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Original Article

Impact of estrogen-to-oocyte ratio on live birth rate in women undergoing in vitro fertilization and embryo transfer

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Abstract: In order to evaluate whether the ratio of serum estrogen level on the day of human chorionic gonadotrophin (hCG) administration to number of oocyte retrieved has any impact on live birth rate in women undergoing *in vitro* fertilization (IVF), we retrospectively analyzed the clinical data from 7,112 women treated with GnRH-a long protocol in our center. Cycles were firstly divided into 6 groups according to the E_2/oocyte ratio (< 150 pg/ml; 150~300 pg/ml; 300~450 pg/ml; 450-600 pg/ml; 600-750 pg/ml; ≥ 750 pg/ml). Live birth rate (39.4%) in women with E_2/oocyte ratio ≥ 750 pg/ml was the lowest compared with that in other groups. We further divided the top 10% of patients into high estrogen group (E_2/oocyte ≥ 740 pg/ml; n = 713). Compared with controls, those with high E_2/oocyte ratio had significantly higher peak E_2 level (6711.85 pg/ml versus 4670.89 pg/ml; $P = 0.000$) on the day of hCG administration; however, the live birth rate (39.27% versus 45.67%; $P = 0.001$) was significantly lower for women with high estrogen level. Thus, we conclude that high E_2/oocyte ratio adversely affects live birth rate in women undergoing IVF treated with GnRH-a long protocol.

Keywords: E_2/oocyte ratio, live birth rate, IVF

Introduction

In order to recruit a sufficient number of follicles to increase pregnancy rate in *in vitro* fertilization (IVF), controlled ovarian hyperstimulation (COH) is used to stimulate multi-follicular growth. Indeed, the employment of ovarian stimulation protocols could increase the number of oocytes retrieved and improve the chance of pregnancy [1]. However, compared with the natural cycles, we all know that serum estrogen level during COH could be more than 10,000 pg/ml. High estrogen level has been shown to have adverse impact not only on oocyte and embryo quality, but also on endometrial receptivity [2-6].

E_2/oocyte ratio, which is thought to reflect accurately the amount of estrogen one oocyte needs during COH, has also been shown to have impact on IVF outcome. However, sample sizes in most previous studies were relatively small. In addition, few studies explore the impact of E_2/oocyte ratio on live birth rate, which has been thought to be the final outcome of IVF treatment.

Thus, the main aim of this large sample size study was to evaluate the impact of E_2/oocyte ratio on live birth rate in women undergoing IVF with gonadotropin releasing hormone agonist (GnRH-a) long protocol.

Materials and methods

Subjects

We retrospectively analyzed the results of 7,112 consecutive infertile women referred to our center for IVF/ICSI treatment from January 2010 to March 2014. Institutional Review Board (IRB) approval was obtained for a retrospective review of the medical charts.

The inclusion criteria were as follows: (1) age between 20-42 years old; (2) the first cycle of IVF treatment using standard GnRH-a long pro-

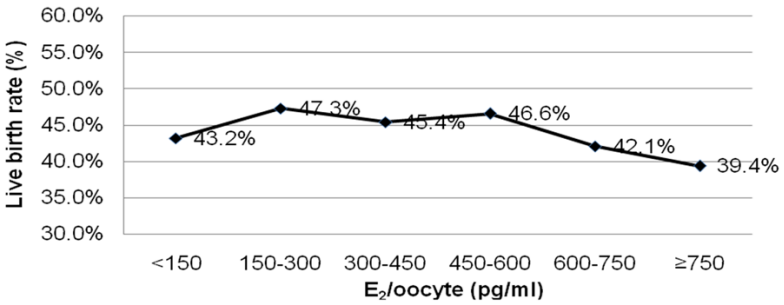

Figure 1. Live birth rate in patients with different E_2/oocyte ratio.

tocol; (3) both ovarian present and normal uterus; (4) only cycles with embryo transfer were included. Oocyte donation, sperm donation, or pre-implantation genetic diagnosis cycles were excluded.

