Prediction of Pregnancy Outcomes by Uterine Artery Impedances on the Day of Embryo Transfer in Human IVF

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인간 체외수정술에서 배아 이식일의 자궁동맥 임피던스에 따른 임신의 예측

정주은 · 조무성 · 김승철 · 주종길 · 최종열 · 이규섭*

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목 적: 본 연구는 배아이식일의 자궁동맥혈류가 인간 체외수정술에서 임신예후의 예측인자가 될 수 있는지 알아보기 위해 시행되었다.

연구방법: 51회의 체외수정술 주기에 대해 전향적인 임상 관찰로 시행되었으며 혈청 estradiol 농도는 hCG 투여일에 측정되었고, 자궁동맥 박동율 (PI)과 저항율 (RI)은 배아이식 3일째에 측정되었다.

결 과: 51회의 주기 중 22주기에서 임상적으로 임신이 확인되었고 (43.1%), 착상률은 14.7%였다. 자궁동맥 PI와 RI는 혈청 estradiol 농도과 의미 있는 음의 상관관계 (p<0.05)를 보였으며 자궁혈류량은 임신된 그룹과 임신 되지 않은 그룹에서 차이가 없었다. 임신율은 PI가 3.0 이상인 군에서 PI가 3.0 이하인 군보다 조금 높게 측정되었으나 통계학적인 유의성은 없었다.

결 론: 배아이식일의 자궁동맥 PI와 RI는 체외수정술에서 임신의 예측인자로는 효용성이 떨어지지만 estradiol 농도와는 음의 상관관계를 보여 자궁혈류와 estradiol 농도가 상관관계가 있음을 알 수 있다.

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중심단어: 자궁동맥 박동율, 저항율, 임신예후, 혈청 estradiol 농도

Angiogenesis plays an important role in growth of endometrium and implantation. A good uterine blood flow is an important factor contributing to uterine receptivity for implantation.[1,2] Since Goswamy et al. suggested an association between decreased uterine perfusion and infertility,[3] many studies have attempted to investigate whether uterine artery impedances such as pulsatiltiy index (PI) or resistance index (RI) are effective in the prediction of endometrial receptivity for successful implantation.[4~9] However, results of these studies have been inconsistent and clinical application of the value of uterine artery impedance have been limited.[10,11]

These inconsistent results may be attributable to the differences in timing of ultrasound examination and the selected uterine region. Ultrasound examination was usually performed on the day of hCG administration,[4] on the day of egg retrieval[6,11,12] or on the day of embryo

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transfer.[7,13] Therefore, it is needed to reevaluate the role of uterine PI and RI as a predictor for the pregnancy outcome considering the time of ultrasouud examination.

Considering the period of embryo transfer is a stage for priming of the uterus for successful implantation, it is most plausible that appropriate times to examine uterine blood flow would be the index for the prediction of pregnancy outcome. Therefore this study was aimed to investigate whether uterine artery impedances on the day of embryo transfer can be a predictor of pregnancy outcome in IVF.

MATERIALS AND METHODS

This was a prospective clinical observation study on a total of 51 cycles from 45 women who underwent IVF-ET with tubal obstruction (16 cycles), male infertility (15 cycles), endometriosis (6 cycles), and unexplained infertility (14 cycles) at the Pusan National University Hospital (PNUH) from March 2006 to June 2007. Of 45 women, 6 underwent IVF-ET two times due to the pregnancy failure in the first cycle.

The criteria consisted of normal uterine cavity and no uterine fibroid on the day of oocyte retrieval. Patients with severe OHSS (ovarian hyperstimulation syndrome) and abnormal uterine cavity were excluded. All women who participated in the study gave written informed consent. The consent form and protocol were approved by the Human Investigation Committee of PNUH. The study was approved by the Institutional Review Board of Pusan National University Hospital.

1. Ovarian hyperstimulation and IVF procedures

Controlled ovarian hyperstimulation (COH) was performed by a standard long protocol with GnRH agonist (leuprorelin acetate; Lucrin). In brief, buserelin acetate was given daily at a dose of 0.1 mL from midluteal phase of the previous cycle for adequate desensitization of pituitary. When the serum estradiol level was 50 pg/mL on the 3rd day of cycle, two or three ampules of recombinant FSH (Gonal-F, Serono, Switzerland) were given subcutaneously depending on the status of follicular development.

