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Studies on Maturation In Vitro of Rat Follicular Oocytes and Fertilization In Vitro of Cumulus-Removed and Intact Oocytes after Maturation

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Summary

Rat oocyte-cumulus complexes were cultured in verious media in order to induce maturation division in vitro. When the complexes were cultured in mKRB containing estrous rat serum (ERS) and FCS of 5% instead of BSA higher proportions (83.3 and 86.7%) of oocytes matured to metaphase II in 20h compared to control (75%) and the maturation rates in mKRB plus FCS were generally higher than those in mKRB plus ERS. Fertilization and early cleavage rates in vitro of the intact oocytes matured in mKRB containing BSA and FCS were generally higher than those of cumulus-removed oocytes and these rates were higher in mKRB containing 5% FCS than those in mKRB containing BSA. These results indicate that maturation rate in vitro was greatly increased by the addition of FCS instead of BSA to mKRB solution and the presence of cumulus cells around oocytes prior to sperm insemination may be responsible for the increase of in vitro fertilization and early cleavage rates.

Introduction

Normal young production by combining in vitro oocyte maturation with in vitro fertilization has desired in laboratory animals (Niwa et al., 1976; Leibfried and Bavister, 1983) and in farm animals (Iritani et al., 1978; Ball et al., 1983; Parrish et al., 1986). However, the developmental potential of in vitro-matured and-fertilized oocytes has reported lower than that obtained in in vivo-matured oocytes (Fukui et al., 1987; Leibfried-Rutledge et al., 1987). Only a very small proportion of these oocytes could develop to normal young (Minato and Toyada, 1983). Little information is available about the reasons for these low developmental abilities of in vitro-matured oocytes. Some investigators have interested in the role of cumulus cells in fertilization of oocytes. Yanagimachi (1981) reported that fertilization rate is much higher if cumulus was not removed and Lenz et al. (1983) demonstrated that cumulus-free oocytes co-cultured with cumulus cells in medium exhibited a higher percentage of penetration rates and two pronuclei compared to cumulus-free oocytes. Several workers have noted that cumulus cells related to sperm capacitation and acrosome reaction (Ball et al., 1982; Bedford, 1983; Meizel, 1985). However, whether the presence of cumulus cells affect successful fertilization in vitro is as yet unclear. The purpose of these studies was to investigate the effect of estrous rat serum and FCS on maturation in vitro of rat follicular oocytes and to determine if difference exists in fertilization and early cleavage in vitro to the presence or absence of cumulus cells in oocytes matured in vitro.

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Materials and Methods

1. Collection of follicular oocytes

Sexually mature rats of Spraques Dawley strain were killed at diestrus and their ovaries were removed to obtain oocytes. Cumulus-oocytes complexes were liberated from follicles by puncturing with a 25 gauge needle and only oocyte with a complete, compact cumulus cells were selected for culture.

2. Culture medium for maturation in vitro

Medium was a modified Kreb's-Ringer-bicarbonate (mKRB) solution with 4mg/ml fraction V bovine serum albumin (BSA, Sigma Chemical Co.) as that used by Iritani et al. (1984). To test the effect of serum on oocytes maturation, three levels (5, 10 and 15%, v/v) of fetal calf serum (FCS, Common Wealth Serum Lab.) and heat-inactivated estrous rat serum (ERS) wer supplemented to mKRB instead of BSA, respectively. The medium was filtered with 0.2μm milipore filter prior to use and was equilibrated for 5 to 6hr in a CO_2 incubator.

3. Maturation in vitro of oocytes

Ten to twenty cumulus-oocyte complexes were transferred into a petri dish (Falcon Plastic 3 # 1006) containing 0.2ml culture medium under paraffin oil and were cultured for 20hr at 38°C in 5% CO_2 in air and 100% relative humidity. At the end of cultivation time, oocytes were mounted on slides, fixed in acetic acid: ethanol (1:3) and stained with 1% aceto-orcein. Oocytes were examined with phase microscopy for signs of maturation and oocytes in metaphase II were termed mature.

4. Collection and capacitation of rat sperm

Cauda epididymal spèrm were removed from mature male rats. Sperm were diluted with mKRB solution and centrifuged twice at 350 x g for 5 min. Sedimented sperm were resuspended with mKRB solution to give a final sperm concentration at in vitro fertilization. Sperm suspension were finally preincubated for 5hr in air-tight capped culture tubes in a CO_2 incubator at 38°C.

5. Fertilization in vitro

In vitro-matured oocytes were washed twice with fresh mKRB solution. All oocytes with expanded cumulus cells were randomly divided into two groups to test the effect of presence (intact) or absence (denuded) of cumulus cells on the rate of fertilization and early cleavage in vitro. Half of oocytes were mechanically denuded by vigorous pipetting through a fine capillary tube. Ten to 12 intact or denuded oocytes were then transferred into a petri dish containing pre-incubated sperm of 4 x 10^6 /ml in 0.2 ml mKRB solution and cocultured for 24 to 26hr in a CO_2 incubator of the same conditions with in vitro oocyte maturation. After culture for fertilization, fixation and staining of oocytes was by the same procedures with the above in vitro oocyte maturation. The presence of pronuclei and normal cleavage was taken as the criteria of fertilization and normal cleavage, respectively.

