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ROLE OF COLOUR DOPPLER IMAGING IN UTERO -OVARIAN BLOOD FLOW IN FERTILE AND INFERTILE FEMALES

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ABSTRACT: *Background: the recent incorporation of color Doppler processing in transvaginal transducer/probes extends the scope of sonographic imaging from an anatomic to a physiological basis. With such versatility, it is tempting to employ the technique for ever more demanding applications and to try to measure increasingly subtle changes in the maternal and fetal circulations. The ability to assess utero-ovarian blood flow has several applications in the assessment of women with fertility problems. Materials and Methods: the present study was conducted in Department of Radiodiagnosis & Imaging, Acharya Shri Chander College of Medical Sciences, Jammu (J&K) from October 2012 to September 2013. In the present observational prospective study, 40 infertile females after exclusion of all the possible causes of infertility and 40 fertile females were recruited. The age of these women ranged from 16-40 years. Doppler blood flow indices studied during days 2-5 of the menstrual cycle included Resistivity index (RI), Peak systolic velocity (PSV, V1) and Minimal diastolic velocity (MDV, V2). Comparative study was performed in the taken parameters between the fertile and infertile females and various conclusions drawn in ovarian vessels, uterine myometrium and endometrium. Results: the Peak systolic velocities (PSV,V1); Minimal Diastolic Velocity (MDV, V2) and resistivity index (RI) in infertile and fertile females expressed as mean +/- standard deviation was taken in the left as well as right ovary and compared. In the left ovary, the (PSV, V1), (MDV, V2) of infertile females was 18.80+/-10.01, 5.99+/-4.28 and 0.68+/-0.13 while that for the fertile group was 10.98+/-3.01, 3.28+/-2.38 and 0.62+/- 0.12 whereas in the right ovary the PSV,V1; MDV, V2 and RI in infertile females was 17.20+/-10.22, 5.95+/-5.20 and 0.65+/-0.10 while that in fertile females was 12.10+/-3.90, 3.28+/-1.08 and 0.64+/-0.10. Conclusions: The infertile and fertile females re-established the fact that there is a variation in the blood flow in the ovarian vessels with the Peak Systolic Velocity and the Minimal Diastolic Velocity being higher in the infertile females on days 2-5 of the menstrual cycle. However, the Resistivity index showed no significant variation in infertile and fertile females. Also, it is also observed that no significant difference in the blood flow parameters was found in the endometrial and myometrial vessels on the days 2-5 of the menstrual cycle in contrast to definite variation observed in the follicular phase of the cycle.*

KEYWORDS: Colour Doppler, Utero-Ovarian Blood Flow, Resistivity Index, Peak Systolic Velocities, Minimal Diastolic Velocity

