

IMPACT OF TRANSITORY HYPERPROLACTINEMIA ON CLINICAL OUTCOME OF *IN VITRO* FERTILIZATION AND EMBRYO TRANSFER

UTICAJ PROLAZNE HIPERPROLAKTINEMIJE NA KLINIČKI ISHOD VEŠTAČKE OPLODNJE I TRANSFER EMBRIONA

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Summary: This study aimed to evaluate the impact of serum prolactin concentration at the day of human chorionic gonadotropin (HCG) administration on the clinical outcome of *in vitro* fertilization and embryo transfer (IVF-ET). A total of 184 patients receiving the IVF-ET/ICSI-ET from October 2005 to March 2008 were retrospectively analyzed. Subjects were divided into four groups according to the serum prolactin concentration [<30 ng/mL (A), 30–60 ng/mL (B), 60–90 ng/mL (C), ≥ 90 ng/mL (D)] on the day of HCG administration during controlled ovarian stimulation (COS). In the Groups A, B, C and D, the implantation rate was 11.76%, 19.71%, 12.72% and 2.22%, respectively, and the pregnancy rate (PR) was 25.00%, 42.70%, 27.30% and 5.88%, respectively. The implantation rate and PR in the Group D were markedly lower than those in the remaining groups ($P=0.011$ and 0.009). During the COS, the serum prolactin concentration was dramatically elevated when compared with the baseline level leading to transient hyperprolactinemia. In addition, the implantation rate and pregnancy rate were significantly markedly decreased when the serum prolactin concentration was remarkably increased (≥ 90 ng/mL). To improve the clinical pregnancy rate of IVF-ET, close monitoring and appropriate intervention are needed for patients with an abnormal prolactin level during the COS.

Keywords: hyperprolactinemia, *in vitro* fertilization and embryo transfer, human chorionic gonadotropin, clinical pregnancy

Kratak sadržaj: Ova studija sprovedena je kako bi se procenio uticaj koncentracije prolaktina u serumu na dan administracije humanog horionskog gonadotropina (HCG) na klinički ishod *in vitro* oplodnje i transfera embriona (IVF-ET). Retrospektivna analiza je obuhvatila ukupno 184 pacijenta podvrgnuta IVF-ET/ICSI-ET između oktobra 2005. i januara 2008. Ispitanici su podeljeni u četiri grupe na osnovu koncentracije prolaktina u serumu [<30 ng/mL (A), 30–60 ng/mL (B), 60–90 ng/mL (C), ≥ 90 ng/mL (D)] na dan administracije HCG tokom kontrolisane stimulacije jajnika (COS). U grupama A, B, C i D stopa implantacije bila je 11,76%, 19,71%, 12,72% i 2,22%, dok je stopa ostvarenih trudnoća (PR) bila 25,00%, 42,70%, 27,30% i 5,88%. Stopa implantacije i PR u grupi D bile su upadljivo niže nego u ostalim grupama ($P=0,011$ i $0,009$). Tokom COS, koncentracija prolaktina u serumu bila je dramatično povišena u poređenju sa početnim nivoom, što je dovelo do prolazne hiperprolaktinemije. Pored toga, stopa implantacije i stopa ostvarenih trudnoća bile su značajno i upadljivo niže kada je koncentracija prolaktina u serumu bila izrazito povišena. Pažljivo praćenje i odgovarajuće intervencije potrebni su kako bi se poboljšala klinička stopa ostvarenih trudnoća u IVF-TE kod pacijenata sa abnormalnim nivoom prolaktina tokom kontrolisane stimulacije jajnika.

