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COLOR DOPPLER AND COLOR DOPPLER ENERGY IMAGING AND MEASUREMENTS OF INTRAOVARIAN VASCULARISATION DURING LUTEAL PHASE OF MENSTRUAL CYCLE

SUMMARY

The aim of this investigation was to determine if there were differences: (1) in color Doppler (CD) ultrasound imaging and measurements of periluteal vascularisation in ovary bearing corpus luteum and stromal blood flow of contralateral ovary in ovulatory cycle, and (2) between intraovarian midluteal vascularisation in the group of ovulatory patients and ovarian stromal blood flow of the 21st cycle day in the patients with anovulatory cycles.

This prospective clinical investigation involved 205 patients divided into two groups: with ovulatory and anovulatory cycles. CD ultrasound examination of intraovarian vascularisation were performed during menstrual cycle, and CD indices were analyzed: pulsatile index PI, resistance index RI, and systolic/diastolic - S/D ratio.

PI was statistically significantly lower in the group of ovulatory patients: 0.8 ± 0.14 vs 1.265 ± 0.41. The same was true for RI (0.51 ± 0.04 vs 0.65 ± 0.07) and for S/D ratio (2.08 ± 0.23 vs 2.91 ± 0.58). Resistance to blood flow in periluteal vessels during the midluteal phase was lower than in stromal vessels of nondominant ovary in ovulatory patients (PI 0.8 ± 0.14 vs 2.08 ± 0.31; RI 0.51 ± 0.04 vs 0.74 ± 0.1 and S/D ratio 2.08 ± 0.23 vs 4.25 ± 1.76).

Our investigation showed that the resistance to intraovarian blood flow was lower in ovulatory compared to anovulatory cycles. The same was true for intraovarian – periluteal vascularisation in the ovary with corpus luteum compared to stromal vascularisation of nondominant ovary.

Key words: **corpus luteum, color Doppler ultrasound**

The function of any organ directly depends on vascularisation. It is logical that this is also true for female reproductive system, especially for ovarium and uterus / endometrium, as the places of mounthly dynamic events. It also seems logical that the changes of vascularisation in reproductive organs must be the a part of these events, and that the vascularisation changes have essential impact on follicular growth and development, ovulation and corpus luteum function. In a few past years, color Doppler (CD) ultrasound examinations became popular for investigation of reproductive tract vascularisation (1-3).

The aim of this investigation was to determine if there were:

- the differences in color Doppler (CD) ultrasound imaging and measurements of periluteal blood flow in ovarium with dominant follicle (corpus luteum) and stromal blood flow of contralateral ovarium in ovulatory cycle;
- the differences in color Doppler (CD) ultrasound imaging and measurements between intraovarian midluteal vascularisation (day +7) in the group of ovulatory patients and ovarian stromal blood flow of the 21st cycle day in the group of patients with anovulatory cycles.

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PATIENTS AND METHODS

Prospective clinical investigation included 205 patients admitted to the Department of Reproductive Endocrinology, Clinic of Gynecology and Obstetrics Niš, for hormone investigation of infertility. After the routine management (history and clinical examination), ultrasound folliculometry was performed for determination of ovulation. The blood was taken for hormonal analysis in basal blood sample (cycle day 2-4), during follicular phase, periovulatory (day O or day 14 of ``ideal`` cycle) and in the middle of luteal phase (day +7 or day 21 – 24 of ``ideal`` 28th day of the cycle). The ovulation was confirmed with the finding of corpus luteum on ultrasound examination in midluteal phase and progesteron level higher than 4 ngr/l in the middle of luteal phase, as has been already described (4).

During folliculometry, CD ultrasuond examination of intraovarian and intrauterine vascularisation were performed. The results of CD measurements of intraovarian luteal blood flow are presented in this article.

