MATURING SINGLY VERSUS IN GROUPS ON OVINE OOCYTE MEIOSIS

PEMBELAHAN MEIOSIS SEL TELUR DOMBA YANG DIMATANGKAN SECARA TUNGGAL DAN KELOMPOK

Endang Tri Margawati

R&D Centre for Biotechnology LIPI, Jl. Raya Bogor Km. 46 Cibinong 16911 INDONESIA,
Telp. (021) 8754587, Faks. (021) 8754588, e-mail : endangtri@hotmail.com

ABSTRACT

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The effect of maturing systems (single vs groups) of ovine oocytes was studied to examine the oocyte development *in vitro*. Oocytes were aspirated from follicle ovaries collected at local abattoirs Cibinong West Java, using a 18-G needle. Aspiration medium consisted of H199 + 2 % FCS + 50 μg/ml Heparin and the maturating medium (IVM) consisted of B199 + 10 % FCS + 10 μg/ml FSH + 10 μg/ml hCG + 1 μg/ml Estradiol. The oocytes collected were divided into three groups and treated separately as follows: T1) oocytes were matured singly in 50 μl IVM medium, T2) every five oocytes was matured in 50 μl IVM medium and T3) every 10 oocytes was matured in 50 μl IVM medium. All oocytes were maintained in incubator with 5 % CO_2 and high humidity at 38 °C for 20 h. The resulting ova were stained in 1 % lacmoid then examined for meiosis division under a microscope. There was a significant effect among treatments on the proportion oocytes reaching metaphase II ($P<0.05$). The oocytes matured singly showed a lower proportion of the metaphase II compared to those matured in groups. Oocytes cultured singly tended to arrest in the metaphase I and anaphase I. Oocytes matured in groups (5 to 10 oocytes per 50 μl IVM drop) resulted a higher proportion in achieving the metaphase II stage compared to those matured singly.

Key Words : ovine oocyte, *in vitro* maturation, maturing system, oocyte meiosis

ABSTRAK

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Pengaruh sistem pematangan (tunggal vs kelompok) oosit telah dipelajari terhadap perkembangan sel telur domba *in vitro*. Sel telur domba diambil dari folikel ovarium yang dikumpulkan dari rumah pemotongan hewan di Cibinong Jawa Barat menggunakan jarum suntik 18-G. Media pengumpul mengandung H199 + FCS 2 % + Heparin 50 μg/ml dan media pematangan (IVM) mengandung B199 + FCS 10 % + FSH 10 μg/ml + hCG 10 μg/ml + Estradiol 1 μg/ml.. Sel telur dibagi tiga kelompok dan diberi perlakuan sebagai berikut: T1) sel telur dimatangkan secara tunggal dalam 50 μl media IVM, T2) setiap lima sel telur dimatangkan dalam 50 μl media IVM dan T3) setiap 10 sel telur dimatangkan dalam 50 μl media IVM. Semua pematangan berlangsung di inkubator 5 % CO_2 dengan kelembaban tinggi pada suhu 38 °C selama 20 jam. Setelah pematangan, sel telur diwarnai dengan lacmoid 1 % dan meiosis diamati dibawah mikroskop. Hasil studi menunjukkan terdapat perbedaan nyata diantara perlakuan terhadap proporsi sel telur yang mencapai tahap metafase II ($P<0,05$). Sel telur yang dimatangkan secara tunggal cenderung tertahan perkembangannya pada metafase I dan anafase I. Sel telur yang dimatangkan secara kelompok (5 sampai 10 sel telur per 50 μl media IVM) menghasilkan sel telur yang mengalami metafase II lebih tinggi dibandingkan yang tunggal.

Kata-kata Kunci : sel telur domba, pematangan *in vitro*, sistem pematangan, pembelahan meiosis sel telur

INTRODUCTION

Development in reproductive technology proved that mass production of *in vitro* produced embryos is enabled by performing a technology of *in vitro* fertilization. However, a major disadvantage of using abattoir ovaries is unavailability of the dam characteristics (Lazzari and Galli, 1996). Recently, a technique has been introduced to aspirate oocyte from living donors which is referred to ovum pick up (OPU). This technique is very commonly applied to human oocyte collection. In cattle, the oocytes can be obtained from juvenile, heifer or cow.