Follicular development was assessed in all patients by monitoring serum estradiol levels and by ovarian ultrasonography. HCG (IVF-C, LG, Korea) was administered when the serum estradiol level reached the maximal peak, and at the same time at least two dominant follicles were 18 mm or larger. Serum estradiol and progesterone concentrations were measured on the day of hCG administration using commercial available kit (Immunlite 2500, DPC Inc., Germany).

Oocyte retrieval by transvaginal ultrasonographic guidance was performed approximately 34~35 hours after the hCG administration. Highly motile spermatozoa collected from three layered percoll gradient were inseminated 5~6 hours after oocyte recovery. Fertilization was confirmed 16~20 hours after insemination by the presence of two pronuclei. All the fertilized embryos were cultured in P1 media from day 1 until embryo transfer. Embryos were routinely transferred on day 3 and werescored based on blastomer size, the presence of fragmentation, and cell number just before transfer. Clinical pregnancy was defined as fetal sacs with heart beat on ultrasonography performed 7 weeks after embryo transfer. Implantation rate was defined as number of fetal sacs per number of transferred embryos.

2. Doppler ultrasonography

Approximately one hour before embryo transfer, uterine blood flow was measured using color and pulsed Doppler transabdominal transducer (SEQUOIA, Siemens, USA). All scans were performed by one operator and checked by a colleague. Transducer was oriented so that the ultrasound beam was in the transverse plane. The spatial peak temporal average intensity for B-mode and

Doppler imaging was < 80 mW/cm^2. Doppler was performed to determine maximum and mean velocities for the uterine arteries by placing the Doppler gate over the area of maximum color until an arterial waveform was obtained. Flow velocity waveformsfrom both right and left ascending branches of uterine arteries lateral to the cervix were recorded on videotape.

Blood flow indices of uterine arteries were expressed as PI and RI. The PI and RI are expressed by PI = (S-D)/M, and (S-D)/S, respectively, where S is the peak systolic shifted frequency, D is the maximum end diastolic frequency, and M is the time-averaged maximum frequency over the whole cardiac cycle. The mean pulsatility indices of the left and right uterine arteries were calculated and used as an index of uterine flow velocity. A reduction in PI reflects an increase in blood flow. RI examines the differences between the peak systolic and end diastolic velocity and is suitable for low resistance vascular beds with continuous flow throughout diastole.

3. Statistical analysis

Unpaired student t-test, one-way ANOVA analysis, and linear regression were used for the statistical analysis using SPSS (statistical package for the social sciences) (version 12.) program. Results were presented as mean ± SD. *P* values of < 0.05 were considered statistically significant.

RESULTS

During the study period, a total of 69 cycles were received controlled ovarian hyperstimulation for IVF treatment, but 8 cycles were cancelled due to poor ovarian response (2 cycles) and severe ovarian hyperstimulation syndrome (6 cycles). Ten cycles were excluded because of congenital uterine abnormality (2 cycles) and uterine fibroid (8 cycles). Therefore, 51 cycles were included in the analysis.

Table 1. Characteristics of patients and IVF outcomes

Age (years)*	34.3±4.2
¶ E_2 (ng/ml)†	3.94 (3.39, 0.92~11.10)
¶ Progesterone (ng/ml)†	1.58 (1.48, 0.45~2.97)
No. of oocytes retrieveds	10.6±3.1
No. of oocytes fertilizeds	9.2±2.7
Fertilization rate (%)*	86.2±9.7
No. of embryos transferred	5.2±0.8
No. of pregnancies	22 (43.1%)
Implantation rate	14.7%

* Values are mean ± SD
† Values are means (median and range)
¶ Levels in the serum were measured on the day of hCG administration

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The average age of patients was 34.3 years old and basal hormone levels of FSH and estradiol were within a normal range. The mean serum estradiol and progesterone levels on the day of hCG administration were 3.94 ng/ml and 1.58 ng/ml, respectively. The average number of oocytes retrieved and embryos transferred were 10.6 and 5.2, respectively. Of 51 cycles, 22 were clinically pregnant (43.1%) and the implantation rate was 14.7% (Table 1).

