

Original Research

Positive Association of the Number of Oocyte Retrieved with the Maternal hCG Concentration in Early Pregnancy Achieved by Vitrified-Warmed Embryo Transfer after *In Vitro* Maturation

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Abstract

Background: Human chorionic gonadotropin (hCG) is synthesized by trophoblast cells and play a critical effect in early pregnancy. The serum hCG concentration is significantly higher in early gestation period achieved through *in vitro* maturation (IVM) than that by traditional IVF. Since IVM does not involve ovarian hyperstimulation, we wonder whether the number of oocyte retrieved affect the hCG concentration in early pregnancy achieved by IVM. **Methods:** The retrospective analysis involved 51 women who delivered singletons were analyzed. The number of oocyte retrieved and the maternal hCG concentration were collected and analyzed. **Results:** 51 women had delivered singletons through IVM. The mean number of oocytes retrieved was 16.78 ± 11.23 , and the hCG concentration was 614.47 ± 445.50 IU/L and 10930.20 ± 7223.17 IU/L at day 14 and 21, respectively. After confounding factors adjusted, the concentration of serum hCG at day 14 and 21 associated significantly with the number of oocyte retrieved. The result of crude linear regression analysis exhibited a positive relationship between the number of oocyte retrieved and the hCG concentration in early gestation. **Conclusions:** Our results suggested that the number of oocyte retrieved associated positively with the serum hCG level in female with early pregnancies that underwent IVM.

Keywords: *in vitro* maturation; human chorionic gonadotropin; oocyte; early pregnancy

1. Introduction

Human chorionic gonadotropin (hCG) is synthesized in trophoblast cells, and its serum level associates with the status of the trophoblastic mass. After embryo implantation, the hCG concentration dynamically increases, and hCG could be detected from the maternal circulation at day 8 [1]. hCG is important for the corpus luteum maintenance during early gestation. In addition, hCG is involved in trophoblast propagation, angiogenesis, placental development, and immunotolerance [2]. In general, the serum hCG level is an indicator of pregnancy progression, and the increase of its concentration is an indicator of ongoing intrauterine gestation. However, a low maternal serum hCG concentration in early gestation is linked to the prevalence of severe pre-eclampsia [3]. Furthermore, the serum hCG concentration is significantly higher in women with multiple pregnancies achieved by IVF-ET than in those with singleton pregnancies [4]. These findings prompted us to ask whether other factors can affect the maternal serum hCG concentration in early pregnancy.

In vitro maturation (IVM), an assisted reproductive technology (ART), refers to the maturation of cumulus

oocyte complexes (COCs) *in vitro* [5]. Since the first IVM baby delivered and reported in 1991, this technology has been widely applied. Since no ovarian stimulation in IVM, it has a great advantage in safety, convenience and reducing the OHSS risk, and attracts more and more attention [6,7]. Therefore, IVM is applicable for PCOS patients, fertility preservation and cost-sensitive people for its unique advantage. Benefit by the biphasic IVM system, the application of IVM has been largely expanded. Additionally, Liu *et al.* [8] reported that during routine gynecological surgery oocytes could be retrieved for *in vitro* maturation, which can be considered as a “fertility insurance” for fertility preservation or *in vitro* fertilization.

Tanbo TG *et al.* [9] reported a negative association between the oocyte retrieved number and the serum hCG concentration when a GnRH agonist or antagonist protocol was used during IVF [9], and Almog B *et al.* [10] reported that the hCG concentration in early pregnancy is notably higher after IVM than after traditional IVF. As we all know, IVM does not involve in ovarian hyperstimulation [11]. Therefore, we wondered whether the number of oocyte retrieved influence the maternal serum hCG concentration



in early pregnancy achieved by IVM. To reconcile the issue, we studied the association between the number of oocyte retrieved and the serum hCG concentration on day 14 and 21 after IVM.

2. Materials and Methods

2.1 Patients

This study was confirmed by the ethics committee of the Peking University Third Hospital (No. 2008013). Patients were performed with IVM as described previously [12]. Briefly, the date of 51 patients with PCOS, who got clinical pregnancy after IVM cycles, were collected and analyzed. Female partner whose age was more than 38 was excluded. Patients received a subcutaneous injection of hCG (Serono, Aubonne, Switzerland; Approval Number: S20110045) when the largest follicle was ≤ 10 mm, and then 36–38 hours later, COCs were collected.

