Original Research Article

A Prospective Observational Study to Evaluate the Findings of Transvaginal Sonography and Endometrial Biopsy in Detecting Ovulation of Infertile Woman

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Abstract

Introduction: Nearly 40% of the infertile women present with ovulation-related causes and 70% of these patients can be diagnosed and treated effectively. Thus, knowing the time of ovulation is crucial in management of infertility. Transvaginal sonography (TVS) and endometrial biopsy are being used to detect ovulation in infertile women. *Aim:* To assess qualitatively the usefulness of serial TVS in detecting ovulation and compare the findings with endometrial biopsy (EB) findings. *Methods:* In thisprospective observational study on 100 infertile women, after obtaining the informed consent, detailed history and clinical examination of the patients was performed. TVS was performed on every alternate day after 11th day of menstrual cycle till the evidence of ovulation or up to 17th day of cycle and EB was done on day 1 of next menstrual cycle for histopathological evaluation of endometrium. The results of TVS and EB for detecting ovulation were compared with Chi square test. *Results:* In this study, the same 100 patients after serial TVS were subjected to EB. Ovulatory cycle was observed in 73 cases with TVS while secretory phase was detected in 63 cases in EB and proliferative phase was seen in 29 cases. Non dominant follicle was seen in 11 cases in TVS out of which only 9 cases in EB showed proliferative endometrium.

Failure to rupture of follicle was seen in 10 cases in TVS. Signs of Polycystic ovarian syndrome (PCOS) were detected in 6 cases by TVS. Thus, there is better detection rate of ovulation by TVS (sensitivity = 100%) compared to EB (P < 0.00354; Chi-square test),while specificity was greater (96%) with EB. *Conclusion:* Our observations indicate that serial TVS along with EB together can be a useful to detect ovulation in the management of infertility. In addition, TVS can also provide details of any uterine, endometrial, cervical or adnexal pathology.

Keywords: Transvaginal Sonography; Infertility; Ovulation; Secretory phase; Proliferatory phase; Endometrial Biopsy.

Introduction

World Health Organization (WHO) has described infertility as the inability to become pregnant, maintain a pregnancy, or carry a pregnancy to live birth [1]. International Committee Monitoring Assisted Reproductive Technologies (ICMART), further modified this definition as "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [2]." Infertility can be primary (non-achievement of 1st pregnancy) or secondary (non-achievement of 2nd or subsequent pregnancy) [1]. As per the National Institute for health and care excellence (NICE) guidelines, a woman of reproductive age not conceiving after 1 year of unprotected sexual intercourse, in the absence of any known cause of infertility, should be assessed and investigated along with her partner and require immediate investigation if the age of woman is above 36

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years [3]. It is known that the infertility can be due to the defects or pathology in male (33%), female (33%) or defects in both male and female (33%) [4]. Thus, nearly 66% of infertile couples have aetiological factors in women. Nearly 40% of these women have ovulatory dysfunction and 40% have tubal and adnexal causes [5,6]. As reported earlier, infertility due to ovulatory dysfunction can be diagnosed easily and has better prognosis with more than 50 % success rate [6].

In addition, ovulation is an obligatory prerequisite and assumes greater importance in assessment of infertile woman [7]. Physiologically, ovulation can be detected by the cervical mucosal changes and by the increased basal body temperature. However, these methods are less reliable and difficult to monitor. Ovulation can also be detected by the hormonal assay of serum progestorone and LH levels during menstrual cycle and are to be repeated on daily basis to detect the time of ovulation. Thus, the patient is subjected to the invasive procedures repeatedly, which are expensive and require specialized laboratory services.

Previously, the occurrence of ovulation was assessed by detecting the secretory phase of the endometrial response by endometrial biopsy. However, this is one time end stage test and cannot assess exact time of ovulation. The use of transvaginal sonography (TVS) in the diagnosis and management of infertility is increasing [8-11]. The improved resolution and better tissue textural differentiation offered by TVS makes this technique useful in monitoring ovarian follicular growth, ovulation, and corpus luteum formation [9, 10, 12]. Therefore, this study was undertaken to evaluate the predictability of serial transvaginal sonography to detect ovulation in infertile women and to compare the findings with endometrial biopsy reports.