And then, we analyzed whether these factors have a significant difference between pregnant and nonpregnant group. Female age was significantly lower and the numbers of oocytes retrieved were significantly higher in the pregnant group compared to the nonpregnant group (p<0.05). However, no statistical significant differences were found in fertilization rate, the number of embryos transferred, serum estradiol and progesterone levels on the day of hCG administration. In addition, uterine PI and RI were not significantly different between both groups (Table 2).

Table 2. Comparisons of Serum E_2 levels and uterine blood flow between pregnant and nonpregnant group

	Pregnant group (n=22)	Nonpregnant group (n=29)
Age (years)*	32.3±3.6[a]	37.2±4.7
No. of oocytes retrieved*	10.6±3.2[a]	8.0±3.1
No. of oocytes fertilized*	9.0±2.9[a]	6.1±2.7
Fertilization rate (%)*	84.5±9.8	74.4±21.4
No. of embryos transferred*	5.6±0.9	4.6±2.1
¶ E_2 (ng/ml)†	4.09 (3.72, 1.79~11.10)	3.79 (2.83, 0.92~8.47)
¶ Progesterone (ng/ml)†	1.74 (1.54, 0.74~2.97)	1.44 (1.31, 0.45~2.46)
Uterine blood flow		
Pulsatility index (PI)*	2.43±1.21	2.58±1.20
Resistance index (RI)*	0.81±0.17	0.85±0.11

* Values are mean ± SD
† Values are means (median and range)
¶ Levels in the serum were measured on the day of hCG administration
a, $p<0.05$ (vs nonpregnant group)

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In analysis of the pregnancy outcomes according to uterine PI, the pregnancy rate wasslightly higher in patients with PI more than 3.0 compared than those with PI of 3.0 or less, but there was no significant difference (Figure 1).

We investigated whether serum estradiol and progesterone levels on the day of hCG administration relate the uterine blood flow at the day of embryo transfer. As shown in Figure 2, uterine PI and RI at embryo transfer of day 3 had a significant inverse correlation with serum estradiol levels ($p<0.05$), but not serum progesterone levels.

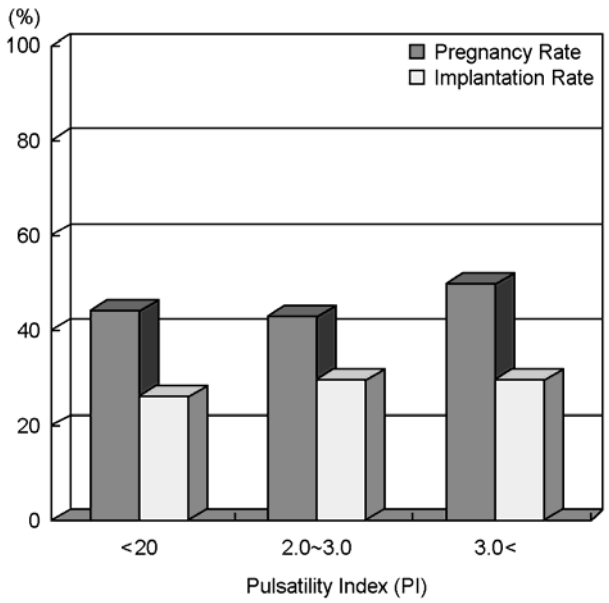

Figure 1. Pregnancy outocmes according to PI values.

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DISCUSSION

The present study demonstrates that both uterine PI and RI had no significant differences between pregnant and nonpregnant groups, indicating uterine PI or RI on the day of embryo transfer cannot be an effective predictor for the pregnancy outcome of IVF by showing that both uterine PI and RI had no significant differences between pregnant and nonpregnant groups. This fact

