Oocyte Maturation and Fertilization *in vitro* in Rhesus Monkey (*Macaca mulatta*)*

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It is not clear how oocyte maturation, embryo development and differentiation are regulated, though they are critical to the mechanism of reproduction. *in vitro* fertilization has been greatly successful in domestic animals and human since the ability of immature mammalian oocytes of undergoing maturation *in vitro* was first demonstrated by Pincus and Enzmann[1]. Although human *in vitro* fertilization has experienced immense popularity and more than 5000 test-tube babies have been born to date[2], serious legal and ethical problems are raised by potential experimentation on these researches. The reproductive physiology of non-human primate is very similar to that of human. Therefore, non-human primate model for IVF is not only useful for solving the problems of infertility for human, but also important to facilitate the study of mechanism of fertilization including the regulation of sperm and oocyte maturation, embryogenesis, implantation and genetic pathology. Successful IVF is dependent upon mature oocytes which are affected by hormone, biochemistry factors and culture conditions *in vitro*[3]. The oocytes from hyerstimulation using exogenous gonadotropins maturated and fertilized *in vitro* have been reported in rhesus monkey, squirrel monkey and cynomolgus macaque[4]. There are few reports about the oocyte obtained from the ovaries of unstimulated monkeys[5]. Promotion of oocyte maturity *in vitro* is closely related to successful fertilization, embryo growth and cleavage[3,6]. This study is designed to evaluate the effects of follicular stimulating hormone (FSH) on the maturation of rhesus oocytes and embryonic development potential following fertilization *in vitro*.

1 Materials and Methods

The ovaries were cut from 12 unstimulated rhesus monkeys used in medical industry. The monkeys had never been used for any experiment before they were sacrificed for the medicine, and their menstrual cycle was unclear. The ovaries were collected into TALP-

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HEPES(TH) 2 h after the animal death. The denuded oocytes or oocytes with few cumulus obtained from the ovaries were pooled in the medium balanced overnight in 5% CO_2 and 95% air at 37°C in a culture room and fallen into two groups: (i) TALP with 20% human placental cord serum (HCS); (ii) TALP with 20% HCS, plus 20% FSH-enriched animal pituitary preparation (FSH-P), respectively. Observation was made at the beginning of the culture and at 24-h intervals. When the oocytes showed a distinct perivitelline space (PVS) or a polar body (PB1), they were inseminated. Rhesus monkey semen was collected by penile electroejaculation. The sperm was prepared as previously described[4, 5] with a little modification. The semen was washed twice with TH at pH 7.4. After the wash procedure, sperm at 10^6 motile cells/ml was resuspended in TALP containing BSA (0.3%) and activated by exposure to dibutyryl cyclic adenosine monophosphate (dbcAMP) and caffeine (0.2 mmol/L each) for 1—1.5 h. After incubation for 6—18 h with sperm (10^5/ml) in TALP containing 0.3% BSA and 2% HCS, eggs were examined for the presence of pronuclei and/or a second polar body (PB2). Fertilized eggs were transferred to growth medium (TALP containing 0.3% BSA and 20% HCS) and the culture was continued. The fertilization rate was determined by counting of the fertilized eggs developed over 2-cell embryo. Chisquire was used for statistical analyses.

2 Results and Discussion

A total of 568 oocytes were obtained from the ovaries of the 12 unstimulated rhesus monkeys. Most of them (65%) were covered in condensed cumulus not used in this study. The 200 denuded oocytes or the ones without cumulus were selected in the research. Fifty-four percent of the oocytes (108/200) showed PVS or PB1 (Fig.1(a)) after incubating for 24—96 h. Most of the oocytes matured after culture for 72h (28.6%) and 96h (50.0%) (Fig.2). There was no significant difference of fertilization between the oocytes treated with FSH (39.8%) and without FSH (30.8%), but the fertilized egg development to 16—32-cell embryo was significantly higher in the group (82.8%) treated with FSH than in the group without FSH (18.2%, $p<0.01$, Table 1). The embryos at different developmental stages (2-cell, 8-cell and early morula) are shown in Fig.1(b)—(d).

