Journal of Assisted Reproduction and Genetics, Vol. 22, No. 1, January 2005 (© 2005)
DOI: 10.1007/s10815-005-0814-z

Assisted Reproduction

A Reduction in Intraovarian Arterial Blood Flow Resistance After Ovulation is Necessary to Achieve Pregnancy in Natural Cycle

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Submitted March 29, 2004; accepted September 24, 2004

***Purpose*: ** Color Doppler imaging permits the accurate localization of vessels and high-frequency pulsed Doppler ultrasonography has improved the resolution of flow velocity waveforms. In this study, intraovarian arterial blood flow before and after follicle rupture in the natural cycle was examined using transvaginal color flow Doppler imaging and changes in intraovarian arterial resistance in relation to the outcome of infertility treatment was analyzed.
***Methods*: ** In a prospective study, 227 spontaneous cycles in 118 infertile patients who were undergoing infertility treatment at the division of Reproductive Medicine in our center were recruited in this study. The impedance to flow in intraovarian vessels was measured by means of transvaginal color flow Doppler imaging during the periovulatory period in the natural cycle of all patients. The pulsatility index (PI) of intraovarian arterial blood flow and pregnancy rate was evaluated.
***Results*: ** On the basis of PI values before and after follicular rupture, 227 cycles were classified into severely decreased (113 cycles) and not-severely decreased groups (114 cycles). The pregnancy rate per cycle in the severely decreased group was 18.6% (21/113), significantly higher than that in the not-severely decreased group (7/114; 6.1%, $p = 0.004$). The miscarriage rate was similar in the two groups.
***Conclusions*: ** A reduction in intraovarian blood vessel resistance is necessary to achieve pregnancy in a natural cycle.

KEY WORDS: Intraovarian arterial blood flow; ovulation; pregnancy; pulsatility index.

INTRODUCTION

Over the last decade, pulsed Doppler ultrasonography has become an established technique in clinical investigations of uteroplacental and fetoplacental circulation. The advent of transvaginal probes has allowed the use of high frequency ultrasonography to produce clear images with lower artifacts thus permitting a better understanding of the female reproductive organs. Transvaginal ultrasonography is currently considered to be an indispensable instrument

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in infertility treatment, worldwide. The addition of color Doppler imaging permits the accurate localization of vessels, and high-frequency pulsed Doppler ultrasonography has improved the resolution of flow velocity waveforms. A number of studies have reported on the use of color Doppler ultrasonography to assess differences in uterine blood flow impedance between fertile and infertile women (1,2) and to study uterine receptivity in women undergoing in vitro fertilization (3). Doppler ultrasonography has also been used to assess changes in blood flow to the uterus and ovaries during menstrual cycles (4–7).

Several investigators have reported that intraovarian blood flow changes dramatically during ovulation (1,7,8). It has been reported that intraovarian blood velocity increases approximately 29 h before the time of follicular rupture and that the mean values for intraovarian PI tend to be lower during the immediate periovulatory period (1,8). However, information concerning changes in intraovarian blood flow in relation to the outcome of infertility treatment is not available.

In all types of infertility treatment including assisted reproductive technology (ART), the accurate evaluation of oocyte or embryo quality is a very important issue, in terms of achieving a good pregnancy outcome and makes it possible to transfer a fewer number of good quality embryos, thus preventing multiple pregnancies. We recently reported that it was possible to directly evaluate oocytes or embryos by measuring or monitoring intraovarian changes; the incidence of apoptotic granulosa cells (9), oxidative stress in granulosa cells (10), alternations in the cell cycle of granulosa cells (11), and concentrations of hyaluronan in follicular fluid (12). These intraovarian changes in the periovulatory phase might affect intraovarian blood flow and arterial resistance.

The present study reports on an examination of intraovarian arterial blood flow before and after follicle rupture in the natural cycle using transvagional color flow Doppler imaging and an assessment of changes in intraovarian arterial resistance in relation to the outcome of infertility treatment.

