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Effect of Insulin-like Growth Factor-I and Cumulus Cells on *In Vitro* Maturation in Porcine Oocytes

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ABSTRACT

The effects of insulin-like growth factor-I (IGF-I) and cumulus cells during *in vitro* maturation in porcine oocytes were examined. When follicular oocytes were cultured in medium with different concentrations of IGF-I, maturation rates were 60, 61 and 62 and 72% for 0, 1 5 and 10ng/ml IGF-I. In medium with 10ng/ml IGF-I, maturation rates were not significantly difference between oocytes with (68%) and without (52%) cumulus cells during the culture. In medium without IGF-I, however, the maturation rates in oocytes with cumulus cells (63%) was significantly ($P<0.05$) higher than oocytes without cumulus cells (32%). On the other hand, when IGF-I was added for first 24 h period or later 24 h period of culture, maturation rates were higher in oocytes with (61 and 49%) that than without (49 and 45%) cumulus cells. In experiment used medium without fetal calf serum (FCS) and porcine follicular fluid (PFF), the maturation rates in oocytes with cumulus cells for 48 h (48 and 67%) or first 24 h (46 and 63%) period after culture were significantly ($P<0.01$) higher than in oocytes without cumulus cells (16 and 18%) in the presence or absence of IGF-I. These results indicated that cumulus cells is essential on maturation *in vitro* in porcine oocytes, but IGF-I can promote oocytes maturation of oocytes without cumulus cells in medium with FCS and PFF.

(Key words: IGF-I, Cumulus cells, *In vitro* maturation, Porcine oocytes)

I. INTRODUCTION

It is evident from many reports in the literature that gonadotrophins, steroids and cellular factors all interact to provide essential support for the oocyte during maturation. Fully grown mammalian oocytes, surrounded by a compact mass of somatic cumulus cells, are maintained in the mature, germinal vesicle stage *in vivo* until a preovulatory gonadotropin surge provokes a dramatic physiological response. In the hours following the ovulation stimulus, the oocyte resumes nuclear maturation, manifested initially by germinal vesicle breakdown, while the cumulus oophorus undergoes mucification and becomes embedded in a glycosaminoglycan matrix, a process termed cumulus expansion. By the time of ovulation, the cumulus cells have fully expanded and encompass an oocyte that has

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progressed meiotically to metaphase-Ⅱ.

The effect of insulin on various aspects of *in vitro* maturation and fertilization of bovine oocytes has been examined. Although work by Stubbings (1989) with insulin on *in vitro* maturation was apparently without effect, a study reported by Zhang et al. (1991) showed that cumulus-enclosed bovine oocytes significantly improved cumulus cell expansion scores and fertilization rates after exposure to insulin-supplemented TC-199 medium. It was also observed that several lines of evidence suggest a positive relationship between insulin and ovarian function in porcine. Insulin administered during preovulatory follicular development increases the ovulation rate (Cox et al., 1987) and decreases follicular atresia (Matamoros et al., 1990) in cyclic gilts, and increases ovarian growth in nutritionally restricted gilts (Britt et al., 1988). Insulin also decreases follicular atresia in preovulatory rat follicles *in vitro* (Chun et al., 1994).

Some studies using growth factors have shown that meiotic resumption in cumulus-oocyte complexes or follicle enclosed oocytes in several species can be induced by epidermal growth factor (Sanbuissho et al., 1990 ; Das et al., 1991 ; Reed et al., 1993), transforming growth factor α (Brucker et al., 1991 ; Tsafriri et al., 1991) and transforming growth factor β (Feng et al., 1988). On the other hand, IGF-I has been reported to be ineffective in stimulating proliferation of rat granulosa cells *in vitro* (Adashi et al., 1985), in contrast to bovine granulosa cells, which are highly responsive to this growth factor (Savion et al., 1981). Insulin-like growth factor-I are small proteins, structurally related to proinsulin, that stimulate growth and differentiation of a wide variety of cell types (Rotwein, 1991; Jones and Clemmons, 1995). IGF-I is a potent mitogen for granulosa cells (Hernandez et al., 1988), even in the absence of FSH, and acts as a biological amplifier of the action of FSH in ovary (Hsu and Hammond, 1987). The major site of action of growth factors that regulate oocyte maturation is the cumulus cells (Coskun and Lin, 1994). It is reported that somatic cells supply nutrients and other substances to the oocytes and communicate with other and the oocyte via gap junction (Dekel et al., 1981). However, previous studies have been limited to granulosa cells (Guthrie et al., 1998.) as a whole, not distinguishing between cumulus cells and more peripheral cells; and there are no reports of IGF-I effects on mitotic activity of cumulus cells of porcine oocytes.