Materials and Methods

Selection of Subjects

This prospective observational study was carried out in the Department of Obstetrics and Gynecology, MLB Medical College, Jhansi over period of 12 months from March 2015 to February 2016. Ethical clearance was obtained from the Institute Ethical clearance committee. A total of 100 subjects were chosen between the age group 18-40 years who visited the OPD with primary or secondary infertility. The sample size was calculated by using *Kappa* coefficient formula [Sample size (n) = $1/r^2(p_o-p_e)^2$; where "r" is the value of relative error taken as 0.2 (20 %); "p_o" is overall agreement

probability taken as 0.5 and "p_e" is chance of agreement probability taken as 0].

Cases with narrow introitus or stenosed vagina, pain or discomfort during TVS procedure were not included in the study. Information regarding, age, duration of marriage, educational status, income group, menstrual history, medical illness, contraceptive used, sexual and marital life, sexually transmitted diseases, previous surgical/medical treatment taken for any menstrual irregularity or infertility was obtained. General, systemic and complete gynecological examinations were done. The patients were investigated for routine blood and urine examination, husband's seminogram, VDRL, TVS follicular study and endometrial biopsy.

TVS- protocol

The convex linear array intravaginal transducer (7-9 MHz) with radius of curvature of 11.0 mm (CLA IVT 7.0) was used for TVS study. TVS study was started on 11th day of menstrual cycle to evaluate uterus, cervix, adnexa and ovarian follicle. Subsequently, TVS was done on every alternate day till the detection of ovulation or up to 17th day of cycle. Ovulation was detected by the disappearance of a dominant follicle along with appearance of free fluid in the pouch of Douglas [10,13]. Absence of ovulation was considered if there was non-development of dominant follicle up to 17th day or non-rupture of dominant follicle up to 17th day in addition to non-appearance of fluid in the pouch of Douglas as reported earlier [10,13]. Follicular size was determined during this period. Normally, 2-3 mm increase in the follicular diameter was seen on each day [10,11].

Endometrial Biopsy

Endometrial biopsy was done on 1st day of next menstrual cycle. Patients were sedated with slow intravenous injection of promethazine (phenargan; 25 mg with 10 time dilution). If the cervix was tightly closed para-cervical block was given. Patients in lithotomy position, vulva was painted and draped. Bladder was catheterized, if not already empty. Bimanual pelvic examination was done to ascertain position of uterus and cervix. Using Sims' speculum, cervix was visualized. The anterior lip of cervix was held with Vulsellum forceps. Uterine sound was passed to know the length of uterine cavity and to exclude polyp. Endometrial biopsy was taken by means of endometrial biopsy curette. Curetted tissue was fixed in 40% formalin for histopathological examination and secretory changes in the

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endometrium gave evidences of ovulation. Even though the ideal time of endometrial biopsy is from 24th to 26th day of the cycle in premenstrual phase, but in our study, the patients were mostly illiterate and were unable to comply with abstinence for sexual intercourse during the cycle. Therefore we performed the biopsy on the first day of next menstrual cycle. In addition, the curettage done in premenstrual phase prevented the chances of pregnancy loss if fertilized.

Statistical Analysis

The data were recorded in EXCEL for analysis. Number of patients showing the signs of ovulation and the percentage values were computed in TVS or EB groups. The concurrent histopathological changes were pooled and analyzed. The qualitative difference for detection of ovulation between the TVS and EB was assessed by 2 X 2 Chi-Square table and $P < 0.05$ was considered as significant.

Results

Demographic Details of Patients

In this study majority of the patients were of the age group 21-25 years (Table 1). Half of the patients (50%) belonged to low socioeconomic group and 36% belonged to middle class. Nearly 68% of the

cases were from the rural background. Only 8% of the patients were having education up to graduation or above, while 26% were illiterate and 42% were below class 10. Thus, the majority of the patients were having lower educational profile (Table 1). About 78% of them were having primary infertility (not conceived earlier). Maximum number of patients (86 %) gave history of infertility < 6 years (Table 1).