MATERIALS AND METHODS

Patients and Ovarian Stimulation

A total of 227 cycles in 118 infertile patients undergoing infertility treatment at the Division of Reproductive Medicine, Department of Perinatal medicine and Maternal Care, National Center for Child Health and Development from July 2002 to July 2003 were recruited in this study. All patients had a normal pelvis, as assessed by transvaginal sonography and patent fallopian tubes, as determined by hysterosalpingography. Cases of male factor infertility and severe endometriosis were excluded. Informed consent was obtained from all patients recruited in this study. This study was approved by the Institutional Review Board of the National Center for Child Health and Development.

Ultrasound and Doppler Examination

All patients were examined by means of transvaginal color flow Doppler imaging before and after follicular rupture within 3 days including the LH positive day. At least one scan was performed within 24 h after the leading follicle had ruptured. The presence of a corpus luteum, thickened endometrium, and ovulation ring were used as ultrasonographic criteria for the presumed ovulation, and the basal body temperature was also measured to confirm ovulation. Testing of daily urine samples with an ovulation predictor kit (Dotest LH, Rohto Pharmaceutical Co., Ltd., Osaka) was initiated on cycle day 11. When the LH surge was detected by urine testing, natural intercourse was instructed on the same day or insemination treatment was scheduled for the following day.

The morphology of the uterus and adnexa was explored by B-mode sonography. Color Doppler was used to visualize intraovarian blood flow, and pulsed Doppler signals were obtained using the 2 mm volume cursor. All examinations were performed with the patients in a lithotomy position, using a Mochida Sonovista-Color II. (Mochida, Tokyo, Japan) 6.5-MHz transvagainal probe for imaging and a 6.5-MHz pulsed Doppler system for blood flow analysis. All scans were performed by one operator (K.N.). The same follicle on daily transvaginal ultrasound monitoring is easily detected from the size and growth of the leading follicle before ovulation, and therefore the examiner is able to detect the same perifollicular artery of a leading follicle on every measurement. After ovulation, the same vessel of the corpus luteum is also easily detected from the size and side of the follicle before ovulation. Moreover, single follicular growth was confirmed in all cases in this study. Therefore the examiner was able to constantly detect the same vessel of the perifollicular artery in every measurement.

Flow velocity waveforms from a perifollicular artery of the leading follicle were recorded. Blood flow impedance was expressed as the pulsatility index (PI), calculated electronically from smooth curves fitted to the waveforms over 3 cardiac cycles according to the formula:

$$\mathrm{PI} = (S - D)/\mathrm{mean}$$

where S is the peak Doppler shifted frequency, D is the minimum Doppler shifted frequency, and mean is the mean maximum Doppler shifted frequency over the cardiac cycle. When the angle between the ultrasound beam and the longitudinal axis of the vessel becomes optimal for a Doppler measurement, the PI is a convenient parameter, in spite of the scanning angle because the angle parameter is not included in the formula for PI. Perifollicular arterial blood flow reflects the cardiac cycle flow. One perifollicular arterial flow corresponds to one cardiac cycle flow and the peripheral arterial blood waveforms become more smoother than the central arteries. Wall filters (100 Hz) were used to eliminate low frequency signals arising from noise. The intraassay coefficient variability was 3.9–4.7%.

Hormone Assay

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL) were assayed using a commercially available ELIZA kit (IMMULIZE 2000; Diagnostic Products Corporation, Los Angeles, CA) from the 2nd to 4th day of the menstruation cycle. The blood samples for FSH, LH, and PRL were taken on the second to the fourth day of the menstrual cycle when the intraovarian arterial PI values were evaluated. Serum estradiol (E2) and progesterone (P) were assayed in the midluteal phase using a commercially available RIA kit (IMMULIZE 2000; Diagnostic Products Corporation, Los Angeles, CA) and a commercially available RIA kit (DPC Progesterone; Diagnostic Products Corporation, Los Angeles, CA), respectively.

At 14–20 days after confirmed follicle rupture, a cycle showing a urinary positive hCG was defined as pregnant.

