Original Article

The Effect of Human Chorionic Gonadotropin on the *In vitro* Development of Immature to Mature Human Oocytes: A Randomized Controlled Study

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Abstract

Context: In controlled ovarian hyperstimulation cycles, 15% of oocytes have been proven to be immature. Key factors include failure in signal transmission from the cumulus cell to the oocyte, insufficient level of luteinizing hormone, and internal conditions of the oocyte itself. **Aims:** The aim of the present study was to investigate the effect of human chorionic gonadotropin (hCG) on the *in vitro* maturity of partially cumulus-denuded immature oocytes collected after controlled ovarian stimulation for *in vitro* fertilization (IVF). **Settings and Design:** This was a prospective, randomized controlled design at the department of obstetrics and gynaecology, university hospital. **Subjects and Methods:** Infertile women underwent gonadotropin-releasing hormone antagonist stimulated protocol for IVF with final maturation triggered by hCG, partially cumulus-denuded immature human oocytes were allocated to two groups: the first was treated with fertilization medium and the second was treated with fertilization medium and hCG. They were cultured for 24 h. Outcomes measured were the oocyte maturation rates to metaphase II (MII) and glucose-6-phosphate dehydrogenase (G6PD) activity of *in vitro* maturation (IVM) mature oocytes which represent the oocyte quality. **Statistical Analysis Used:** The Mann–Whitney *U*-test and One-way ANOVA were used to compare continuous variables, and Chi-square was used for categorical data. **Results:** In all, 250 immature stimulated oocytes were allocated (125 per group). The maturation rate was higher in the hCG supplement group (48% vs. 39.2%) without significance. The positive brilliant cresyl blue results among the MII oocytes developed from the metaphase I (MI) were significantly higher in the hCG group ($P = 0.001$). **Conclusions:** Rescue IVM in fertilization culture medium plus hCG was slightly better than that in the only fertilization culture. MII oocytes developed from MI in hCG supplemented medium had a higher quality based on the measured G6PD activity.

Keywords: *Glucose-6-phosphate dehydrogenase activity, human chorionic gonadotropin, in vitro maturation, partially cumulus-denuded immature oocyte*

Introduction

Infertility is a common problem in poor ovarian reserve women.[1] For *in vitro* fertilization (IVF), 15% of collected oocytes are immature (4% of them are in the metaphase I [MI] and 11% are in the germinal vesicle [GV] stage).[2-4] Factors that affect oocyte

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maturity include signal transmission failure between the cumulus cell and the oocyte, luteinizing hormone (LH) insufficiency, and intrinsic factors of the oocyte.[5]

Basically, *in vivo*, LH plays an important role in regulating oocyte growth and triggering the resumption of the meiosis and nuclear maturation of oocytes. During controlled ovarian stimulation (COS), human chorionic gonadotropin (hCG) replicates the surge of LH to stimulate the maturation and growth of oocytes due to its long half-life and greater potency.[6,7] The effect of gonadotropin is based on its physiologic role in oocyte-cumulus cell communication and is highly beneficial to nuclear and cytoplasmic maturation of the cumulus-oocyte complex.[8]

Successes concerning rescue *in vitro* maturation (IVM) oocytes are limited; hence, in the current clinical practice, they are not widely used and remain a nonstandard protocol. We attempted to explore factors that improved the quality of IVM rescued method.[9-12]

This study aimed to compare the results of *in vitro* development of immature oocytes cultured in cleavage culture medium and those cultured in hCG supplemented medium.

Subjects and Methods

The study was approved by the Ethics Clearance Committee on Human Rights Related to Research Involving Human Subjects. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and the Helsinki Declaration of 1975. All 250 immature stimulated oocytes from the 97 patients were allocated for IVM. These volunteered women, aged 30–42 years with primary or secondary infertility, underwent COS between June 1, 2018 and May 31, 2019. Participants were recruited using suggestions from nurses and physicians. The eligible participants were selected for infertility treatment and underwent COS using the antagonist protocol. Then, oocytes were picked up under intravenous anesthesia in the operating room according to the standard protocol of the hospital. Exclusion criteria included polycystic ovary syndrome, ovarian cysts during ovum pick-up (OPU), and total oocytes retrieved was <3. The number of immature oocytes was limited to the maximum of five oocytes per patient.