The purpose of this study was to examine the effect of supplementing the culture medium with IGF-I during *in vitro* maturation when oocytes with or without cumulus cells was used.

Ⅱ. MATERIALS AND METHODS

1. Oocyte Recovery and *in vitro* Maturation

Porcine ovaries were collected from a local slaughter-house and kept in saline (NaCl, 0.9% W/V ; Penicillin 100,000 IU/L ; Streptomycin 100mg/L and Amphotericin B 250μg/L ; Sigma Chemical, St-Louis, MO, USA) at 30 to 32℃. Cumulus-oocytes complexes were aspirated from 2 to 6 mm follicles with a 10-ml syringe with an 18-G needle. The collected oocytes were washed three times in Hepes-buffered Tyrode's medium (TLH) and once in maturation medium, oocytes with a compact and complete cumulus cells were introduced to droplets of maturation medium (10 oocytes/50μl droplet), covered with mineral oil and were cultured under an atmosphere of 5% CO_2 in air at 39℃. The maturation medium consisted of TCM-199 with Earle's salts (Gibco-BRL, NY, USA) supplemented with 3.05mM glucose, 0.32mM Ca-lactate, 2.5mM Hepes (Sigma), 10% fetal calf serum (FCS), 0.2

mM Na-pyruvate (Sigma), 50μg/ml gentamycin (Sigma), 1μg/ml FSH (Sigma), 5μg/ml LH (Sigma), 1μg/ml estradiol 17β (Sigma) and 10%(v/v) porcine follicular fluid (PFF).

2. Experimental Design

The first experiment was undertaken to assess effect of IGF-I (Sigma) concentrations (0, 1, 5 and 10ng/ml) during *in vitro* maturation for about 48 h using the oocyte culture system described above.

In the second series of experiments, porcine oocytes with or without cumulus cells were cultured in presence or absence of IGF-I (10ng/ml) during the culture. At 48 h after culture, the oocytes were examined for maturation status.

In the third series of experiments, oocytes with or without cumulus were cultured in medium with IGF-I (10ng/ml) for first 24 h period or later 24 h period during *in vitro* maturation.

In the last series of experiments, oocytes with cumulus cells for 0, 24 or 48 h were cultured in medium with or without IGF-I during *in vitro* maturation. These maturation medium was used in the absence of FCS and PFF.

3. Assessment of Oocyte Maturation

At the end of culture, oocytes were mounted, fixed to ascertain the influence of IGF-I on nuclear maturation, and the effect of the presence or absence of cumulus cells surrounding the oocyte. Both cumulus-oocyte complexes and denuded oocytes were used. Cumulus-oocyte complexes were placed in maturation medium containing 0.1% hyaluronidase. Cumulus cells were removed mechanically by repeated aspiration with a fine-bore pipette. The oocytes were then pipette onto a slide. A coverslip, spotted with a paraffin wax-vaseline (10:1) mixture at each corner, was placed directly over the centre of the drop containing the oocytes. Fixation of oocytes was carried out by placing the slides in acetic acid : ethanol (1:3) for 2~3 days, and staining with aceto-orcein (1% orcein in 60% acetic acid) for 1~2 min. Nuclear maturation was evaluated under a phase-contrast microscope at × 200 and ×400 magnification, and was expressed as the percentage of oocytes that had achieved metaphase-Ⅱ.

4. Statistical Analysis

Chi-square analysis with the Yates correction was used to test the significance of individual comparisons for rates of maturation status.

