Differences in Development of Bovine Oocytes Recovered by Aspiration or by Mincing

Y. Takagi*, K. Mori†, T. Takahashi*, S. Sugawara*, and J. Masaki*

*Department of Animal Science, Tohoku University, Sendai 980, Japan
and †Laboratory of Embryo Transplantation, Snow Brand Milk Products Co., Ltd., Tomakomai 059-13, Japan

ABSTRACT: This experiment was performed to clarify relationships between conditions of bovine ovaries and developmental capacity of the follicular oocytes recovered from them and to compare two methods of oocyte collection, aspiration and mincing. Follicular oocytes with surrounding intact, unexpanded cumulus recovered by follicular aspiration or by mincing of tissue from 24 pairs of ovaries were matured and fertilized in vitro. The number of follicular oocytes recovered from pairs of ovaries averaged 32.1 ± 3.2, but the number recovered varied greatly among the 24 pairs of ovaries (range, 7 to 71). The overall rate of development to the blastocyst stage was 18% (137/771), and the average number of blastocysts produced from a pair of ovaries was 5.7 ± 1.1 (range, 0 to 17). No relationships were found between the presence of corpora lutea or large follicles and the proportion of oocytes capable of reaching the blastocyst stage in vitro. However, a positive correlation was observed between the number of oocytes obtained from each pair of ovaries and subsequent in vitro development; the correlation was especially high for oocytes obtained by aspiration. These data suggest that the developmental capacity of bovine follicular oocytes after in vitro maturation and fertilization is correlated to the number of antral follicles aspirated from the pair of ovaries.

Key Words: Cattle, Ovaries, Fertilization, Embryos, Blastocyst

Introduction

Techniques for in vitro fertilization (IVF) of mammalian oocytes matured in vitro, especially in cows, have advanced greatly. In bovine IVF programs, follicular oocytes are generally recovered from ovaries excised from slaughtered cattle. Excised ovaries contain a large number of follicles at various stages of development (e.g., vesicular, growing, and primordial follicles; Erickson, 1988). Oocytes at various stages of oogenesis either spontaneously resume meiosis or degenerate after culture in vitro (Iwasaki et al., 1987). It has been reported that the capacity of follicular oocytes to undergo spontaneous nuclear maturation in vitro is not lost until late in the process of oocyte degeneration (Leibfried and First, 1979). Thus, oocytes that complete nuclear maturation in vitro differ from each other. Furthermore, Leibfried and First (1979) and Suss et al. (1988) demonstrated that there is no relationship between the donor's estrous cycle stage and the frequency of nuclear maturation, although any relationship to the subsequent capacity to develop to the blastocyst stage is still not clear. The present experiment was conducted 1) to clarify relationships between the condition of a pair of ovaries and the developmental capacity of oocytes after maturation and fertilization in vitro and 2) to compare two methods of oocyte recovery, aspiration and mincing.

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3To whom correspondence should be addressed.
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Materials and Methods

Collection of Follicular Oocytes. Pairs of ovaries were excised from 24 Japanese Black cows and heifers killed at a local slaughterhouse. Pairs of ovaries were placed in physiological saline (PS; 0.85% NaCl + 100 pg/mL of streptomycin) at 33 to 37°C and brought to the laboratory within 3 h. The weight and numbers of corpora lutea (≥ 1 cm in diameter) and follicles (≥ 1 cm in diameter) of each pair of ovaries were recorded. The ovaries were then washed three times with PS, and the contents of the visible follicles (3 to 5 mm in diameter) and of follicles were aspirated with a disposable syringe using an 18-gauge needle. After aspiration of follicular oocytes, ovaries were cut into two pieces with scissors and transferred to 100-mm culture dishes filled with appropriate amounts of Whittingham's modified Dulbecco's phosphate-buffered saline (m-PBS) supplemented with 5 mg/mL of polyvinylalcohol (PVA, P-8136, Sigma Chemical, St. Louis, MO). The divided ovaries were then minced with a surgical knife and the small vesicular follicles ruptured. Only oocytes with an intact, unexpanded cumulus (Shioya et al., 1988) were selected for the experiment.

