayer of cumulus cells to support embryo culture.

2.2.2. In vitro fertilization and IVC

After the 18-hour maturation period, COCs were co-incubated with frozen-thawed bull sperm (*Bos taurus*