

## Study on relationship between perfollicular blood flow and *in vitro* fertilization-embryo transfer

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### Abstract

**Objective:** To study the relationship between perfollicular blood flow and follicle development, oocyte maturing rate, fertilizing rate, cleaving rate, embryo quality and the outcomes of embryo transfer. **Methods:** The samples were selected from 66 suffers who underwent in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Each patients' perfollicular blood flow (diameter  $\geq 12$  mm) was estimated on the day of human chorionic gonadotropin (HCG) administration. **Results:** Among 66 cycles, 26 (39.4%) cycles resulted in pregnancy. perfollicular blood flow resistance index (RI), peak systolic velocity/end diastasis velocity (S/D) of non-pregnant group was significantly higher than that of the pregnant group ( $P < 0.004$ ). When  $RI < 0.49$ , the pregnancy rates, fecundation rates, fertilization rates, metaphase numbers for the of second meiosis oocytes increased evidently ( $P < 0.05$ ), but there were no statistical difference in gonadotropin dosage, cycle frequency, infertility years, ages, estradiol (E2) on the day of HCG administration, numbers of oocyte retrieved and high-quality embryo rates ( $P > 0.05$ ). There were no statistical difference between non-pregnant group and pregnant group in S and D ( $P > 0.05$ ). There was no correlation between perfollicular blood flow RI and follicular diameter by linear regression analysis. **Conclusion:** Our study shows that perfollicular blood flow RI and S/D are effective indices of predicting the pregnancy outcome of IVF-ET.

**Key words:** perfollicular blood flow; resistance index (RI); pregnancy outcome.

### INTRODUCTION

In recent years, different reports have shown the results between perfollicular blood flow and IVF-ET pregnant rate. There are so many factors to influence IVF-ET; however oocytes quality is the core reason to verify if in vitro fertilization embryo transfer (IVF-ET) embedded successfully. The research is based on transvaginal color doppler sonography, this (TV-CDS) is used to inspect perfollicular artery blood flow parameters which could be the reference to forecast the pregnant of the results IVF-ET.

### MATERIAL AND METHODS

#### Subjects

The samples were selected from 66 patients (58 cycles

of female fallopian tube factor; 8 cycles for male factor) aged 26-41 years old, ( $32 \pm 4$ ) years old in average, infertility year from 2 years to 18 years, ( $7 \pm 3$ ) in average who underwent IVF or ICSI in our Reproduction Medical Center from June 2005 to December 2005.

#### Directions for the cure cycle

Use GnRHa decrease modulation and GnRHa Decapeptil (German Linhui) 0.05-0.10 mg/d during 2th day of the ovulation promotion cycle. In the 3th day cycle use Gonal-F (Switzerland Serono) 75-300 IU/d to promote ovulate, then use the HMG (Switzerland Serono). Under the inspection by vagina B ultrasound, inject HCG 10 000 IU, take the ovum after 36 h, *in vitro* fertilized and embryo transfer after taking the ovum 6-8 h, in 16-18 h wipe of the grain cell to observe fecundation situation; transfer 2-3 quality embryos to the cavity of uterus after 48-72 h. When B ultrasound shows there

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are more than 1 perifollicular diameter exceeded (equal 18 mm or more than 2 of it exceed and equal 17mm the bi-side ovary). Start to use progesterone to support corpus luteum after the surgery. After fertilized and embryo transfer 12th day *in vitro*, check blood HCG and confirm pregnant situation by ultrasound at around 6 weeks. Physiologically and chemically pregnant: Blood HCG > 10 m IU/ml; Clinically pregnant concept: ultrasound cavity of uterus can see the pregnant bursa.

**Directons for Sample collection**

Survey E2 on the day of HCG administration: all suffers need to collect empty stomach vein blood 5 ml in limosis during the day of HCG administration duration in 8-10AM.

**Reagent and inspection measure**

Inspection for E2 uses the ACS180 SEI immunity radiation analyzation instrument(provided by Bayer America). Per follicular blood parameter inspection adopts Acuson Sequoia 512 type transvaginal color Doppler sonography,TV-CDS, explore caput rate 5.0-7.0 MHz, expand angle 120°. The patients were observed to determine if the diameter exceeds or equals 12 mm perifollicular through the vagina and therefore obtain perifollicular artery blood parameter, peak systolic velocity, peak systolic velocity(S) and end diastasis velocity, end diastasis velocity(D),resistive index, RI, S/D value(Fig 1). Average for the three measure results are shown as the final value.

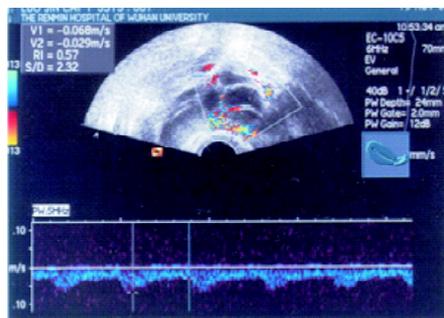


Fig 1 Per follicular blood flow ultrasound chart and flood flow diameter on HCG injection day

**Statistic analysis**

To adopt SPSS13.0 statistic software process statistic transact and select  $\alpha = 0.05$  as the statistic docimastic

significance level.