Ključne reči: hiperprolaktinemija, veštačka oplodnja i transfer embriona, humani horionski gonadotropin, klinička trudnoća

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List of abbreviations: PRL – prolactin, IVF-ET – *in vitro* fertilization and embryo transfer, COS – controlled ovary stimulation, HCG – human chorionic gonadotropin, PR – pregnancy rate, GnRH-a – gonadotropin-releasing hormone analogues, Gn – gonadotropin, FR – fertilization rate, CR – cleavage rate, IR – implantation rate, PR – pregnancy rate, PRF – prolactin releasing factor, PIF – prolactin inhibitory factor, P – progesterone, E₂ – estradiol, PGE₂ – prostaglandin E₂

Introduction

Prolactin (PRL) is a single chain peptide hormone secreted by the anterior pituitary acidophile cells, pregnancy deciduas and immune cells, and consists of 198 amino acids with a molecular weight of about 23 kD. The human PRL gene is located on the chromosome 6 and is encoded by a single gene. The PRL mRNA is about 1 kb in length and encodes 227 amino acids with 28 amino acids as a signal peptide. The normal reference range of the PRL level is <30 pg/mL in the women of child-bearing age (1).

Under normal circumstances, the secretion of PRL by the anterior pituitary is regulated by the PRL releasing factor (PRF) and PRL inhibitory factor (PIF). The PIFs include dopamine, γ -aminobutyric acid and gonadotropin-related peptide, and dopamine is the main PIF. The dominant function of PRL is to stimulate the growth and milk production of breast tissues, regulate the osmotic pressure and the composition and volume of amniotic fluid (1). In recent years, an animal study showed PRL played an important role in the ovarian function and pregnancy maintenance (2), however, few studies have reported the effects of serum PRL level on the outcome of *in vitro* fertilization and embryo transfer (IVF-ET) during the controlled ovary stimulation (COS) and the conclusions are inconsistent. In the present study, a total of 184 patients receiving the IVF-ET/ICSI-ET from October 2005 to March 2008 were retrospectively analyzed.

Materials and Methods

Subjects

A total of 184 infertility patients undergoing IVF-ET/ICSI-ET were recruited from the Reproductive Medicine Center of the First Affiliated Hospital, Sun Yat-sen University. Exclusion criteria included patients with abnormal PRL secretion or history of pituitary adenoma. The causes of infertility involved male sterile factors ($n=56$), tubal abnormalities ($n=83$), pelvic adhesion ($n=17$), endometriosis ($n=17$) and other (abnormal ovulation, repeated failure of artificial insemination and unknown causes; $n=11$).

Procedures of IVF-ET

IVF-ET was performed according to the conventional procedures conducted in our department (3). The long protocol for pituitary down-regulation was used with gonadotropin-releasing hormone analogues (GnRH-a; Diphereline; France)/gonadotropin (Gn; Gonal-F; Switzerland)/chorionic gonadotropin (HCG; Switzerland) in a sequential regimen for ovulation induction. Transvaginal ultrasound examination was performed regularly with an ALOKA IP-1233 ultrasonic instrument (frequency of the probe: 7.2 MHz) to monitor the follicular development and endo-

metrial thickness. When the ovarian follicles (more than 1 ovarian follicle greater than 18 mm in diameter under ultrasonography) were mature, 10 000 IU of HCG were intramuscularly injected and the oocytes were harvested 36 h later followed by culture for 4–6 h for *in vitro* fertilization. The quality of embryos was evaluated with an embryo scoring system and high-quality embryos were collected 42–45 h later for embryo transfer.

Detection of hormone levels

At 8–9 am of the day 2–3 of menstrual cycle and the day of HCG injection, 3 mL of venous blood were obtained and serum was separated. The levels of FSH, LH, E2 and PRL were determined with the microparticle enzyme immunoassay (Architect AxSYM System, USA).

The sensitivity for FSH was 0.37 IU/L. The intra-assay variation was 3.65–7.64% and interassay variation was 2.13–5.93%. The sensitivity for LH was 0.07 mIU/L. The intraassay variation was 4.28–4.57% and interassay variation was 0.00–2.49%. The sensitivity for E₂ was 20 pg/mL. The intraassay variation was 4.0–10.9% and interassay variation was 0.00–6.40%. The sensitivity for PRL was 0.02 ng/mL. The intraassay variation was 3.33–9.66% and interassay variation was 0.00–3.91%.