Ⅲ. RESULTS

In the first experiment, when complexes oocytes cumulus cultured with different concentrations of IGF-I, the proportions of oocytes matured to M-Ⅱ stage were 60, 61, 62 and 72% for 0, 1, 5 and 10ng/ml of IGF-I, respectively (Table 1). However, significant differences were not observed.

In the second experiment (Fig. 1), oocytes were cultured in medium with or without IGF-I (10ng/ml). In medium with IGF-I, the maturation rate was higher in oocytes with (68%) than without (52%) cumulus cells, but there were not significant differences in the maturation rates. In the absence of IGF-I, the proportions of oocytes matured to Metaphase-Ⅱ were significantly ($P<0.05$) higher in oocytes with (63%) than without (32%) cumulus cells.

In the third experiment (Fig. 2), when IGF-I was added different periods during the culture, maturation rates in medium with IGF-I for first 24 h period or later 24 h period of culture was higher in medium with (61 and 49%) that than without (49 and 45%) cumulus cells.

In the last experiment (Fig. 3), oocytes were

Table 1. Effect of IGF-I concentrations during *in-vitro* maturation in porcine oocytes

Concentrations of IGF-1(ng /ml)	No. of oocytes examined*	No. of oocytes†		
		GV	P-I ~T-I	M-II (%)
0	91	0	36	55(60)
1	100	1	38	61(61)
5	91	0	35	56(62)
10	64	0	18	46(72)

* Data from three replicates were pooled.

† GV : germinal vesicle, P-I : prophase-I, T-I : telophase-I, M-II : metaphase-II

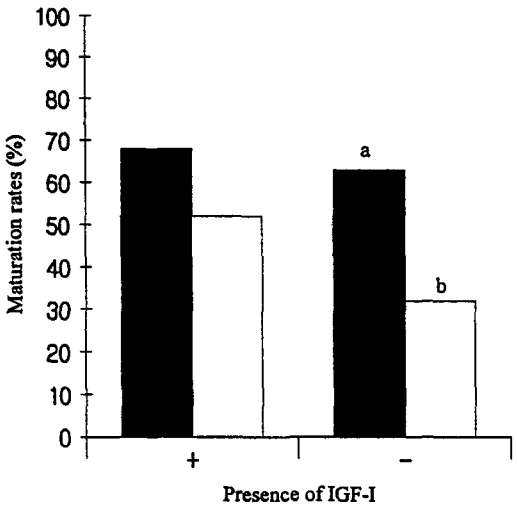

Fig. 1. Effect of cumulus cells on *in vitro* maturation of porcine oocytes in medium with or without IGF-I. Porcine oocytes with(n=144 and 118, ■) or without (n=128 and 120, □) cumulus cells were cultured for maturation. Between oocytes with and without cumulus cells in medium without IGF-I, means with different subscripts differ significantly (P<0.05).

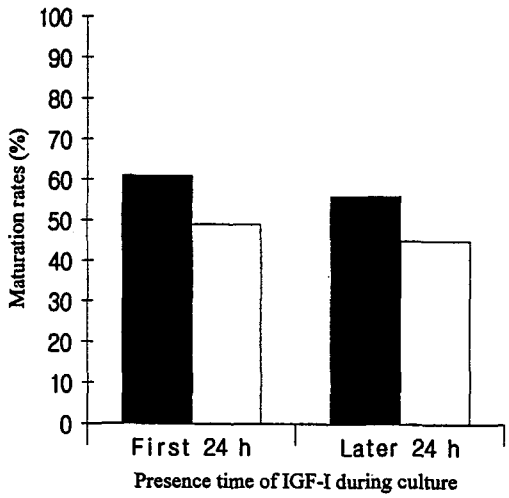

Fig. 2. Effect of exposure time of IGF-I on *in vitro* maturation of porcine oocytes with or without cumulus cells. Porcine oocytes with (n=164 and 162, ■) or without (n=114 and 142, □) cumulus cells were cultured for maturation. IGF-I was added for first 24 h period or later 24 h period during the culture for 48 h.

cultured in medium without FCS and PFF. The maturation rates in oocytes with cumulus cells for 48 h (48 and 67%) or 24 h (46 and 63%) period after culture in medium with or without IGF-I were significantly ($P<0.01$) higher than in oocytes without cumulus cells cultured in the presence (16%) or absence (18%) of IGF-I.

