In vitro Maturation of Bovine Oocytes Recovered from Follicles of Various Sizes

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Abstract

In vitro maturation of bovine oocytes recovered from follicles of various sizes were investigated. Oocytes from ≧5 mm (A), 6–10 mm (B), 11–15 mm (C) and 16–20 mm (D) follicles were cultured in TCM 199 with 4 mg/m*l* bovine serum albumin and antibiotics for 36 h with 3 h intervals. The cultured oocytes were fixed with acetic-alcohol (1 : 3) and stanied with 0.75% acetic-orcein. After culture, the oocytes that reached the metaphase II stage were found first at 18 and 12 h for follicles A and B, respectively. Some oocytes from follicles C and D had resumed the meiotic division before the culture started. The follicular sizes had no significant influences on the proportion of matured oocytes in the culture of 18 to 36 h. However, oocyte maturation was affected by the culture time (18–36 h: $P < 0.001$) i.e., 30 h culture gave the highest proportion of matured oocytes.

Introduction

FUKUI and SAKUMA (1980) showed that there was no significant difference in the proportion of matured oocytes cultured for 24 to 33 h between oocytes from smaller follicles (≦5 mm) and larger follicles (6–10 mm and 11–20 mm). Oocytes recovered from larger follicles, however, appeared to reach the metaphase II earlier than those recovered from smaller follicles. Although many workers have studied maturation rates of oocytes recovered from follicles of various sizes in mice (ERICKSON and SORENSON, 1974), rabbit (BAE and FOOTE, 1975), pigs (TSAFRIRI and CHANNING, 1975; MCGAUGHEY, 1977), cattle (JAGIELLO et al., 1974) and rhesus monkey (SMITH et al., 1978), there have been no studies to determine the precise time for *in vitro* maturation of bovine oocytes recovered from follicles of various sizes. The present experiment was conducted to investigate the resumption of meiotic division in bovine oocytes recovered from four different sizes of follicles and cultured *in vitro*.

Materials and Methods

Ovaries were taken from cows at a local slaughterhouse and brought to the laboratory

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in 0.86% saline solution at 37°C within 2 h. Follicular oocytes were recovered by puncturing the follicles (A: ≦5 mm, B: 6–10 mm, C: 11–15 mm, D: 16–20 mm) with a 22 gauge needle. Since the ovaries were obtained from a slaughterhouse, the stage of the estrous cycle or whether the animals were pregnant or not was unknown.

The recovered oocytes were washed twice in Dulbecco's phosphate buffered saline at 37°C and cultured in a small test tube containing 1 m*l* of TCM 199, 4 mg/m*l* and antibiotics (pH 7.1) following the methods described by FUKUI and SAKUMA (1980). Only oocytes completely surrounded by the cumulus cells were cultured for 36 h. The culture was terminated at 3 h intervals and the stage of maturation in each oocyte was examined by a phase contrast microscopy after fixation and statining (FUKUI and SAKUMA, 1980). Some oocytes recovered from the follicles of all sizes were immediately fixed and stained to examine meiotic division at "0 h" culture.

Results

Table 1 shows the stage of meiotic division of bovine oocytes recovered from follicles of the four different sizes at "0 h" culture. For follicle A, 91.9% (113/123) of the oocytes examined immediately after recovery from follicles were at the diplotene stage with germinal vesicle (GV), whereas some oocytes

Table 1. Stages of the meiotic division of bovine oocytes recovered from follicles of various sizes

Follicular*1 sizes	No. of oocytes examined	Maturation stages*2						Degenerative
		GV	P–I	M–I	A–I	T–I	M–II	
A	123	113	9	0	0	0	0	1
B	68	40	14	7	0	1	1	5
C	32	14	6	3	2	0	1	6
D	28	9	0	1	0	5	9	4

*1: Follicular sizes of A, B, C and D indicate≦5 mm, 6–10 mm, 11–15 mm and 16–20 mm, respectively.