Clinical information

During the IVF-ET, the age, cause of infertility, duration of infertility, cycles of IVF-ET, the number of oocytes, fertilized oocytes, cleavage oocytes and embryos for transfer and the outcome of pregnancy were recorded.

Determination of results

Clinical pregnancy was defined as HCG positive and intrauterine gestational sac under ultrasonography or obvious villi demonstrated by the post-abortion pathological examination (3).

Grouping

According to the serum PRL levels on the day of HCG injection [reference range of PRL was <30 ng/mL (1)], the patients were divided into four groups: Group A: PRL <30 ng/mL; Group B: 30–60 ng/mL; Group C: 60–90 ng/mL and Group D: ≥ 90 ng/mL.

Statistical analysis

Statistical analysis was performed with the SPSS 11.0 statistic software and data were expressed as means \pm standard deviation ($\bar{x} \pm SD$). The qualitative data were analyzed with t test or analysis of variance

and the categorical data were evaluated with Kruskal-Wallis test or Chi square test. In addition, Spearman rank correlation analysis and multiple linear regression (Stepwise method) were used. A value of $P < 0.05$ was considered statistically significant.

Results

Demographics

In the present study, a total of 184 cycles of ET were involved, including 127 cycles of IVF and 57 cycles of ICSI. The mean age of these patients was 32.42 ± 4.75 years (range: 22–47 years). The mean duration of infertility was 5.34 ± 3.83 years (range: 1–16 years). The mean baseline serum levels of FSH, E₂, and PRL were 6.16 ± 2.91 IU/L (range: 1.02–26.37 IU/L), 41.73 ± 24.59 pg/L (10–161 pg/L) and 16.60 ± 6.34 ng/mL (2.42–29.98 ng/mL), respectively. Analysis of variance indicated there were no marked differences in the age, duration of infertility, and baseline serum levels of FSH, E₂, and PRL

between the four groups ($P > 0.05$) (Table I). The constituent ratios of causes and the cycles of IVF were analyzed with chi square test and the results showed no significant differences between these patients ($P > 0.05$).

PRL levels on the day of HCG injection and outcome of IVF

The mean PRL level on the day of HCG administration was 45.24 ± 29.22 ng/mL (range: 3.50–211.05 ng/mL). The levels of E₂ and PRL, number of oocytes and embryos for transfer, fertilization rate (FR), the cleavage rate (CR), implantation rate (IR), pregnancy rate (PR), are shown in the Table II and III.

There was heterogeneity of variance between the PRL levels at baseline and on the day of HCG administration ($F = 109.2$, $P < 0.01$). Therefore, t-test with Bonferroni correction was performed ($t = 13.61$, $P < 0.01$) and results revealed the PRL level on the day of HCG administration was higher than that at

Table I Parameters observed during the IVF-ET ($\bar{x} \pm SD$).

Group	Cycles	Age (years)	Duration of infertility	Baseline FSH (IU/L)	Baseline PRL (ng/mL)	Baseline E ₂ (pg/mL)
A	56	33.07 ± 3.60	5.64 ± 0.54	6.52 ± 0.39	17.31 ± 0.89	44.37 ± 2.75
B	89	32.26 ± 3.93	5.32 ± 0.40	6.19 ± 0.32	15.85 ± 0.62	41.16 ± 2.52
C	22	33.04 ± 2.72	5.83 ± 0.75	5.83 ± 0.39	16.31 ± 0.62	39.50 ± 6.95
D	17	30.35 ± 4.14	4.31 ± 0.76	5.06 ± 0.41	19.68 ± 1.78	38.53 ± 3.99
Total	184	32.31 ± 4.75	5.34 ± 3.83	6.16 ± 2.91	16.60 ± 6.34	41.73 ± 24.59
P		>0.05	>0.05	>0.05	>0.05	>0.05

Table II PRL levels on the day of HCG injection and the outcome of IVF (1) ($\bar{x} \pm SD$).