INTRODUCTION

In recent years, the capabilities of ultrasound flow imaging have increased enormously. Color flow imaging is now commonplace and facilities such as 'power' or 'energy' Doppler provide new ways of imaging flow. With such versatility, it is tempting to employ the technique for ever more demanding applications and to try to measure increasingly subtle changes in the maternal and fetal circulations [1]. Colour Doppler imaging is an important tool to check utero-ovarian blood flow in infertile and fertile females. With advancement in age of women, both spontaneous conceptions [2] and conception due to assisted reproductive methods are reduced. The decrease in fertility with ageing is probably due to a decreasing number of primordial follicles after birth. The cyclic development of follicles is finely controlled by a sequence of hypothalamic–pituitary–ovarian interactions, and angiogenesis is an important component of both the follicular and luteal phase of an ovarian cycle [3]. Early follicular phase FSH level is increased with the advancement in age by a reduced inhibin-mediated feedback towards the pituitary gland. A number of parameters known as ovarian reserve markers are studied in many assisted reproduction technology (ART) programmes to predict ovarian responses prior to ovarian stimulation with gonadotrophins [4]. Hormonal markers include basal FSH, inhibin B, and anti-mullerian hormone (AMH) levels. Other less commonly used hormonal markers such as serum estradiol level, FSH/LH ratio, GnRH agonist stimulation test and exogenous FSH ovarian reserve test are of limited value in the prediction of ovarian response [5]. Recently, ultrasound assessment of the ovarian volume (Syrop *et al*., 1999), total antral follicle counts (AFC) and ovarian stromal blood flow was also found to be useful in predicting ovarian responses. Infertility, whether involuntary or induced, is a distressing condition which prevents reproduction in a couple in the reproductive age. The infertility could be primary when the couple has not conceived even once or secondary when the couple has conceived at least once but is unable to conceive again for two years or more [6]. Only up to 5% of the infertility is due to anatomical, genetic, endocrinological and immunological problems while the rest is due to preventable conditions such as sexually transmitted diseases (STDs), parasitic diseases, harmful health care practices and policies, unsafe abortions, and exposure to potentially toxic substances. In women, the causes of infertility include tubal disease, hormonal abnormalities, ovulatory dysfunction, endometriosis, immunological factors, congenital abnormalities and sexual dysfunction [7]. Many women in developed countries delay childbearing to fulfill their personal commitments. An accurate assessment of reproductive age would be of help in counselling these women about their fertility potential and perhaps in scheduling pregnancies. The majority of ovarian reserve tests are performed in women presenting with infertility problems [8]. There are very few studies addressing the effects, we included, in a group of infertile females who had undergone all known and possible investigations which were normal but had failed to conceive despite all efforts [9]. This group comprises about 10-15 percent of our population and are known to suffer from what is called **Idiopathic or Unexplained Infertility.** It is in these patients where various researchers have proposed the uterine and ovarian blood flow as an important possible factor that could affect non-conception. Various blood flow parameters have been studied in infertile females in different days of the menstrual cycle [10,11]. The studied parameters include the pulsatility index, resistivity index, peak systolic velocity and minimal diastolic velocities in uterine and ovarian vessels. While many studies have been performed in the periovulatory phase and demonstrate increased pulsatility and resistivity indices in this phase in infertile females as compared to

fertile females, varied changes have been demonstrated in the menstrual phase which is a less exploited and researched phase of menstrual cycle.

Materials and Methods

The present study was conducted in Department of Radiodiagnosis & Imaging, Acharya Shri Chander College of Medical Sciences, Jammu (J&K) from October 2012 to September 2013. In the present observational prospective study, 40 infertile females after exclusion of all the possible causes of infertility and 40 fertile females were recruited. The age of these women ranged from 16-40years. The duration of infertility was from 1-20 years. Study population cases and controls were subjected to the same investigations and procedures. Doppler blood flow indices studied during days 2-5 of the menstrual cycle included Resistivity index (RI), Peak systolic velocity (PSV, V1) and Minimal diastolic velocity (MDV, V2) in ovarian vessels, uterine myometrium and endometrium. Comparative study was performed in the taken parameters between the fertile and infertile females and various conclusions drawn.

RESULTS

In the present study, the mean+/-standard deviation of the Peak systolic velocity (PSV, V1) Minimal Diastolic Velocity (MDV, V2) and resistivity index (RI) were obtained from 40 fertile and 40 infertile females in the Left ovary were seen. The (PSV, V1), (MDV, V2) and (RI) of infertile females was 18.80+/-10.01, 5.99+/-4.28 and 0.68+/-0.13 while that for the fertile group was 10.98+/-3.01, 3.28+/-2.38 and 0.62+/- 0.12. There was a difference in the PSV (V1) of the infertile and fertile in the left ovary. Hence, confirming the fact that the PSV was higher in the left ovary in infertile females. The MDV was significantly higher in infertile group while there was no significant difference in the resistivity index of the left ovary of fertile and infertile females. The (PSV,V1); (MDV, V2) and (RI) in infertile and fertile females expressed as mean +/- standard deviation was taken in the right ovary and compared. The PSV,V1; MDV, V2 and RI in infertile females was 17.20+/-10.22, 5.95+/-5.20 and 0.65+/-0.10 while that in fertile females was 12.10+/-3.90, 3.28+/-1.08 and 0.64+/-0.10. Thus, the comparison resulted in the conclusion that the PSV and MDV was higher in the infertile group than the fertile group.

