FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol.13, No 2, 2006, pp. 104 - 108 UC 618.177-089.888.11

COLOR DOPPLER AND COLOR DOPPLER ENERGY IMAGING AND MEASUREMENTS OF OVARIAN STROMAL BLOOD FLOW IN CONTROLLED OVARIAN HYPERSTIMULATION FOR IN VITRO FERTILIZATION

Ranko Kutlešić[1], Aleksandar Ljubić[2], Mila Milosavljević[1], Milan Stefanović[1], Predrag Vukomanović[1]

[1]Clinic of Gynecology and Obstetrics Clinical Center Niš, Serbia
[2]Institute of Gynecology and Obstetrics, Clinical Center Serbia, Belgrade, Serbia
E-mail: kutlesicr@medianis.net

Summary. *The aim of this investigation is to determine the correlation of color Doppler (CD) and color Doppler Energy (CDE) imaging and measurements of ovarian stromal blood flow prior to controlled ovarian hyperstimulation (COH), predictors of success of COH and numbers of oocyte retrieved, especially in group of poor responders. Fifty-two patients on IVF treatment were included in this prospective clinical trial. The long protocol of controlled ovarian hyperstimulation was administered in all 52 patients. Prior to COH, at basal ultrasound examination, the ovarian stromal vascularisation was registered by color Doppler and color Doppler energy imaging with 6.5 MHz endovaginal ultrasound probe. In general population of infertile patients submitted to IVF treatment, CD and CDE imaging and measurements do not correlate with the result of controlled ovarian hyperstimulation in IVF cycles and the rate of pregnancy. "Poor responders" during COH with long protocol, show less expressed and poorer ovarian stromal blood flow on CD and CDE imaging, associated with higher resistance to blood flow in ovarian stromal vessels at the beginning of COH. This group of patients should be switched to some other stimulation protocol, which could spare them from unsuccessful IVF cycle and give them more chance for pregnancy.*

Key words: *In vitro fertilization, controlled ovarian hyperstimulation, Doppler*

Introduction

The function of any organ directly depends on vascularization. Therefore, it is logical that follicular blood supply could exert influence on the success of controlled ovarian hyperstimulation (COH) during in vitro fertilization treatment. The main predictive parameters for success of COH are age of patients, volume of ovaries, basal antral follicle count (AFC), and estradiol level on the day of HCG administration (1-6). There are controversial reports regarding the significance of color Doppler (CD) and color Doppler energy (CDE) imaging and measuring of ovarian stromal blood flow and perifollicular vascularization.

The aim of the study

The aim of this investigation is to determine the correlation of CD and CDE imaging and measuring of ovarian stromal blood flow prior to COH, predictors of success of COH and numbers of oocyte retrieved, especially in group of poor responders.

Method

Fifty-two patients on IVF treatment were included in this prospective clinical trial. This work has been done during author's specialization in fertility and sterility at the Institute of Gynecology and Obstetrics in Belgrade, from May 2002 to November 2003. Oral contraceptives were administered in all patients for two months before the controlled ovarian hyperstimulation. The long protocol was administered to all 52 patients. Pituitary suppression with GnRH agonist was started in the midluteal phase of subsequent cycle, and confirmed by basal estradiol levels, done on days 2 - 4 of the treatment cycle. At the same day the basal ultrasound examination was also performed. The dimensions of uterus and ovaries were measured; antral follicle counted, the endometrial thickness and eventual abnormalities were also registered. The ovarian stromal vascularisation was registered by color Doppler and color Doppler energy imaging with 6.5 MHz endovaginal ultrasound probe. CD imaging was used for mapping the ovarian stromal blood vessels. The cursor was positioned on the most prominent ovarian stromal blood vessel, with the gate width as much as possible closed to the diameter of the vessel, after which the pulse wave was started. Measuring was performed when the minimum of three clear waveforms was obtained. Pulsatile index (PI), resistance

index (RI), S/D ratio and peak systolic velocities (v max) were measured. The same investigator (R.K.) performed all ultrasound exams.

Statistical significance was tested on commercial software by t-test, χ^2 test and Student's t-test with Cohren and Cox's corrective approximate method for small samples, when it was appropriate.