*2: GV; Germinal vesicle, P–I; Prometaphase I (germinal vesicle breakdown), M–I; Metaphase I, A–I; Anaphase I, T–I; Telophase I, M–II; Metaphase II.

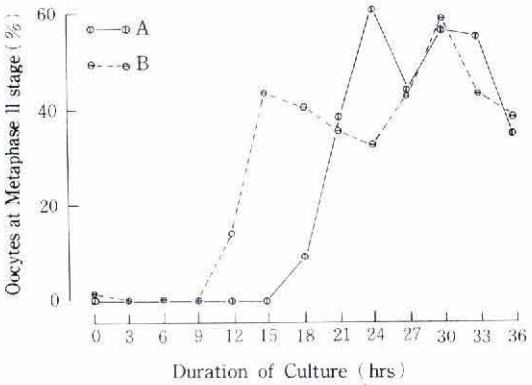

Fig. 1. Pattern of *in vitro* maturation rates of bovine oocytes recovered from follicles A (≦5mm) and (6–10 mm)

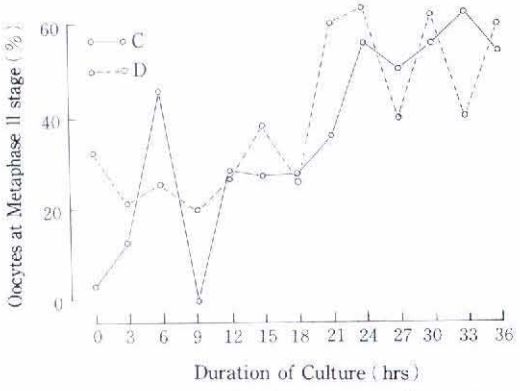

Fig. 2. Pattern of *in vitro* maturation rates of bovine oocytes recovered from follicles C (11–15mm) and D (16–20mm)

recovered from follicles B, C and D were between metaphase I and metaphase II of meiotic division. Among 28 oocytes recovered from follicle D, 9 (32.1%) were at metaphase II. Some oocytes from large follicles (B, C and D) were degenerative. Tables 2 and 3 show the results of *in vitro* maturation of bovine oocytes recovered from follicles A and B, and follicles C and D, respectively. Oocytes at metaphase II were first observed 18 and 12 h in culture for follicles A and B, respectively (Fig. 1). On the other hand, some oocytes from larger follicles (C and D) had reached the metaphase II before culture. However, as shown in Fig. 2, a high rate of oocyte maturation which was similar in oocytes from smaller follicles was obtained after 21 h in culture. In follicle D, oocytes with GV were not observed after 12 h in culture.

There was no significant difference in the rates of oocyte maturation ($x^2=3.59$, d.f. 3) among four different follicular sizes. However, a highly significant difference was found in the rate of degenerative oocytes ($x^2=13.26$, d.f.

Table 2. *In vitro* maturation of bovine oocytes recovered from follicles of A (≤ 5 mm) and B (6–10 mm)

Follicular*1 sizes	Culture times (hr)	No. of oocytes examined	Maturation stages*2						Degenerative (%)
			GV	P-I	M-I	A-I	T-I	M-II (%)	
A	3	20	15	2	0	0	0	0	3
	6	19	9	10	0	0	0	0	0
	9	20	12	5	2	0	0	0	1
	12	26	5	16	4	0	0	0	1
	15	20	4	5	11	0	0	0	0
	18	21	3	6	6	0	1	2	3
	21	21	1	5	4	0	1	8	2
	24	46	0	3	3	1	2	28	9
	27	48	0	3	7	0	5	21	12
	30	101	3	6	9	2	16	57	8
	33	51	0	6	7	1	4	28	5
	36	60	7	8	6	1	3	21	14
	Total	453	59	75	59	5	32	165(36.4)	58(12.8)
B	3	20	16	2	1	0	0	0	1
	6	13	4	4	2	0	0	0	3
	9	16	0	2	9	1	1	0	3
	12	14	0	1	10	0	1	2	0
	15	16	0	0	1	0	3	7	5
	18	10	0	1	3	1	1	2	2
	21	14	2	1	0	3	2	5	1
	24	12	0	1	4	0	1	4	2
	27	14	1	3	0	0	1	6	3
	30	49	5	4	0	1	4	29	6
	33	14	1	3	1	0	1	6	2
	36	21	1	4	3	0	0	8	5
	Total	213	30	26	34	6	15	69(32.4)	33(15.5)