The MDV, RI in myometrial vessels was 4.32+/-2.90, 0.75+/-0.10 and 3.42+/-1.72, 0.78+/-0.11 for infertile and fertile females respectively. The PSV, V1; MDV,V2 and RI of the endometrium of the uterus was compared between infertile females and fertile females as expressed by the mean+/- standard deviation of the values. The V1, V2 and RI for the infertile group was 9.48+/-8.34, 4.46+/-4.01 and 0.60+/-0.14, while that for the fertile group was 7.68+/-3.14, 3.18+/-2.38 and 0.66+/-0.12. The difference of the peak systolic velocity, Minimal Diastolic Velocity and RI in the two groups that is the infertile and fertile females was insignificant and was described in Table 1.

The fertile females were divided into those with age less than 30 years and those with age greater than 30 years. There were 21 females less than 30 years and 19 females greater than 30 years. The peak systolic velocity, minimal diastolic velocity and resistivity index of left ovary, Myometrium and Endometrium, were obtained expressed as mean +/-standard deviation for each parameter. The PSV, MDV and RI (left ovary) for female less than and greater than 30 years were 13.64+/-6.84, 6.66+/-3.34, 0.66+/-0.10 and 14.28+/-7.89, 7.14+/-

5.18, 0.67+/-0.11 respectively. The PSV, MDV and RI of myometrium were obtained expressed as mean +/-standard deviation for each parameter. The PSV, MDV and RI for female less than and greater than 30 years were 16.40+/-6.12, 3.88+/-2.08, 0.72+/-0.08 and 15.74+/-6.24, 3.90+/-3.10, 0.74+/-0.08 respectively. The PSV, MDV and RI of endometrium were obtained and expressed as mean +/- standard deviation for each parameter and for female less than and greater than 30 years were 8.58+/-6.24, 3.68+/-2.88, 0.65+/-0.10 and 8.88+/-6.68, 3.88+/-2.22, 0.64+/-0.11 respectively and described in Table 2 and Table 3

The infertile females were divided into those with body mass index less than 25kg/m^2 and those with body mass index greater than 25 kg/m^2. There were 20 females with BMI less than 25 kg/m^2 and 20 females with BMI greater than 25 kg/m^2. The PSV, MDV and RI of right ovary were obtained and expressed as mean +/-standard deviation for each parameter were found to be 14.38+/-10.49, 5.02+/-3.42, 0.66+/-0.10 and 14.26+/-5.18, 4.42+/-2.52, 0.71+/-0.10 respectively. The PSV, MDV and RI for infertile females with Body mass index less than and greater than 25kg/m^2 in left ovary were found to be 15.82+/-8.96, 7.50+/-5.24, 0.66+/-0.12 and 16.26+/-7.33, 6.94+/-3.30, 0.64+/-0.10. The results for the two groups were comparable and no significant difference was found. The PSV, MDV and RI of myometrium were found to be 17.69+/-5.54, 3.88+/-2.64,0.74+/-0.12 and 16.28+/-7.96, 3.92+/-2.40, 0.75+/-0.10 respectively. The PSV, MDV and RI of endometrium was found to be 9.9+/-6.24, 3.66+/-2.62, 0.62+/-0.12 and 8.70+/-6.72, 3.83+/-2.16, 0.64+/-0.14 respectively and shown in Table 4.

The fertile females were divided into those with body mass index less than 25kg/m^2 and those with body mass index greater than 25 kg/m^2. There were 17 females less than 25 kg/m^2 and 23 females greater than 25 kg/m^2. The peak systolic velocity, minimal diastolic velocity and resistivity index of right ovary were obtained and expressed as mean +/- standard deviation for each parameter. The PSV, MDV and RI for fertile females with BMI less than and greater than 25kg/m^2 were found to be 13.60+/-7.52, 4.68+/-3.52,0.78+/-0.12 and 14.04+/-6.22, 4.02+/-2.04, 0.68+/-0.22 respectively. The PSV, MDV and RI of left ovary were obtained and expressed as mean +/-standard deviation for each parameter and was found to be 16.30+/-7.94, 6.49+/-3.49, 0.62+/-0.11and15.84+/-7.68, 6.91+/-4.32, 0.69+/-0.11 respectively. The PSV, MDV and RI in myometrium for fertile females with BMI less than and greater than 25kg/m^2 were found to be 17.22+/-6.12, 3.88+/-2.10, 0.74+/-0.12 and 17.04+/-5.24, 4.02+/-2.62, 0.70+/-0.12 respectively. The PSV, MDV and RI for fertile females in endometrium with BMI less than and greater than 25kg/m^2 were found to be 10.1+/-6.2, 3.94+/-2.32, 0.66+/-0.10 and 9.20+/-6.20, 4.01+/-3.32, 0.62+/-0.12 respectively and described in table 5.

