Maturation Rate of Cattle Oocytes in Different Quality

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Abstract: This study was conducted to analyze the relation between maturation success and oocyte quality. Overall 1235 cattle oocytes, which were classified as quality A (n=420), B (n=400) and C (n=415), were matured for 24 hours in the maturation medium, which consisted of tissue culture medium (TCM 199) commercial solution with 3 mg/ml bovine serum albumine (BSA Frak. V), and 100 ng/ml epidermal growth factor (EGF). Maturation rates were 94.28, 62.75, and 0% in quality A, B and C oocytes, which were classified according to cumulus expansion, respectively. Additionally the maturation rate was 89.52, 55.75, and 1.45% in quality A, B and C oocytes according to first polar body excretion classification, respectively. As a result, it was found that the maturation rate decreased as the oocyte quality decreased.
Keywords: *Cow oocyte, oocyte quality, maturation*

Farklı Kaliteli Sığır Oositlerinde Maturasyon Oranları

Özet: Bu çalışma, sığır in vitro embriyo üretiminin en önemli aşaması olan maturasyon başarısının oosit kalitesi ile ilişkisinin araştırılması için yapılmıştır. Bu amaçla 420 adet A, 400 adet B ve 415 adet C kalite olmak üzere 1235 sığır oositi, doku kültür medyumu (TCM 199) ticari solüsyona 3 mg/ml oranında sığır serum albumini (BSA Frak. V) ve 100 ng/ml epidermal growth faktör (EGF) ilave edilmiş maturasyon vasatında 24 saat mature edildi. Maturasyon oranları; tam kumulus ekspansiyonu şekillenme kriterine göre, A kalite oositlerde % 94.28, B kalite oositlerde % 62.75, C kalite oositlerde % 0 olarak, birinci kutup hücresi atılım kriterine göre ise sırasıyla % 89.52, % 55.75, % 1.45 olarak bulundu. Sonuç olarak sığır oosit maturasyon oranının oosit kalitesi düştükçe azaldığı kanaatine varıldı.
Anahtar Kelimeler: *İnek oositi, oosit kalitesi, maturasyon*

Introduction

Assisted reproductive techniques are frequently used for improvement of livestock productivity and embryo production process. However, the success of in vitro embryo production and pregnancy rates are low in laboratory conditions. This is generally associated with absences in maturation process and inadequacy in culture media used for embryo production (Akyol and Sulu, 2005). Generally, the maturation is defined as the conversion of diploid (2n) chromosomes to haploid (n) form and having fertilization ability depend on organelle formation during the process (Xu et al., 1986).

The fertilization ability and embryonic development of the bovine oocytes are influenced by in-vitro conditions (Bavister et al., 1992; Brackett and Zuelke, 1993; Gordon, 2003). In the maturation process, the granulosa and theca cells, which are named as cumulus oocyte complex (COC), support to oocyte for in resting and growing phase (Gordon, 2003). The oocyte is in both direct and indirect interaction with these cells via gap junctions, which allow the intercellular passage of ions, and hormones and growth factors, respectively (Hagemann, 1999). Furthermore, the cumulus cells have a regulatory role in RNA synthesis (Soom et al., 2002).

In all maturation process, the maturation and fertilization abilities of an oocyte are evaluated with the follicular environment such as follicular size, presence of cumulus size and COC morphology, which are used as criteria for prediction of oocyte maturation capacity (Gordon, 2003). In evaluation, oocytes, which have homogenous cytoplasm, intact and compact cumulus cells that surround the oocyte are accepted to be suitable for a success maturation process (Akyol, 2005; Kanagawa, 1995). Following to culture conditions, the treated oocytes, which achieved to cumulus expansion (the dissemination of cumulus cells when manipulated with the tip of a pipette) and have first polar body, are considered as maturated oocytes. As a result, oocyte

morphology is accepted an important factor on success of maturation process (Gordon, 2003).

The aim of this study was to analyze the relationship between maturation success of bovine oocytes and initial oocyte quality.