Results

The mean age of our patients was 35.14 ± 5.2 year, all of them had basal FSH levels less than 10 IU/l. The mean body mass index (BMI) was 23.1 ± 1.47. There were no significant cysts or uterine fibroids on basal (day 2 – 4) ultrasound examination. The mean antral follicle count (AFC) were as follows: 4.2 ± 1.8 (right ovary), 3.86 ± 1.93 (left ovary). The long protocol was applied in all patients. The mean gonadotrophin dosage was 39.86 ± 13.01 ampoules per cycle. The mean number of oocyte retrieved was 7.24 ± 5.98. The mean number of transferred embryos was 2.22 ± 1.89. The mean values of color Doppler indices are shown in table 1.

Table 1. The mean values of CD indices

	Right ovary	Left ovary
v_{max} (cm/sec)	7.59 ± 2.58	7.49 ± 3.24
RI	0.62 ± 0.10	0.54 ± 0.12
S/D	2.85 ± 0.90	3.30 ± 1.50

The age of the patient is the most predictive parameter in IVF cycles, according to previous studies. In our patients, there were no significant differences in CD indices of ovarian stromal blood flow according to the age of the patients (table 2).

Table 2. CD indices of ovarian stromal blood flow according to age of the patient

Age	RI left ovary	RI right ovary	v_{max} left ovary	v_{max} right ovary
< 35 years n =20	0.60±0.11	0.64±0.10	6.86±2.32	7.73±2.73
≥ 35 years n=32	0.66±0.12	0.60±0.11	7.81±3.64	7.52±2.56
t-test	p=0.1899	p=0.3999	p=0.4871	p=0.8343

However, in the group of the patients younger than 35 years, stromal blood flow was easily detected on CD and CDE imaging and much more expressive according to the examiner's subjective estimate than in the group of older patients. In the group of patients younger than 35 years there were 7 pregnancies (7/20 – 35%), in the other group two patients got pregnant (2/32 – 6.25%), (p = 0.048).

The number of retrieved oocytes correlated with the antral follicle count (AFC) (r = 0.56 for right ovary and r = 0.67 for left ovary). The number of oocytes retrieved has shown only a tendency of negative correlation with the age of the patients, but statistically insignificant. There were no statistical significant correlations with CD indices of ovarian stromal blood flow (table 3).

Table 3. Correlation between the number of oocyte retrieved and predictors of success of COH and CD measurements of ovarian arterial stromal blood flow

Age of patients	r = –0.27	v_{max} right ovary	r = 0.13
BMI	r = 0.37	v max left ovary	r = –0.01
AFC right ovary	r = 0.56*	RI right ovary	r = –0.07
AFC left ovary	r = 0.67*	RI left ovary	r = –0.17

* Statistically significant correlation

The subgroup of "poor responders" was analyzed separately. In this subgroup, we included patients with less than three oocytes retrieved. The differences between "poor responders" and "normoresponders" are shown in the table 4.

There were no statistically significant differences in age and BMI between "poor responders" and "normoresponders", but "poor responders" had significantly less antral follicle count on basal ultrasound examination than group of "normoresponders". The group of "poor responders" had the less preferable CD indices of ovarian stromal blood flow: patients with less than three oocytes retrieved, had greater resistance to blood flow in ovarian stroma.

Discussion

It is well known that the age of the patient, basal FSH concentration, AFC and BMI are predictors of the number of oocyte retrieved. AFC is the only predictive factor for serum estradiol concentration on the day of HCG administration, and BMI is predictor of gonadotrophine

Table 4. The differences between "poor responders" and "normoresponders"

	age	BMI	AFC	v max (cm/sec)	RI
< 3 oocytes retrieved n = 7	36.4±3.7	23±1.38	2.5±0.7	5.0±0.75	0.73±0.02
≥ 3 oocytes retrieved n = 45	35.2±5.3	23.7±1.59	5.31±1.35	7.6±3.69	0.61±0.1
t-test	P=0.5550	P=0.9455	P=0.0394*	P=0.0004*	P=0.0074*