Collected cumulus-oocyte complexes were cultured in fertilization medium and placed in 5% CO_2 incubation for 4–6 h and stripped by mechanical pipetting and hyaluronidase. Immature oocytes (GV and MI), with cumulus cells left from 10% to 20% after stripping, were employed in the study and randomized. Serial numbered opaque and sealed envelopes were established according to a computer-generated block of four randomizations and opened by a physician at the artificial reproductive technology laboratory after enrollment. In the control group, immature oocytes were cultured in Sydney IVF fertilization medium (Cook Medical) 0.2 ml. In the study group, immature oocytes were cultured in Sydney IVF fertilization medium (Cook Medical) with hCG 0.5 IU/ml 0.2 ml and placed in 5% CO_2 incubation for 24 h. Oocyte maturation was observed 24 h later.

Developed mature oocytes (metaphase II [MII] stage) from both groups were transferred; 13 μmol/L brilliant cresyl blue (BCB) was diluted in Sydney IVF fertilization medium and incubated for 90 min in 5% CO_2 incubation for 90 min. Oocyte staining was examined by inverted microscopy.

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp, Armonk, NY, USA). For baseline demographics, clinical and laboratory characteristics, mean ± standard deviation, and median (interquartile range) were used for continuous data, and the percentage was used for categorical data. The Mann–Whitney *U*-test and one-way ANOVA were used to compare continuous variables, whereas Chi-square was used for categorical data. Median, minimum, and maximum values were shown when the data presented an abnormal distribution. The level of statistical significance was set at $P < 0.05$.

Results

In this study, 250 immature stimulated oocytes from 97 patients were allocated for IVM. The characteristics of the patients are shown in Table 1. Every patient received the antagonist protocol, and the ovulation was then triggered by hCG. The medium averages of the immature oocytes from the OPU comprised: GV Stage 1 oocytes, MI Stage 1 oocytes, and MII stage 10 oocytes.

Table 1: Demographic data

Characteristic	Patient
Age (years)	36.51±3.46
BMI (kg/m2)	22.65±3.26
Nulliparous (%)	87.6
AFC (follicle)	8.45±3.21
Stimulation duration (days)	10.11±1.07
Gonadotropin dosage (IU)	2754.12±654.41
Antagonist duration (days)	3.88±1.11
Oocyte yield, median (maximum, minimum)	
GV	1 (0.5)
MI	1 (0.4)
MII	10 (3.19)

AFC=Antral follicle count, GV=Germinal vesicle, MI=Metaphase I, MII=Metaphase II, BMI=Body mass index

In all, 125 immature oocytes (GV, 73 oocytes, MI, 52 oocytes) were allocated for IVM with hCG added to the fertilization culture medium and another 125 immature oocytes (GV, 75 oocytes, M1, 50 oocytes) were allocated for IVM with only fertilization culture medium. The maturation rate at 24 h was higher in the hCG group supplement when compared with the control group (48% vs. 39.2%) without significance [Table 2].

After that the MII oocytes received from IVM from both groups were employed to investigate glucose-6-phosphate dehydrogenase (G6PD) activity using BCB dye. The result showed that BCB positive results among the MII oocytes developed from the MI group were significantly higher in the hCG group those from the control group ($P = 0.001$), but no difference was found in the MII oocytes developed from the GV in both groups [Table 3].

Discussion

In this study, the maturation of immature oocytes was achieved in hCG supplemented culture medium. Optimal meiotic resumption was elicited by hCG action even though the difference was without significance. When adding the supplement hCG, GV would evolve to MII more compared with the control group (32.8% vs. 24%). Furthermore, MI would evolve to MII more when added with the supplement hCG (69.2% vs. 62%). In summary, our results were in agreement with related studies, where gonadotropin in rescue IVM or hCG in standard IVM may have contributed to the nuclear maturation.[13-15]

In vivo, the preovulatory LH surge is essential to both oocyte meiotic resumption and ovulation.[6] At present, more studies have pointed out that recombinant LH or hCG has a more essential role in oocyte maturation in *in vitro* culture because recombinant LH or hCG added to the culture medium could promote a meiotic reinitiating, adding more oocyte nuclear maturation and cytoplasmic maturation and promoting protein synthesis and gene expression including facilitating oocyte metabolism and nutritional environment.[7,16]

Table 2: Maturation rate

Characteristic	hCG (%)	Control (%)	*P*
Immature oocytes			
GV	73	75	
MI	52	50	
Maturation rate	60 (48)	49 (39.2)	
GV to MII	24 (32.8)	18 (24)	0.365
MI to MII	36 (69.2)	31 (62)	0.288
Oocyte arrest			
GV	23 (31.5)	31 (41.3)	0.214
MI	16 (30.8)	19 (38)	0.442

hCG=Human chorionic gonadotropin, GV=Germinal vesicle, MI=Metaphase I, MII=Metaphase II