Materials and Methods

Recovery and transport of ovaries

This study was conducted on 1235 bovine oocytes, which were collected from 6416 follicles from 786 ovaries from the slaughtered cows and heifers by aspiration method. The ovaries, which were removed from the cow and heifer carcasses, were brought to the laboratory within 3 hours in a vacuum flask which contained 500 ml of 0.9% NaCl solution at 37°C and supplemented with 100 IU penicilin/ml and 100 μg streptomycin/ml. The ovary tissues which were brought to the in vitro fertilization laboratory were washed twice using 0,9% NaCl at 37°C for separation from blood and transport medium and were left to dry on blotting paper which were laid on heating trays with their temperature set to 37°C.

Preparation of transport and culture media

Preparation of the ovary transport medium: 0,9% isotonic solution was prepared as transport medium, by adding 4,5 gr NaCl into 500 ml distilled water 12 hours before going to the slaughterhouse, according to Chung (1999). After the solution was incubated at 37°C incubator for, 0.5 ml of antibiotic-antimycotic solution (Biol. Ind. 03-033-18) was added just before ovary collection (1ml/lt).

Preparation of the ovary-washing medium: The collected ovaries were washed with 0.9% NaCl, which is stored at 37°C 12 h before aspiration Chung (1999).

Preparation of oocyte maturation medium: The maturation medium was prepared by adding 3 mg/ml bovine serum albumine (S4190325, Biol. Ind.) into TCM 199 commercial solution (M7528, Sigma-Aldrich Co.), according to Guang Peng Li et al., (2004). An antibiotic solution, which consisted of 100 IU penicillin and 100 μg streptomycin, was added into the IVM (in vitro maturation) media, 12 hours before use. Furthermore, 100 ng/ml epidermal growth factor (EGF) (E9644, sigma-Aldrich Co.) was added into the media as supplements. The pH of the maturation medium was kept between pH 7.2-7.4 and its osmolarity was kept between 270-295 mOsm.

Follicle aspiration and oocyte selection: The aspiration procedure was performed using 10 ml plastic syringes onto which 18 G cannulas were attached, according to Keskintepe and Bracket (2000). Peripheral follicles between 2-8 mm were preferred for aspiration. 0Maturation medium (0. 5 ml) was drawn into the syringe before aspiration. The follicle fluids, which were collected inside 15 ml conical centrifuge tubes, were kept in water bath. These tubes were kept at 39°C incubator for 5 minutes for the oocytes to sink to the bottom of tube. At the end of this period, the supernatant liquid was removed and 5 ml of IVM medium was added. Thus the oocytes were washed and separated from other cells. The washing process was repeated 3 times.

One ml of the IVM medium, which was left at the bottom of the tube, was transferred via pipettes to sterile petri dishes, which were sized 60x15 mm and heated to 37°C on the heating tray on laminar flow.

The collected oocytes were classified under stereo microscope (Leica M205C, Germany) according to procedure described by previous reports (Brackett and Zuelke, 1993). The oocytes, which had an intact zona pellucida, a cytoplasm with uniform sandy appearance, and surrounded by 4 or more layers of cumulus cells were classified as A quality (n= 420). The oocytes, which were surrounded by 2-4 layers of cumulus cells or a few layers of cumulus cells and had an ooplasm with sandy or light sandy appearance were classified as B quality (n=400), and the oocytes which were not surrounded by cumulus cells and had a homogenous gel appearance were classified as C quality (n=415).

Maturation of oocytes: The prepared maturation medium was cultured in 5% CO_2 environment at 38.5 °C with maximum humidity for 6-8 h before maturation. During the maturation process, each 10 oocytes were inserted into a 50 µl drop of IVM medium and incubated under sterile mineral oil and the oocytes were incubated in in 5% CO_2 environment at 38.5 °C with maximum humidity. for 24 h. Maturation rates

were determined according to first polar body procedure, which described above.

Statistical Evaluation

The variation between oocyte quality and the maturation rates were evaluated using chi square test in the SPSS (Statistic Packet of Social Science) 14.01 (serial: 9869264) program and statistically significance were evaluated at P<0.01

Results

The maturation rates were 94.2, 62.75 and 0% for quality A, B, C oocytes according to the full cumulus expansion criterion (figure 1). On the other hand, maturation rates were 89.52, 55.75, 1.45% for quality A, B, C oocytes according to first polar body excretion (Table 1).