TVS Data

Table 2 shows the groupings of patients with TVS findings. The grouping was done as described in the table. The TVS findings revealed normal ovulatory cycle in 73% of patients (Group 1). Non dominant follicle was seen in 11% of cases (Group 2). In 10% of cases follicular development was seen but they did not show the signs of rupture (Group 3). In 6% of cases, PCOS picture were detected on serial transvaginal sonography (Group 4; Table 2).

EB Data

The endometrial histology of the patients showed secretory phase of endometrium (denoting ovulatory cycle) in 63% of cases and proliferatory endometrium was seen in 29% of cases (Table 3). Besides, 2% cases showed simple endometrial hyperplasia and benign cystic hyperplasia and 4% cases showed tubercular endometritis on histopathology.

Table 1: Demographic data of the patients recruited in the study

Demographic parameters	Number of patients
Age in years	
<20	11
21-25	54
26-30	23
31-35	10
36-40	2
Socioeconomic status(as described earlier)[14]	
Upper	14
Middle	36
Low	50
Residential area	
Rural	68
Urban	32
Educational status	
Illiterate	26
Primary school (Class1 to 7)	30
Lower Secondary (Class 8 to 10)	12
Higher Secondary (Class 11 to 12)	24
Graduate and postgraduate	8
Duration of infertility (years)	
1-3	53
3-6	35
6-10	8
>10	4

Table 2: Categorization of patients in various groups as per the findings in serial transvaginal sonography findings

Group	Follicular Pattern	Number of patients
Group 1	Signs of Ovulation present	73
Group 2	Non-dominant follicle and no signs of ovulation	11
Group 3	Development of dominant follicle but failing to rupture even after 28 mm size	10
Group 4	Polycystic ovarian disease	6
	Total	100

Table 3: Distribution of number cases according to the histopathological findings on endometrial biopsy tissue

Endometrial Character	Number of patients
Proliferatory	29
Secretory (denoting ovulation)	63
Simple endometrial hyperplasia	2
Benign cystic hyperplasia	2
Tubercular endometritis	4

Table 4: Endometrial biopsy findings in various TVS groups are mentioned below. The data obtained in the TVS groups was analysed according to the groups

Endometrial features by EB ↓	TVS findings as grades mentioned below				
	Group 1 (73)	Group 2 (11)	Group 3 (10)	Group 4 (6)	Total (100)
Proliferatory	6	9	9	5	29
Secretory	62	-	1	-	63
Simple Hyperplasia	1	-	-	1	2
Benign cystic hyperplasia	-	2	-	-	2
TB endometritis	4	-	-	-	4
Total	73*	11	10	6	100

Table 5: Sensitivity, specificity, positive predictive value and negative predictive value are shown in the table. The data for calculation are taken from Table 4

Parameters	TVS Data	EB Data
True Positive	67	62
False Positive	6	1
True Negative	11	23
False Negative	0	6
Senstivity (%)	100	91
Specificity (%)	65	96
Positive Predictive Value (%)	92	98
Negative Predictive Value (%)	100	79

EB Data in Comparison to Various Groups of TVS

The data of histopathological findings EB were recorded in various groups of TVS and are provided in Table 4. Secretory phase (sign of ovulation) was seen in 85% of patients of group 1 of TVS group (Table 4). In group 2 (no ovulation no dominant follicle formation), none of the patients in EB group showed secretory phase but 81% showed proliferatory endometrium. In group 3, EB report showed 90% patients having proliferative phase and only in 1 case there was secretory phase.

The TVS and EB groups for showing the absence or presence of the ovulatory signs were analysed using 2X2 tables for Chi-square analysis (73% in TVS and 63% in EB, non–ovulators were 11% and 29% for TVS and EB, respectively). There was significant difference in ovulation detection in TVS and EB group (P < 0.00354, Chi-square test). The findings indicate qualitative superiority of TVS over EB for prediction of ovulation.

Further we analysed the TVS and EB data for sensitivity and specificity in Table 5. Our analysis revealed 100% sensitivity in TVS data as compared to 91% sensitivity in EB data. Even the negative predictive value of TVS data was 100% as compared to 79% in EB data. On the other hand, EB data was found to have greater specificity.