Color Doppler ultrasound examination and measurements

Color Doppler ultrasound examinations were performed on color Doppler ultrasound system Acuson 128 XP 10i (Acuson Co., Mountain View, California, US), from 2000 to November 2005, with 7 MHz transvaginal ultrasound probe, with acustic power less than 50 mW/cm^2 (as recommended by Bioeffects Committee of the American Institute of Ultrasound in Medecine (5,6). The same investigator performed all ultrasound examinations.

The presence and form of corpus luteum, appearance and width of endometrium, the shape of cavum uteri were registered on ultrasound examination during the midluteal phase. CD imaging and measurements of periluteal and intrathecal blood flow were performed during the same examination as well. Ovarian stromal blood flow, periluteal and intrathecal vascularization were mapped using color Doppler ultrasound image. The sample gate was placed on the most prominent blood vessel in the desired area, the sample gate width adjusted to be as close as possible to the diameter of measured blood vessel, pulse wave was started, and after three consecutive clear waves color Doppler measurements were taken: pulsatile index PI, resistance index RI, systolic and diastolic blood velocity ratio - S/D ratio and maximal systolic velocity – v max.

Data base was formed on commercial software. Statistical significance was tested using the Student's t-test. Correlation was tested with calculated coefficient of correlation (r). The level of 5% has been considered as upper limit of statistical significance.

RESULTS

The results are shown in tables.

Resistance to blood flow in periluteal vessels during the midluteal phase was lower than in stromal vessels of nondominant ovary in ovulatory patients (*Table 1*).

Table 1. CD prameters of periluteal blood flow (dominant side) and stromal flow of contralateral ovarium (nondominant side) on ultrasound examination in the middle of luteal phase in ovulatory patients (day +7 or 21st day of ``ideal`` cycle)

	PI (X±SD)	RI (X±SD)	S/D (X±SD)	V max (X±SD)
dominant side n = 120	0.8 ± 0.14	0.51± 0.04	2.08 ±0.23	14.0 ± 6.6
nondominant side n = 120	2.08 ± 0.31	0.74 ± 0.1	4.25 ± 1.76	14 ± 11.31
p	p = 0.0002729**	p = 0.005891**	p = 0.0256915*	p = 0.4926634

* statistically significant difference, ** highly statistically significant difference

Table 2. CD measurements of intraovarian blood flow in the middle of luteal phase (day +7) in the group of ovulatory patients and in the 21st cycle day in the group of anovulatory patients

	PI	RI	S/D	V max cm/sec
Ovulatory patients n=120	0.8 ± 0.14	0.51 ± 0.04	2.08 ± 0.23	14.0 ± 6.6
Anovulatory patients n=85	1.265 ± 0.41	0.65 ± 0.07	2.91 ± 0.58	7.5 ± 3.53
p	p = 0.027712*	p = 0.006302**	p = 0.008029**	p = 0.236526

* statistically significant difference, ** highly statistically significant difference

PI was statistically significantly lower in the group of ovulatory patients: 0.8 ± 0.14 vs 1.265 ± 0.41. The same was true for RI (0.51 ± 0.04 vs 0.65 ± 0.07) and for S/D ratio (2.08 ± 0.23 vs 2.91 ± 0.58) (*Table 2*).

Resistance to blood flow in ovarian stromal arteriolar vessels is higher with age, according to the correlation of age and CD indices: with PI (r = 0.52), with RI (r = 0.65), with S/D ratio (r = 0.517). The age did not correlate with v max (r = -0.20).

DISCUSSION

The function of any organ directly depends on vascularization. Dynamics of cyclic changes in female reproductive system is unique in human body. The changes in uterine and ovarian vascularisation must be a part of this process: blood flow through small vessels of reproductive organs, regulating mechanisms and angiogenesis have essential role. In adult human body, angiogenesis is physiological only in three situations: during reparation of wounds and fractures, during endometrial reparation in proliferative phase of menstrual cycle and in folliculogenesis and formation of corpus luteum in ovarium (7). Physiological angiogenesis also takes place during placentogenesis and embryo development (8).