Group	PRL (ng/mL)	E ₂ (pg/L)	oocytes (n)	FR (%)
A	19.77 ± 0.89	2032.61 ± 198.24	10.36 ± 0.84	70.32 ± 3.16
B	42.79 ± 0.83	2516.58 ± 159.85	12.75 ± 0.80	72.73 ± 2.19
C	70.93 ± 1.78	2743.68 ± 236.42	16.04 ± 1.73	66.32 ± 5.60
D	118.47 ± 7.01	3024.53 ± 341.41	16.12 ± 1.69	78.65 ± 3.94
Total	45.24 ± 29.22	2452.39 ± 1539.71	$13.5 \pm 7.9^*$	71.02 ± 23.71
P	<0.01	<0.05	<0.05	>0.05

Note: * $P < 0.05$ Group A vs Group C or Group D after the Bonferroni test

Table III PRL levels on the day of HCG injection and outcome of IVF (2) ($\bar{x}\pm$ SD).

Group	CR (%)	embryos(n)	IR (%)	PR (%)
A	67.21 \pm 3.17	2.19 \pm 0.10	16/136(11.76)	14/56(25.00)
B	66.81 \pm 2.28	2.20 \pm 0.79	43/218(12.5)	38/89(42.70)
C	67.00 \pm 4.50	2.33 \pm 0.18	7/56(12.5)	6/22(22.00)
D	74.33 \pm 4.87	2.65 \pm 0.12	1/45(2.22)*	1/17(5.88)**
Total	67.61 \pm 22.57	2.25 \pm 0.78	14.91 \pm 23.81	59/184(32.10)
P	>0.05	>0.05	0.011	0.009

Note: * $P<0.01$ Group D vs remaining groups after the Bonferroni test

** $P<0.01$ Group D vs remaining groups after the Bonferroni test

baseline. Spearman rank correlation analysis was conducted to analyze the correlation between the PRL level and E_2 level on the day of HCG injection, and the results showed a positive relationship ($r=0.354$, $P<0.01$).

Significant difference in the number of oocytes was observed between the groups. Bonferroni test showed the number of oocytes in the Group A was markedly different from that in the Group C and Group D ($P<0.05$). That is to say, the higher the PRL level on the day of HCG administration, the larger the number of oocytes obtained. Spearman rank correlation revealed a positive relationship between the number of oocytes and the PRL level ($r=0.305$, $P<0.01$). To explore whether the PRL level on the day of HCG administration can predict the number of oocytes, the number of oocytes served as a dependent variable and the PRL level and E_2 level served as independent variables followed by stepwise multiple linear regression analysis. The multiple regression equation ($Y=4.882+0.003038X$, $F=110.94$, $P<0.01$, $R^2=0.358$, where X was the E_2 level) did not include the PRL level. Therefore, we speculated that the PRL level on the day of HCG administration could not serve as a predictor of the number of oocytes. Additionally, there were no marked differences in the FR and CR between different groups ($P>0.05$). Significant difference in the IR was observed between the four groups ($P<0.05$) and the Bonferroni test showed the IR in the Group D was significantly different from that in the remaining groups ($P<0.01$). That is to say, the PRL level on the day of HCG administration of no less than 90 ng/ml predicts a low IR. In addition, there was no marked difference in the number of embryos for transfer between the four groups ($P>0.05$). Significant difference in the PR was observed between the four groups ($P<0.05$) and Bonferroni test showed the PR in the Group D was significantly different from that in the remaining groups ($P<0.01$). That is, the PRL level on the day of

HCG administration of no less than 90 ng/mL predicts a low PR.

Discussion

Effects of PRL on the reproductive physiology

The decidualized human endometrial stromal cells, decidual cells during the normal pregnancy and ectopic pregnancy, human follicle cells, and granulosa cells are the dominant cells expressing PRL and PRL receptors (4, 5). The local PRL of deciduas can regulate the development of ovarian follicles and intrauterine environment through autocrine or paracrine mechanisms and plays important roles in the regulation of ovarian follicle maturation, secretion of E_2 and progesterone (P) by granulosa cells, decidual nutrition and implantation of fertilized eggs.

