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Oocyte Quality and Subsequent *In Vitro* Maturation of Sheep Oocyte-Cumulus Complex from Ovary with Presence and Absence of *Corpus Luteum*

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Abstract

In vitro maturation is the crucial step for in vitro embryo production. It needs a large number of oocytes as source gamet cells recovered. The present study is aimed to assess the influence of corpus luteum on the average number oocytes harvested, COCs quality and subsequent maturation of immature oocytes recovered from sheep ovaries. Sheep ovaries were collected from local slaughterhouse and COCs were collected by using slicing method. Collected COCs were graded into three categories dependent upon cumulus cells surrounding them and the homogenous of cytoplasm. COCs were maturated in maturation media at 5% CO2 for 24 hours. Maturation of oocytes evaluated base on the expansion of cumulus cells and extrusion of the first polar body. There was significantly higher on average of COCs harvested from ovaries with corpus luteum compared without corpus luteum. The presence of Corpus luteum did not affect the COCs quality and ability to reach the maturation stage. However, there was a dramatic effect of cultured COCs quality on maturation rate both groups. Collectively, these results indicate that COCs quality is the main factor affecting the subsequent of oocytes matured in vitro.

Keywords: Corpus luteum; cumulus oocyte complex; in vitro maturation; maturation rate; ovaries.

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1. Introduction

In vitro embryo production (IVP) is a valuable tool for commercial livestock production, because increase the number offspring that can be produced from genetically valuable animals. However, IVP is also widely known in human fertility clinics to provide infertile couples with children. In vitro embryo production involves three steps: In vitro maturation (IVM), in vitro fertilization (IVF), and in vitro culture (IVC) [1]. IVM is the crucial step and an integral part of IVP In vitro maturation (IVM) is the crucial step and an integral part of IVP, because it influences oocyte quality, which subsequently affects embryonic development, fetal development, and even the health of the offspring [2]. In vitro maturation (IVM) is a reproductive technology whereby to collect oocytes from follicles in the ovary and be matured in a laboratory setting.

IVM is very important for human IVP to avoid the use of potentially harmful drugs to stimulate ovarian function. On human infertility treatment programs, candidate women require super ovulatory treatment for several days with high doses of hormones to acquire the optimum number of mature oocytes. This process can cause ovarian hyperstimulation syndrome (OHSS), which in severe cases can result in some complications such as thrombo-embolic disease [2] and kidney failure [3]. IVM also can be utilized in a number of research commercial and application technology such as nuclear transfer in cloning research, preservation of endangered species and transgenic animal production [4].

In IVM, a large number of good quality and high developmental competence oocytes must be recovered from ovaries to produce good embryos. The oocytes are recovered from both ovaries with presence or absence of corpus luteum. The presence of corpus luteum is associated with the luteal phase of the oestrus cycle or pregnancy if a conceptus is present. The previous study confirmed that there was a relationship between the development of corpus luteum and the development of follicles that may cause the heterogeneity in the developmental competence of oocytes [6].

Studies have been conducted to investigate the effect of the presence of corpus luteum on recovery rate of oocytes [5] and maturation rate in vitro [5a, 6]. However the results were still unclear and need further investigation. The aim of this study is to investigate the effect of corpus luteum on the recovery of oocytes in sheep and the relation between initial qualities of the oocytes to their subsequent maturation under in vitro conditions.

2. Materials and Methods

2.1. Collection of Cumulus-Oocytes Complex

Two independent experiments were conducted. In the first experiment, the effect of the presence or absence of corpus luteum on COCs quality was assessed, while in the second experiment the relation between initial quality of COCs and in vitro maturation was investigated. All sheep ovaries were collected from a local slaughterhouse and took to the laboratories in physiological saline (0,9%, w/v, NaCl) supplemented with 50 ug/ml gentamicin sulfate (Gibco; USA) at 35°C within 4 hours. COCs were collected from follicles in ovaries with or without corpus luteum by slicing method in Dulbecco's Phosphate Buffered Saline (DPBS; Gibco, USA) media supplemented with 5% Fetal Bovine Serum (FBS; Gibco, USA) and 50 ug/ml gentamicin sulfate/ ml (Gibco, USA). COCs were classified depending upon the number of cumulus cells surrounding them and homogeneous of cytoplasm. COCs that have more than 4 layers of cumulus cells were graded as grade 1, 3-4 layers were grade 2 and 0-2 layer were grade 3. COCs were transferred to 35 mm petri dish and then washed twice in maturation media. COCs selection and manipulation were performed at room temperature

2.2. In Vitro Maturation

COCs were washed twice in maturation medium (25mM HEPES-buffered Tissue Culture Media 199 (TCM 199; Sigma USA) supplemented with 10 IU / ml pregnant mare serum gonadotrophin (PMSG) (Intergonan, Intervet Deutschland GmbH), 10 IU / ml human chorionic gonadotrophin (hCG) (Chorulon, international Intervet BV Boxmeer-Holland) and 50 ug / ml gentamycin (Sigma, USA)). Five to ten COCs from all grades were introduced to 50µl maturation media and covered by mineral oil (Sigma, USA) in a 35 mm petri dish. The COCs were cultured for 24 hours in incubator (5% CO_2; 90% humidity; 38°C). Each COCs was examined under a stereomicroscope and evaluated for maturation based on cumulus cell expansion and extrusion of the first polar body.

2.3. Data Analysis

All data were analyzed using SPSS software version 16 (SPSS Inc, Chicago, IL, USA). The effect of the presence of corpus luteum on recovery, grade and maturation rate of COCs were analyzed using Student's t-test. For the effect of initial quality of COCs on subsequent IVM were analyzed by analysis of variance (ANOVA). Tukey's HSD test. post-hoc

multiple comparison tests were performed to evaluate the differences between the control and experimental groups Different in mean were considered significant at $p < 0.05$.