IV. DISCUSSION

The specificity of IGF-I action on oocyte maturation supports the possibility of IGF involvement in the control of meiotic maturation *in vivo*. Consistent with this idea are previous studies

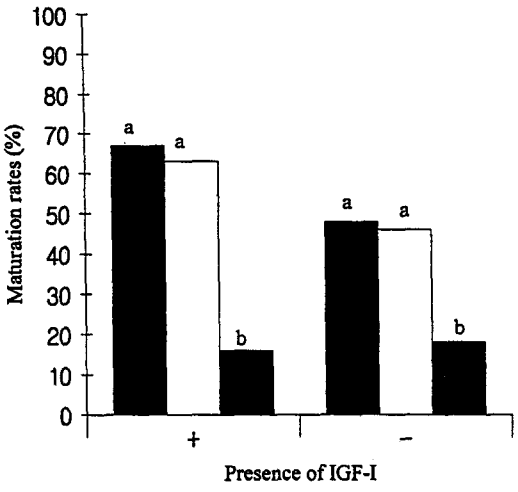

Fig. 3. Effect of periods with cumulus cells on *in vitro* maturation in medium with or without IGF-I in porcine oocytes. Porcine oocytes with cumulus cells for 48 h (n=84 and 92, ■), first 24 h period (n=112 and 92, □) or 0 h (n=126 and 110, ▨) were cultured for *in vitro* maturation in medium without FCS and PFF. Different superscripts are significantly different (P<0.01).

demonstrating a positive action of IGF on rabbit (Lorenzo et al., 1996) and bovine (Rieger et al., 1998) oocyte maturation as well as a physiological role in ovarian steroidogenesis and plasminogen activator activity in the rabbit (Yoshimura et al., 1996). The present study has demonstrated a interaction of IGF-I and cumulus cells on *in vitro* maturation of porcine oocytes. While IGF-I tested herein stimulated little or no differences in cumulus cells expansions between with and without IGF-I under the microscopic observation, a pronounced induction of maturation was observed during the oocyte culture. This growth factor has been reported in rat ovarian in the acceleration of progesterone accumulation in cultured granulosa cell (DeMoura et al., 1997). Conversely, or additionally, local production of IGF-I and other growth factors could function in an autocrine or paracrine manner within the developing follicle. Whitley et al. (1998) has demonstrated that insulin influenced the IGF-I system in a manner consistent with slowing follicular growth and possibly allowed more follicles to become available for ovulation. There are also IGF-binding protein production by primary culture of ovine granulosa and theca cell (Armstrong et al., 1996a) that IGF-binding protein production in the developing ovarian follicle is dependent on both cell type and follicle size and is regulated by IGF-I and gonadotropins. Furthermore, IGF-I has a well-established mitogenic effect on cultured cumulus cells (Armstrong et al., 1996b) and dramatically modifies the response of these cells to other hormones (Armstrong et al., 1996b ; Khamsi and Armstrong, 1997). Thus, while it is possible that IGF-I could act alone, it could also interact with cumulus cells to alter the meiotic state of the oocyte.

In the present study, the effect of IGF-I and cumulus cells was examined on nuclear maturation during *in vitro* maturation of porcine immature oocytes, and this factor was affected at GVBD and M-II on nuclear maturation of oocytes without cumulus cells. Cumulus oophorus expansion in mammalian oocytes occurs in response to an ever-changing milieu of gonadotrophins, growth factors, steroids, factors secreted by the oocyte and other unknown molecules (Buccione et al., 1990). These compounds could be contributing to maturational changes that occur in the oocyte mediated by intracellular messengers such as cAMP, calmodulin or diacylglycerol (Goncalves and Graves, 1992).

