Influence of Midluteal Serum Prolactin on Outcome of Pregnancy After IVF-ET: A Preliminary Study

TOMOYA OZAKI,^{1,2} KENTARO TAKAHASHI,¹ HIROKO KURIOKA,¹ and KOHJI MIYAZAKI¹

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Purpose: To investigate the relationship between serum prolactin levels in the midluteal phase and the prognosis of pregnancy after IVF-ET.

Methods: Design: Prospective study; Setting: Shimane Medical University Hospital; Patients: 29 patients completing their 34th IVF cycle; and Main outcome measures: Serum levels of prolactin, estradiol-17 β , and progesterone in the midluteal phase.

Results: Midluteal prolactin levels were significantly lower in patients with early pregnancy loss than in the successful pregnancy group. The mean progesterone/prolactin ratio and the estradiol-17 β /prolactin ratio were highest in patients with early pregnancy loss and were lowest in patients with successful pregnancies.

Conclusions: Findings of this study suggest that measurement of the serum prolactin concentration in the midluteal phase is useful for predicting the outcome of IVF-ET.

KEY WORDS: decidual prolactin; early pregnancy loss; IVF-ET; serum prolactin.

INTRODUCTION

Despite consistently high rates of fertilization following IVF-ET, the pregnancy rate continues to be sub-optimal because of the high rate of spontaneous abortion. Because ovarian hyperstimulation is induced by hMG and hCG during IVF cycles, levels of estradiol-17 β (E2), progesterone (P), and prolactin (PRL) differ from those in normal cycle (1). The use of a GnRH analog to suppress the pituitary-ovarian axis improves

the ovarian response to gonadotropin stimulation for IVF, resulting in very high preovulatory and midluteal ovarian steroid levels (2). These elevated levels of ovarian steroids are believed to affect implantation. In a hyperstimulated cycle, the serum level of PRL is much higher in the midluteal phase than in the preovulatory phase (1), and Seppola *et al.* have reported that high PRL levels can induce luteal insufficiency (3). In this study, we examined hormonal profiles in the midluteal phase of superovulated IVF cycles and evaluated whether a predictive relationship exists between PRL levels of midluteal phase and pregnancy outcome after IVF-ET.

MATERIALS AND METHODS

We studied 29 patients who completed their 34th IVF cycle in 1996 or 1997 at Shimane Medical University Hospital. Into each patient were transferred three embryos that were in good morphological quality, according to Veeck's preembryo grading system (4). Ages of these patients ranged from 27 to 43 years (mean $\pm$ SD = 33.3 $\pm$ 3.1). On the basis of clinical features and laboratory examination, infertility caused by tubal occlusion was diagnosed in 15 patients, infertility caused by male factors in 11, and endometriosis in 3. Patients with unexplained infertility or known endocrine disorders including ovulatory disorders, and obese or lean women were excluded. Multiple follicular stimulation, cycle monitoring, and oocyte retrieval were performed using standard protocols. Venous blood samples for basal hormonal measurements were obtained on Day 3 or 4 of the previous menstrual cycle. All women received, by way of a GnRH analog protocol, 400 μ g/day of nafarelin acetate (Nasanyl[®]; Yamanouchi, Tokyo) starting in the midluteal phase of the previous cycle. Gonadotropic stimulation of the ovaries was started

¹ Department of Obstetrics and Gynecology, Shimane Medical University, Izumo, Japan.

² To whom correspondence should be addressed at Department of Obstetrics and Gynecology, Shimane Medical University, 89-1, Enya-cho, Izumo 693-8501, Japan.

when the serum E2 level declined to <30 pg/mL and the serum FSH level declined to <10 mIU/mL. From Day 1 to Day 3 of ovarian stimulation, 150 or 300 IU of FSH (Fertinorm P[®]; Serono Japan, Tokyo) was administered. Beginning on Day 4, 150 or 300 IU of hMG (Humegon[®]; Organon, Tokyo) was administered based on the ovarian response. The criteria for hCG administration (HCG Mochida[®]; Mochida, Tokyo) were the presence of follicles >18 mm in diameter and serum E2 levels >200 pg/mL per the number of follicles >15 mm in diameter. Venous blood samples for E2 levels were drawn at the day of hCG administration. Nafarelin treatment was discontinued on the day of hCG administration.

Oocyte aspiration was performed with vaginal ultrasonography 36 h after hCG administration. Three embryos that cleaved into 4–8 blastomeres were transferred into each subject. All embryos were graded 1 or 2, according to Veeck's preembryo grading system (4). An additional 50 mg of progesterone (Luteum[®], Teikoku, Tokyo) was administered by intramuscular injection on alternate days, beginning on the day of follicular aspiration, and 3000 IU of hCG (HCG Mochida[®]; Mochida, Tokyo) was administered 3 and 7 days after oocyte aspiration.