**RESULTS**

In 66 cycles, 50 cases were the first cycle and 16 cases from the second. IVF cycle numbers were 40 cases and ICSI cycle numbers were 26. During the cycle, average adoption ovum number was  $9 \pm 4$ , with the average fecundation rate being 68.03%, and embryo transfer number( $2 \pm 1$ ). There were also 26 cycle clinically pregnant (accounting for 39.4%) and 6 biochemistry pregnant cases.

**Relationship between Perifollicular blood Flow and pregnancy eventual results**

Were separated the patients into two groups: pregnant and non-pregnant, then measured each item parameter around the perfollicular blood flow and took the average value as comparison. The results show that RI and S/D of pregnant group is lower than that of the non-pregnant group but has no statistically significant difference between S and D based on their ages(Tab 1).

**Relationship between RI and IVF-ET in different perifollicular artery**

According to the results, 95% of the pregnant group were perifollicular RI (believable scope-0.38, 0.49). We split the patients into 3 groups by different perifollicular RI values (on HCG administrated day) and analyzed the pregnancy results.  $RI < 0.38$ , the 4 cases are all pregnant; when  $0.38 \leq RI < 0.49$  the pregnancy rate was 50%; when  $RI \geq 0.49$  it decreased to 23%, with statistic difference meaning( $P = 0.004$ ). Fecundation rate,cleaving rate,numbers of metaphase of second meiosis decreased significantly( $P < 0.05$ ) in  $RI = 0.49$  group. However, Gn dosage, non-pregnant years, age, E2 consistence in HCG administrate, numbers of gained ovum, high quality embryo rate had no statistical difference found between the three groups(Tab 2).

**Relationship between perifollicular artery RI and the diameter of the perifollicular**

There are no statistic difference between each perifollicular RI and each perifollicular diameter when process Pearson related analysis. ( $r = 0.025, P = 0.840$ )

Tab 1 Relationship between follicle blood flow and pregnancy outcome ( $\bar{x} \pm s$ )

Group	No. of cycles	Age(years)	Blood flow parameter on the day of HCG administration(cm/s)			
			S	D	RI	S/D
Pregnant	26	$31 \pm 1$	$11.0 \pm 1.9$	$6.4 \pm 3.2$	$0.4 \pm 0.1$	$1.9 \pm 0.3$
Non-pregnant	40	$32 \pm 4$	$10.3 \pm 2.7$	$5.3 \pm 1.6$	$0.5 \pm 0.1$	$2.2 \pm 0.4$
F value	-	2.041	1.327	3.418	15.738	9.280
P value	-	0.125	0.254	0.069	0.000	0.003

S:Maximum systolic Doppler shift, D: End-diastolic Doppler shift, RI: Resistent index.

Tab 2 Relationship between different Perifollicular blood flow RI and IVF-ET ( $\bar{x} \pm s$ )

Group of RI	<0.37	0.38-0.48	0.49~	$\chi^2$ value	Pvalue
No	4	28	34	-	-
No.of ampoules Gn used	24 $\pm$ 9	24 $\pm$ 8	20 $\pm$ 7	1.898	0.158
Infertility duration	7.0 $\pm$ 2.3	6.2 $\pm$ 2.1	7.1 $\pm$ 4.1	0.591	0.557
Age(years)	34 $\pm$ 1	31 $\pm$ 3	32 $\pm$ 4	1.121	0.332
E2 on the day of HCG administration(pg/ml)	1 465 $\pm$ 1 212	1 667 $\pm$ 1 033	2 224 $\pm$ 1 299	2.029	0.140
No. of oocytes retrieved	10 $\pm$ 5	10 $\pm$ 4	9 $\pm$ 5	0.245	0.783
No. of M II	3 $\pm$ 4	5 $\pm$ 5	1 $\pm$ 3	12.182*	0.002
No.of Fertilization(%)	92.9 $\pm$ 8.3	74.6 $\pm$ 18.3	59.7 $\pm$ 30.1	7.124*	0.028
No.of Merogenesis(%)	92.9 $\pm$ 8.3	71.9 $\pm$ 21.1	52.2 $\pm$ 32.7	10.155*	0.006
No.of high quality embryos(%)	54.2 $\pm$ 14.4	64.8 $\pm$ 25.3	48.5 $\pm$ 33.9	2.222	0.117
No.of clinical pregnancies(%)	100	50	23	11.057**	0.004

\*\*chi square test.

## DISCUSSION

Blood vessels' form and effective blood flow are highly important in female procreate physiology. For instance perifollicular growth, superiority perifollicular foundation, corpus luteum form, endometrial differentiation, embryo establish, etal. all need the formation of new blood vessels. Thus, effective blood flow is the basic condition to forming the mature perifollicular region. At present, under the support of the procreate technical assistance, the main way to judge perifollicular's maturity is measuring perifollicular's size, morphologic exchange and blood plasma estrogen level. However the definition of perifollicular maturity is not credible by just considering the configuration, as it is hard to determine estrogen in most Reproduction Medical Center.

