Journal of Assisted Reproduction and Genetics, Vol. 19, No. 12, December 2002 (© 2002)

Clinical Assisted Reproduction

Human Chorionic Gonadotropin Combined with Progesterone for Luteal Support Improves Pregnancy Rate in Patients with Low Late-Midluteal Estradiol Levels in IVF Cycles

Akihisa Fujimoto,[1] Yutaka Osuga,[1,3] Toshihiro Fujiwara,[1] Tetsu Yano,[1] Osamu Tsutsumi,[1,2] Mikio Momoeda,[1] Koji Kugu,[1] Kaori Koga,[1] Yutaka Morita,[1] Osamu Wada,[1] and Yuji Taketani[1]

Submitted May 21, 2002; accepted June 19, 2002

***Purpose*:** To investigate how late-midluteal estradiol levels relate to the pregnancy outcome in IVF cycles, and to assess whether human chorionic gonadotropin (hCG) for luteal support benefits the pregnancy outcome of patients with low late-midluteal estradiol levels.
***Methods*:** The pregnancy rate of 436 women undergoing first IVF cycles with long protocol and luteal support with progesterone alone were analyzed. Unsuccessful women with low late-midluteal estradiol levels (<100 pg/mL) proceeded with the exploratory second IVF cycles where they were randomly given with either progesterone alone (P protocol) or hCG +progesterone (P+hCG protocol) for luteal support.
***Results*:** Pregnancy rate in women with low late-midluteal estradiol levels was significantly lower compared to that with medium (100–500 pg/mL) and high (>500 pg/mL) levels (13.3, 26.8, and 36.3%, respectively). P+hCG protocol increased late-midluteal estradiol levels and produced a significantly higher pregnancy rate (31.7%) than P protocol (13.7%).
***Conclusions*:** hCG in combination with progesterone for luteal support was suggested to benefit women undergoing IVF with low late-midluteal estradiol levels.

KEY WORDS: Estradiol; hCG; IVF-ET; luteal support; progesterone.

INTRODUCTION

Appropriate hormonal conditions are necessary for the preparation of a receptive endometrium. While progesterone has been demonstrated to be a prerequisite for achieving implantation, a role of estradiol in the event of implantation remains to be elucidated. In an oocyte donation program, depletion of estradiol in the luteal phase resulted in poor pregnancy outcome (1) although the morphology of the endometrium was seemingly not affected (2,3).

In IVF cycles, despite estradiol levels at the oocyte pickup being usually well above the physiological range, its levels during implantation, that is, at the midluteal phase, may vary considerably depending on ovarian stimulation protocols and individual sensitivities to them. However, the issue that midluteal estradiol levels have any impact on IVF outcomes is as yet unsettled.

[1] Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
[2] CREST, Japan Science and Technology, Kawaguchi, Saitama 332-0012, Japan.
[3] To whom correspondence should be addressed; e-mail: yutakaos-tky@umin.ac.jp.

1058-0468/02/1200-0550/0 © 2002 Plenum Publishing Corporation

Some reports showed that midluteal estradiol levels were not related to IVF outcomes (4,5) and that an addition of estradiol for luteal support did not improve pregnancy rate (6). On the other hand, another report showed that higher estradiol levels were observed in pregnant cycles compared with nonpregnant cycles (7). In parallel with these studies, other studies looked at whether a rapid decline in estradiol concentrations from the day of human chorionic goradotropin (hCG) to the midluteal phase was associated with impaired endometrial receptivity. This also remained divided such that one study suggested that a precipitous decline in estradiol levels after hCG injection resulted in low pregnancy rate (8), which was not seen in another study (9). Thus, at this time, it is largely undefined whether modulating estradiol levels in the luteal phase might affect pregnancy rate in IVF-ET.

Luteal estradiol levels depend on how the luteal phase is managed. Many favor progesterone alone for luteal support, in part, for the fear of developing ovarian hyperstimulation syndrome, a serious event frequently associated with the use of hCG for luteal support in hope of its stimulatory effect on the production of both progesterone and estradiol. We, therefore, surmised that clinical relevance of luteal estradiol levels should be viewed in the context of luteal support regimen.

With this background in mind, we asked how estradiol levels at late-midluteal phase in IVF cycles using long protocol with progesterone for luteal support relate to the pregnancy outcome. Our next interest was to see whether unsuccessful women accompanied by low late-midluteal estradiol levels benefit from hCG in combination of progesterone for luteal support.