Group 1- Follicle showing the signs of Ovulation by TVS. Group 2- Detection of no dominant follicle. No signs of OvulationGroup 3- Dominant follicle

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failed to rupture even after size ≥ 28mm. No signs of ovulation. Group 4- Polycystic ovarian disease.

Discussion

In the present study, we were able to detect the ovulation in greater number of cases using serial TVS than seen with endometrial biopsy. Our observations are consistent with Yee et al [15] where they have shown the positive correlation of number of follicles detected by TVS with the laparoscopic findings [15] and also with others where they reported increase in diameter of the ovarian follicle can be seen in from day 10 onwards of menstruation [10,11]. Further, TVS has been successfully used to identify the endometrial receptivity in infertile women [16].

Serial TVS testing is a non-invasive and OPD procedure. In addition to follicular changes (for ovulation), this investigation allows us to detect uterine, endometrial, cervical or adnexal pathology and fluid collection in pouch of Douglas fairly and accurately [7, 8, 10, 15]. TVS is also shown to provide better indicator of uterine cavity abnormalities in abnormal uterine bleeding [17]. Similarly, TVS was fairly accurate in detecting uterine cavity abnormalities in infertile women [18]. Further, follicular growth can be monitored on daily basis (real time) by TVS. When we compared TVS with endometrial biopsy for detection of ovulation, we found that TVS is a more accurate diagnostic tool for infertility (with 100 % sensitivity). Also, TVS can be performed in a pregnant or could be pregnant woman without the losing a pregnancy [19]. TVS can also be used to assess the ovulation after medication with ovulation-inducing drugs in infertile women or in women opting for in vitro fertilization [20]. However, endometrial biopsy has an inherent limitation and it does not directly evaluate the ovulation rather would detect the hormonal responses of endometrium produced after ovulation. In our study, one patient showed secretory changes with EB but did not show the ovulation in serial TVS.

Serial TVS has an added advantage for continuous monitoring of the growth of the follicle and to detect the time of ovulation. In nearly 10% of cases, the ovum attained a maturity but was not able to rupture. Similar phenomenon was described earlier [10]. They described it on the basis of decreased blood flow and vascularity to the follicle leading to failure of progesterone clearance [10]. In such situation, hormonal assay to assess the LH activity is of greater value. It was shown that the TVS findings of ovulation and serum LH levels were peaked in 92% of the individuals [21]. Considering these findings, ovulation can be induced by increasing LH activity endogenously or exogenously to trigger the ovulation in group 3 patients. This could be achieved by optimally timed injection of hCG 5000-10000 IU as suggested elsewhere [20]. Thus, these patients can easily be treated.

In 6 of 73 patients (8-9%) showing ovulation by TVS did not show secretory changes by endometrial biopsy. This indicates possible endometrial pathology such as tuberculosis or endometrial hyperplasia. This is supported by the fact that 4 of 73 patients showing ovulation by TVS, showed tubercular endometritis. In this situation antitubercular treatment may restore the uterine functions. Thus, it is advisable to perform endometrial biopsy to rule out the endometrial pathology. Such observations are made in earlier studies on abnormal uterine bleedin or in detecting uterine cavity abnormalities in infertile women using TVS along with hysterscoy or endometrial biopsy [17,18,22]. These authors suggested that greater information about the uterine pathology can be obtained by combining the data with TVS and other tests [17,18,22].

Limitations of the Study

The follow up of patients in terms of infertility treatment in ovulating woman would have given the outcome of the investigations. However in our study, follow up of patients was not feasible as the majority of the patients were from rural background belonging to low socioeconomic group and lower educational profile (Table 1).

Secondly, the correlation of hormonal profile along with the TVS data would have been ideal. This could not be achieved because of the hormonal assays were expensive and would have to be repeated several times to correlate with the TVS changes.

Conclusion

Our observations reveal that sensitivity of TVS was 100% to detect ovulation. However, the specificity of EB data greater than TVS. Considering these observations, it is concluded that TVS findings are able to detect the ovulation in infertile woman at higher rate. Further, combination of TVS and endometrial biopsy results when taken together will provide more reliable indication for the detection of ovulation thus can be useful in the management of infertility.

Conflict of Interest

None

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