Following the development of TV-CDS, it is possible to measure blood exchange of the ovary and uterine through the vagina. Transvaginal color Doppler sonography, TV-CDS have many advantages such as being convenient, hard to break, economical and are high replicable, so we are able to continue observing it and the perifolliculars's process of development and blood perfusion. This technology starts to be applied in IVF to assess the development and quality of the perifollicular in recent years. It is reported that perifollicular blood flow parameter could evaluate the development and mature degree of it and could be considered as the potential factor to influence success rate of transfer<sup>[1]</sup>.

Coulam et al<sup>[2]</sup>. found that the more perifollicular blood flow information is the maturer it is. The strength of information around the follicular, illustrate that more blood vessels were formed and were content rich in liquid oxygen, low chromosome abnormality rate and low triploid embryo rate. The higher the drag index of the blood flow around the perifollicular is, the lower the press release in it, which could influence cleaving rate, implanting rate, and overall pregnancy rate. Du et al<sup>[8]</sup> considered

that the PI and RI of the intraovarian artery blood flow measured on the day of follicle aspiration may be good indicators of the follicle luteinization and oxidation as well as of oocyte quality. Costello et al<sup>[9]</sup> found that a significant negative correlation between age and ovarian PFBF in women undergoing IVF which was only observed very late in the follicular phase of ovarian stimulation. Nargund et al<sup>[3]</sup> considered that follicular whose PSV has obvious elevator before take the ovum could be fecundation easier. 75% of the follicular could gain the high quality embryo when PSV=10 m/s. Lozano et al<sup>[10]</sup> considered that A qualitative flow index rather than quantitative vascularization index relationship exists between vascular status and functional quality of the follicle after HCG administration. Oyesanya et al<sup>[7]</sup> offered the concept of the follicular flowin-dex, FVI that is the ratio between the follicular with blood flow information and the sum of the follicular has obviously higher FVI who gain the ovum in the HCG administration day. Gorokhovskiy et al<sup>[11]</sup>, Kim et al<sup>[4]</sup> compared follicular blood flow with pregnancy eventual results, follicular blood flow increase and PI exponent decreases noticeably in pregnant group. It considered that follicular is the effective guide line to predict pregnancy eventually results. Shrestha et al<sup>[12]</sup> found that high grade ovarian perifollicular blood flow in the early follicular phase during IVF is associated with both high grade perifollicular blood flow in the late follicular phase and a higher clinical pregnancy rate. Ng<sup>[13]</sup>, Ragni G<sup>[14]</sup>, Shrestha et al<sup>[5]</sup> also believed that follicular blood flow has a direct ratio with pregnancy results without a relationship to endometrial blood flow<sup>[6]</sup>.

This report finds that follicular artery RI decreases obviously in both pregnant and non-pregnant groups. For age; follicular artery S and D have no statistic difference. When RI<0.49, pregnancy rate, fertilization rate, merogenesis rate, numbers of metaphase of second meiosis enhance largely. Gn dosage, cycle sequence,

infertility years, age, E2 concentration in HCG administration day, oocytes retrieved rate, quality embryo had no statistical difference. The results support what Kim<sup>[4]</sup>, Shrestha<sup>[5]</sup> and Nargund<sup>[3]</sup> reported: That there are some physical relationships in the follicular blood flow, providing implant potential capability, oocytes retrieved, and quality embryos gained. So, the experiments verify that follicular blood flow(RI) is a strong guide line to predict pregnancy eventual results.

Nargund et al<sup>[3]</sup> found that whether follicular blood flow exists is decided by ovum gained rate other than the size of the follicular before injecting HCG. Follicular with the same size could have a quite different blood supply. Oyesanya et al<sup>[7]</sup> thought that follicular blood flow's foundation has a higher relationship with the oocytes retrieved rate. And Kim<sup>[4]</sup> considered the ratio between follicular blood flow RI and the size is negative. The author compared the relationship with each follicular diameter with each follicular artery RI and each suffer follicular average diameter with follicular artery RI in average, the results have no statistic difference which is consistent with the conclusion of Nargund<sup>[3]</sup> and Oyesanya<sup>[7]</sup> but different to Kim<sup>[4]</sup>. This text Author found that blood flow and RI will decrease influenced by the growth in good quality follicular but there's no influence in low quality follicular(so it will affect Mero-genesis rate, implantation rate and pregnancy rates). They also find there is no statistic difference respectively between pregnant groups and non-pregnant groups when they analyzed follicular artery RIs and diameters. Thus we should combine follicular diameter, E2 level in blood serum and blood flow RI around follicular to appraise the quality and mature level of follicular before HCG is administered.

The research illustrates that blood flow RI around follicular is an effective guideline to predict pregnancy eventually results. When  $RI < 0.49$ , pregnancy rate, Fertilization rate, merogenesis rate, numbers of M II enhance obviously.

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