Using the OPU procedure suffers from a highly variable and unpredictable yield of oocytes both collected from treated or untreated animals prior to collection and it often result in a low number of oocytes. This problem has contributed to the idea of this study. A local problem in obtaining abattoir

ovaries has also contributed to support this study since only culled or non-reproductive cows are allowed to be slaughtered in Indonesia (Margawati *et al.*, 1997). Therefore, to maximize the potential applications of OPU procedures, and to cope with the local problem in collecting abattoir ovaries, it is necessary to elucidate the effect of maturing oocyte systems on the oocyte development *in vitro*. Oocytes collected either from live or death animals are generally immature and unpredictable in the preovulatory development of follicles and as a consequence, those oocytes need to be matured prior to fertilization *in vitro*. This *in vitro* maturation of oocytes are to meet the IVF requirements of availability of the matured oocytes, capacitated sperm and acrosome reaction of sperm (Parrish and First, 1993).

Based on the above statements, maturing oocyte systems singly vs in groups were conducted in the ovine oocyte to provide a number of the matured oocytes originated from immature oocytes collected from abattoir ovaries. This study was also prepared for further study in embryo development derived from a small number of oocytes collected from living animal.

MATERIALS AND METHODS

Oocyte Collection

Ovine oocytes were collected from unidentified ewe ovaries slaughtered at the local abattoirs at Cibinong of West Java. The ovaries were transported in the saline held at 30 °C to the laboratory. Oocytes were aspirated from any diameter of follicles using a 18-G needle with aspiration medium of Hepes buffered 199 (H199) + 2 % Fetal Calf Serum (FCS) + 50 μg/ml Heparin. The oocytes were handled in H199 + 10 % FCS before washed in bicarbonate buffered 199 (B199) + 10 % FCS and finally washed in IVM medium prior to maturation *in vitro*.

In vitro Maturation of Oocytes

Maturation medium used comprised of B199 + 10 μg/ml Follicle Stimulating Hormone (FSH) + 10 μg/ml human Chorionic Gonadotropin (hCG) + 1 μg/ml Estradiol (E2). Only selected oocytes were allowed to undergo the designed experiments. Oocytes were divided into three groups and treated separately as follows: T1) oocytes were matured singly in 50 μl of maturation drop (IVM drop), T2) oocytes were matured in group of five oocytes per 50 μl IVM drop and T3) oocytes were matured in group of 10 oocytes per 50 μl IVM drop. All oocytes were maintained for 20 hours at 38 °C in the incubator with 5 % CO_2 in air and high humidity.

Fixing and Staining Oocytes

Adherent cumulus cells were removed mechanically using a flame-drawn pipette tip or by vortexing. Denuded oocytes were washed in washing or handling oocyte medium. Every 10-oocyte was placed in a very small volume on a slide glass and covered by a coverslip. A fixative solution of acetic acid and ethanol (1 : 3) was allowed to flow under the coverslip prior to complete immersion in fixative solution for 48 hours. The oocytes were stained with 1 % lacmoid solution for about 1 to 2 minutes then washed with 45 % acetic acid. Determination of meiosis division was undertaken under a microscope.

Statistical Analysis

The experiments were designed in a randomized block design with three treatments (one, five or 10 oocytes in 50 μl of IVM drop) and seven replications of each treatment. Block was referred as replication, each block contained of each treatment of 1, 5 or 10 oocytes. Collected data in percentages of metaphase I, anaphase I, telophase I and metaphase II were analyzed using ANOVA in the Statistical Analysis Systems (SAS) package to calculate means and standard errors for the means (SEM). Differences among treatments were tested by Tukey's Test at the significant levels of 5 % to 1 % ($P<0.05$ to $P<0.01$).