Ovarian stimulation protocol

In all cycles, women were treated with standard GnRH-a long protocol [7]. Stimulation was performed with rec-FSH and hMG after pituitary down-regulation with GnRH-a, which was started from mid-luteal phase of previous menstrual cycle. GnRH-a was given every day at a dose of 0.1 mg until ovarian suppression was achieved when serum E_2 and LH levels were < 30 pg/mL and 3 mIU/mL, respectively. After the initiation of gonadotrophin stimulation, GnRH-a was given daily at a dose of 0.05 mg, together with IH injection of 112.5-300 IU rec-FSH or IM injection of 150-300 IU hMG. Gonadotropin dosage was adjusted based on the follicular development.

Follicular size was assessed by transvaginal ultrasound and human chorionic gonadotropin (hCG) was given to trigger ovulation at a dose of 10,000 IU IM. Criteria for hCG administration were the presence of two or more leading follicles ≥ 17 mm in diameter, with the majority of follicles being ≥ 14 mm. Transvaginal ultrasound-guided oocyte retrieval was performed 36-37 hours after hCG administration. Routine IVF/ICSI was performed as appropriate. Usually 2 or 3 embryos were transferred 3-5 days after oocyte retrieval.

The luteal phase was supported with 60 mg IM of progesterone in oil, starting on the day of embryo transfer until 8 weeks' gestation if pregnancy was achieved. Live birth rate was defined as a successful delivery resulting from embryo transfer.

Hormone measurement

Basal ovarian reserve studies, including serum FSH and E_2 in addition to LH levels, were obtained on day 2 or 3 of a natural menstrual cycle. On the day of hCG administration, serum E_2 was also measured. The intra-assay and inter-assay coefficients of variation (CV) were < 3% and < 4% for LH, < 3% and < 6% for FSH, < 5% and < 7% for hCG, and 5% and < 10% for E_2 respectively.

Statistical analysis

We analyzed the data using the Statistical Package for Social Sciences (SPSS 17.0; SPSS, Chicago, IL). Baseline demographic and fertility-related variables between groups were analyzed using one-way ANOVA. Differences in live birth rate were analyzed by Chi-square test. A $P < .05$ was considered statistically significant.

Results

The mean patient age was 30.7 years. The mean number of oocytes retrieved was 10.7 (range, 1-32). The mean E_2 level on the day of hCG administration was 4875.53 pg/mL (range, 289-19,569 pg/mL). Of 7,112 patients, 3,202 live births were achieved.

Patients were divided into six groups according to their E_2/oocyte ratio: E_2/oocyte ratio < 150 pg/ml (n = 44); 150 pg/ml ≤ E_2/oocyte ratio < 300 pg/ml (n = 1,048); 300 pg/ml ≤ E_2/oocyte ratio < 450 pg/ml (n = 2,565); 450 pg/ml ≤ E_2/oocyte ratio < 600 pg/ml (n = 1,899); 600 pg/ml ≤ E_2/oocyte ratio < 750 pg/ml (n = 889); 750 pg/ml ≤ E_2/oocyte ratio (n = 665). Live birth rates in each group were shown in **Figure 1**. The average live birth rate was more than 45.0% in patients with 150 pg/ml ≤ E_2/oocyte ratio < 600. However, less than 40.0% of women with E_2/oocyte ratio ≥ 750 pg/ml achieved live birth.

We further divided the top 10% of patients into high estrogen group (E_2/oocyte ≥ 740 pg/ml; n = 713). Compared with controls, those with high E_2/oocyte ratio had significantly higher Gn duration (11.43 versus 10.99; $P = 0.000$) and dose (2228.46 versus 2086.64; $P = 0.000$),

Table 1. Basic characteristics and live birth rate in 7,112 patients

	Controls (E_2/oocyte < 740 pg/ml)	High estrogen group (E_2/oocyte ≥ 740 pg/ml)	P
No. of patients	6,399	713	
Age (year)	30.69 ± 4.94	31.47 ± 5.11	0.000
Infertility duration (year)	4.61 ± 3.39	4.84 ± 3.76	0.123
BMI (kg/m^2)	22.55 ± 3.12	21.98 ± 3.01	0.000
Gn duration (days)	10.99 ± 1.74	11.43 ± 1.71	0.000
Gn dose (IU)	2086.64 ± 810.67	2228.46 ± 886.75	0.000
No. of oocytes retrieved	11.12 ± 4.83	7.32 ± 3.41	0.000
Peak E_2 level (pg/ml)	4670.89 ± 2261.79	6711.85 ± 3065.46	0.000
No. of embryos transferred	2.07 ± 0.28	2.06 ± 0.34	0.681
Live birth rate (%)	2,922 (45.67%)	280 (39.27%)	0.001