Venous blood samples for hormonal measurements in the luteal phase were obtained on Day 7 or Day 8 after oocyte retrieval. All the samples were drawn at almost the same time (2:00 p.m.–3:00 p.m.). We measured serum levels of E2, P, and PRL.

Appropriate permission from the university hospital committee was obtained for the sample-collection protocol.

Assays

Serum levels of hormones were measured using direct radioimmunoassays for E2 (DPC Estradiol Kit[®], Diagnostic Products Corporation, Los Angeles, CA), P (DPC Progesterone Kit[®], Diagnostic Products Corporation), and PRL (SPAC-S Prolactin Kit[®], Daiichi Radioisotope, Tokyo, Japan); the intra-assay and interassay coefficients of variation were 5.0% and 4.5%, 2.0% and 3.2%, and 2.6% and 2.0%, respectively. Serum E2 and P4 levels are expressed in picograms per milliliter and in nanograms per milliliter, respectively. We also determined the ratio of the serum levels of P to the serum levels of E2 (P/E2), the ratio of the P levels to the PRL levels (P/PRL), and the ratio of the PRL levels to the E2 levels (PRL/E2).

Statistical Analysis

The data are expressed as the mean $\pm$ SD. The study group was divided into three subgroups as follows: successful pregnancy ($n = 12$), early pregnancy loss ($n = 8$), and nonconception group ($n = 14$). The early pregnancy loss group included patients who aborted within the first trimester. The successful pregnancy group included patients who gave birth to healthy infant. We analyzed between-group differences in the values of E2, P, PRL, the P/E2 ratio, the P/PRL ratio, and the E2/PRL ratio, using the Kruskal–Wallis non-parametric analysis and Bonferroni's multiple comparison test.

RESULTS

There were no significant differences in the mean age, basal serum levels of LH, FSH, E2, and PRL, the total dose of hMG, the numbers of follicles >16 mm and >10 mm, E2 levels on day of hCG administration, the number of oocytes retrieved, or the fertilization rate among the groups (Table I).

There were no significant differences in the midluteal E2 levels among the groups (Table II). The midluteal P levels was significantly lower in the successful pregnancy group than in the non-conception group ($p < .05$). The midluteal PRL level was significantly lower in the early pregnancy loss group than in the non-conception group ($p < .05$). The mean P/PRL ratio and E2/PRL ratio were significantly higher in the early pregnancy loss group than in the successful pregnancy group ($p < .05$).

DISCUSSION

In this study, the serum level of PRL predicted the IVF-ET result. Other investigators reported that there was no difference in midluteal PRL levels in IVF-ET cycles of different outcome (1,5). Kaplan *et al.* reported the high incidence of luteal-phase hyperprolactinemia during ovarian stimulation with hMG and no attenuation of luteal-phase hyperprolactinemia by GnRH analog (5). In this study, all patients were administered GnRH analog. There were no differences in follicle number (<10 mm, <16 mm) and E2 levels on day of hCG administration in follicular phase among the three groups studied. There were significant differences in midluteal PRL levels in cycles of different outcome. Therefore, it is suggested that

Table I. Basal Hormone Measurements and Clinical Characteristics

	Nonconception (n = 14)	Early pregnancy loss (n = 8)	Ongoing pregnancy (n = 12)
Age	32.3 ± 2.3	32.9 ± 3.6	34.6 ± 3.4
Basal hormone levels			
LH (mIU/mL)	3.9 ± 1.2	5.7 ± 4.4	4.7 ± 2.4
FSH (mIU/mL)	8.6 ± 2.0	8.1 ± 2.1	8.0 ± 4.4
E2 (pg/mL)	30.8 ± 9.5	32.5 ± 16.0	33.9 ± 26.8
PRL (ng/mL)	9.6 ± 2.7	11.9 ± 11.8	12.6 ± 4.6
Follicle numbers			
>10 mm	12.0 ± 4.6	10.2 ± 5.9	10.0 ± 3.6
>16 mm	5.6 ± 1.2	4.8 ± 0.8	5.1 ± 1.7
Estradiol levels on the day of hCG administration (pg/mL)	1819 ± 872	1446 ± 473	1741 ± 737
hMG (IU)	1931 ± 610	1978 ± 666	2062 ± 358
Oocytes retrieved	9.8 ± 4.3	8.6 ± 4.8	9.5 ± 4.1
Fertilization rate (%)	66.7 ± 20.0	79.6 ± 15.4	67.6 ± 26.2

Note. Data are the mean ± SD.

other factors may affect midluteal PRL levels and the sequential outcomes.

