

RELATIONSHIP BETWEEN CORPUS LUTEUM AND POSSIBILITY DAIRY COWS OOCYTES TO MATURATION *IN VITRO*

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Abstract. The aim of this study was to investigate the effects of corpus luteum (CL) on the developmental potential of cows oocytes. The ovaries of dairy cows were cut out immediately after slaughter and transported within one hour. After ovarian classification based on presence or absence of CL. We selected follicles which diameter, were medium (6-9 mm). Quality grading (A, B) of the oocytes was performed on the basis of cumulus cell development and homogeneity of cytoplasm according to Chaubal et al. (2006). A total of 125 COCs were aspirated from 124 ovaries. Among 72 COCs were aspirated of ovaries presence of CL, and 53 COCs absence of CL. Only Grade A and B oocytes COCs were used for maturation. A higher percentage of oocytes was collected from medium size follicles of ovaries with presence of corpus luteum (34.15 percent) than absence of corpus luteum ($p < 0.05$). In ovaries group with presence of CL, COCs matured 84.72 percent (Grade A and B). In ovaries group with absence of CL, COCs matured 64.15 percent (Grade A and B). A significant difference was detected between the presence and absence of CL group ($p < 0.05$).

Keywords: IVF, oocytes, cumulus cells, maturation.

Introduction. There are distinct problems associated with *in vitro* embryo production of bovine (Shabankareh et al., 2015). It has been clearly established that oocyte quality (intrinsic quality of primary oocyte coupled to maturation conditions used) determines the rate of blastocyst production (Alm et al., 2005). *In vitro* maturation (IVM) is the crucial step and an integral part of *in vitro* embryo production, because it influences oocyte quality, which subsequently affects embryonic development, fetal development, and even the health of the off spring (Eppig, 1996). In studies confirmed that be a relationship between the development of corpus luteum (CL) and the development of follicles which may cause asymmetry in the function of the reproductive organs in dairy cows. The presence of corpus luteum is associated with the luteal phase of the oestrus cycle or pregnancy if a conceptus present (Penitente-Filho et al., 2014). A. Boediono (1993) and A.H. Sugulle (2008) reported some discrepancies about positive and negative effects of CL on oocyte quality (Boediono et al., 1993; Sugulle et al., 2008). It appears that there are other factors associated with heterogeneity in the developmental competence of oocytes such as different stages of growth, atresia and different follicle sizes (Mirshamsi et al., 2013). In cows, it was reported that the size and the quality of the follicle of origin influence the developmental capacity of bovine oocytes (Beker-van Woudenberg et al., 2006). Therefore, interaction between follicle size and the phase of follicular wave affected the efficiency of embryo production (Machatkova et al., 2004).

In this study we wanted to research the effects of CL, interactions on the developmental potential of bovine oocytes were investigated.

Objective. The aim of this study was investigate the effects of corpus luteum on the *in vitro* developmental potential of cows oocytes from medium size of follicles.

Material and methods

The research was conducted in accordance with the

provisions of the Law of the Republic of Lithuania No. 1-2271 on Protection, Keeping and Use of Animals, dated 03/10/2012 (Valstybės žinios (Official Gazette) No. 122-6126 dated 20/10/2012) and of the by-laws, Education and training purposes of animals used in storage, maintenance and conditions of use No. B1-866, dated 31/10/2012 (Valstybės žinios (Official Gazette) No. 130-6595 dated 10/11/2012).

The ovaries of the cows were excised immediately after slaughter and transported to the laboratory, within 1 h. There was no information available regarding the health or physiological status of donors, except that they were the Lithuanian Black-and-White dairy cows (4 lactation).

After ovarian classification based on presence or absence of CL. We selected follicles which diameter, were medium (6-9 mm). Follicles were aspirated using a sterile syringe and 22 G needle. In such cases, fluid collected from follicles of the same category from the same ovary of the same animal was pooled.

Grading of the oocytes was done on the basis of cumulus cells investment and homogeneity of cytoplasm according to Chaubal et al. (2006). Grade A: Those with over 3 layers of cumulus cells encompassing the oocyte and uniform cytoplasm. Grade B: Those with less than 3 layers of cumulus cells encompassing the oocyte and uniform cytoplasm. Multilayered cumulus cells were selected, washed five times in the BO-IVM medium (Bioscience). Groups of cumulus oocyte complexes (COCs) were matured in 400 μ L of ready to use BO-IVM medium (Bioscience), covered with mineral oil, in four well plates (Minitub, Germany), for 24 h at 38.5 $^{\circ}$ C, and 5% CO₂ in air. After maturation (Day 0), matured COCs were washed and appreciate.

Results

A total of 125 COCs were aspirated from 124 ovaries. Among 72 COCs were aspirated of ovaries presence of

CL, and 53 COCs absence of CL. Only Grade A and B oocytes COCs were used for maturation. A higher percentage of oocytes was collected from medium size follicles of ovaries with presence of corpus luteum (34.15 percent) than absence of corpus luteum ($p<0.05$) (Figure 1).

In ovaries group with presence of CL, COCs matured

84.72 percent from 72 imatured COCs (Grade A and B). In ovaries group with absence of CL, COCs matured 64.15 percent from 53 imatured COCs (Grade A and B). A significant difference was detected between the presence and absence group ($p<0.05$). The mean of matured oocytes from ovaries on presence or absence of CL are presented in Figure 2.