Although some qualified oocytes can be obtained by hyperstimulation in non-human primate, there are some disadvantages in the hyperstimulation, e.g. the steroid hormone in the follicle fluid by hyperstimulation is not normal in comparison with that in natural cycle and the animals cannot be used in hyperstimulation repeatedly as exogenous gonadotropins produced neutralizing antibodies[5]. Undoubtedly it is useful for the understanding of the regulation of oocyte maturation and mechanism of fertilization if the oocytes obtained from the ovaries of unstimulated monkey can mature under the control conditions. Morgan *et al.*[6] reported the oocytes obtained from ovariectomies of unstimulated rhesus monkeys. Our study shows that oocytes obtained from the ovaries of unstimulated rhesus monkeys sacrificed for 2 h can resume maturation *in vitro*, they can be fertilized, and then, early morular embryos can be obtained by *in vitro* culture.

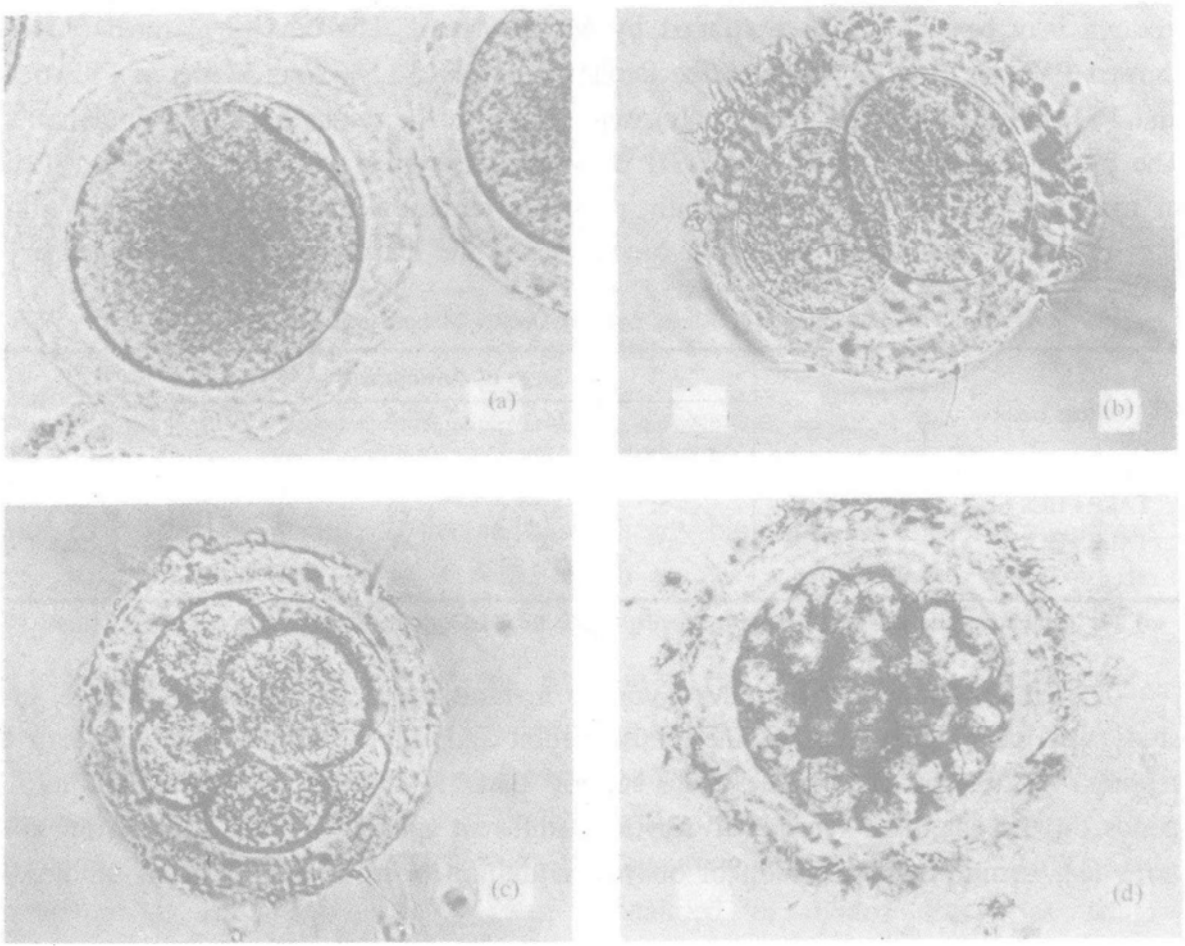

Fig.1. *in vitro* maturation of ovarian oocytes from unstimulated rhesus monkeys. (a) A mature egg after 72 h of *in vitro* maturation in TALP alone with PB1 in perivitelline space, ×400; (b) 2-cell embryo 30 h after insemination, ×400; (c) 8-cell embryo, ×400; (d) an early morular embryo, ×400.

Maturity of oocyte includes nuclear and cytoplasmic maturation. The superficial morphology of nuclear maturity is germinal vesicle breakdown or extrusion of PB1, but crucial to examine cytoplasmic maturation[7]. The immature cytoplasm of oocyte can affect fertilization, resulting in polyspermy decrease viability and/or retard embryo development, and even resulting in embryonic death[5]. It is indicated by both *in vivo* and *in vitro* study that gonadotropin promotes oocyte maturation[4 – 7]. Morgan *et al.* reported that FSH enhances maturation, fertilization and embryonic cleavage of the cumulus oocytes (at least 2 layers cumulus covered) obtained from