Table 3: Glucose-6-phosphate dehydrogenase activity using brilliant cresyl blue dye

Group	BCB status	hCG (%)	Control (%)	*P*
GV to MII	BCB+	14/24 (58)	6/18 (33)	0.098
MI to MII	BCB+	24/36 (67)	7/31 (23)	0.001

BCB=Brilliant cresyl blue, hCG=Human chorionic gonadotropin, GV=Germinal vesicle, MI=Metaphase I, MII=Metaphase II

A study conducted in 2011 did the immature human oocytes culture by treating the cumulus-partially denuded oocytes in 75 IU follicle stimulating hormone (FSH) and 75 IU LH added IVM medium. The authors reported that GV would turn to MII more than others when compared with the control group (68% vs. 60%) and would consume less time to maturation in IVM medium with 75 IU FSH/LH (22.7 vs. 23.7 h). From MI to MII, small differences were observed in the maturation rates in IVM medium with 75 IU FSH/LH and the control group.[15] Similar to the outcome of this study, we found a higher maturation rate, but without significance, in the hCG supplemented group both from GV and MI to MII. This might have been due to the effect of hCG on the LH receptor located on the surrounding cumulus cell leading to GV breakdown and meiotic resumption. Activated LH receptor regulates oocyte maturation and meiosis resumption through several mechanisms. One of the proven mechanisms acts through the epidermal growth factor (EGF) / epidermal growth factor receptor (EGFR) network. LH surge activates the EGF/EGFR signaling that decrease Cyclic adenosine monophosphate (cAMP) and Cyclic guanosine monophosphate (cGMP) level. Low levels of cAMP and cGMP result in releasing the inhibition of phosphodiesterase 3A, which activates a maturation promoting factor. Finally, the MPA induces oocyte chromosome segregation, meiosis I completion, and meiosis II oocyte formation.

After adding 0.5 IU/ml of hCG to the oocyte culture medium, we observed a small nonsignificant benefit. The dose of hCG supplement used in this study has been widely used in human oocyte IVM. This is also in accordance with several studies of animal oocytes.[14,17]

Significant oocyte quality is known to affect the developmental competence and implantation potential of the derived embryo. A popular method for identifying competent oocytes relies on staining with BCB to measure G6PD activity. BCB is promoted as a nontoxic method to identify oocyte potential. Furthermore, other research studies in animals such as cows, pigs, and sheep have concluded that oocytes with the BCB dye exhibit low G6PD activity and thus, can be fertilized, leading to a higher live birth rate than oocytes without BCB dye.[18-20] Therefore, in our

research, G6PD activity was studied using BCB dye to determine MII quality.

In this research, the MII stage, which develops from the MI group, possesses a higher chance of presenting the color from the BCB dye than that of the control group. However, the MII developed from GV has the same level of BCB dye in the hCG and control groups. In IVM, the culture system adequately supports nuclear maturation but fails to produce oocytes with cytoplasmic competency. Therefore, MII developed from GV is more asynchronous regarding the progression of nuclear and cytoplasm maturation.[21] HCG added to culture medium might perform similarly as in the *in vivo* group, which can fasten the nuclear maturation process while slightly promoting cytoplasm maturation.

For individuals with low ovarian reserves, a low amount of MII will develop from the stimulation cycle. Thus, rescue IVM of immature oocytes is highly valuable as it could become an embryo and the embryo transfer leading to higher chances for pregnancy.[22] From this research, once hCG was added to the culture medium for IVM, the percentage of maturation increased, but without significance. Furthermore, MII oocytes, having developed from MI, would have a higher quality base for G6PD activity. This might benefit the rescue IVM in women with low MII from stimulation cycles.

This study was the first where hCG supplements were used in the culture medium to increase the maturation of immature oocytes from stimulated cycles. However, limitations of this study included stripping the cumulus cell to examine the oocyte stage, which would also remove LH and FSH receptors on top of the cumulus cell, which are necessary for oocyte maturation. The number of oocytes used in this study was small, and this would have affected the likelihood of detecting any difference. In future, we suggested that further studies should determine whether the MII from stimulated immature oocytes could be used to fertilize and produce live births as effective as *in vivo* MII.

Conclusions

Rescue IVM in fertilization culture medium plus hCG was slightly better than that in the only fertilization culture. However, please note that the difference did not meet the statistic significance. MII oocytes developed from MI in hCG supplemented medium had a higher quality based on the measured G6PD activity. According to the results, adding hCG into the fertilization culture medium might benefits stimulation cycles with high number of immature oocytes

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Conflicts of interest

There are no conflicts of interest.

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