* Statistically significant difference

dose (1). The conclusions of some other studies are similar (3,4,5,7,8). The reports regarding the predictive value of CD and CDE imaging and measurements of ovarian stromal and perifollicular blood flow are conflicting. In the early studies, the difference between blood flows through ovarian artery was detected, with the less resistance on the side of dominant follicle (6). With technical development, the measurements of ovarian artery are abandoned, and the investigations are focused on ovarian stromal and perifollicular blood flow. According to the previous report (9,10,11,12), there is clear difference in ovarian stromal and perifollicular blood flow between patients who conceived on IVF, and patients who did not, with less resistance and more expressive blood flow detected by CD and CDE in patient who considered on IVF. The conclusions of many other studies are similar (5,9,12,13,14). It was also reported that pregnancy rate during cycles with in vitro maturation of immature oocytes was correlated not only with the well known predictive parameters (AFC, the number of immature oocyte retrieved, absence of dominant follicle at the time of immature oocyte retrieved and endometrial thickness at embryo transfer), but also with the peak ovarian stromal blood flow velocity at the basal ultrasound scan as well (14,15). On the other side, there are reports that CD measurements did not correlate with the success of IVF treatment, especially not with the rate of pregnancy (1). There were no differences in PI of ovarian perifollicular and stromal blood flow between women with normal and polycystic ovaries during COH in IVF cycles (16). However, PCO patients had lower PI of ovarian stromal blood flow on the ultrasound (US) examination before FSH stimulation than the patients with US normal ovaries (8,17,18,19).

We also did not find significant correlations between the success of COH and CD and CDE imaging and measurements, if the group of our IVF patients is analyzed in general. Pregnancy is the result of the harmony of many known and unknown factors, so we consider that this is the reason why CD imaging and measurements of ovarian stromal blood flow do not correlate completely with the pregnancy rate. Therefore, we pay the special attention to the number of oocyte retrieved, although it is clear that there is the influence of many other factors (e.g. age of the patient and laboratory conditions). The whole material is processed in the same laboratory, by the same embryologist, so we consider that the influence of the laboratory conditions was the same in all cases. "Poor responders" (with the less than 3 oocytes retrieved) had less antral follicles on basal US examination, less expressed ovarian stromal vascularisation on CD and CDE imaging, and greater resistance to the blood flow in ovarian stroma. In the previously mentioned report (1) that denied correlation between CD indices and success of COH, the group of poor responders was not analyzed separately. In the group of our patients, there were no significant differences in age and BMI between "poor responders", and "normoresponders", but there were differences in CD and CDE imaging and measurements of ovarian stromal blood flow on basal ultrasound examination. The conclusions of some other investigators are similar to ours: CD indices of ovarian stromal blood flow in "poor responders" are significantly less preferable than in "normoresponders". Moreover, preferable ovarian stromal vascularisation is associated with better endometrial perfusion on day of embryo transfer and higher pregnancy rate (3), which could be the consequence of favorable endometrial surrounding for successful implantation. This might be the fact of practical significance: in patients with the long protocol, that have higher impendence to ovarian stromal blood flow on basal US examination, COH could be delayed, and in some other cycle, another protocol could be started. Moreover, it is possible to switch on other protocol even in the same cycle. The first possibility is "the early GnRH cessation protocol" involving classical pituitary desensitization with luteal phase start of GnRH agonist for 10 – 14 days, and the cessation of GnRH agonist with the onset of the treatment with FSH. The second possibility is "micro dose flare protocol", with oral contraceptive priming followed by diluted doses of GnRH agonist. Two days later, stimulation is started with high doses of gonadotrophin treatment, and micro doses of GnRH and high doses of gonadotrophin are continued until the day of HCG administration.

There are only a few reports regarding the effects of pituitary suppression with GnRH agonists on antral follicle count and CD flow indices. Conclusions are that GnRH agonists did not significantly change these parameters (8,20). Further investigations are needed for definitive conclusions.

The age of the patients is one of the most important predictors of the success of IVF treatment. Older patients with infertility had reduced ovarian stromal blood flow (21,22). In women with proven fertility, ovarian blood flow is significantly reduced after 41 year (23).

On the other side, "hyper responders" during COH have significantly higher indices of ovarian vascularisation than "normoresponders" (5), which points to the importance of ovarian stromal vascularisation for success of COH. Increased ovarian stromal blood flow in patients with PCO (that respond well to the controlled ovarian hyperstimulation), could explain the increased response to COH (7,17,18,19).

Conclusions

In general population of infertile patients submitted to IVF treatment, CD and CDE imaging and measurements do not correlate with the result of COH in IVF cycles and the rate of pregnancy. "Poor responders" during COH with long protocol, show less expressed and poorer ovarian stromal blood supply on CD and CDE imaging, associated with higher resistance to blood flow in ovarian stromal vessels at the beginning of COH. This group of patients should be switched to some other stimulation protocol, which could spare them from unsuccessful IVF cycle and give them more chance for pregnancy.