Statistics

All data are presented as the mean ± SEM (standard error of the mean). The significance of the differences was determined by unpaired t-test, the Mann–Whitney U test, and chi-square test.

RESULTS

During this study period, 28 pregnancies were obtained, and the overall pregnancy rate per cycle was 12.3%.

The increasing degree of intraovarian arterial PI values after follicular rupture compared with that before follicular rupture is defined as the PI increasing rate, and this rate was calculated as follows; the intraovarian arterial PI value after follicular rupture was divided by that before follicular rupture. According to the PI increasing rate, the 227 cycles could be classified into 8 groups and the pregnancy rates per cycle in each group are shown in Figure 1. The pregnancy rate in the group (group A) whose PI increasing rate was 30% and more was 4.5%. Pregnancy rates in group B (20% $\leq$ PI increasing rate <30%), group C (10% $\leq$ PI increasing rate <20%), group D (0% $\leq$ PI increasing rate <10%), group E (−10% $\leq$ PI increasing rate <0%), group F (−20% $\leq$ PI increasing rate <−10%), group G (−30% $\leq$ PI increasing rate <−20%), and group H (PI increasing rate

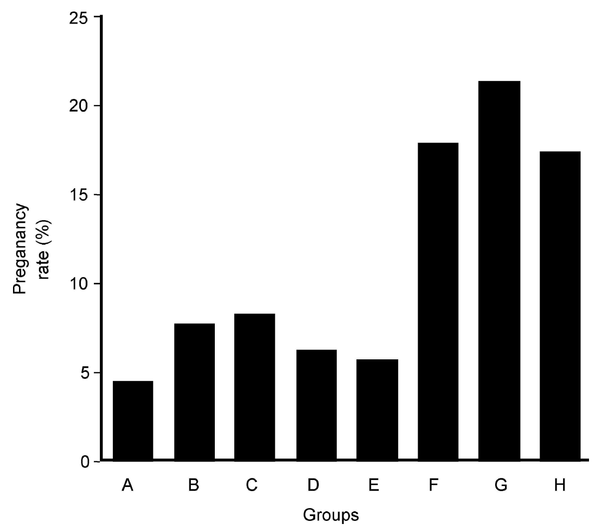

Fig. 1. The pregnancy rate in each group that was classified by a PI increasing rate. The pregnancy rate in the group (group A) in which the PI increasing rate was 30% and more was 4.5%. Pregnancy rates in group B (20% $\leq$ PI increasing rate <30%), group C (10% $\leq$ PI increasing rate <20%), group D (0% $\leq$ PI increasing rate <10%), group E (−10% $\leq$ PI increasing rate < 0%), group F (−20% $\leq$ PI increasing rate <−10%), group G (−30% $\leq$ PI increasing rate <−20%), and group H (PI increasing rate <−30%) were 7.7, 8.3, 6.3, 5.7, 17.9, 21.4, and 17.4%, respectively. The pregnancy rate in groups in which the PI values decreased after follicular rapture by more than 10% tends to be higher than those in the other groups.

Table I. Profiles and Serum FSH, LH and PRL Levels on day 3 of each Group

	Severely decreased group	Not-severely decreased group	p-Value
Number of cycles	113	114	—
Mean age (years)	34.1 ± 0.4	33.2 ± 0.4	0.110
Duration of infertility (years)	2.5 ± 0.2	3.2 ± 0.4	0.08
FSH (mIU/mL)	7.3 ± 0.4	8.6 ± 1.1	0.264
LH (mIU/mL)	4.7 ± 0.3	4.5 ± 0.4	0.713
PRL (ng/mL)	10.8 ± 0.6	10.9 ± 0.9	0.926

Note. Values are mean ± SEM.

$<-30\%$) were 7.7, 8.3, 6.3, 5.7, 17.9, 21.4, and 17.4%, respectively. The pregnancy rate in groups whose PI values decreased after follicular rupture by more than 10% tended to be higher than those in the other groups.