Oocytes maturation in the present work was effect in medium with high concentrations (10ng/ml) of IGF-I in oocytes with cumulus ce-

lls (Table 1). It is possible that there is a relationship between IGF-I and oocyte maturation, particularly as high concentrations of IGF-I were found in the cumulus oophorus of rats (Oliver et al., 1989). The most successful *in vitro* maturation systems used fetal serum or oestrous or pro-oestrous serum to optimize development of oocytes (Schellander et al., 1990). However, when the maturation medium used in the present study (Fig. 3) was not supplemented FCS and PFF, maturation rates were low in oocytes without cumulus cells. By using a practically FCS and PFF-free media for the *in vitro* maturation in the work presented here, it was possible to examine the relationship between IGF-I and regulation of nuclear maturation in oocytes with or without cumulus cells, while effectively ruling out the influence of unknown factor(s) in FCS and PFF.

The results presented here show that IGF-I enhance maturation ability of oocytes without cumulus cells in medium with FCS and PFF, and effect of presence period of IGF-I were examined in oocytes with cumulus cells. Cumulus expansion usually refers to the dispersion of cumulus cells by mucification during oocyte maturation. Relatively few reports have been published on the maturation of denuded bovine oocytes (Leibfried-Rutledge et al., 1989 ; Lorenzo et al., 1994). These reports refer to oocytes found to be nude on aspiration and not to normal complexes oocytes-cumulus that subsequently denuded as in the presented. Lorenzo et al. (1994) reported a significant improvement over control values when bovine complexes oocytes -cumulus- were matured in the presence of IGF-I or EGF. This effect was not apparent for denuded oocytes. In this study, the maturation rates in cumulus free oocytes was extremely low (16 and 18%). Why such a low rate was observed is unclear, but the reason may be related to absence of serum and follicular fluids. Serum and follicular fluid are highly complex combination of components including proteins, fatty acids, vitamins, hormones, trace elements and growth factors.

In conclusions, the present study shows that, at least in porcine, the addition of IGF-I to the maturation medium results in more oocytes undergoing nuclear maturation than without IGF-I. However, all these stimulatory actions are possible during culture of oocytes with or without cumulus cells in medium with FCS and PFF.

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요 약

돼지난자의 체외성숙시 Insulin-like Growth Factor-I과 난구세포의 영향

박춘근 · 조재원[1] · 신명균[2] · 정희태 · 양부근 · 김정익

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본 연구는 돼지난자의 체외성숙동안 insulin-like growth factor-I (IGF-I)과 난구세포의 영향을 검토하기 위하여 실시하였다. 미성숙난자를 0(60%), 1(61%), 5(62%) 및 10ng /ml(72%)의 서로 다른 IGF-I의 농도로 첨가하여 배양했을 때 체외성숙율은 큰 차이를 나타내지 않았다. 또한 10ng /ml의 IGF-I이 첨가된 배양액내에서 난자를 배양한 경우, 난구세포 부착시(68%) 제거(52%)된 난자에 비해 높은 성숙율을 나타냈지만 유의적인 차이는 인정되지 않았다. 그러나 IGF-I이 첨가되지 않은 경우에는 난구세포 부착(63%)난자가 제거(32%)된 난자에 비해 유의적($P<0.05$)으로 높은 성숙율을 나타냈으며, IGF-I를 성숙배양기간중 전반기 24시간 또는 후반기 24시간 동안만 첨가했을 때의 난구세포 부착시(61과 49%) 제거된 (45와 49%) 난자에 비해 높은 성숙율을 나타냈다. 한편, IGF-I의 존재 여부에 관계없이 FCS와 돼지난포액(PFF)이 무첨가된 배양액에서 48시간동안 또는 배양전반기 24시간동안 난구세포를 제거한 경우(16과 18%)로 난구세포 부착시 (46과 63%)에 비해 유의적($P<0.01$)으로 낮은 성숙율을 나타냈다. 이와 같은 결과는 난구세포가 IGF-I의 존재 유무에 관계없이 난자의 체외성숙에 필수적이며, FCS와 PFF첨가시 난구세포가 제거된 난자의 체외성숙을 촉진하는 것으로 밝혀졌다.

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