MATERIALS AND METHODS

Patients Selection

A total of 436 patients treated with IVF in the assisted reproductive technology unit of the Department of Obstetrics and Gynecology, University of Tokyo between January 1998 and December 2000 participated in the study.

IVF Treatment Protocol

The patients were treated with buserelin nasal spray (Sprecur, Aventis Pharma, Tokyo, Japan), 300 μg three times a day, starting at the midluteal phase of the previous cycle. After confirmation of pituitary down regulation by observing the ovaries and endometrium with transvaginal ultrasound, daily injection of HMG (Nikken HMG, Nikken Kagaku Co., Tokyo, Japan) was started. Then, hCG at a dose of 10,000 IU was injected on the day when at least two follicles >17 mm in diameter were detected. Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 h after the hCG injection. Follicles were flushed once or twice by modified human tubal fluid (mHTF, Irvine, CA) to secure oocyte retrieval. A maximum of three embryos were transferred into the uterine cavity 48–72 h after oocyte retrieval.

In the first cycle, all the patients were given daily injection of 25-mg progesterone as luteal phase support, starting on the day after oocyte retrieval (P protocol). One hundred and fourteen patients who failed to conceive and exhibited lower late-midluteal estradiol levels (<100 pg/mL) proceeded with the exploratory second cycle where they were randomly treated with either the same protocol as the first cycle or with hCG (3000 IU i.m. on day 1, 4 and 7 after ET) additionally (P+hCG protocol) under informed consent.

A pregnancy test was performed 14 days after ET. The presence of a gestational sac was examined by transvaginal-ultrasound 7 days later. In the present study, we defined pregnancy as a positive gestational sac on the examination.

Hormone Measurement

Seven days after ET, serum was collected for the measurement of estradiol and progesterone. Serum estradiol and progesterone levels were measured using a radioimmunoassay kit (Coat-A-Estradiol, Diagnostic Products Corporation, Los Angels, CA). The sensitivity of these assays was 8 pg/mL for estradiol and 0.02 ng/mL for progesterone. The intra- and inter-assay coefficients of variation were less than 10% in both assays.

Statistical analysis

Tests of statistical significance for comparison between the groups of different serum estradiol levels were performed using a one-way analysis of variance (ANOVA) with Scheffe's F test as a post hoc test. For comparison of hormone levels, number of retrieved oocytes and transferred embryos between P group and P+hCG group, t test was used. Pregnancy rates were analyzed using chi-square test.

RESULTS

A total of 436 patients undergoing the first cycle IVF-ET were divided into three groups according to serum estradiol levels 7 days after ET: Group A– <100 pg/mL; Group B – 100–500 pg/mL; Group C – >500 pg/mL (Table I). Age, the number of oocytes retrieved, the number of embryos transferred, estradiol levels on the day of hCG, and progesterone levels 7 days after ET were not significantly different between Group A and Group B. However, the pregnancy rate of Group B (26.8) was significantly higher than that of Group A (13.3%). There was also a significant difference in pregnancy rate between Group A and Group C (36.3%), whereas no difference was detected between Group B and Group C. In Group C, significantly more oocytes were retrieved and more embryos were transferred than in Group A. Estradiol levels on the day of hCG and progesterone levels 7 days after ET were also significantly higher in Group C than those in Group A.

Comparison between P protocol and P+hCG protocol in the second IVF cycles is shown in Table II. Unsuccessful women of Group A went on to undergo the exploratory second cycle. There were no significant difference in age, the number of oocytes retrieved, and the number of embryos transferred between the two protocols. Both estradiol and progesterone levels 7 days after ET were significantly higher in P+hCG protocol (estradiol, 1504 ± 122 pg/mL; progesterone, 172.0 ± 14.0 ng/mL; mean ± SEM) than in P protocol (107 ± 26 pg/mL; 20.8 ± 4.9 ng/mL). A significantly higher pregnancy rate was observed in P+hCG protocol (31.7%) than in P protocol (13.7%) ($p < 0.05$). Two women (2.7%) in P+hCG group developed moderate OHSS that needed albumin administration to stimulate diuresis.