From these data, a total of 227 cycles were classified into two groups as a severely decreased or a not-severely decreased group. One hundred and thirteen cycles, in which the postovulatory PI values showed more than a 10% decrease were classified as severely decreased, and 114 cycles in which the postovulatory PI values were 10% and less decrease or increase were classified as not-severely decreased. Several clinical and hormonal profiles were compared between the two groups. The mean age and duration of infertility in the severely decreased group were similar to those in the not-severely decreased group. Serum FSH, LH, and PRL levels were also similar in the two groups (Table I).

The endometrial thickness after follicular rupture, serum E2, and p-levels in the midluteal phase in the severely decreased group were similar to those in the not-severely decreased group (Table II). The pregnancy rate per cycle in the severely decreased group was 18.6% (21/113), was significantly higher than for the not-severely decreased group (7/114; 6.1%, $p = 0.004$). The miscarriage rate was similar for the two groups (Table II). All pregnant patients had a single GS. Therefore, without intraovarian arterial PI values before and after ovulation there was no parameter in relating to the pregnancy rate.

Between the pregnant (28 cycles) and nonpregnant groups (199 cycles), the intraovarian arterial PI values before follicular rupture (mean ± SEM) were 1.00 ± 0.07 and 0.91 ± 0.02, respectively, which did not represent a significant difference. However, after follicular rupture the intraovarian arterial PI value in the pregnant groups (0.72 ± 0.02) was significantly lower than in the nonpregnant group (0.82 ± 0.02 $p = 0.045$) (Fig. 2).

DISCUSSION

It has been reported that intraovarian blood flow changes dramatically during ovulation (1,7,8). Kurjack and coworkers reported that the resistance index in the postovulatory phase showed a significant decrease from the preovulatory phase (1). Campbell *et al.*, also reported that intrafollicular PI values decreased after follicular rupture but the differences were not significant (8). Thus, previous reports have indicated that intraovarian blood flow resistance is decreased after ovulation. However, the findings in this study indicate that there were several cycles that showed increased intraovarian PI values after follicular rupture, thus permitting us to divide the infertility treatment cycles into two groups according to intraovarian arterial PI values before and after follicular rupture.

In 114 of 227 treatment cycles (50.2%) intraovarian PI values increased or did not decrease severely

Table II. Outcomes and Hormonal Proliles for each Group

	Severely decreased group	Not-severely decreased group	p-Value
Endometrial thickness after ovulation (mm)	11.4 ± 0.3	12.2 ± 0.5	0.534
Estradiol on midluteal phase (pg/mL)	151.5 ± 11.3	141.5 ± 9.3	0.505
Progesterone on midluteal phase (ng/mL)	13.0 ± 0.5	12.6 ± 0.5	0.609
Pregnancy rate (/cycle; %)	18.6	6.1	0.004
Abortion rate (/pregnancy; %)	19.0	14.2	0.601

Note. Values are mean ± SEM.

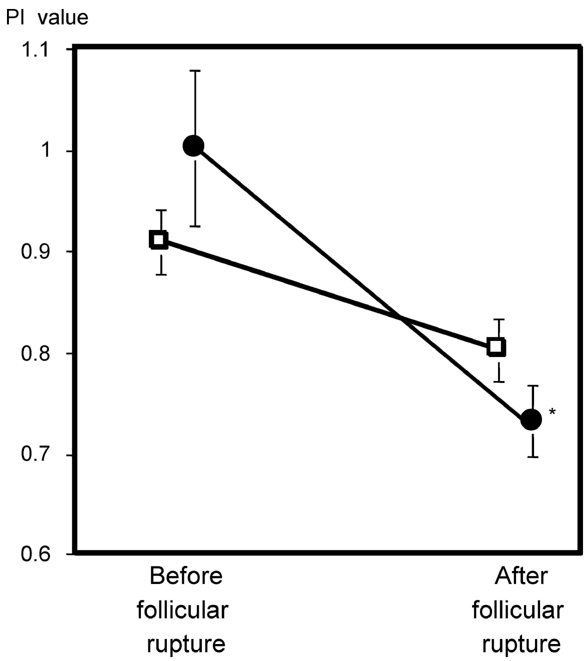

Fig. 2. Changes in intraovarian arterial pulsatility index (PI) values before and after follicle rupture. The closed circles and open squares indicate pregnant and nonpregnant groups, respectively. Values are the mean ± SME PI values after follicle rupture in the pregnant group are significant higher than those in the nonpregnant group ($p = 0.045$, Mann–Whitney's U test).