TABLE NO.1-Comparison of Peak Systolic Velocity (V1), Minimal Diastolic Velocity(V2) and Resistivity Index (RI) Between Infertile and Fertile Females in the Left and Right Ovary, Myometrium and Endometrium.

	Fertility State	PSV,V1 (cm/sec)	MDV,V2 (cm/sec)	RI
Left Ovary	Infertile N=40	18.80 ± 10.01	5.99 ± 4.28	0.68 ± 0.13
	Fertile N=40	10.98 ± 3.10	3.28 ± 2.38	0.62 ± 0.12
Right Ovary	Infertile N=40	17.20 ± 10.20	5.95 ± 5.20	0.65 ± 0.10
	Fertile N=40	12.10 ± 3.90	3.28 ± 1.08	0.64 ± 0.10
Myometrium	Infertile N=40	15.75 ± 7.80	4.32 ± 2.90	0.75 ± 0.10
	Fertile N=40	15.24 ± 5.1	3.42 ± 1.72	0.78 ± 0.11
Endometrium	Infertile N=40	9.48 ± 8.34	4.46 ± 4.01	0.60 ± 0.14
	Fertile N=40	7.68 ± 3.14	3.18 ± 2.38	0.66 ± 0.12

Table 2: Effect of Age on Blood Flow in Fertile Females in Age groups Less Than and Greater Than 30 Years in the Right and Left Ovary.

	Fertility State	Age	PSV,V_1 (cm/s)	MDV,V_2 (cm/s)	RI
Right Ovary	**Fertile**	<30 years N=21	12.90 ±7.28	3.58 ± 2.10	0.68 ± 0.20
		> 30 years N=19	13.01± 6.14	3.6 ± 2.01	0.72 ± 0.10
Left Ovary		<30 years N=21	13.64 ± 6.84	6.66 ± 3.34	0.66 ± 0.10
		> 30 years N=19	14.28 ± 7.89	7.14 ± 5.18	0.67 ± 0.11
Myometrium		<30 years N=21	16.40 ± 6.12	3.88 ± 2.08	0.72 ± 0.08
		> 30 years N=19	15.74 ± 6.24	3.90 ± 3.10	0.74 ± 0.10
Endometrium		<30 years N=21	8.58 ± 6.24	3.68 ± 2.88	0.65 ± 0.10
		> 30 years N=19	8.88 ± 6.68	3.88 ± 2.22	0.64 ± 0.11

Table 3: Effect of Age on Blood Flow in Infertile Females in Age Groups Less Than and Greater Than 30 Years in The Right and Left Ovary.

	Fertility State	Age	PSV,V_1 (cm/s)	MDV,V_2 (cm/s)	RI
Right ovary	**Infertile**	<30 years N=20	14.95 ± 8.20	6.04 ± 4.80	0.68 ± 0.1
		> 30 years N=20	12.80 ± 7.04	4.01 ± 2.99	0.70 ± 0.9
Left ovary		<30 years N=20	15.98 ± 6.92	7.04 ± 3.58	0.62± 0.10
		> 30 years N=20	13.99 ± 9.34	6.80 ± 5.66	0.64± 0.11
Myometrium		<30 years N=20	15.85 ± 6.99	4.09 ± 2.46	0.78± 0.13
		>30 years N=20	18.48 ± 6.40	3.48 ± 2.24	0.76± 0.18
Endometrium		<30 years N=20	9.85 ± 6.86	4.14 ± 3.41	0.62± 0.13
		>30 years N=20	8.60 ± 6.08	3.52 ± 2.31	0.66± 0.11

Table 4: Effect of Body Mass Index on Infertile Females with BMI <25 kg/m^2 and > 25 kg/m^2 in Right and Left Ovary, Endometrium, Myometrium.