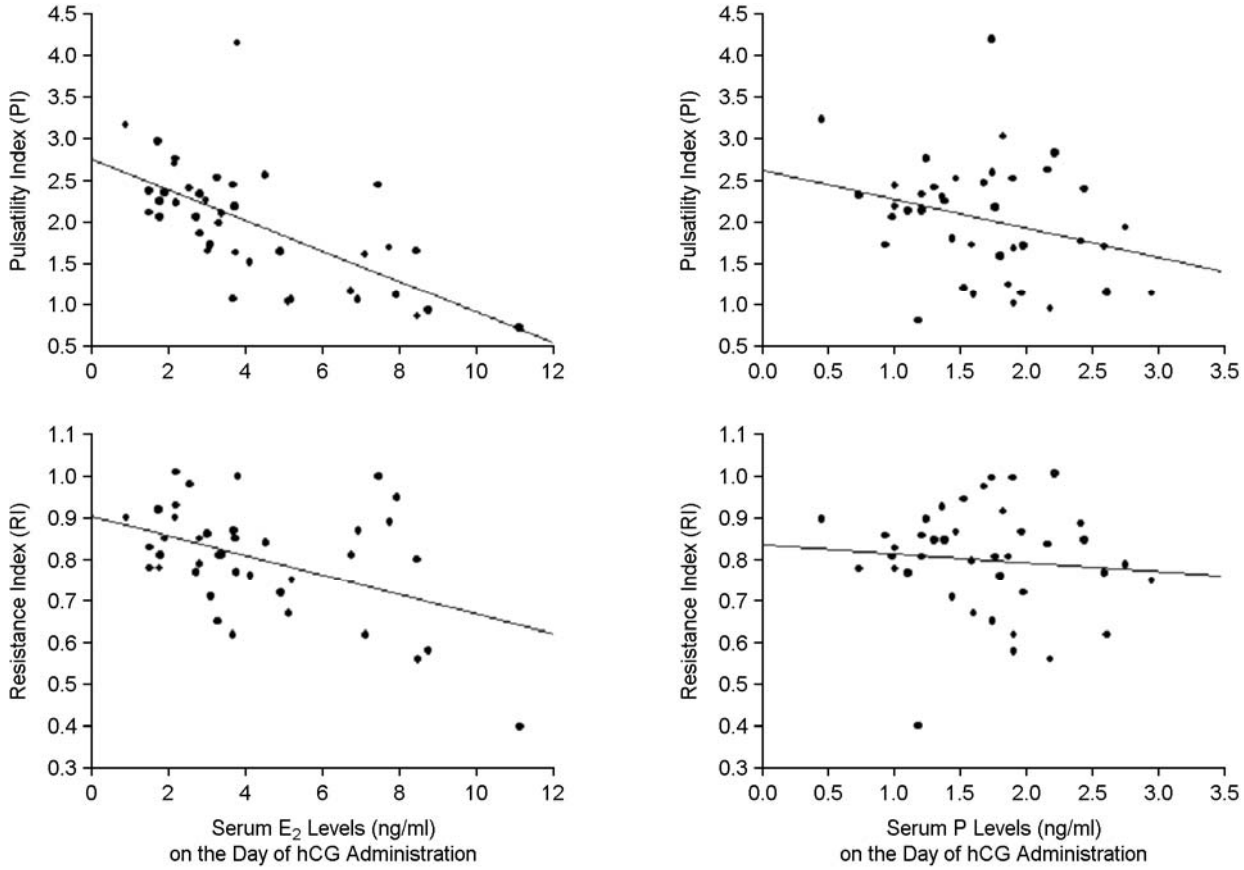

Figure 2. Serum Estridiol and Progesterone concentration on the day of hCG administration

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can be obviously seen in the result of 6 women who underwent IVF-ET two times due to the failure of pregnancy in the first cycle. Of them, 2 women were pregnant in the second cycle. However, on the contrary their uterine PI and RI were increased from 1.68 and 0.80 (first woman) and 2.13 and 0.81 (second woman) in the first cycle to 3.29 and 1.0, and 5.01 and 1.0 in the second cycle, respectively. There were no significant differences in serum estradiol levels on the day of hCG administration, the numbers of oocytes retrieved, and the numbers of embryos transferred between the two cycles in both women.