Table I. Pregnancy Rates and Related Parameters According to Late-Midluteal Estradiol Levels

Estradiol 7 days after ET (pg/ml)	Group A (<100)	Group B (100–500)	Group C (>500)
Age	35.3 ± 0.2	35.1 ± 0.3	35.1 ± 0.4
No. of cycles	218	127	91
No. of oocyte retrieved	6.9 ± 0.3***	7.9 ± 0.4**	9.6 ± 0.6
No. of embryos transferred	2.5 ± 0.1***	2.7 ± 0.1	2.8 ± 0.1
Pregnancy rate (%)	29/218*,*** (13.3)	34/127 (26.8)	33/91 (36.3)
Estradiol on the day of hCG (pg/mL)	1578 ± 127**	2028 ± 171	2262 ± 167
Estradiol 7 days after ET (pg/mL)	43 ± 2*,***	227 ± 10***	1399 ± 90
Progesterone 7 days after ET (ng/mL)	11.7 ± 0.9***	26.0 ± 3.3***	141.7 ± 15

Note. ET: embryo transfer. Values are mean ± SEM.
* $p < 0.01$ vs. Group B, ** $p < 0.05$ vs. Group C, *** $p < 0.01$ vs. Group C.

Table II. Pregnancy Rates and Related Parameters in Different Luteal Support Protocols

	Luteal support protocol	
	Progesterone	Progesterone + hCG
Age	35.2 ± 0.5	35.3 ± 0.5
No. of cycles	51	63
No. of oocyte retrieved	7.5 ± 0.6	6.7 ± 0.5
No. of embryos transferred	2.6 ± 0.1	2.6 ± 0.1
Pregnancy rate (%)	7/51* (13.7)	20/63* (31.7)
Estradiol on the day of hCG (pg/mL)	1496 ± 143	1383 ± 109
Estradiol 7 days after ET (pg/mL)	107 ± 26**	1504 ± 122**
Progesterone 7 days after ET (ng/mL)	20.8 ± 4.9**	172.0 ± 14.0**

Note. Subjects are those who showed low late-midluteal estradiol levels (<100 pg/mL) in the first IVF cycles. ET: embryo transfer. Values are mean ± SEM.
* $p < 0.05$; ** $p < 0.01$.

DISCUSSION

Here we demonstrated that lower estradiol levels at the late-midluteal phase in IVF cycles using long protocol with progesterone for luteal support were associated with a lower pregnancy rate. In addition, the failed women who underwent an IVF employing this protocol benefited from luteal support consisting of hCG and progesterone. These findings seem to have implications that a subset of women undergoing IVF may fail to conceive because of inappropriate estrogenic effects on the endometrium during the implantation period, which could be corrected by the addition of hCG for luteal support.

Luteal estradiol levels are influenced by numerous factors including ovulation induction protocols and a way of oocyte aspiration, multiple flushing being known to reduce a granulosa cell mass and thereby impair luteal function. Especially both the number of the corpus luteum and estradiol-producing ability of each corpus luteum directly contribute to luteal estradiol levels. In our study, significantly more oocytes were collected in the high late-midluteal estradiol group

(>500pg/mL) compared with groups with lower estradiol. Therefore, an increased number of the corpus luteum may be responsible for high late-midluteal estradiol levels. Furthermore, higher pregnancy rate in this group may be, in part, ascribable to an increased number of oocytes collected.

It is to be noted that the intermediate estradiol level group (100–500 pg/mL) showed a significantly higher pregnancy rate than the low estradiol group (<100pg/mL) despite comparable number of oocytes collected and embryos transferred. The late-midluteal progesterone levels were also not significantly different between the two groups, prompting the suggestion that late-midluteal estradiol levels may determine the outcome of IVF-ET. This idea encouraged further study to see whether hCG supplement to stimulate estradiol production could improve pregnancy rate in the low estradiol group. As expected, hCG supplement resulted in a significantly higher pregnancy rate associated with higher late-midluteal estradiol levels.

Previous studies regarding the effect of hCG supplement during the luteal phase were controversial. In GnRHa/HMG IVF cycles, Buvat *et al.* showed a superior pregnancy rate in hCG supplement group than in progesterone supplement group (10), whereas no difference was detected in a study conducted by Claman *et al.* (11). On the other hand, a randomized study performed by Mochtar *et al.* (12), who compared vaginal progesterone alone with a combination of vaginal progesterone and hCG for luteal support, demonstrated a lower pregnancy rate in hCG supplement group despite the higher midluteal estradiol levels. A likely explanation for the discrepancy as compared with our findings is that the subjects of their studies were not the patients displaying lower late-midluteal estradiol levels. Cumulatively, we favor the notion that hCG, if properly used, may facilitate the implantation process. In other words, an appropriate range of late-midluteal estradiol level seems necessary to prepare a receptive endometrium in ovarian stimulated IVF cycles.

