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FSH priming improves oocyte maturation, but priming with FSH or hCG has no effect on subsequent embryonic development in an in vitro maturation program

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Abstract

Aim: To determine whether maturation and subsequent blastocyst development of in vitro matured oocytes can be improved by in vivo follicle stimulating hormone (FSH) or human chorionic gonadotrophin (hCG) priming, using a mouse model.

Experimental design: Five groups of oocytes were used: in vivo control, in vitro matured (IVM) control, IVM after 24 h in vivo priming with FSH, IVM after 48 h in vivo priming with FSH and IVM after 16 h in vivo priming with hCG. In vitro fertilization (IVF) was performed on all groups.

Oocyte maturation, fertilization, blastocyst development rates and blastocyst cell numbers were assessed for all groups.

Results: Significant improvement in oocyte maturation was observed in the two FSH priming groups compared with the IVM control group ($P < 0.005$ and $P < 0.001$, respectively). There were no significant differences in fertilization between all five groups. Blastocyst development was significantly higher in the in vivo control compared to the IVM groups ($P < 0.001$). No significant differences were observed in blastocyst cell numbers among all five groups.

Conclusions: While FSH priming improves the maturation rate of IVM oocytes, FSH or hCG priming does not improve development to the blastocyst stage.
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Keywords: In vitro maturation; Blastocyst; Mouse; Priming

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1. Introduction

The maturation of mammalian oocytes in vivo is the culmination of a number of factors. A cohort of follicles develops at the beginning of the menstrual cycle and a dominant follicle grows under the influence of follicle stimulating hormone (FSH). As a result of increasing luteinizing hormone (LH) levels, the oocyte in the dominant follicle matures and develops to the metaphase II (MII) stage of meiosis and is released from the ovary by the process of ovulation [1].

In vitro fertilization (IVF) technology has utilized the principles of FSH administration for follicle and oocyte recruitment and growth and of human chorionic gonadotrophin (hCG) for final oocyte maturation prior to ovulation [2]. These simple regimens have been successful in generating mature oocytes in clinical programs for humans as well as in the agricultural industry. Such oocytes are capable of fertilization, and given the appropriate culture conditions, are able to develop normally to the blastocyst stage [3,4]. However, when immature oocytes are isolated from the ovary and matured in vitro under the influence of FSH and hCG, the resulting MII oocytes obtained have poor rates of embryo viability after fertilization [5–10].

In vitro maturation (IVM) potentially, offers many advantages over exogenous gonadotrophin stimulation, including reduced costs and side effects and eliminating the risk of ovarian hyperstimulation syndrome [11]. It is therefore important that IVM technology be improved so that embryo competence is at least equivalent to that observed following exogenous gonadotrophin stimulation.

A number of researchers have used in vivo priming with FSH or hCG prior to IVM, with variable findings. Younis et al. [12] observed a significant increase in the number of oocytes maturing to the MII stage of meiosis in their IVM system in cynomolgus monkeys (*Macaca fascicularis*) following a single 1000 IU dose of pregnant mare's serum gonadotrophin (PMSG) in the follicular phase. Similarly, a significantly improved yield in the number of MII oocytes after IVM of cumulus-enclosed oocytes retrieved from follicles greater than 4 mm in diameter has been observed in humans following FSH priming in the follicular phase [13]. Low-dose administration of FSH in the luteal phase has also been observed to result in efficient maturation and fertilization of immature human oocytes after IVM [14]. In contrast, in human studies Mikkelsen et al. [15,16] found no improvement in the number of oocytes maturing to MII after FSH priming and IVM. Interestingly, they also found that the MII oocytes did not show any improvement in developmental competence in terms of fertilization and the first 2 to 3 days of embryo cleavage.

Priming with hCG in the late follicular phase (Days 10–14) has been performed in women prior to oocyte aspiration [17,18]. The results of these studies show significantly higher percentages of oocytes maturing to MII after hCG priming. However, hCG priming was not able to provide the oocytes any improvement in fertilization or early embryo cleavage.