Culture of Oocytes. Selected cumulus-oocyte complexes (COC) were rinsed twice with m-PBS and rinsed again with maturation medium (TCM199, Gibco, Grand Island, NY) supplemented with 10% (vol/vol) heat-inactivated fetal calf serum (FCS, Flow Laboratories, North Ryde, Australia) and antibiotics (100 IU/mL of penicillin and 100 μg/mL of streptomycin). The COC were then transferred into .35 mL of TCM199 maturation medium, covered with mineral oil (Squibb, Princeton, NJ) in a 35-mm culture dish (Falcon, Lincoln Park, NJ), and cultured for 21 to 23 h in 5% CO2 and 95% air with high humidity at 38.5°C.

Sperm Preparation and Insemination. Sperm from a Japanese Black bull was donated by Miyagi Animal Husbandry Experimental Station (Iwadeyama, Miyagi, Japan). The experimental procedure used for insemination was similar to that used by Takagi et al. (1991). A straw of frozen semen was thawed in warm water (32 to 35°C) and the spermatozoa were processed to induce capacitation in vitro. An aliquot of semen was transferred into BSA-free BO medium (Brackett and Oliphant, 1975) containing 10 mM caffeine. Spermatozoa were washed twice by centrifugation at 200 × g for 5 min and resuspended in the BSA-free BO medium supplemented with caffeine. This suspension was diluted twice with BO medium containing 2 mg/mL of BSA (Sigma Chemical) immediately after 1 min of treatment with .1 μM Ca ionophore A23187 (Sigma Chemical; Aoyagi et al., 1988). The COC were transferred to a 50-μL droplet of BSA-free BO medium after incubation for 21 to 23 h. Then, 50 μL of the spermatozoa suspension was added to the droplet containing COC. Final spermatozoal concentration was approximately 1.5 × 107/mL.

In Vitro Development. The experimental procedure used for in vitro development was similar to that of Goto et al. (1998). After 6 h of insemination, oocytes were transferred to .35 mL of TCM199 supplemented with 5% FCS and antibiotics. At 72 h after insemination, the cumulus cells surrounding the embryos were removed by gentle pipetting, and the naked embryos were cocultured with the remaining monolayer of cumulus cells. Development medium was replaced with fresh medium every 48 h. The embryos were examined under a microscope for development every 24 h after the removal of cumulus cells from the embryos.

Statistical Analysis. Differences between individual means were analyzed by Student's t-test, or, if the variances of the two groups were significantly different, by Bartlett's test for unequal variances. Frequencies of the embryonal development were analyzed by the chi-square test. Correlation coefficients were obtained by linear regression analysis and evaluated by Student's t-test.
**Results**

Weight and numbers of corpora lutea and follicles for 24 pairs of ovaries are shown in Table 1. There was no significant correlation between the number of corpora lutea (≥ 1 cm) or follicles (≥ 1 cm) and the weight of each pair of ovaries.

For aspirated follicles, the number of follicular oocytes with an intact and unexpanded cumulus averaged 17.8 ± 1.9 and ranged from 3 to 37 (Table 2). Following mincing of ovarian tissue after aspiration, an additional 14.3 ± 1.9 oocytes were recovered. There was no significant correlation between the number of oocytes and weight (r = -0.30) or numbers of corpora lutea (r = 0.05) or large follicles (r = -0.25) of each pair of ovaries.

Numbers and percentage of oocytes that developed to the blastocyst stage are given in Table 3. The data indicate that 70% (300/427) of oocytes obtained by aspiration cleaved and 21% (90/427) developed to the blastocyst stage. This compares with 51% (174/344) and 14% (47/377) for oocytes obtained by mincing. This difference in the developmental rate of oocytes obtained by the two methods was significant (P < 0.05).

Correlations between the number of oocytes collected from a pair of ovaries and the developmental rate of oocytes are shown in Table 5. For aspirated oocytes, significant positive correlations were observed between the number of oocytes and percentages at the 2-cell (r = 0.76, P < 0.001), 8-cell (r = 0.72, P < 0.001), and blastocyst (r = 0.56, P < 0.01) stages. Oocytes obtained by mincing showed no significant correlations. In addition, no relationships were found between the presence of corpora lutea or large follicles and subsequent in vitro development.