In the present study, late-midluteal progesterone levels were also significantly higher in hCG supplement group than in nonsupplement group. Therefore, elevated progesterone levels along with elevated estradiol levels might relate to improved pregnancy rate in hCG supplement group although the extent of its contribution could not be elucidated from the present data.

HCG supplement during the luteal phase was shown to increase the risk of severe OHSS (12%) than progesterone (13). It is also known that high serum estradiol levels before oocyte pickup increase the risk of OHSS. In our study, the serum estradiol levels on the day of hCG were significantly lower in the low late-midluteal estradiol group than in the high late-midluteal estradiol group, a plausible explanation for an observed low rate (2.7%) of OHSS in the hCG supplement group. Thus, hCG supplement, when attempted selectively in low late-midluteal estradiol women, may carry a lower risk of OHSS.

In summary, like progesterone, estradiol levels in the luteal phase in IVF cycles with long protocol may be crucial for achieving pregnancy. In addition, the patients given progesterone alone for luteal support and displaying low late-midluteal estradiol levels may benefit from hCG in combination with progesterone, possibly through an elevation in luteal estradiol levels.

REFERENCES

1. Younis JS, Simon A, Laufer N: Endometrial preparation: lessons from oocyte donation. Fertil Steril 1996;66:873–884
2. de Ziegler D, Bergeron C, Cornel C, Medalie DA, Massai MR, Milgrom E, Frydman R, Bouchard P: Effects of luteal estradiol on the secretory transformation of human endometrium and plasma gonadotropins. J Clin Endocrinol Metab 1992;74:322–331
3. Younis JS, Ezra Y, Sherman Y, Simon A, Schenker JG, Laufer N: The effect of estradiol depletion during the luteal phase on endometrial development. Fertil Steril 1994;62:103–107
4. Muasher S, Acosta AA, Garcia JE, Jones GS, Jones HW, Jr: Luteal phase serum estradiol and progesterone in in vitro fertilization. Fertil Steril 1984;41:838–843
5. Nylund L, Beskow C, Carlstrom K, Fredricsson B, Gustafson O, Lunell NO, Pousette A, Rosenborg L, Slotte H, Akerlof E: The early luteal phase in successful and unsuccessful implantation after IVF-ET. Hum Reprod 1990;5:40–42
6. Smitz J, Bourgain C, Van Waesberghe L, Camus M, Devroey P, Van Steirteghem AC: A prospective randomized study on oestradiol valerate supplementation in addition to intravaginal micronized progesterone in buserelin and HMG induced superovulation. Hum Reprod 1993;8:40–45
7. Hutchinson-Williams KA, Lunenfeld B, Diamond MP, Lavy G, Boyers SP, DeCherney AH: Human chorionic gonadotropin, estradiol, and progesterone profiles in conception and nonconception cycles in an in vitro fertilization program. Fertil Steril 1989;52:441–445
8. Sharara FI, McClamrock HD: Ratio of oestradiol concentration on the day of human chorionic gonadotrophin administration to mid-luteal oestradiol concentration is predictive of in-vitro fertilization outcome. Hum Reprod 1999;14:2777–2782
9. Yu Ng EH, Yeung WS, Yee Lan LE, So WW, Ho PC: High serum oestradiol concentrations in fresh IVF cycles do not impair implantation and pregnancy rates in subsequent frozen-thawed embryo transfer cycles. Hum Reprod 2000;15:250–255
10. Buvat J, Marcolin G, Guittard C, Herbaut JC, Louvet AL, Dehaene JL: Luteal support after luteinizing hormone-releasing hormone agonist for in vitro fertilization: Superiority

of human chorionic gonadotropin over oral progesterone. Fertil Steril 1990;53:490–494
11. Claman P, Domingo M, Leader A: Luteal phase support in in-vitro fertilization using gonadotrophin releasing hormone analogue before ovarian stimulation: A prospective randomized study of human chorionic gonadotrophin versus intramuscular progesterone. Hum Reprod 1992;7:487–489
12. Mochtar MH, Hogerzeil HV, Mol BW: Progesterone alone versus progesterone combined with HCG as luteal support in GnRHa/HMG induced IVF cycles: A randomized clinical trial. Hum Reprod 1996;11:1602–1605
13. McClure N, Leya J, Radwanska E, Rawlins R, Haning RV, Jr: Luteal phase support and severe ovarian hyperstimulation syndrome. Hum Reprod 1992;7:758–764