Table 1. The rate of cumulus expansion and first polar body excretion in different quality oocytes maturations.

Quality of oocytes	n	FCE (%)	PPC (%)
A	420	396 (9.28)[a]	376 (89.52)[a]
B	400	251 (62.75)	223 (55.75)
C	415	0 (0)[b]	6 (1.45)[b]

FCE: Full cumulus expansion, PPC: Primary poll cell, a, b and c: Rates with different letters in the same column are statistically significant (P<0.01; P=0.0061).

Figure 1. Cumulus expansion (a) and primary poll cell excretion (b) (40X0,50)

Discussion

At the study that was presented, the aspiration success rate was 57.28%, the number of oocytes which were obtained from a single ovary tissue was 4.68. It was observed that the values which were found were similar to the findings of Fry et al. (1997), Küplülü and Ün (2001) and Ün (2002). If the majority of the ovary oocyte potential is made of B and C quality oocytes when the distribution of the aspired and classified oocytes is considered (A quality 33.08%; B quality 30.56%; C quality 36.65%), the opinion that the potential can be an important and cost efficient source for IVF and in vitro embryo studies arises. The number of the oocytes with full cumulus expansion was the highest for quality A oocytes (94.28 %). The cumulus expansion rates in quality A oocytes were parallel to the findings, which was declared by Cetin (2004), Lonergan et al. (1996), Polat (2005) and Stock et al. (1997). The cumulus expansion rates which were observed in the quality B and C oocytes that were used were lower than all mentioned researchers. The excretion rate of first polar bodys of the quality A oocytes after maturation process was higher than previous reports by Keefer et al. (1993), Macun (2004) and Polat (2005) whereas

it was compatible with Park et al. (1999). The excretion rate of first polar bodys of the quality B and C oocytes was much lower than before mentioned researchers. In this study, it was found that the first polar body excretion was lower than the cumulus expansion rates. This difference was supported by previous reports (Çetin, 2004; Harper and Brackett, 1993; Macun, 2004; Park et al., 1999; Polat, 2005).

In the presented study, the maturation rate was higher in quality A oocytes than the others as expected. This might be due to the variations between the maturation media and oocyte resource. Such that, the maturation in quality B oocytes are lower. Besides, no maturation in quality C oocytes was observed as compatible with previous reports. This result was associated with the phenomenon that surrounding cumulus cells, which was thin or inadequate in quality B and C oocytes, supported to maturation process. Thus, researchers used quality A oocytes in IVF studies due to the positive relation between oocyte quality and maturation and fertilization rates. Furthermore, survival of in vitro embryos and further development stages were depended to oocyte quality and choosing process.

Selection of proper oocytes was accepted as the most important step in in vitro embryo production. In a study, which analyzed the division ability of the oocytes that were classified according to cumulus morphology (Shioya, 1993), 63.7, 29.5 and 17.7% division rates were observed in quality A, B, and C oocytes, respectively. The conditions, which were provided for in-vitro maturation, affected the fertilization ability and the embryonic development of the cattle oocytes, significantly (Bavister et al., 1992; Brackett and Zuelke, 1993; Gordon, 2003).

The granulosa and theca cells help the oocyte to supply its needs during the meiotic rest period and to gain the ability to develop during the maturation period. The cumulus cells, which provide a support by allowing the intercellular passage of ions through the gap junctions (Hagemann, 1999), are also have regulating role in RNA synthesis (Akyol, 2006; Soom et al., 2002) in the oocytes by releasing of growth factors and hormones.

As a result, maturation rate decreases as the oocyte quality, which is evaluated by cumulus structure, decreases. So that, selection of quality A oocytes, which is an initial step for further development, is critical to provide a successful maturation and to have transferable embryos in in vitro conditions.

The findings about maturation rates in various quality oocytes support this hypothesis in the presented study. In future studies, molecular properties of the cumulus cells can also be adapted to the oocyte selection criteria rather than microscopic properties.

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