The aims of the current study were two-fold: first, to determine whether FSH or hCG priming can improve the maturation rate of IVM oocytes; and second, whether FSH or hCG priming are able to improve developmental competence through to the blastocyst stage of embryos generated from IVM oocytes. An immature mouse model was used for this purpose.

2. Materials and methods

Three-week old F1 hybrid (C57 × CBA) females and 2-month old males (CBA) of proven fertility were used in this study. The Murdoch University Animal Ethics Committee gave approval for the use of animals in this project. The females were placed in five groups: in vivo superovulation control, IVM control, FSH priming 24 h prior to IVM, FSH priming 48 h prior to IVM, and hCG priming 16 h prior to IVM.

2.1. In vivo superovulation of mice

The superovulation regime of mice was as described previously [19]. Briefly, females were treated with 7.5 IU of PMSG (Sigma Chemical Co., St. Louis, MO, USA) and 48 h later with 10 IU hCG (Profasi, Serono, French's Forest, NSW, Australia). Sixteen hours later, the mice were humanely killed and their ovaries and oviducts placed into Dulbecco's phosphate buffered medium (PBS) (Gibco, Grand Island, NY, USA) supplemented with 3 mg/ml bovine serum albumin (BSA) (Sigma). Cumulus masses containing the oocytes were aspirated from the distal end of the oviducts and observed carefully for meiotic maturity. The MII oocytes (within their cumulus masses) from all groups were placed in human tubal fluid (HTF) medium (made in-house in our laboratory) supplemented with 3 mg/ml BSA. These oocytes acted as the in vivo controls for the study.

2.2. Priming with FSH or hCG

There were two groups receiving FSH priming. The first was treated with 7.5 IU PMSG 24 h prior to ovary removal, while the second was treated with 7.5 IU PMSG 48 h prior to ovary removal. The hCG group was administered 10 IU hCG 16 h prior to ovary removal. The antral follicles were dissected manually from the ovaries of mice of each of these groups using 26 gauge insulin needles (Becton Dickinson, Franklin Lakes, NJ, USA) and placed into the IVM system.

2.3. In vitro maturation of oocytes

Follicles from all groups except the in vivo control group underwent IVM as described previously [19]. The dissected preantral follicles were placed into 20 ul droplets of MEM-alpha medium (Gibco) supplemented with 10% fetal calf serum (FCS) (Sigma), 0.1 IU/ml FSH (Gonal F, Serono, French's Forest, NSW, Australia), 0.5 IU/ml hCG and 0.05 mg/ml penicillin (Commonwealth Serum Laboratories, Parkeville, Vic., Australia) under sterile paraffin (BDH, Poole, Dorset, UK) at 37 °C in 5% CO_2 atmosphere for 24 h. Oocytes within their cumulus masses that had ovulated from the follicles were checked for meiotic maturation and all MII stage oocytes within their defined groups were placed into temporary cultures of HTF supplemented with 3 mg/ml BSA.

2.4. In vitro fertilization and embryo culture

In vitro fertilization was performed on the MII oocytes from all groups. The oocytes were placed into separate aliquots of HTF supplemented with 5 mg/ml BSA, with

the addition of capacitated epididymal sperm at a concentration of 1 million/ml for 5 h. The oocytes were then removed from the insemination medium and placed into fresh HTF, supplemented with 3 mg/ml BSA. Fertilization was confirmed 16–20 h later by the presence of two-cell embryos. Blastocyst development was assessed after 5 days.

2.5. Differential blastocyst staining

All blastocysts were stained to quantify total cell number, as well as inner cell mass (ICM) and trophectoderm (TE) cell numbers based on the methods of Handyside and Hunter [20]. The blastocysts were removed from culture and placed in heat-inactivated rabbit anti-mouse antiserum diluted 1:5 with hepes buffered HTF (HHTF) (made in-house in our laboratory) supplemented with 4 mg/ml BSA for 15 min. After washing, the blastocysts were transferred to guinea pig complement (collected in our laboratory) diluted 1:5 with the HHTF supplemented with 10 ug/ml propidium iodide (Sigma) for 30 min at 37 °C. The blastocysts were washed in PBS and fixed with absolute ethanol (BDH). They were transferred to absolute ethanol with the addition of 10 ug/ml bisbenzimide (Hoechst 33258, Sigma) for 1.5 h at 4 °C. The blastocysts were incubated in absolute ethanol once again for a period of 1 h at 4 °C before mounting on slides in glycerol (Sigma).