When the PRL level is extremely high, it can act on the local PRL receptor and attenuate or block the response of the ovary to gonadotropin and inhibit the development and maturation of ovarian follicles. Therefore, the estrogen peak and LH peak before ovulation cannot be achieved. In addition, a high level of PRL may also inhibit FSH-induced generation of estrogen and the LH-induced production of P (3). In clinical practice, women with a high PRL level have the characteristics of shortened luteal phase, continuous non-ovulation, oligomenorrhea or amenorrhea and infertility is a main feature of women of childbearing age with a high PRL level.

Elevated serum PRL level in controlled ovarian stimulation

Our results showed, in the long protocol for pituitary down-regulation, the serum PRL level on the day of HCG administration was higher than that at the baseline during the controlled ovarian stimulation when the castration of hypothalamus and pituitary

was performed. Furthermore, the amplitude of increase in the PRL level was positively related to the E₂ level.

The hypothalamus can confer suppressive effects on the secretion of PRL by the pituitary through one or more PRL inhibitors (PRI). In the normal menstrual cycle, the PRL level reaches a high level in the luteal phase (6) and changes with the E₂ level. A small PRL peak is observed after the E₂ peak. Wang et al. (7) found that the level of PRL with biological activity and immune activity in the middle menstrual cycle of healthy women was markedly increased, which was not observed in women with infertility of unknown cause. During the pregnancy and lactation period, the PRL level increases under the stimulation of estrogen. However, in the controlled ovarian stimulation, gonadotropin can remarkably elevate the serum levels of E₂ and PRL. Balasch et al. (8) also revealed, during the ovulation induction with HMG, the prevalence of hyperprolactinemia in the luteal phase was increased and this hyperprolactinemia could not be improved by GnRH analogues. There may be a feedback loop between E₂ and PRL: after binding to the nuclear receptor, estrogen can activate the transcription factor in the PRL secreting cells leading to a feedback inhibition of dopamine release and subsequent increase in PRL synthesis (9). Our results were consistent with the above-mentioned findings.

Effects of transitory hyperprolactinemia on the pregnancy in IVF-ET

Our results demonstrated that the number of oocytes obtained elevated with the increase in the PRL level on the day of HCG administration. When the PRL was < 30 ng/mL (Group A), the number of oocytes was the lowest, which may be associated with the relatively low E₂ level. These findings suggested there was a feedback loop between E₂ and PRL. The estrogen secreted by the ovarian follicles can regulate the secretion of PRL by the PRL secreting cells in the pituitary. Therefore, the PRL level is indirectly related to the number of oocytes obtained, which should be further confirmed by more studies.

Different from previous studies, our research indicated the PRL level on the day of HCG administration was not related to the FR. When the PRL level was 30–60 ng/mL (Group B), the IR and PR were the highest. Both high and low PRL levels are not beneficial for the IVF and pregnancy. Especially the extremely high PRL level could affect the outcome of pregnancy.

Some researchers have reported that an increased serum PRL level on the day of HCG administration can affect the clinical PR of IVF. Mendes MC et al. (10) investigated patients with ovarian hyperstimulation after superovulation and the results

showed the PRL level reached a maximal level before the harvest of fertilized eggs. In addition, the amplitude of the increase in PRL level was 2 times higher than the PRL level at baseline. Moreover, the number of big ovarian follicles (≥ 12 mm in diameter) and mature eggs and the success rate of IVF were higher than those in the groups with the increase in the PRL level of no more than 2 times higher than the baseline PRL level. Thus, we speculated that the transitory hyperprolactinemia may be associated with the IVF outcome.