The vascularisation of reproductive organs is a subject of cyclic changes regulated by steroid hormones and local peptides. This is true for big vessels supplying reproductive organs: uterine and ovarian arteries, even more for small vessels inside these organs. The changes in small vessels in ovarian stroma, myometrium and endometrium are directly associated with the process of folliculogenesis, ovulation and function of corpus luteum.

More than fifty years ago, Edward Marqee described cyclic changes of spasms and dilatation of arteriolas, which happened just before endometrial shedding during menstruation, followed by growth of new blood vessels during regeneration of endometrium. These cyclic changes were observed in endometrium implanted in anterior eye chamber of rhesus monkey (9, 10). After forty years, it was determined that vascularisation of dominant follicle at the ninth cycle day is twice developed than vascularisation of other follicles, enabling delivery of more gonadotropins to dominant follicle (11). The importance of vascularisation changes is emphasized in luteogenesis. Endothelial cells make one half of all maturated cells in corpus luteum (12). Angiogenesis and production of vasoactive components in vascular endothelium of corpus luteum is under direct influence of luteal cells products which take part in steroidogenesis, and, the endothelial products have direct impact on luteal function: endotelin 1 is a possible mediator in luteolysis (13,14). The functional lifespan of corpus luteum depends on autocrine and paracrine mechanisms that regulate steroid hormone production and neovascularization (15). Angiogenesis, the process of new capillary formation from previously existing mature vessels, is regulated by ovarian follicle and corpus luteum factors. These factors are controlled by endocrine, paracrine, autocrine regulation and metabolic signals as intracellular oxygen content and ageing. The possible cause of vascular dysfunction and ovarian disorders is aberrant production of these angiogenic factors (16).

These facts came from animal experiments. 1990 color Doppler ultrasound enabled noninvasive in vivo observation of blood flow through uterine and ovarian arteries during human menstrual cycle (17-20). The early CD investigations have determined decreasing resistance to blood flow in these vessels during menstrual cycle, with lower resistance on the side of a dominant follicle (21). Resistance to blood flow through uterine artery during luteal phase decreased, especially during "implantation window" from the 19th to 25th cycle day, when endometrium is prepared for implantation. These hemodynamic changes are characteristic only for uterine and ovarian arteries, and there are no such changes in other peripheral arteries (17). After all, uterine and ovarian arteries are big vessels, and the blood flow through these arteries is under the influence of many systemic factors, not only local products. There are also technical difficulties due to the angle of insonation for ovarian artery. For these reasons, most of the investigators today measure blood flow through small vessels: ovarian and uterine arteriolas. Use of high frequency transvaginal ultrasound with high resolution makes possible investigations of endometrial, myometrial and ovarian stromal, perifollicular, periluteal ond intrathecal blood flow (21-27).

Measurements of perifollicular and luteal blood flow during menstrual cycle also determined the decrease of resistance index in regular luteal phase (28). There is also the hypothesis that this fall in resistance is ultimate for pregnancy in natural spontaneous cycle (29).

Transvaginal color Doppler ultrasound measurement is a reliable way to determine dimension and vascularisation of corpus luteum (30). This investigation also found that during corpus luteum aging the resistance to blood flow rose in periluteal blood vessels. Pulsatile index of arteriola supplying corpus luteum is in inverse correlation with vascular density inside corpus luteum, which is supported by anatomic investigation of corpus luteum removed on surgery (during elective

hysterectomy or tubal sterilisation) (30).