2.2 In Vitro Maturation Protocol

The COCs were collected by a single-lumen aspiration needle (19 G; Cook, Queensland, Australia) with the help of transvaginal ultrasound. The suction force was approximately 80 mm Hg. The aspirated complex was collected and filtered by a cell strainer (Falcon, MA, USA). Then we washed the cell strainer with warmed G-mops-plus media and the COCs would be re-suspended in the washed media. All the COCs were picked up and then cultured in the IVM medium (Sage, CT, USA), in which the hormones, follicle-stimulating hormone (FSH, 0.075 IU/mL) and luteinizing hormone (LH, 0.075 IU/mL) (Menopur, Kiel, Germany), had been added and mixed in advance.

2.3 Oocyte Fertilization

After 28–32 hours of culture, cumulus cells were removed and the oocytes in the stage of metaphase II (MII) were selected for intracytoplasmic sperm injection (ICSI) according to the laboratory protocol. 17 ± 1 hours later after injection, the pronuclei was observed and the zygotes with two pronuclei were then transferred to a pre-equilibrated GM cleavage medium (LifeGlobal, Guilford, CT, USA). Embryo characteristics (for example: cell number, fragmentation, and symmetry) were evaluated at Day 3 (68 ± 1 hours later after injection). The characteristics of the embryo we evaluated include cytoplasmic fragmentation rate, blastomere's number and symmetry, and the cytoplasm quality.

2.4 Embryo Vitrification and Warming

Embryo was vitrified and warmed just as previously described [12]. Briefly, the embryo was firstly balanced in S1 media (HEPS supplemented with 10% HSA) for 1 min at 37 °C, and then transferred into S2 media [S1 media added with 7.5% (v/v) dimethyl 7.5% (v/v) ethylene glycol (EG) and sulfoxide (DMSO) (Sigma-Aldrich, USA)] for 2 min, and transferred to S3 media [S1 media supplemented with

0.65 mol/L sucrose, 15% (v/v) EG and 5% (v/v) DMSO] for 30 seconds before laid on the Cryotop strip (Kitazato Corp., Fujji, Japan). After loaded, the Cryotop laid with embryos was instantly placed into liquid nitrogen for long-time storage. For warming, the vitrified embryo was instantly transferred into T1 media (HEPS with 0.33 mol/L sucrose and 10% HSA) from liquid nitrogen at 37 °C, where the vitrified embryos would be floated and incubated for 2 mins, and then equilibrated into T2 media (HEPS with 0.2 mol/L sucrose and 10% HSA) for 3 mins, and then into HEPS media (supplemented with 10% HAS) for 5 mins. After 2 hours of culture, the quality of warmed embryos was evaluated. The embryos which contained more than 4 blastmeres would be viable for transplantation.

2.5 Endometrial Preparation and Embryo Transplantation

From the second day of the menstrual cycle, all patients received supplementary oestradiol valerate 6 mg each day (Schering, Berlin, Germany) for endometrial preparation. When the thickness of endometrium got to 8 mm, progesterone (Merck Serono, USA) and dydrogesterone was provided. Embryo was transferred by a soft catheter (K-Soft 5100; Cook, Queensland, Australia). The number of embryos transplanted was determined upon many reasons, for example the maternal age, number of IVF cycles, the quality and developmental stage of embryo. The serum hCG concentration was detected on day 14 and 21 after embryo transplantation using ELISA kits by a Beckman DxI800 immunoassay at the endocrine laboratory attached to the Reproductive Centre of Peking University Third Hospital.

2.6 Statistical Analyses

Statistical analysis was processed by SPSS software (version 16.0, IBM Corp., Armonk, NY, USA). Date is represented as the mean \pm standard deviation. The relationship between the number of oocyte retrieved and the concentration of serum hCG was assessed and determined through multiple linear regression analysis after adjusting for the maternal age, male age, sub-fertility duration, maternal BMI, oocytes retrieved number, and number of transferred embryos.

3. Results

51 women had delivered singletons through IVM and the data were collected and analyzed in the present study. The clinical characteristics were shown in Table 1. The mean female age was 29.94 ± 3.73 . The mean number of oocytes retrieved was 16.78 ± 11.23 , and the hCG concentration was 614.47 ± 445.50 IU/L on Day 14 and 10930.20 ± 7223.17 IU/L on day 21 after embryo transfer, respectively.

Table 1. Basic characteristics of 51 women who delivered singletons after IVM.