The blastocysts were observed under fluorescent microscopy at magnification 1000× with oil immersion (Leitz Laborlux, fitted with UV excitation). The TE cells appeared red, while the ICM appeared blue. The total cell numbers, along with cell type were recorded.

2.6. Statistical analysis

Chi-square analyses were used to compare the maturation rates of the IVM groups, while a chi-square contingency table was used to compare fertilization and blastocyst development rates for all groups. One-way ANOVA was performed to test the difference in the blastocyst cell numbers among the five groups.

3. Results

3.1. In vitro maturation of oocytes

The number of MII oocytes achieved for each of the IVM groups are shown in Table 1. Both FSH priming groups had significantly higher rates of oocyte maturation than either the IVM control or the hCG priming groups.

3.2. In vitro fertilization

The fertilization rates for each of the groups are shown in Table 2. There were no significant differences in fertilization among the five groups.

Table 1
Maturation rates (MII oocyte per follicle cultured) for each of the IVM groups tested

Group	Follicles cultured (*n*)	MII oocytes after 24 h of IVM (*n*) (%)
IVM control	148	94 (63.5)*
24 h FSH priming	71	62 (87.3)†
48 h FSH priming	88	78 (88.6)‡
16 h hCG priming	130	82 (63.1)

χ^2: * vs. †: $P < 0.005$; * vs. ‡: $P < 0.001$.

Table 2
Fertilization rates for each of the five groups

Group	Fertilization (*n*) (%)
In vivo control	86/104 (82.7)
IVM control	68/94 (72.3)
24 h FSH priming	46/62 (79)
48 h FSH priming	58/78 (74.4)
16 h hCG priming	57/82 (69.5)

3.3. Blastocyst development

Blastocyst development rates are shown in Table 3. Blastocyst development rates for the IVM groups were significantly reduced when compared with the in vivo control group ($P < 0.001$).

Table 3
Blastocyst development rates for each of the five groups

Group	Blastocyst development (*n*) (%)
In vivo control	61/85 (70.9)
IVM control	14/68 (20.6)
24 h FSH priming	12/49 (25.5)
48 h FSH priming	13/58 (22.4)
16 h hCG priming	10/57 (17.5)

Table 4
Total and differential blastocyst cell numbers for all five groups

Group	Trophodectoderm cell number (mean ± S.D.)	Inner cell mass cell number (mean ± S.D.)	Total cell number (mean ± S.D.)
In vivo control	48.76 ± 4.02	16.15 ± 2.28	65.00 ± 6.08
IVM control	47.50 ± 3.48	15.36 ± 1.12	62.86 ± 4.42
24 h FSH priming	49.91 ± 3.73	16.64 ± 1.63	66.55 ± 5.03
48 h FSH priming	49.76 ± 3.53	16.50 ± 1.73	66.17 ± 5.11
16 h hCG priming	46.90 ± 3.51	14.80 ± 1.69	61.70 ± 4.9

3.4. Differential blastocyst staining

Differential blastocyst staining was possible in 54 of the in vivo controls, 14 of the IVM controls, 11 of the 24-h FSH priming, 12 of the 48-h FSH priming and 10 of the hCG priming groups. Total blastocyst cell numbers along with ICM and TE cell numbers did not differ significantly among all five groups (Table 4).