Doldi N et al. (11) divided the patients with transitory hyperprolactinemia in the ICSI into two groups. The patients in one group were given cabergoline or bromocriptine to reduce the PRL level, and those in the other group were treated with placebo (controls). In the control group, the amount of FSH used in the procedure was lower, the number of embryos with high quality was larger, the FR was higher and the number of embryos for implantation was larger. These findings implied transitory hyperprolactinemia was positively relevant to the outcome of ICSI, especially the quality of eggs and the FR.

There are two hypotheses to explain the hyperprolactinemia-induced reproductive dysfunction: (1) Central action theory: through a short-loop feedback, PRL can inhibit the secretion of GnRH; (2) Peripheral action theory: hyperprolactinemia can cause the dysfunction of granulosa cells and down-regulate the production of steroid hormones affecting the development of ovarian follicles.

An extremely high PRL level can modulate the functions of the hypothalamus–pituitary–gonadal axis and corpus luteum, the endometrial receptivity and the immune tolerance affecting the outcome of pregnancy.

Hyperprolactinemia can influence the hypothalamus–pituitary–gonadal axis at different levels: a high PRL level can inhibit the secretion of GnRH from the GT1 hypothalamic neurons through suppressing the expression of PRL receptor and decrease the secretion of LH through attenuating the amplitude and frequency of the secretion pulse.

Experiments have revealed that PRL can exert trophic effects on the corpus luteum function and PRL is also defined as a trophic hormone of corpus luteum. McNatty et al. (12) found the PRL at a physiological level was essential for the synthesis of P in human granulosa cells. *In vitro* experiments indicated a high PRL level conferred suppressive effects. Feltus et al. (13) have shown PRL could activate the expression of 3β -hydroxysteroid dehydrogenase II, which is a terminal enzyme in the biosynthesis of P. Previous studies have showed oral bromocriptine could decrease the serum PRL level in healthy women, accompanied by a marked decrease in the P level in the luteal phase (14). Additionally, corpus luteum insufficiency was also noted in the endometrial biopsy.

These findings suggested the PRL at a normal level is critical in maintaining the normal corpus luteum function. But hyperprolactinemia can result in the dysfunction of granulosa cells and reduce the production of steroid hormones affecting the development of ovarian follicles. Experiments on rats also confirmed that a high PRL level could dramatically attenuate the HCG induced egg maturation and ovulation and regulate the local release of β -endorphin and prostaglandin E₂ (PGE₂) in the ovary influencing the exogenous Gn induced ovulation.

During the IVF-, the endometrium can generate PRL which provides an optimal microenvironment for blastocysts through acting on the PRL-R, and maintains the endometrial receptivity. However, a high PRL level can inhibit the proliferation of luteinizing granulosa cells and the corpus luteum function resulting in shortened luteal phase and P insufficiency, as well as subsequent abnormal implantation and early development of embryos (15, 16).

PRL is not only a lactation promoting hormone, but an important immunoregulatory factor playing key roles in the survival, activation and proliferation of lymphocytes. An extremely high PRL level may affect the immune intolerance of the mother to the implanted fetus through influencing the immune function. In addition, PRL can also impact the maturation of Th1 cells which can receive the stimulation from TCR, is involved in the differentiation of T cells and may reg-

ulate the Th1/Th2 shift towards Th1 cell response. In normal pregnancy, the Th1/Th2 balance is shifted towards T2 cell response and the production of Th1 cytokines is increased. Therefore, the cell-mediated immunity is enhanced which may affect the immune tolerance during the IVF and damage the pregnancy or even result in abortion (17).

Therefore, a high PRL level after ovulation is detrimental to the outcome of IVF. During the IVF-ET, the IR and PR are the highest when the PRL level on the day of HCG administration is 30–60 ng/mL. However, when the PRL level is dramatically increased (PRL \geq 90 ng/mL), the IR and clinical PR are markedly decreased. Therefore, in the controlled ovarian stimulation, the patients with an abnormal PRL level should be closely monitored and appropriate measurements can be taken if necessary, which may be beneficial for improving clinical PR.

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Conflict of Interest statement

None. No pharmaceutical/diagnostic company was involved in funding this project.

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