Characteristic	n = 51
Female age (years)	29.94 ± 3.73
Male age (years)	32.14 ± 4.42
Duration of sub-fertility (years)	4.63 ± 2.86
Body mass index (BMI, kg/m ²)	25.84 ± 4.31
Number of oocytes retrieved	16.78 ± 11.23
Number of embryos transferred	2.07 ± 0.39
hCG concentration (IU/L) on day 14	614.47 ± 445.50
hCG concentration (IU/L) on day 21	10930.20 ± 7223.17

Data are presented as the mean ± SD.

After confounding factors adjusted, the number of oocyte retrieved was positively associated with the concentration of the maternal serum hCG on days 14 ($p = 0.046$, Table 2) and 21 ($p = 0.022$, Table 3). Furthermore, crude linear regression analysis showed a positive relationship between the number of oocytes retrieved and the serum concentration of hCG on days 14 (Fig. 1A) and 21 (Fig. 1B).

Table 2. Factors related with the maternal serum hCG concentration at day 14 after IVM.

Parameter	β	t	p-value
Female age (years)	-0.072	-0.358	0.722
Male age (years)	0.422	2.250	0.030*
Duration of sub-fertility (years)	0.106	0.690	0.494
BMI (kg/m ²)	-0.130	-0.892	0.377
Number of oocytes retrieved	0.306	2.057	0.046*
Number of embryos transferred	-0.071	-0.458	0.649

β is the regression coefficient; * means $p < 0.05$.

Table 3. Factors related with the maternal serum hCG concentration at day 21 after IVM.

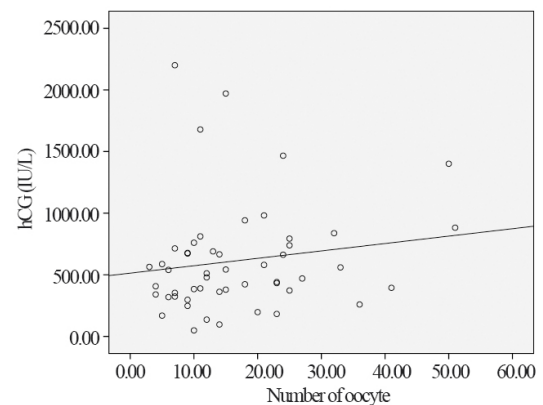
Parameters	β	t	p-value
Female age (years)	0.095	0.465	0.644
Male age (years)	0.306	1.619	0.113
Duration of sub-fertility (years)	-0.055	-0.357	0.723
Body mass index (kg/m ²)	-0.164	-1.122	0.268
Number of oocytes retrieved	0.356	2.371	0.022*
Number of embryos transferred	-0.066	-0.428	0.671

β is the regression coefficient; * means $p < 0.05$.

4. Discussion

As we all know, hCG is critical for the corpus luteum, trophoblast proliferation and placental development in early gestation, and the serum hCG is an important indicator for early pregnancy. The factors which would influence the serum hCG level were concerned and studied. In

A



B

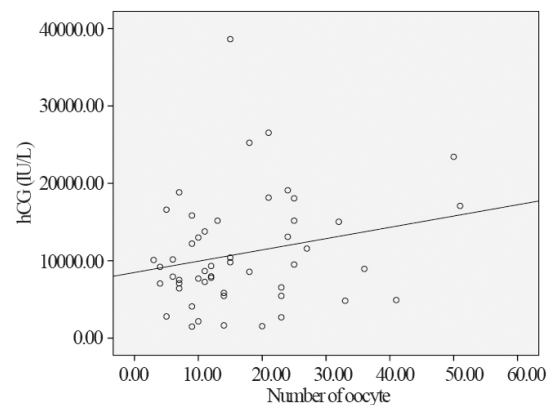


Fig. 1. Crude linear regression analysis of the relationship between the number of oocytes retrieved and the maternal serum concentration of hCG on days 14 (A) and 21 (B). hCG: IU/mL.

the present study, we analyzed the data of 51 women who had successfully delivered singletons after IVM, and the results showed that there was a positive association between the number of oocyte retrieved and the serum concentration of hCG at days 14 and 21 after embryo transplantation.