References

1. Hung Ya N E, Shun Tang O, Wai Chan C, Chung H. Ovarian stromal blood flow in prediction of ovarian response during in vitro fertilization treatment. Hum Reprod 2005; 24.
2. Karande YC. Management and predicting of low response to standard IVF therapy: a review of the options. Semm Endocrinol 2003; 2(4), 67-72.
3. Kupesic S. Three-dimensional ultrasonographic uterine vascularisation and embryo implantation. J Gynecol Obstet Biol Reprod (Paris) 2004; 33(1 Pt 2): 518-20.
4. Jarvela IY, Sladkevicius P, Kelly S, Oiha K, Campbell S, Narguad G. Effect of pituitary down regulation on the ovary before in vitro fertilization measured using three-dimensional power Doppler ultrasound. Fertil Streil 2003; 79(5): 1124-35.
5. Pan HA, Wu MH, Chang YC, Chung FM. Quantification of ovarian Doppler signal in hyper responders during in vitro fertilization treatment using three-dimensional power Doppler ultrasonography ultrasonography. Ultrasound Med Biol 2003; 29(7): 921-7.
6. Kim SU, Ku SY, Suh CS. Clinical significance of transvaginal color Doppler ultrasonography of the ovarian artery as a predictor of ovarian response in controlled ovarian hyperstimulation for in vitro fertilization and embryo transfer. J Assist Reprod Geneti 2002; 19(3): 103-12.
7. Zaidi J Blood flow changes in the ovarian and uterine arteries in women in normal and polycystic ovaries. Hum Fertil (Camb) 2000; 3(3): 194-198.
8. Altundag M, Levi B, Adakan S. Intraovarian stromal arterial Doppler indices in predicting ovarian responses. J Reprod Med 2002; 47(11): 886-90.
9. Kupesic S, Kurjak A. Predictors of IVF outcome by three-dimensional ultrasound. Hum Reprod 2002; 17(4): 950-5.
10. Engelmann L, Sladkevicius P, Agrawal B, Bekir Y, Campbell S, Tun SL.The pattern of changes in ovarian stromal and uterine artery blood flow velocities during IVF treatment and its relations with outcome of the cycle. Ultrasound Obstet Gynecol 1999; 13(1): 26-33.
11. Engelmann L, Sladkevicius P, Agrawal B, Bekir Y, Campbell S, Tun SL. Value of ovarian stromal blood flow velocity measurements after pituitary suppression in the predicition of ovarian responsiveness and outcome of in IVF treatment. Fertil Steril 1999; 712(1): 22-29.
12. Yalti S, Gurbuz B, Ficicicoglu C, Canova H J. Doppler evaluation of the uterine, intraovarian, stromal and spiral arteries on the day of human chorionic gonadotrophin administration in controlled ovarian hyperstimulation. J Obstet Gynecol 2003; 23(4): 402-6.
13. Popovic Todorovic B, Loft A, Lindhurd A, Bangsboll S, Anderson AM, Anderson AN. A prospective study of predictive factors of ovarian response in "standard" IVF/ICSI patients treated with recombinant FSH, a suggestion for a recombinant FSH dosage normogram. Hum Reprod 2003; 18(4): 781-7.
14. Kupesic S, Kurjak A, Bjelos D, Vujisic S. Three-dimensional ultrasonographic ovarian measurements and in vitro fertilization outcome are related to age. Fertil Steril 2003; 79(1): 190-7.
15. Vicino M, Lorusso F. In vitro maturation and fertilization of oocytes from unstimulated ovaries: predicting the number of immature oocytes retrieved by early follicular phase ultrasonography. Am J Obstet Gynecol 2002; 186(4): 684-9.
16. Costello MF, Shrecha S, Sjoblom P, Mc Nally G, Beunett Mj, Steigrund JS, Hugher GJ. Power Doppler ultrasound assessment of ovarian perifolicular blood flow in women with polycystic ovaries and normal ovaries during in vitro fertilization treatment. Fertil Steril 2005; 83(4): 945: 54.
17. Pan H, Wu MH, Chung YC, Li CH, Chang FM. Quantification of Doppler signal in polycystic ovary syndrome using three-dimensional power Doppler ultrasonography: a possible new marker for diagnosis. Hum Reprod 2002; 17(9): 2484.
18. Loverro G, Vicino M, Lorusso F, Vimarcati A, Guco P, Selvaugi L. Polycystic ovary syndrome: relationship between insulin sensitivity, sex hormone levels and ovarian stromal blood flow. Gynecol Endocrinol 2001; 15(2): 142-9.
19. Buckett WM, Bouranen R, Wathin K, Tulandi T, Tan SL. Ovarian stromal echogenity in women with normal and polycystic ovaries. Hum Reprod 1999; 4(3): 618-21.
20. Yu Ng EH, Chi WAI, Chan C, Tang OS, Shu Biu, Yang W, Chung Ho P. Effect of pituitary down regulation on antral follicle count, ovarian volume and stromal blood flow measured by three-dimensional ultrasound with power Doppler prior to ovarian stimulation. Hum Reprod 2004; 19(12): 2811-5.
21. Ng EH, Chan CC, Yeang WS, Ho PC. Effect of age on ovarian stromal flow measured by three-dimensional ultrasound with power Doppler in Chinese women with proven fertility. Hum Reprod 2004; 19(9): 2132-7.
22. Pan HA, Wu MH, Chang YC, Chung FM. Quantification of ovarian stromal Doppler signals in poor responders undergoing in vitro fertilization with three-dimensional power Doppler ultrasonography. Am J Obstet Gynecol 2004; 190(2): 338-44.
23. NgEH, Yeung WS, Fong DJ, Ho PC. Effects of age on hormonal and ultrasound marquers of ovarian reserve in Chinese women with proven fertility. Hum Reprod 2003; 18(10): 2169-74.