Results and Discussion

1. Maturation in vitro of follicular oocytes

Maturation rates of oocytes after in vitro culture for 20hr are listed in Table 1. Seventy-five percentage of oocytes in mKRB solution containing BSA reached metaphase II. When ERS and FCS of 5% (v/v) to mKRB solution instead of BSA were supplemented, 83.3 and 86.7% of oocytes matured to metaphase II, respectively. However, when the level of ERS supplement in-

Table 1. In vitro maturation of rat follicular oocytes cultured for 20hr in mKRB containing estrous rat serum (ERS) and FCS

Culture medium (mKRB) containing			No. of oocytes	Stage of meiosis					
BSA (mg/ml)	ERS (%)	FCS (%)		Met. I	Ana. I	Tel. I	Met. II	Deg.	% Met. II
–	–	–	30	3	4	3	17	3	56.7
4	–	–	16	2	1	1	17	0	75.0
–	5	–	30	1	0	3	25	0	83.3
–	10	–	30	1	0	2	23	0	76.3
–	20	–	28	7	2	2	14	3	50.0
–	–	5	30	0	1	3	26	0	86.7
–	–	10	27	1	1	1	21	3	77.8
–	–	20	30	0	1	4	24	1	80.0

Table 2. In vitro fertilization and early cleavage of oocytes with or without cumulus cells

Muturation medium	Presence (+) of cumulus cells at sperm insemination	No. of oocytes	24 hr culture			48 hr culture			62 hr culture		
			Pronuclei	Fragment	%IVF	2-cell	4-cell	%Cleaved	2-cell	4-cell	%Cleaved
mKRB+BSA	–	49	14	3	34.7	3	0	6.1	5	0	10.2
	+	55	15	17	45.5	2	0	3.6	8	0	14.5
	Parthenogenesis (+)	20	3	11	15.0	1	0	5.0	1	0	5.0
BSA—free mKRB	–	51	17	5	30.0	3	0	5.9	8	0	17.6
+5% FCS	+	55	32	10	58.2	6	0	10.9	14	0	25.1
	Parthenogenesis (+)	20	3	8	15.0	1	0	5.0	2	0	10.0

creased there was decreased in the percentage of matured oocytes and was increased in degenerated oocytes. Maturation rates in mKRB plus FCS were generally higher than that in mKRB plus ERS. The beneficial effect of ERS in this study was similar to those obtained for mouse oocytes (Minato and Toyada, 1982a), for porcine oocytes (Minato and Toyoda, 1982b; Kim et al., 1988). Also, a similar result in maturation rate in medium containing FCS has been reported for porcine oocytes (Minato and Toyoda, 1982b; Lee, 1888) and for bovine oocytes (Kim et al., 1988).

2. Fertilization and early cleavage in vitro of oocytes with or without cumulus cells

Table 2 is a presentation of fertilization and early cleavage in vitro of ocytes which removed or did not removed cumulus cells prior to sperm insemination. In vitro fertilization rates of intact oocytes with cumulus cells were generally higher than those of cumulus-removed oocytes and intact oocytes with cumulus cells tended to have a higher percentage of early cleavage than those without cumulus cells. Also, in vitro fertilization and early cleavage of oocytes matured in mKRB containing BSA.

The increased fertilization rate of intact oocytes was similar to that reported by Yanagimachi (1981) and Lenz et al. (1983). Yanagimachi (1981) demonstrated that fertilization incidence was usually much higher if cumulus cells was not removed. Lenz et al. (1983) indicated that oocytes with cumulus cells exhibited a higher sperm penetration and formation of two pronuclei compared to cumulus-free oocytes. On the other hand, Ball et al. (1983) found that fertilization frequency tended to be increased when expanded cumuli were removed. Fraser et al. (1971) and Brackett et al. (1971) reported that there was no significant difference between in vitro fertilization rates of denuded and intact oocytes with sperm capacitated at uterus. Niwa et al. (1983) indicated that no significant difference was noted in the penetration rate of rabbit oocytes with and without cumulus cells and they also suggested that cumulus cells did not play a role in in vitro penetration of oocytes.

Observation from our study does not fully support the role of cumulus cells in fertilization or subsequent early cleavage. However, the fact that oocytes with cumulus cells showed a higher fertilization rate and subsequent cleavage rate correspond with the previous assumptions that capacitation and acrosome reaction are presumably related to mature cumulus mass (Bedford, 1983; Lenz et al., 1983; Meizel and Turner, 1986). Lenz et al. (1983) and Meizel and Turner (1986) has demonstrated that glycosaminoglycans in cumulus oophorus can rapidly stimulate sperm acrosome reaction in vitro. Although further studies are needed to determine the role of cumulus cells in fertilization, the results of this study indicate that the presence of cumulus cells may be worth consideration for increasing fretilization rates.

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