After embryo implantation, placental trophoblasts invade the endometrium until the first trimester [13]. During trophoblast invasion, the placenta secretes hCG, progesterone, erythropoietin, angiotensin II, and adrenomedullin [14]. hCG, which consists of alpha and beta subunits, as well as various carbohydrate moieties, is the first message secreted from the embryo to the mother. Additionally, the hCG mRNA is detected in 2-cell stage embryos [15], and hCG is considered as a good predictor of early gestation outcomes. To induce priming, 10,000 IU of urinary hCG was injected subcutaneously at 36 hours before oocyte retrieval. Since the half-life of hCG is 2.3 days, the injected hCG did not affect our results. Although the level of serum hCG increases dramatically during early pregnancy, its level is subject to large inter-individual variations [16]. During traditional IVF-ET, the estradiol level is high in pre-ovulatory follicles after treatment with a GnRH agonist or antagonist. Estradiol can also inhibit the protein expression of leukemia

inhibitory factor (LIF), which is critical for embryo implantation [17]. Therefore, a high estradiol concentration led to slower trophoblast proliferation and lower hCG concentration because of the decreased receptivity of the endometrium. The number of oocytes retrieved by traditional IVF associates negatively with the serum hCG concentration in early gestation [9]. In the present analysis, however, we found a positive association between the high oocytes retrieved number and the serum hCG concentration in early pregnancy after IVM. In general, traditional IVF allows for the retrieval of oocytes that are 20 mm in diameter. However, IVM allows for the retrieval of oocytes that are only 10 mm in diameter. It is possible that the estradiol concentration is low in IVM follicles, which promotes trophoblast proliferation and stimulates hCG secretion via the optimal receptivity of the endometrium. However, further research is needed to understand its underlying mechanism.

Multiple linear regression analysis was applied to assess the association among the maternal serum concentration of hCG and the confounding factors. The number of oocyte retrieved was positively associated with the hCG concentration at day 14 and 21 after embryo transplantation. Crude linear regression analysis also showed that there was a positive association between the number of oocyte retrieved and the concentration of serum hCG. In this study, we transferred 2 cleavage stage embryos for most patients (Table 1). It's reported that the multiple pregnancy rate of IVF was 20–30 times higher than natural pregnancy [18,19]. Therefore, single elective embryo transfer was recommended for many centers [20]. Single cleavage embryo transfer, however, was associated with lower on-going pregnancy rate and delivery rate in comparison with single blastocyst transfer [21]. After comprehensive assessment of financial pressure, pregnancy rate and other risks, two cleavage stage embryos were transferred in our center. In fact, previous studies revealed that maternal age, embryo grade and the number of embryo transfer all were factors that would affected hCG levels [22–24]. The transfer of multiple embryos was associated with higher hCG value compared with the transfer of single embryo [24–26]. Although in the current study we had only one gestational sac observed after the transplantation of two cleavage stage embryos, it was also possible that the non-implanted embryo affected the hCG level before vanishing. Therefore, more studies with single embryo transfer and larger number of samples are needed in the future. We also checked the relationship between the serum concentration of hCG and the birth weight (3366.4 ± 93.7 g) of these singletons and the results showed that there was no positive relationship ($p > 0.05$). This result was consistent with the research performed by Thomas Strowitzki [27]. In addition, we found an association between the male age and the serum hCG concentration on day 14. Although we do not know the reason for this association, future studies will address the association between male age and the serum hCG concen-

tration using more patients.

Pingping Qiu *et al.* [28] reported that the concentration of serum β -HCG on day 10 were associated with the outcomes of vitrified-warmed blastocyst transplantation at different ages. Many models have been built to predict the pregnancy outcomes based on the serum hCG values [29–32]. Our findings provide a new idea for the study and prediction of the pregnancy outcomes after IVM, and can better help us to understand the hCG values after IVM treatment. Our results also suggested that the number of oocytes retrieved should be considered when we predicted the pregnancy outcome of IVM patients through the hCG level, and a new model different from IVF should be established for IVM patients. Our data analysis was limited because of the small number of patients that were available and the retrospective design of this study. Therefore, further research is needed and the underlying mechanisms call for further investigation.

5. Conclusions

In summary, the present study showed a positive association between the number of oocyte retrieved and the concentration of serum hCG in early pregnancies after IVM. Our results partially explain the transient changes in the concentration of maternal serum hCG after IVM. But for the small number of patients, further researches with larger samples and multiple centers were needed.

Author Contributions

Conceived and designed the study—PL; coordinated data collection—YPW, SLL, XYZ, YL and RY; analyzed the data—YPW and SLL; wrote the paper—YPW and SLL; all authors interpreted the data. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was confirmed by the ethics committee of the Peking University Third Hospital (No. 2008013). Patients received a subcutaneous injection of hCG (Serono, Aubonne, Switzerland; Approval Number: S20110045).

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Conflict of Interest

The authors declare no conflict of interest.

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