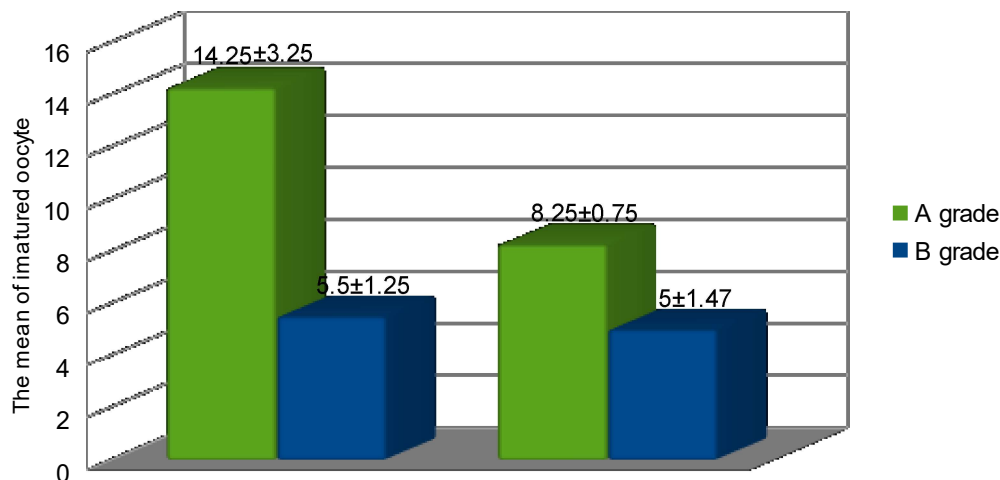


Fig. 1. The mean of imatured oocytes from different ovaries groups

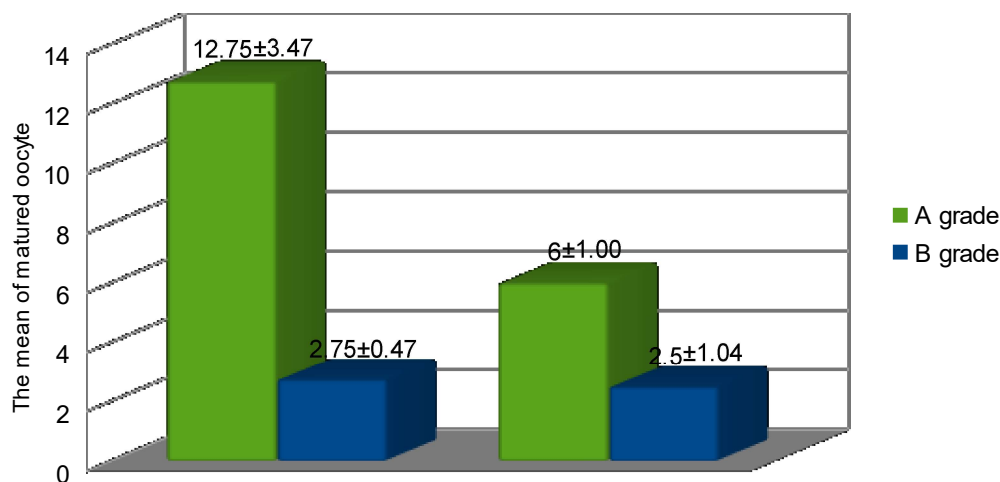


Fig. 2. The mean of matured oocytes from different ovaries groups

Discussion. Many factors have effect on *in vitro* maturation of cattle oocytes. There are many discrepancies about positive or negative effects of CL on reproductive parameters and the developmental potential of oocytes (Pirestani et al., 2010). J.M. Penitente-Filho (2015) reported that ovaries with CL showed greater numbers of good quality oocytes than ovaries without CL (Penitente-Filho et al., 2015).

I. Contreras-Solis (2008) reported the existence of not only a systemic, but also a possible intraovarian effect from the CL on follicular dynamics, with a greater decrease in the number of follicles growing to large sizes

in the ovary to the CL (Contreras-Solis et al., 2008). The same authors results support the existence of local inhibitory factors released from the CL. Inhibin, which is secreted by the CL of goats and cattle and secreted into ovarian venous blood of ewes, is widely known to affect ovarian follicular growth (Contreras-Solis et al., 2008; Sangha et al., 2002).

Our results showed that the presence of corpus luteum on ovary adversely affected total number oocytes and an average number of oocytes recovered compared with ovaries without corpus luteum, but the proportion of different quality oocytes was similar between both of

group. A higher percentage of oocytes was collected from medium size follicles of ovaries with presence of corpus luteum (34.15 percent).

Based on cumulus cells surrounding and homogeneous cytoplasm of oocytes, there was no significantly different between with or without corpus luteum groups based on the grade. On the contrary, the previous study in ruminant reported that the average number of good quality COCs recovered from ovaries with an absence of corpus luteum was comparably higher to the ovaries with a present of corpus luteum. In ovaries group with presence of CL, COCs matured 65.63 percent while in group absence of CL, COCs matured 67.68 percent. The negative effect of corpus luteum on the developmental competence of oocyte depends on follicle size (Kumar et al., 2004).

By our results there was significantly higher on average of COCs harvested from ovaries with corpus luteum compared without corpus luteum. In ovaries group with presence of CL, COCs matured 84.72 percent. In ovaries group with absence of CL, COCs matured 64.15 percent. The presence of corpus luteum did not affect the COCs quality and ability to reach the maturation stage.

Conclusion

Results showed that, the presence corpus luteum in the ovaries affect the average number of o availability oocytes. It was no correlation between COCs quality and subsequent with developmental potential oocytes *in vitro*. Moreover, ovaries with presence of corpus luteum considered as a suitable source a large number oocytes for *in vitro* embryo production.

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Received 20 May 2018

Accepted 11 June 2018