Notes: BMI = body mass index; Gn = gonadotrophin; IU = international unit.

but had less oocytes retrieved (7.32 versus 11.12; P = 0.000). Interestingly, peak E_2 level on the day of hCG administration was higher in high estrogen group; however, the live birth rate (39.27% versus 45.67%; P = 0.001) was significantly lower for women in high estrogen group (**Table 1**).

Discussion

In the present study, our data clearly showed an association between E_2/oocyte ratio and live birth rate in IVF, and confirmed that the live birth rate was the lowest when E_2/oocyte ratio ≥ 750 pg/mL.

To the best of our knowledge, one of the earliest studies about the optimal E_2/oocyte ratio was performed by Loumaye et al. [8], who showed that the optimal ratio was 70-140 pg/mL and declared that the ratio was predictable for IVF outcome in women treated with GnRH agonist protocol. Our findings are similar with their results. Meanwhile, this phenomenon could also be observed from the study conducted by Yang and colleagues, who discovered that the pregnancy and implantation rates were significantly decreased in cycles with an E_2/oocyte ratio ≥ 350 pg/mL as compared with those with a ratio < 350 pg/mL [9].

Ovarian stimulation is important and necessary to IVF. However, there are more and more evidences showing that super-physiologic E_2 levels resulting from ovarian stimulation have deleterious influence on endometrial development and subsequent embryo implantation. Thus, people are beginning to advocate the conception of mild stimulation to select 'fittest' oocyte 'naturally' [10].

According to a study performed by Pellicer et al., implantation rates were highest in women with 1-5 oocytes retrieved and declined as the number of retrieved oocytes increased. Meanwhile, the fertilization rates were markedly lower when > 10 oocytes were retrieved, even though the pregnancy rates were not different [11]. In another study, Simon found that both the implantation rate and pregnancy rate were significantly reduced when > 15 oocytes were retrieved compared with < 15 oocytes [12]. In addition, the highest pregnancy rates per embryo transfer and per started cycle were observed when 13 oocytes were obtained according to a large cohort study of 7,422 women undergoing oocyte retrieval for IVF carried out by van der Gaast [13].

Another study advocating mild stimulation regime was performed by Joo [14], who retrospectively reviewed data of 455 cycles of fresh IVF-ET with COH. In that study, the authors divided patients into five groups according to the serum E_2 levels on the day of hCG administration: group A (< 1000 pg/mL), group B (1000-2000 pg/mL), group C (2000-3000 pg/mL), group D (3000-4000 pg/mL), group E (> 4000 pg/mL). The number of oocytes obtained increased with increasing serum E_2 levels. However, the pregnancy rate gradually increased from group A to D as E_2 levels increased but decreased in group E.

As we all know, embryo quality and endometrial receptivity were identified as two important fac-

tors that influence the outcomes of IVF [15]. As early as the 1990s, investigators observed that ovarian stimulation was associated with gland-stroma dys-synchrony and a shift in the window of receptivity [16]. In a recent study in which DNA microarrays was used to evaluate endometrial receptivity, both GnRH agonist and GnRH antagonist protocols adversely affected endometrial receptivity in comparison with their natural cycles in the same patient [17]. As shown in our study, despite a significant increase in peak E_2 level on the day of hCG administration for women with high E_2/oocyte ratio level, the live birth rate in that group was significantly compromised.

In conclusion, the results of this study indicate that E_2/oocyte ratio have impact on live birth rate in women undergoing IVF treated with GnRH-a long protocol. These findings support the notion that mild ovarian stimulation protocols, which aim for oocyte 'quality' rather than 'quantity', should be advocated to improve clinical outcomes in IVF-ET treatment.

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Disclosure of conflict of interest

None.

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