4. Discussion

The significant improvement in oocyte maturation following FSH priming and IVM observed in this study supports the results of previous studies [12,13,21]. Other studies, however, have found no improvement in oocyte maturation following FSH priming and IVM [15,16,22]. It is possible that the improvements observed in mature oocytes in the current study resulted from a bias in the selection process at the time of follicle dissection. The follicles dissected from both the 24- and 48-h FSH priming groups were noticeably larger than those in the IVM control and hCG priming groups. The influence of FSH upon follicle recruitment and development in vivo may therefore have contributed to an increased likelihood in selection of follicles destined to produce mature oocytes.

Intact follicle IVM was used in this study, compared with isolated oocyte culture in previous studies. Cells within the follicle are known to exhibit close associations through their gap junctions [23]. Interactions between the granulosa and thecal cells of the follicle have been shown to be involved in regulating the meiotic maturity of the oocyte [24]. Factors within the follicle compartment, therefore, may have contributed to an improvement in the meiotic competence of the cultured oocytes.

In contrast to the current study, Chian et al. [18] found significant improvements in oocyte maturation following hCG priming. Their study used immature oocytes retrieved on cycle days 10–14 of a woman's cycle, whereas those collected in the present study were from preantral follicles of prepubertal mice. Luteinizing hormone (hCG) receptors are enhanced in the late follicular phase due to a sustained presence of FSH and increasing estradiol levels [25]. It is therefore possible that the follicle–oocyte complexes in the present study were collected prior to becoming physiologically functional.

There were no significant differences in fertilization observed between the in vivo controls, IVM control and priming groups in this study. The zona pellucida of IVM oocytes is known to undergo hardening, which may reduce the chance of fertilization [26]. Intracytoplasmic sperm injection (ICSI) has therefore been employed in many human IVM programs to reduce this risk [7,9,27]. Moreover, ICSI has been employed as the insemination method to achieve fertilization in programs using FSH [15,16] and hCG [17,18] priming prior to IVM. Interestingly, however, the mature oocytes following FSH or hCG priming and IVM were fertilized using traditional IVF techniques in the current study.

Embryo viability is invariably reduced with oocytes cultured by IVM, with implantation rates lower than for the traditional IVF procedure [7,18,22,27,28]. Priming with FSH or hCG was not able to improve embryo development to the blastocyst stage in this study. Gonadotrophins are able to control nuclear maturation and the resumption of meiosis of the

immature oocyte by influencing concentrations of cAMP within the oocyte and follicle [29]. However, the role of gonadotrophins in cytoplasmic maturation is less clear. Recent evidence would suggest that cytoplasmic maturity of the oocyte is of major importance for subsequent embryonic development [30]. Izadyar et al. [31] used growth hormone in their IVM culture system to improve the developmental competence of oocytes. Their study suggested that these improvements were a result of improved cytoplasmic maturation due to cortical granule migration. Similarly, inhibin A and activin A have been reported to enhance cytoplasmic maturation in an IVM culture system [32]. The injection of donor cytoplasm from in vivo matured oocytes to in vitro matured oocytes has also been found to significantly improve embryonic development to the blastocyst stage [19].

The total blastocyst cell numbers, together with the differential blastocyst cell numbers did not differ significantly among all groups. It is possible that there is a selection process occurring during the early cleavage stages, and those embryos progressing through to the blastocyst stage have equal embryonic competence. Though viability was not assessed by the transfer of blastocysts to pseudopregnant mice, total blastocyst cell number [33] and differential blastocyst cell number [34] are considered to be effective predictors of embryonic development capacity.

In conclusion, this study has found that while a significant improvement of maturation of immature oocytes was observed in IVM following FSH priming, the use of either FSH or hCG priming does not afford the subsequent embryo any improvement in fertilization or development to the blastocyst stage. We believe that the animal model used in this study is relevant to human IVM. Previous priming studies in the human may not reflect the true nature of the effect of priming in IVM. Women recruited into such studies are from various infertility backgrounds and their oocyte and follicle complexes have been exposed repeatedly to endogenous, and possibly also exogenous gonadotrophins. The present study ensured that the oocyte and follicle complexes had no previous exposure to gonadotrophins from either endogenous or exogenous sources.

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