CD measurements of perifollicular blood flow during controlled ovarian hyperstimulation for in vitro fertilization also showed decrease in perifollicular blood flow around follicules with mature oocyte (31). Better follicular vascularisation enables better delivery of gonadotropins, which is essential for follicular growth and development during follicular phase. During late follicular phase there is a fall in gonadotropin blood levels due to rising levels of estradiol and peptides – products of dominant follicle. The consequence of this fall in gonadotropins is suppression of growth of subdominant follicles: in most of human cycles there is only one dominant follicle. At the same time, dominant follicle rescues itself from the decrease of gonadotropins with high concentration of estradiol in non-primates and with high production of local peptides in primates. Perifollicular vascularisation is also more developed in dominant follicles than in other follicles from the same cohort, due to angiogenesis supported by local peptide – vascular endothelial growth factor (VEGF) produced by granulosa under the influence of LH (32-34). In late follicular phase, there is also a fall in resistance to blood flow in vascularisation around the dominant follicle (35). 3-D color Doppler investigation showed higher vascularisation index in the area of 2 mm around dominant follicle compared to the rest of the dominant ovary (36). Such difference is more expressed when spontaneous ovulatory and anovulatory cycles are compared, which is shown in our present investigation.

In the group of our patients with spontaneous ovulatory cycles, we also compared the resistance to blood flow during luteal phase in intraovarian blood vessels in ovary with dominant follicle / corpus luteum and in contalateral ovary. The lower resistance to blood flow was registered in dominant ovary which corresponds to the results of our former investigations of intraovarian blood flow resistance in ovulatory and anovulatory cycles (37-39).

Color Doppler ultrasound imaging has a great potential for assessing microvascularisation, especially high frequency ultrasound (> 20 MHz), which use is still in experimental phase (40, 41). The clinical use of these imaging systems will give many answers regarding the changes of reproductive tract vascularisation during menstrual cycle.

CONCLUSION

Our investigation shows that the resistance to intraovarian blood flow is lower in ovulatory than in anovulatroy cycles. The same is true for intraovarian – periluteal – vascularisation in the dominant ovary with corpus luteum compared to stromal vascualarisation of nondominant ovary.

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KOLOR DOPPLER I KOLOR DOPPLER ENERGIJSKI PRIKAZ I MERENJA INTRAOVARIJALNE VASKULARIZACIJE U TOKU LUTEALNE FAZE MENSTRUALNOG CIKLUSA

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SAŽETAK

Cilj istraživanja bio je da se odredi da li postoje razlike u kolor Doppler (CD) ultrazvučnom prikazu i merenjima: (1) perilutealne vaskularizacije u ovarijumu sa žutim telom i stromalnog protoka krvi suprotnog jajnika u ovulatornim ciklusima i (2) između intraovarijalne vaskularizacije u sredini lutealne faze bolesnica u ovulatornom ciklusu i stromalnog protoka krvi 21-og dana ciklusa bolesnica sa anovulatornim ciklusima.

Ovo prospektivno kliničko istraživanje obuhvatilo je 205 bolesnica podeljenih u dve grupe: sa ovulatornim i anovulatornim ciklusima. U toku menstrualnog ciklusa rađen je CD ultrazvučni pregled intraovarijalne vaskularizacije i analizirani su sledeći CD parametri: pulsatilni indeks PI, indeks rezistence RI, sistolno/dijastolni – S/D – odnos.

PI je bio statistički značajno niži u grupi bolesnica sa ovulacijom: 0.8 ± 0.14 vs 1.265 ± 0.41. To je važilo i za RI (0.51 ± 0.04 vs 0.65 ± 0.07) i za S/D odnos (2.08 ± 0.23 vs 2.91 ± 0.58). Otpor protoku krvi u perilutealnim sudovima u sredini lutealne faze bio je niži nego u stromalnim krvnim sudovima nedominantnog jajnika bolesnica sa ovulacijom (PI 0.8 ± 0.14 vs 2.08 ± 0.31; RI 0.51 ± 0.04 vs 0.74 ± 0.1 i S/D odnos 2.08 ± 0.23 vs 4.25 ± 1.76).

Naše istraživanje pokazuje da je otpor protoku krvi kroz jajnik niži u ovulatornim nego u anovulatornim ciklusima. Isto važi i za intraovarijalnu – perilutealnu – vaskularizaciju u dominantnom jajniku u poređenju sa stromalnom vaskularizacijom nedominantnog jajnika.

Ključne reči: **corpus luteum, kolor Doppler ultrazvuk**