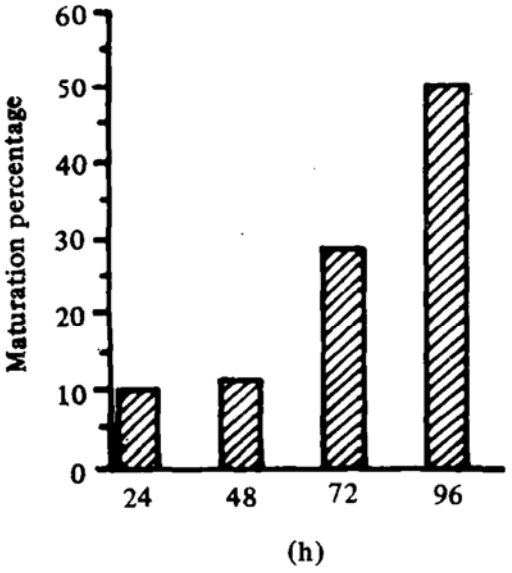

Fig.2. The maturation percentage of rhesus monkey oocytes in different durations *in vitro*.

the ovaries of unstimulated rhesus monkey *in vitro*. The maturation of oocytes used in this research may be higher than that used by Morgan *et al.* The oocytes matured (108 eggs showed PVS or PB1) *in vitro* and the fertilization rates (72.5% and 57.9% in FSH-treated and FSH-untreated groups, respectively) were similar to the results by hyperstimulation[4, 6, 7]. The present results also show that FSH does not have great effect on increasing fertility of maturing oocytes, but it significantly promotes developmental potential of the fertilized eggs (Table 1). It indicates that FSH can accelerate the maturation of oocyte plasma.

Table 1 Development of Rhesus Monkey Oocytes Matured and Fertilized *in vitro*

Culture medium	Egg number	Stage of development				Fertilization rate (%)[a)]
		PVS/PB1	2—4 cells	5—8 cells	16—32 cells	
TALP+HCS+FSH	93	51	—	6	31	39.8
TALP+HCS	107	57	7	20	6	30.8
Total	200	108	7	26	37	35.0

a) The fertilization rate was determined by counting of the fertilized eggs developed over 2-cell embryo stage.

The culture duration to achieve complete maturation of rhesus monkey oocytes in the study was longer than those of the mouse, rabbit and sheep[3], and also longer than that reported by Morgan *et al.*[6] The results support that the duration of oocyte maturation depends on the length of reproductive cycle in different species[3]. Howe does culture conditions (e.g. serum) affect duration of oocyte maturation *in vitro* will be further studied.

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