	Fertility State	BMI	PSV,V1 (cm/s)	MDV,V_2 (cm/s)	RI
Right Ovary	**Infertile**	<25 kg/m^2 n=20	14.38 ± 10.49	5.02 ± 3.42	0.66 ± 0.10
		>25 kg/m^2 n=20	14.26 ± 5.18	4.42 ± 2.52	0.71 ± 0.10
Left Ovary		<25 kg/m^2 n=20	15.82 ± 8.96	7.50 ± 5.24	0.66 ± 0.12
		>25 kg/m^2 n=20	16.26 ± 7.33	6.94 ± 3.30	0.64 ± 0.10
Myometrium		<25 kg/m^2 n=20	17.69 ± 5.54	3.88 ± 2.64	0.74 ± 0.12
		>25 kg/m^2 n=20	16.28 ± 7.96	3.92 ± 2.40	0.75 ± 0.10
Endometrium		<25kg/m^2 n=20	9.9 ± 6.24	3.66 ± 2.64	0.62 ± 0.12
		>25 kg/m^2 n=20	8.70 ± 6.72	3.83 ± 2.16	0.64 ± 0.14

Table 5: Effect of Body Mass Index on Fertile Females with BMI <25 kg/m2 and > 25 kg/m2 in Right and Left Ovary

	Fertility State	BMI	PSV,V1 (cm/s)	MDV,V_2 (cm/s)	RI
Right Ovary	**Fertile**	<25 kg/m^2 n=20	13.60 ± 7.52	4.68 ± 3.52	0.78 ± 0.12
		>25 kg/m^2 n=20	14.04 ± 6.22	4.20 ± 2.04	0.68 ± 0.22
Left Ovary		<25 kg/m^2 n=20	16.30 ± 7.94	6.49 ± 3.49	0.62 ± 0.11
		>25 kg/m^2 n=20	15.84 ± 7.68	6.91 ± 4.32	0.69 ± 0.11
Endometrium		<25 kg/m^2 n=20	17.22 ± 6.12	3.88 ± 2.10	0.74 ± 0.12
		>25 kg/m^2 n=20	17.04 ± 5.24	4.02 ± 2.62	0.70 ± 0.12
Myometrium		<25 kg/m^2 n=20	10.1 ± 6.2	3.94 ± 2.32	0.66 ± 0.10
		>25 kg/m^2 n=20	9.2 ± 6.20	4.01 ± 3.32	0.62 ± 0.12

DISCUSSION

With the advance of ultrasonography, the use of ultrasonic technique has increased as a way for tracking the changes in endometrial thickness, in assessment of the developmental potential of basal layer of endometrium. With the increased resolving power and sensitivity of ultrasonography more studies were conducted on the endometrial flow as a factor in implantation and hence in fertility. Upto-date advantages of ultrasonography including its non –invasiveness, repeatability, real time monitoring and predictivity have gained medical attention [12]. Also, the Doppler study of ovarian blood flow has been studied as the utero-ovarian anastomosis has been established to play a role in endometrial receptivity [13]. With the advent of transvaginal scanning the tracing of endometrial and myometrial flow has been possible and the ovarian vessels have been traced with greater confidence thus revolutionising the Doppler assessment of these parameters [14]. Our study re-established the fact that there is a variation in the blood flow in the ovarian vessels with the Peak Systolic Velocity and the Minimal Diastolic Velocity being higher in the infertile females on Days 2-5 of the menstrual cycle. However, the Resistivity index showed no significant variation in infertile and fertile females. Also, it is seen from the study that no significant difference in the blood flow parameters that is the PSV, MDV and RI was found in the endometrial and myometrial vessels on the days 2-5 of the menstrual cycle in contrast to definite variation observed in the follicular phase of the cycle. Hence, there is a definite relationship between unexplained/idiopathic infertility and the utero-ovarian blood flow. Our study further proved that in the reproductive age group no variation is seen in the utero-ovarian blood flow