RESULTS AND DISCUSSION

The distribution of meiotic division at maturing oocytes singly versus in groups is shown in Table 1. There were significant differences for the proportion of metaphase I and anaphase I ($P<0.05$) while maturing systems did not affect for the proportion of telophase I derived from the immature ovine oocytes matured singly or in groups ($P>0.05$). The result shows when oocytes matured singly, the oocytes were arrested at the division phases of metaphase I or anaphase I, and only 33 % oocytes underwent metaphase II (matured oocyte). These patterns of meiotic division indicate that maturation oocytes singly may need some extra substances out of FSH, LH and estradiol. As reported by Baker *et al* (1977), those components of maturation medium are effective in inducing meiotic division and progression to metaphase II. Supplementation of growth factors has been proposed to promote oocyte maturation *in vitro* in the presence of steroids and gonadotropin (Dekel and Sherizly, 1985; Downs *et al.*, 1988; Feng *et al.*, 1988). A recent study reported that concentration of 1000 or 2000 units/ml Leukemia Inhibitory Factor (LIF) in a modified TCM-199 containing FSH, LH and estradiol improved the proportion of immature oocytes that reached metaphase II (Margawati, 1995).

There was a significant difference ($P<0.05$) in maturing oocytes singly and groups in the proportion of immature oocytes achieving the matured oocytes (metaphase II). The table shows that the oocytes matured in groups (T2 and T3) increased the proportion of oocytes at the metaphase II (60 and 70 %, respectively) compared to the oocytes matured singly (T1= 33 %). As suggested by O'Doherty *et al* (1997), co-operation between each other is needed for better development of oocytes. In addition, co-culture with granulose

Table 1. The effect of maturing oocytes singly or in groups on the achievement of ovine oocytes into matured oocytes (Mean±SEM)

Treatment	Number Of Oocytes	Meiosis Division (%)			
		Metaphase I	Anaphase I	Telophase I	Metaphase II
T1	7	21.54±3.24[a]	41.90±2.29[a]	2.86±1.84[a]	33.69±2.16[a]
T2	35	10.03±2.19[b]	24.21±1.78[b]	4.30±2.01[a]	60.17±3.36[b]
T3	70	7.14±2.86[b]	15.71±2.97[c]	7.14±1.84[a]	70.00±3.78[c]

Means with different superscripts within column differ significantly ($P<0.05$), SEM = Standard error for the means

cells is needed for optimum development. This support system may be in the form of autocrine or paracrine factors of growth factors which are lacking in smaller groups. A recent opinion, however, has argued the use of co-culture in media since in general there is no evidence for any embryotrophic activity and it may be needed for inappropriate culture media or problem in their preparation (Bavister, 1992).

The use of maturation medium for oocyte maturation has strong contribution in the development of embryo production. Bavister *et al* (1992) promoted the use of defined culture media (without serum or somatic cell conditioning) for bovine oocyte maturation on subsequent embryo development. Their findings showed that SFRE, TCM-199 and the three MEM-containing media were similar in the proportion of oocytes able to reach the 2-cell stage following IVF.

Even though the role of cumulus cells during *in vitro* maturation remains obscure, there was no maturation or a low maturation rate of bovine oocytes when the cumulus cells were removed prior to oocyte culture *in vitro* (Leibfried and First, 1979; Fukui and Sukuma, 1980; Dahlhausen *et al.*, 1981). Crister *et al* (1986) reported that compared to nude or corona-enclosed oocytes, *in vitro* maturation of cumulus enclosed bovine oocytes yielded a significantly higher proportion of embryos following IVF.

Growth factors found within the ovary (Hammond *et al.*, 1985; Skimmer *et al.*, 1987), may act both as *autocrine* and *paracrine* regulators of ovarian function (Hammond *et al.*, 1988; Carson *et al.*, 1989). There is a possibility of collaborative action of gonadotropins with growth factors (Harper and Brackett, 1993b). The positive influence of epidermal growth factor (EGF) during *in vitro* maturation of bovine oocytes resulted in an increase of the proportion of oocytes that were able to undergo cleavage and development to the blastocyst stage (Harper and Brackett, 1993a). A combination of platelet-derived growth factor (PDGF) with FSH increased the proportion of matured and fertilized oocytes developing into blastocysts (Harper and Brackett, 1993b).

CONCLUSION

Using maturation medium of bicarbonate buffered 199 containing FSH, LH and estradiol, ovine oocytes matured in groups, resulted better number in achieving oocytes into matured oocytes compared to oocytes matured singly. It may need to try to use co-culture or to add growth factors into maturation medium in promoting ovine immature oocytes matured singly into metaphase II (matured oocytes).

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