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The presence of corpus luteum may have a negative impact on *in vitro* developmental competency of bovine oocytes

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ABSTRACT

The aim of the current study was to investigate the effects of the presence or absence of corpus luteum (CL) on *in vitro* developmental competence of bovine oocytes. In experiment 1, cumulus oocyte complexes (COCs) were collected from slaughterhouse ovaries and divided according to the presence (CL^+ oocytes) or absence (CL^- oocytes) of a CL in the ovary. Control oocytes (C group) were obtained from ovaries which were not selected toward the presence or absence of CL. All oocytes were submitted to *in vitro* maturation, fertilization and culture. In experiment 2, the oocytes from the CL^+ and CL^- ovaries were divided into grown (BCB^+) and growing (BCB^-) categories by means of the brilliant cresyl blue (BCB) test. The oocytes from all groups (CL^+/BCB^+, CL^-/BCB^+, CL^+/BCB^-, CL^-/BCB^- and control oocytes) were subjected to *in vitro* embryo production. In experiment 1, the cleavage and blastocyst rates of CL^- oocytes were higher than those of CL^+ oocytes (83.9% and 43% *vs.* 69.3% and 22.5%, respectively). In experiment 2, there was less BCB^+ oocytes (more competent oocytes) in the group of CL^+ oocytes than in the group of CL^- oocytes. Furthermore, developmental competence of all CL^+ oocytes (CL^+/BCB^+ and CL^+/BCB^-) was lower than that of all CL^- oocytes (CL^-/BCB^+ and CL^-/BCB^-). Thus, the presence of a corpus luteum in the ovary may have negative effects on developmental competence of ipsilateral oocytes.

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1. Introduction

Previous studies confirmed asymmetry in the function of reproductive system in dairy cows due to differences in ovarian activity and probably because of physiological differences in the tubular parts of reproductive organs which result from the side of the previous gestation [1]. It was found that there are both systemic and local (intraovarian) CL effects on follicular population [2,3]. An interaction between the development of bovine CL and the development of follicles was reported previously [4]. In dairy cows, the presence of CL was believed to affect estradiol concentration [5] as well as quantity and quality of follicles [6]. Follicles from the ovaries containing CL from a previous gestation had a smaller diameter than follicles from the ovaries without previous CL [7]. It is possible that CL directly inhibits folliculogenesis since more medium follicles was found during the bovine estrous cycle in the ovaries without previous CL than in the ovaries containing CL [8]. Moreover, the presence of CL was shown to locally suppress the number of antral follicles not growing beyond 3 mm in diameter in Western White Face ewes [2]. These observations indicate the existence of suppressive effects of progesterone from the CL on lifespan of dominant follicles [9].

Oocytes harvested from the ovaries of slaughtered cattle are commonly used to study *in vitro* embryo production (IVEP). The heterogeneity of the oocytes collected from growing follicles remains a challenge for *in vitro* maturation (IVM) success and limits the rate of embryo development. Due to unknown stage of estrous cycle, the quality of oocytes collected in slaughterhouse is variable [10]. Factors associated with heterogeneity in the developmental competence of oocytes have been extensively studied. It was reported that there was a significant effect of the stage of the estrous cycle and follicular status on *in vitro* developmental competence of buffalo oocytes [11]. More follicles and normal cumulus oocyte complexes (COCs) were found in the CL-absent ovaries than in the CL-present ovaries [12]. However, another study showed that the proportion of bovine oocytes that developed into blastocysts was higher in the absence of a dominant follicle (Days 2 and 10) comparing to the presence of a dominant follicle (Days 7 and 15) [13]. Reports on the effects of CL on the developmental potential of oocytes are still limited. Therefore, the aims of the current study were: (i) to assess the developmental competence of bovine oocytes originating from ovaries bearing a CL (CL^+ oocytes) or not bearing a CL (CL^- oocytes) and (ii) to examine heterogeneity in the developmental competence of CL^+ oocytes or CL^- oocytes with the use of the brilliant cresyl blue (BCB) test.

2. Materials and methods

This experiment was approved and performed under the guidelines of Ethics Committee for Animal Use of Razi University. Unless otherwise stated, all chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.1. Oocyte collection

The bovine ovaries were harvested from slaughtered cows (Holstein Friesian; 4–7 years old) with clinically normal reproductive tracts. COCs were harvested from ovarian follicles 3–8 mm in diameter [14]. Ovaries from pregnant females (pregnancy was diagnosed after slaughter by visual examination of fetal membranes, amniotic vesicle, cotyledons, fetus or changes in uterine tone and shape) and those with pathological lesions such as cystic follicles were not included in the current study. Only animals with a diestrus CL were included in the study [15]; the cows in diestrus had a CL on either right or left side. Moreover, artretic follicles or those with red fluid were discarded from the experiment [15]. The selected ovaries were transported to our laboratory within 2 h after slaughter in a thermos flask containing saline (30–35 °C) with penicillin (100 IU/mL) and streptomycin (50 mg/mL). The ovaries were divided into three groups: $1/CL^+$ (with CL) group, $2/CL^-$ (without CL) group and 3/C group (control group–oocytes were obtained from the ovaries which were not selected toward the presence or absence of CL). Control ovaries represent ovaries submitted to a routine ovarian classification which is used in bovine IVEP. The COCs were aspirated from follicles using 18-gauge needles attached to a 10 mL syringe. Only oocytes with a compact, multilayer cumulus cells and homogeneous ooplasm were used in the study.

2.2. Experimental design

Two experiments were conducted in the current study. Experiment 1 was conducted to assess the developmental competence of bovine oocytes ($n = 1581$) originating from ovaries bearing a CL (CL^+ oocytes), ovaries not bearing a CL (CL^- oocytes) and control ovaries. In experiment 2, ovaries different from those used in experiment 1 were divided into three groups: CL^+, CL^- and control ovaries to examine heterogeneity in the developmental competence of oocytes with the use of the brilliant cresyl blue (BCB) test. The BCB test was successfully used to differentiate grown and growing oocytes [10,16]. The BCB test determines the activity of glucose-6-phosphate dehydrogenase (G6PDH), an enzyme of the pentose phosphate pathway which is synthesized in growing oocytes (active G6PDH: BCB^-) but inactive in grown oocytes (inactive G6PDH: BCB^+) [17]. Oocytes lacking G6PDH reduce the blue dye. Previously, several studies showed that the percentage of BCB^+ oocytes developing to the morula and blastocyst stage was significantly higher than that of the control and BCB^- oocytes (cattle [18,19]; sheep [10,16]).

Immediately after collection, the oocytes were washed three times in Dulbecco's PBS, modified by addition of 0.4% BSA (mDPBS). Then, the COCs were exposed to 26 μM of BCB diluted in mDPBS for 90 min at 38.5 °C in humidified air [10]. This BCB concentration was found to be effective for cattle and sheep oocytes [10,19]. Following exposure to BCB, the COCs were transferred to mDPBS, washed twice and examined under a stereo zoom microscope (magnification 50×). The COCs were divided into two classes according to the cytoplasm staining; those with or without blue staining of the cytoplasm were designated as BCB^+ (CL^+/BCB^+ oocytes and CL^-/BCB^+ oocytes) and BCB^- oocytes (CL^+/BCB^- oocytes