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**Discussion**

In this study, we recovered an average of 32.1 COC per pair of ovaries by aspiration of small follicles visible on the ovarian surface followed by mincing of the ovary. The bovine IVF system, in which follicular oocytes are usually aspirated from visible follicles at the ovarian surface using a syringe and needle, generally yields approximately 10 compact cumulus oocytes per pair of ovaries (Leibfried and First, 1979; Iwasaki et al., 1987; Suss et al., 1988). Katska (1984) stated that...
the number of oocytes recovered by follicle aspiration represents only 43.2% of the entire number of follicular oocytes within the ovary, and that this low recovery rate may be due to difficulties in separating the cumulus cell-layer oocyte from the cumulus oophorus. Furthermore, Iwasaki et al. (1987) reported that more compact cumulus oocytes were recovered by mincing the ovary using razors and a food grater after aspiration of visible follicular oocytes. Consequently, mincing of the ovary was thought to be an effective method of obtaining a large number of COC.

Frequencies of blastocyst formation by oocytes obtained by the two methods were significantly different. For example, 21% of aspirated oocytes and 14% of oocytes from minced ovarian tissue developed to the blastocyst stage ($P < .05$, Table 3). These results indicate that the developmental capacity of oocytes recovered by aspiration is different from that of oocytes recovered by mincing the ovary. In this experiment, the mincing of ovarian tissue was performed after the completion of the aspiration of surface follicles. This automatically removes oocytes that have the highest potential to develop, thereby potentially biasing the results against mincing. When oocytes were recovered by mincing alone, the percentage of oocytes that developed to blastocyst stage was 17% (64/377, $n = 9$; our unpublished observations). This value is approximately equal to the overall rate of development to the blastocyst stage (18%, Table 3). However, we found no differences in the ratio of blastocysts to cleaved embryos (blastocyst/2-cell) between two methods (Table 3). This suggests that, although the cleavage rate of oocytes recovered by aspiration was higher than that of oocytes recovered by mincing, the developmental capacity of cleaved embryos recovered by the two methods is equal.

Recently, Goto et al. (1990) reported that an average of three blastocysts per cow were obtained using the aspiration method. We obtained an average of 3.8 blastocysts per pair of ovaries by the same method. However, the mincing method yielded an additional 2.0 blastocysts, for a total of 5.7 blastocysts per cow. This result suggests that the mincing method is useful in commercial bovine IVF systems.

The number of oocytes recovered from individual pairs of ovaries in this study varied from 7 to 71. Suss et al. (1988) reported that the number of oocytes aspirated from paired ovaries ranged from 4 to 32, and that no significant correlation was observed between yield of compact COC and the age of the animal, estrous cycle stage, or number of small follicles visible on the ovary. Similarly, we found no correlation between yield of oocytes and presence of a large corpus luteum or any other measure of ovarian status. Nevertheless, we did find significant correlations between the yield of compact COC and the developmental ability of oocytes matured and fertilized in vitro; that is, the greater the number of oocytes recovered from a pair of ovaries, the higher the percentage of embryos that developed to the blastocyst stage. Recently, Goto et al. (1990) similarly reported that the rate at which embryos developed to the blastocyst stage was affected by the number of oocytes recovered from individual cows, although this relationship was not significant. Furthermore, the results summarized in Table 5 show that this relationship is particularly strong for oocytes recovered by aspiration. These data imply that the number of visible follicles (3 to 5 mm in diameter) of the pair of ovaries is related to the developmental capacity of follicular oocytes after in vitro maturation and fertilization.

It has been reported that the ability of oocytes to undergo nuclear maturation in vitro and to undergo fertilization with male pronuclear formation are not dependent on the estrous cycle stage of the donor cow (Leibfried and First, 1979; Leibfried et al., 1985). We also could not demonstrate any relationships between the presence of corpora lutea or large follicles and the proportion of oocytes capable of reaching the blastocyst stage in vitro.

More detailed investigations are required to clarify the mechanism that accounts for the correlation between number of oocytes recovered by follicular aspiration and in vitro developmental capacity.
Implications

A significant positive correlation was observed between the number of oocytes obtained from each pair of ovaries and subsequent in vitro development; the correlation was especially high for oocytes obtained by aspiration. These findings establish that the developmental capacity of bovine follicular oocytes after in vitro maturation and fertilization is correlated to the number of antral follicles aspirated from the pair of ovaries.

Literature Cited