3. Result and Discussion

The oocyte collected for this study was immature oocyte and graded base upon cells cumulus surrounding them and cytoplasm homogeneity. Total number of COCs, average number of COCs recovered each ovary, different grades of COCs from ovaries with presence and absence of corpus luteum are presented in Table 1.

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. Our observation also have been supported by the previous studies in buffalo [5b-d, 6], bovine [7] and goat [8]. However, they recovered fewer oocytes per ovary compared with the number oocytes harvested in our experiment. The different may be caused by the different of species, season and method of collection.

The presence of corpus luteum in the ovaries was almost identical that source of COCs obtained from luteal phase or pregnant female[9]. The lutein cell of corpus luteum increases synthesis progesterone which inhibits oestrus and gave the negative effect on the anterior pituitary to secrete follicle stimulating hormone (FSH) [10]. Inhibin, that known increase the follicular androgen production synergistically with luteinizing hormone (LH), was also secreted in high concentration during the luteal phase. Furthermore, there is a relationship between the development of corpus luteum and the development of follicle [11]. The presence corpus luteum in the ovary may contribute to unfavorable condition for follicular growth [12]. As a result, follicle regressed and led to lower COCs recovered from ovaries with a presence of corpus luteum.

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 [5a]. The difference may be caused by the different follicle size when oocytes collected. The negative effect of corpus luteum on the developmental competence of oocyte depends on follicle size. It means that the negative effect of corpus luteum just influence the oocytes from small and medium follicle [7].

All of the oocytes recovered from both of groups were cultured for 24 hours in maturation media to observed oocyte maturation rate. The number of oocytes matured from ovaries with presence and absence of corpus luteum showed in Table 2.

Our result showed that the present of corpus luteum on the ovaries significantly did not affect the oocyte ability to reach the maturation stage. Our results were in agreement with previous studies that found the presence of corpus luteum and pregnancy did not affect the maturation and meiotic competence of oocytes [5b, 6]. The two important aspect of oocytes meiotic competence are the maturation of cytoplasmic and nuclear [13]. Both of these processes are essential for the formation of an oocyte with the capacity to undergo fertilization and development to live offspring. Nuclear maturation encompasses the processes of reserving meiotic arrest at prophase I and driving the progression of meiosis to metaphase II. Cytoplasmic maturation refers to the processes that prepare the oocytes for activation and preimplantation development [14].

To determine the effect of COCs morphology with or without corpus luteum, COCs were classified based on surrounding cumulus cells and cytoplasm homogeneity. The effect of initial quality of COCs on subsequent IVM in ovaries with presence of corpus luteum is depicted in Table 3.

There was no difference in the proportion of matured oocytes after IVM of cultured grade 1 and grade 2 of COCs but the maturation rate was further reduced in grade 3 of COCs ($p<0.05$). Our results showed that the presence of cumulus cells and homogenous cytoplasm significantly affected the maturation rate of the oocytes. In the previous study, it was found that the culture of COCs with more than 3 layers of cumulus cells and homogenous cytoplasm obtained a higher maturation rate compared to oocytes with less than 3 layers or without of cumulus cells with irregular cytoplasm in some of species[15], example: cattle [16], cats [17], canine [18] and bovine [19].

Our study showed that there is positive correlation between of number of cumulus cells layers and homogeneity of cytoplasm to maturation rate of COCs. The gap junction between cumulus cells and oocytes are thought to be essential for oocyte maturation and fertilization. During oocyte nuclear maturation, gap junction is the main connection between cumulus cells and oocytes [20]. The cumulus cells provide the oocyte with nutrients and regulatory signals to facilitate the progression of maturation.

Table 1: The Effect of Corpus Luteum on Oocytes Recovery and Grade of COCs.

Group	Number of Ovaries	Number of Recovered Oocytes	Average Number of Oocytes / Ovary	COCs Quality		
				Grade 1	Grade 2	Grade 3
Corpus luteum (+)	22	143	6.48±0.30[a]	61 (42.54%)[a]	60 (42.56%)[a]	22 (14.90%)[a]
Corpus luteum (-)	22	232	10.55±2.48[b]	87 (37.50%)[a]	91(39.22%)[a]	54 (23.28%)[a]
Note: A different superscripts values indicates statistically significant different ($p < 0.05$).						

Table 2: The Effect of Corpus Luteum on Oocytes Maturation.

Group	Number of Cultured Oocytes	Number. of Matured Oocytes	Percentage of Matured Oocytes matured
Corpus luteum (+)	143	94	65.63 %[a]
Corpus luteum (-)	232	158	67.68%[a]
Note: A different superscripts values indicates statistically significant different ($p < 0.05$).			

4. Conclusion

As a conclusion, the presence or absence corpus luteum in the ovaries affect the average number of oocytes availability with no correlation to COCs quality and subsequent of oocytes matured in vitro. Maturation is correlated with initial quality of COCs

Table 3: The Effect of Initial Quality of COCs on Subsequent IVM in Ovaries with Presence of Corpus Luteum.

Group	Number of Cultured COCs	Number of Matured COCs	Percentage of Matured COC
COC recovered with corpus luteum			
Grade 1	61	44	71.58%[a]
Grade 2	60	42	70.20%[a]
Grade 3	22	8	36.81%[b]
COC recovered without corpus luteum			
Grade 1	87	67	77.22%[a]
Grade 2	91	65	71.15%[a]
Grade 3		24	44.98%[b]
Note: A different superscripts values indicates statistically significant different ($p < 0.05$)			

cultured. Moreover, ovaries with an absence of corpus luteum considered as a suitable source a large number oocytes for in vitro embryo production.

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