parameters in ages above and below 30 years in infertile and fertile females. Similarly, the study has shown that the BMI above and below 25 kg/m does not affect the utero-ovarian blood flow in infertile and fertile females. Tekay *et al.,* (1995) conducted a study which showed measured ovarian artery blood flow by Doppler ultrasound, using an abdominal transducer, and by an invasive technique employing a 10 MHz directional continuous wave flow meter applied directly to the ovarian, iliac, and uterine arteries and concluded that ovarian and uterine arteries, waveforms and PI were identical for both techniques, and that the PI and RI were lower on the side with the dominant follicle which is similar to our study [15]. Taylor *et al*., (1985) established that women who are infertile for whatever cause demonstrate pulsatility and resistance indices that are significantly higher than those of fertile women. They studied and concluded that Peak systolic velocity underwent the most significant change in amenorrhoeic patients, being significantly lower in comparison with that of controls, both in the uterine (P = 0.0009) and ovarian (P = 0.001) arteries. Compared with controls, the end-diastolic velocity of the ovarian artery was significantly lower (P = 0.039) in amenorrhoeic patients, and was also lower in the uterine artery (though not statistically significantly so). A reduction in blood flow was also evident in the ovarian stroma in amenorrhoeic patients. They further established the fact that estrogen play an important role in regulating both uterine and ovarian blood flow. In a study they measured spiral artery and uterine artery PI and RI during the periovulatory period in 27 patients with spontaneous cycles and 51 patients with stimulated cycles, but parity was not stated and found consistent differences between the side with the dominant follicle and the contralateral side. Diastolic flow was absent in both uterine arteries in 15% of spontaneous cycles [16]. At their nadir the day before the average uterine artery PI and spiral artery PI were 2.22 and 1.13 respectively. No periodical variations in PI were noted in patients stimulated with clomiphene citrate or human menopausal gonadotrophin (HMG). Turnbull *et al.,* (1995**)** reported Maximum peak systolic velocity (MPSV), or simply peak systolic velocity (PSV) and minimum diastolic velocity (MDV) findings were in concordance with our study. No significant variation in the Resistivity index in the menstrual phase of the menstrual cycle was seen in their study [17]. Dina Jamal Eldeen *et al.,* (2007) performed a case control study to study the endometrial perfusion and receptivity in unexplained infertility in 35 females of unexplained infertility and 16 fertile females [18]. Our study of vascularisation of this area showed that blood flow was detected in 51.4 percent of females with unexplained infertility and 93.8 percent of control group. Mean Pulsatility Index (PI) in the subendometrial arteries, if detected was 2.65$\pm$0.37 in infertile group and 1.95$\pm$0.24 in fertile group. The Mean Peak Systolic Velocity (PSV) in subendometrial arteries in infertile group was 0.29+-0.002m/sec and in the control, group was 0.42+-0.03m/sec. The difference is statistically significant. Also, the study showed that PI, RI are higher and PSV lower in unexplained infertility group. Hence the study established the blood flow parameters as important determinants in unexplained infertility and process of conception.

CONCLUSION

It is concluded that the infertile and fertile females re-established the fact that there is a variation in the blood flow in the ovarian vessels with the Peak Systolic Velocity and the Minimal Diastolic Velocity being higher in the infertile females on Days 2-5 of the menstrual cycle. However, the Resistivity index showed no significant variation in infertile and fertile females. Also, it is deduced from the study that no significant difference in the blood flow

parameters that is the Peak Systolic Velocity, Minimal Diastolic Velocity and Resistivity Index was found in the endometrial and myometrial vessels on the Days 2-5 of the menstrual cycle in contrast to definite variation observed in the follicular phase of the cycle. Hence there is a definite relationship between unexplained/idiopathic infertility and the utero-ovarian blood flow. Also, it is proved in the study that in the reproductive age group no variation is seen in the utero-ovarian blood flow parameters in ages above and below 30 years in infertile and fertile females. Similarly, the study has shown that the Body Mass Index above and below 25 kg/m does not affect the utero-ovarian blood flow in infertile and fertile females.

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