after follicular rupture and the PI value was not severely deceased in only about half of the cycles. We hypothesize that a process of neoangiogenesis or activity of vasoactive compounds around the follicle occurred successfully or adequately in the severely decreased group but not successfully or inadequately in the not-severely decreased group. If this hypothesis is correct, in the severely decreased group, a good environment for developing good quality oocytes might be in the ovary. Therefore, we compared the pregnancy outcome in the two groups.

Although the background and hormonal profiles were similar in the two groups, and the pregnancy rate in the severely decreased group (18.6%) was significantly higher than in the not-severely decreased group (6.1%). The PI values of both the intrauterine artery which denotes the artery detected by vaginal ultrasonograpy immediately outside of the endometrium and the uterine artery were also measured before and after follicular rupture, these were not useful in indicating the significant difference in relation to pregnancy rate. Moreover, there was no significant difference in PI values for the intrauterine artery and both uterine arteries between in intraovarian arterial PI for the severely decreased and not-severely decreased groups (data not shown). A decrease in intraovarian blood vessel resistance is necessary to achieve pregnancy. From these results, we hypothesize that good quality oocytes appear to be produced and are ovulated in the decreased group.

Recently, Yokota and coworkers reported that the PI values of uterine arteries on the day of ovulation in conception cycles were significantly lower than those in nonconception cycles (13). They indicated that, in conception cycles, the PI values, not only of the uterine arteries but also the ovarian arteries of active ovaries decreased before and after ovulation, but no significant difference was detected between conception and nonconception cycles relative to the arterial PI values of active ovaries. In this study, we found that the intraovarian arterial PI values after ovulation in the pregnant group were significantly lower than those in the nonpregnant group.

Yokota *et al*. proposed that a decrease in the impedance of uterine arteries occurred during the conception cycles providing evidence for an increase in blood supply during the day of ovulation. It is possible that the same phenomenon might occur around the dominant follicle during the periovulatory period. That is, blood flow in the ovulatory phase is a fundamental prerequisite for the structural changes and increased vascularization around the dominant follicle. Very recently, we showed that in ART cycles, no oocyte could be obtained from patients who showed no reduction in intraovarian arterial PI values before and after hCG administration (submitted). From this point of view, the reduction in intraovarian arterial PI values is a necessary condition for the production of a good quality oocyte.

Our previous studies involved investigating the quality of oocytes exclusively through the study of intraovarian changes in the ART cycles. The incidence of apoptotic granulosa cells in a cumulus mass containing a subsequently fertilized oocyte was significantly lower than that in a cumulus mass with an unfertilized oocyte (9). Oxidative stress in granulose cells lowered fertilization rates and subsequently led to a decrease in the quality of the embryo (10). The concentration of hyaluronan in follicular fluid is an indicator of oocyte viability (12). That is, the monitoring of these parameters enabled us to evaluate the quality of oocytes or embryos directly. However, useful information concerning these parameters cannot be obtained without invasive treatment, such as oocyte pickup. In this study, although we did not demonstrate directly a reduction in perifollicular arterial PI values during ovulation in relation to oocyte quality, this parameter seemed to reflect the oocyte quality and might be useful information concerning oocyte quality obtained by means of pulsed Doppler ultrasonography, i.e., using noninvasive procedure.

In conclusion, a reduction of intraovarian arterial blood flow resistance appears to be necessary for achieving pregnancy in natural cycles. This represents a new parameter for the noninvasive evaluation in predicting a successful outcome.

ACKNOWLEDGMENTS

This study was supported, in part, by a committed grant to National Center for Child Health and Development (14 KOU-4) from the Ministry of Health, Labor, and Welfare, Japan.

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