Like the present study, Cacciatore et al.[7] and Chien et al.[14] assessed uterine PI and RI at the day of embryo transfer. However, they found a significant lower uterine PI and RI in the pregnant group than that of the non-pregnant group unlike our results. This inconsistent result with our study can be explained in three ways. First is a definite difference of the day of embryo transfer. We transferred embryos on day 3 after oocyte retrieval whereas they transferred on day 2. Uterine contractility and blood flow have a definite alteration depending on the menstrual cycle. Second, the size of our study is too small to compare with other studies. Third reason may be associated with the different serum estradiol levels resulting from different superovulation regimens because the endometrial angiogenesis depends on the serum estradiol concentrations.[13,15,16]

The present study also showed no significant differences in the pregnancy outcomes according to

uterine PI and RI. This result is closely similar to the observation of Ng et al.[11] who showed no significant differences in demographic data among these three groups when they classified uterine PI into three groups - < 2.0, 2.0~2.99, and ≥ 3.0. However, Dickey reported that the implantation in IVF cycles was decreased when uterine PI was ≥ 3.3~3.5, and uterine resistance index (RI) was ≥ 0.95 at embryo transfer.[17] Steer et al.[18] reported no pregnancies in those with uterine PI > 3.0. Cacciatore et al.[7] showed overall pregnancy rate decreased significantly when PI was > 3.0 and RI > 0.92. Therefore, further studies are needed to elucidate clearly the pregnancy outcome according to the value of PI or RI.

Another notable finding of our present study is that uterine PI and RI on day of embryo transfer had an inverse relationship with serum estradiol levels on the day of hCG administration. This result suggests that serum estradiol levels in periovulatory phase during ovarian hyperstimulation are associated positively with uterine blood flow at a time around embryo transfer considering that a reduction in PI reflects an increase in blood flow. This conclusion can be supported by the following properties of estrogens.

Estrogens are a potent angiogenic and vasodiative factor secreted directly from growing follicles[19] and play a crucial role in the mechanisms that improve uterine and endometrial perfusion for uterine preparation to embryo implantation.[12,20,21] Adequate uterine estrogenization is a necessary condition for uterine receptivity. [22,23] Indeed, vaginal estradiol administration improves endometrial proliferation and uterine perfusion.[24]

However, the exact mechanism by which estrogens exert its function on the process of angiogenesis and vasodilation of the endometrium is unclear.[25] It can be inferred that vascular endothelial growth factor (VEGF) may be involved in the stimulation of angiogenesis by estrogen because VEGF is a critical regulator of angiogenesis and its expression is stimulated by estrogen.[26,27]

Estrogens seem to have as a biphasic effect on uterine blood flow and VEGF expression in endometrium uterine blood flow via VEGF expression was enhanced by optimal estradiol concentration (1~10 nmol/l), but decreased by supraphysioligic estradiol concentration (100 nmol/l).[28] These results suggested that ovarian hyperstimulation for the production of adequate estradiol concentrations has to be controlled strictly in order to induce a maximal uterine blood flow for a favorable uterine receptivity in IVF cycle.

Uterine PI and RI at embryo transfer on day 3 were not good predictors of pregnancy outcome of IVF treatment. They had an inverse relationship with serum estradiol levels on the day of hCG administration, meaning a significant correlation between uterine blood flow and serum estradiol levels during COH in human IVF.

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= **Abstract** =

Objective: This study was aimed to investigate whether uterine blood flow on the day of embryo transfer can be a predictor of pregnancy outcome in human IVF.

Methods: Fifty-one patients undergoing IVF program were included in this study. Serum estradiol levels were measured on the day of hCG administration and uterine pulsatility index (PI) and resistance index (RI) was examined for at embryo transfer of day 3.

Results: Of 51 cycles, 22 cycles were clinically pregnant (43.1%) and the implantation rate was 14.7%. Uterine PI and RI had a significant inverse correlation with serum estradiol levels ($p<0.05$). These uterine blood flows were not significantly different between pregnant and nonpregnant groups. The pregnancy rate was slightly higher in patients with PI more than 3.0 compared to those with PI of 3.0 or less, but there was no significant difference.

Conclusion: These results suggest that uterine PI and RI at the day of embryo transfer could not be a good predictor of pregnancy in IVF treatment. But they had an inverse correlation with serum estradiol levels on the day of hCG administration.

Key Words: Uterine pulsatility index, Resistance index, Pregnancy outcome, Serum estradiol levels