Prolactin is a polypeptide hormone secreted from the anterior pituitary gland. High concentrations of PRL have also been found in human amniotic fluid (6). The source of amniotic fluid PRL is the maternal decidualized endometrium (7). The amino acid sequence of endometrial PRL is identical to that of pituitary PRL, although endometrial PRL mRNA is 150 nucleotides longer than pituitary PRL mRNA (8). Wu *et al.* have shown that the endometrial PRL gene is expressed in decidual cells (9). Prolactin has also been shown to be produced in late secretory endometrium of women with normal cycles (10). The amount of PRL in secretory endometrium increases with the advancing histologic day. The production of PRL in the secretory endometrium begins between histological Day 22–25 (10). Kauma and Shapiro demonstrated by immunoperoxidase staining that PRL production in the secretory endometrium begins on histological Day 25 (11). It is unclear whether decidual PRL has an influence on the serum concentration of PRL. Some

investigators reported that decidual prolactin is not secreted into the circulation in significant quantities (12,13). However, we speculated the possibility that the elevated serum concentration of PRL in this study had an effect on the production of decidual PRL. A previous study has shown that the production of PRL is deficient in secretory endometrium with a luteal phase defect compared with normal secretory endometrium in tissue culture (14). The physiological role and regulation of endometrial PRL have not yet been fully elucidated. Prolactin has been shown to suppress the immune response and is involved in the maintenance of T-cell immunocompetence (15). It is detected in endometrium after Day 23 of the menstrual cycle and its production increases as decidualization spreads throughout the endometrial stroma (10), which corresponds to the time at which implantation would normally occur.

In this study, the midluteal serum PRL level was lowest in the early pregnancy loss group. This result may reflect a defect or delay in endometrial decidualization in patients with early pregnancy loss. However, we could not show the detailed effect of prolactin on the implantation mechanism. Additional studies will be needed to establish that endometrial and pituitary PRL is important for implantation and maintenance of pregnancy after IVF-ET.

The midluteal P level was lowest in the successful pregnancy group in this study, and the mean P/E2 ratio in the successful pregnancy group and the early pregnancy loss group were closer to 100 (successful pregnancy group: 105.6, early pregnancy loss group: 94.9) than in the non-conception group. Some authors have found higher luteal E2 levels and a low P/E2 ratio in

Table II. Midluteal Hormone Levels

	Nonconception	Early pregnancy loss	Ongoing pregnancy
E2 (pg/ml)	1593 ± 867	1385 ± 665	1156 ± 1130
P (ng/ml)	236.6 ± 145.6 ^a	133.6 ± 96.4	83.5 ± 77.1 ^b
PRL (ng/ml)	16.9 ± 5.2	15.0 ± 11.7 ^c	22.4 ± 8.1 ^d
P/E2 ratio	147.4 ± 55.8	105.6 ± 82.8	94.9 ± 55.7
P/PRL ratio	14.2 ± 8.6	16.9 ± 22.1 ^e	4.3 ± 4.8 ^f
E2/PRL ratio	0.10 ± 0.06	0.18 ± 0.24 ^g	0.06 ± 0.04 ^h

Note. Data are the mean ± SD. *p* < .05, a vs. b, c vs. d, e vs. f, g vs. h.

IVF-ET patients who failed to impact compared with those who became pregnant (16,17). On the other hand, other investigators have found no significant differences between conceptual and non-conceptual cycles and emphasize that high P and high P/E2 ratio in the luteal phase of IVF-ET cycles are not necessarily connected with pregnancy (18,19). We considered on the basis of our results that the appropriate levels of midluteal P and P/E2 ratio are needed for implantation after IVF-ET.

At our institutions, we usually use hMG and hCG for ovarian hyperstimulation for IVF, which usually results in high levels of E2. The serum PRL level increases in the luteal phase following hyperstimulation (20). The increase in the serum PRL level may reflect a direct effect of E2 on pituitary PRL production (21). In this study, all patients received hMG, hCG, and a GnRH analog. There was no significant difference in the midluteal E2 level among the groups. However, the midluteal PRL level was lowest and the E2/PRL ratio was highest in the early pregnancy loss group, suggesting that the pituitary response to serum E2 was weak in this group and that adequate serum PRL levels may be needed to maintain pregnancy after IVF-ET.

In summary, the midluteal serum PRL level was low in the early pregnancy loss group after IVF-ET. The low PRL level may have reflected a delay or defect in endometrial decidualization. These findings suggest that measurement of the serum PRL level at the midluteal phase may be useful for estimating the outcome of IVF-ET.

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