*1, *2: The same as shown in Table 1

Table 3. *In vitro* maturation of bovine oocytes recovered from follicles of C (11–15 mm) and D (16–20 mm)

Follicular*1 sizes	Culture times (hr)	No. of oocytes examined	Maturation stages*2						Degenerative (%)
			GV	P-I	M-I	A-I	T-I	M-II (%)	
C	3	16	4	3	5	0	0	2	2
	6	11	1	1	1	0	1	5	2
	9	11	0	0	3	1	1	0	6
	12	14	0	0	7	0	1	4	2
	15	11	1	0	1	0	2	3	4
	18	18	0	1	3	0	3	5	6
	21	14	1	1	2	1	2	5	2
	24	16	0	1	2	0	2	9	2
	27	12	0	2	0	0	0	6	4
	30	36	7	4	2	0	0	20	3
	33	16	0	1	1	0	1	10	3
	36	11	0	1	1	0	0	6	3
	Total	186	14	15	28	2	13	75(40.3)	39(21.0)
D	3	14	3	2	0	0	0	3	6
	6	11	1	1	2	0	1	3	3
	9	10	1	0	0	1	3	2	2
	12	11	0	0	1	1	2	3	4
	15	13	0	0	4	0	2	5	1
	18	15	0	5	5	0	0	4	1
	21	10	0	0	0	0	1	6	3
	24	11	0	0	1	0	2	7	1
	27	10	0	1	1	0	0	4	4
	30	16	0	2	1	0	1	10	2
	33	10	0	0	1	0	1	4	4
	36	10	0	0	1	0	0	6	3
	Total	141	5	11	17	4	13	57(40.4)	34(24.1)

*1, *2: The same as shown in Table 1

3: $P < 0.005$). The proportions of degenrative oocytes from larger follicles (C and D, Table 3) were higher than those of smaller follicles (A and B, Table 2).

Because matured oocytes of follicle A was first observed 18 h in culture, analysis of variance was performed to investigate the significance in the difference due to culture times (18 to 36 h) and the four follicular sizes. The results shown in Table 4 revealed that there was again no significant difference among the follicular sizes, but a highly signif-

Table 4. Analysis of variance for the oocytes matured to metaphase II during culture for 18–36 hours

Source of variation	DF	MS	F
Culture times	6	220.13	4.36***
Follicular sizes	3	77.09	1.53
Interaction	18	30.47	0.60
Error		50.54	

*** $P < 0.001$.

icant difference was found among the culture times ($P < 0.001$). The culture for 30 h showed

a significantly higher maturation rates than culture times ($P<0.01$).

Discussion

The present study shows that the oocytes recovered from larger follicles (C and D) had resumed meiotic division and some of the examined oocytes had reached the metaphase II stage before culture. These oocytes would have been recovered from preovulatory follicles and degenerated oocytes would have been recovered from atretic follicles. The proportions of degenerated oocytes from larger follicles were high as compared with those from smaller follicles in both before (Table 1) and after (Table 3) culture. In the present study, the presence of corpora lutea was not taken in account, because FUKUI and SAKUMA (1980) reported that the presence of corpora lutea had no relations to the in vitro maturation of bovine oocytes recovered from follicles of various sizes.