KOLOR DOPPLER (CD) I KOLOR DOPPLER ENERGIJSKI PRIKAZ I MERENJA PROTOKA KRVI KROZ STROMU JAJNIKA U KONTROLISANOJ HIPERSTIMULACIJI TOKOM IN VITRO FERTILIZACIJE

Ranko Kutlešić[1], Aleksandar Ljubić[2], Mila Milosavljević[1], Milan Stefanović[1], Predrag Vukomanović[1]

[1]Klinika za ginekologiju i akušerstvo, Klinički centar, Niš
[2]Institut za ginekologiju i akušerstvo, Klinički centar Srbije, Beograd
E-mail: kutlesicr@medianis.net

Kratak sadržaj: *Cilj ovog istraživanja je da se odredi korelacija kolor Doppler (CD) i kolor Doppler energijskog (CDE) prikaza i merenja protoka krvi kroz stromu jajnika pre početka kontrolisane hiperstimulacije jajnika (KOH) tokom ciklusa in vitro fertilizacije (IVF), prediktora uspeha KOH i broja dobijenih oocita, posebno u grupi*

pacijentkinja sa lošim odgovorom na kontrolisanu hiperstimulaciju jajnika. Ovo prospektivno kliničko istraživanje je obuhvatilo 52 pacijentkinje, podvrgnute »dugom protokolu« kontrolisane hiperstimulacije jajnika tokom ciklusa in vitro fertilizacije.Pre početka KOH kolor Doppler i kolor Doppler energijskim prikazom, na početnom ultrazvučnom pregledu, je registrovana vaskularizacija strome jajnika, pomoću endovaginalne sonde od 6.5 MHz. Posmatrano u celini, u populaciji infertilnih pacijentkinja tokom ciklusa IVF, CD i CDE prikaz i merenja protoka krvi kroz stromu jajnika, ne koreliraju sa rezultatima kontrolisane hiperstimulacije jajnika tokom IVF ciklusa, niti sa stopom trudnoća. Pacijentkinje sa lošim odgovorom na kontrolisanu hiperstimulaciju jajnika »dugim protokolom« u toku IVF ciklusa, imaju manje izražen protok krvi kroz krvne sudove strome jajnika na CD i CDE prikazu, što prati i viša rezistenca protoku krvi kroz arteriole strome jajnika na početku KOH. Pacijentkinje ove grupe bi trebalo prebaciti na neki drugi protokol stimulacije, što bi ih poštedelo neuspešnog IVF ciklusa i dalo im više šansi da postignu trudnoću.

Ključne reči: *in vitro fertilizacija, kontrolisana hiperstimulacija jajnika, doppler*