Matured oocytes from follicle A were first observed after 18 h in culture. This was similar to other reports in bovine (JAGIELLO et al., 1974) and ovine (CROSBY and GORDON, 1971) oocytes. In the pig, MCGAUGHEY et al. (1979) suggested that only oocytes recovered from large, preovulatory follicles may be developmentally competent to undergo normal maturation including the first meiotic division, although a high incidence of oocytes from small follicles are capable of undergoing the early stage of maturation *in vitro*. From this respect, it was necessary to investigate precise time for oocytes from large follicles to reach the metaphase II stage *in vitro*, although SATO et al. (1978) reported maturation time of bovine oocytes recovered from small follicles (2-5 mm). However, for the oocytes from large follicles, it was difficult to determine the precise time to the metaphase II, because some oocyres from large follicles had resumed meiotic division before culture (Table 1). It is considered that the oocytes from large follicles could reach the metaphase II stage by a shorter time of culture than those of small follicles. Moreover, the proportions of matured oocytes from large follicles after 21 h in culture were similar to those of oocytes from small follicles (Figs. 1 and 2). Overall maturation rates among the four follicular sizes were not significantly different. This confirms the previous result of FUKUI and SAKUMA (1980). The oocytes from large follicles could reach the metaphase II stage earlier than those of small follicles, but the proportion of degenerated oocytes could also increase. Oocytes isolated from follicles and cultured *in vitro* were not dependent on the follicular size in contrast to the *in vivo* conditions.

Among the culture times investigated, 30 h was the most appropriate duration for oocyte maturation from any sizes of follicles. This agrees with the reports of other workers (EDWARDS, 1965; SHEA et al., 1973; SATO et al., 1977). However, there was no significant difference among the culture times of 24, 27, 30 and 33 h when the oocytes from follicles A were compared. In the present study, the number of oocytes from follicles C and D was small due to the difficulty in obtaining these materials from a slaughterhouse and in recovering oocytes from the follicles. FUKUI and SAKUMA (1980) indicated that the proportion of oocytes which were collectable from large follicles and suitable for culture were only 50% and 30%, respectively when compared with those from small follicles.

In conclusion, the present study indicates that bovine oocytes from follicles A (≦5 mm) and B (6-10 mm) were first reached the metaphase II stage after 18 and 12 h in culture, respectively. On the other hand, it was likely difficult to determine the precise time of *in vitro* maturation for the oocytes from large

follicles, since some oocytes had resumed meiotic division and had matured before culture. In the present culture conditions, 30 h in culture appears to be an appropriate duration to maximize the resumption and completion of bovine oocyte maturation *in vitro*.

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大きさの異なる卵胞から採取されたウシ卵母細胞の体外成熟に関する研究

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摘　　要

4種の大きさの異なる卵胞（A：≦5 mm，B：6–10 mm，C：11–15 mm，D：16–20 mm）から採取されたウシ卵母細胞（卵胞卵子）の体外成熟について検討した。使用した培養液は TCM 199，ウシ血清アルブミン（4 mg/m*l*）および抗生物質を含んだものである（pH. 7.1）。卵胞より採取した卵子の内，卵丘細胞塊を有するもの5～10個を1 m*l*の培養液が入っている小試験管に入れ，5% in air を封入，密栓後3時間毎の間隔で36時間まで培養した。培養終了後，常法により固定・染色を行ない，位相差顕微鏡下で成熟分裂の段階を調べた。

卵胞Aから採取された卵子は培養18時間で，卵胞Bから採取された卵子は培養12時間で各々第2成熟分裂の中期に達した。一方，卵胞CとDから採取された卵子は培養前，または9時間までの短時間培養でも第2成熟分裂の中期に達しているものが観察された。このことから，大きな卵胞から採取された卵子は小さな卵胞から採取されたものより早く第2成熟分裂中期に達することが示唆されるが，正確な時間については明らかにできなかった。また，培養18–36時間までの卵子成熟率を分散分析（7×4）で検討したところ，第2成熟分裂に達した卵子の割合は卵胞の大きさには影響されず，培養時間において有意差（$P < 0.001$）が認められた。そして，30時間の培養がウシ卵母細胞の体外成熟に最適であると考えられた。

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