ORIGINAL ARTICLE

Histopathological study of endometrial biopsy in infertility: A cross sectional study in a teaching hospital

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Abstract

Background: Endometrium is dynamic tissue which responds to hormones hence is the most sensitive indicator of ovarian function. Uterine pathology and cervical pathology like chronic infections or quality of cervical mucus plays a role in failure of conception. Hence endometrial biopsy is one of the most important investigations in infertility. Aim and Objectives: To document the morphological changes seen in endometrial biopsies and their demographic distribution in patients with infertility. Secondly to examine cervico-vaginal pap smear changes in infertility cases. Material and Methods: The study is a prospective cross-sectional study carried out during the period of June 2018 to July 2020. Married women visiting infertility clinic, who have undergone endometrial biopsy as an infertility evaluation were included in the study. Informed consent for the procedure was taken. Only premenstrual endometrial biopsy was included and inadequate biopsy samples were excluded from the study. Cervico-vaginal pap smear of these cases was processed under liquid-based cytology and reported using Bethesda system for cervical cytology. The histopathological findings and pap smear findings were statistically analyzed. Results: Ten percent of endometrial biopsies were indicated in evaluation of infertility among all biopsy registries. Primary infertility accounted 64% and secondary infertility as 36%. Sixty-six cases of endometrial biopsy were studied during study period. The most common histopathological findings were secretory endometrium, followed by proliferative (anovulatory and 2 cases luteal phase defect) endometrium, progestin induced changes, endometrial polyps, chronic endometritis and benign hyperplasia. Seventy-seven percentage of pap smears were negative for intraepithelial lesions (no squamous or endocervical abnormalities), predominantly showing signs of cervicitis and bacterial vaginitis. Conclusion: Histopathological study of endometrium forms an important, safe and cheaper diagnostic tool. Uterine pathology contributes to major pathogenesis of infertility and thus endometrial biopsy plays a significant role in preliminary evaluation of cases with infertility.

Keywords: Endometrial Biopsy, Pap Smear, Infertility

Introduction

Infertility is a global public health problem due to its complexity and the difficulty in diagnosing, treating and preventing it. The incidence of infertility ranges from 8-12% [1]. In India, 10.2 million couples are infertile. According to WHO, prevalence of infertility in India is between 3.9 to 16.8% [2]. Infertility results from physiological and pathological factors. Inspite of many investigatory tools available endometrial histology is a sensitive indicator of ovarian function which reflects changes based on hormones like estrogen and progesterone. Premenstrual endometrial biopsy plays an important diagnostic role in cases of infertility to rule out common uterine pathology [1]. The purpose of investigating the infertile couple is to assess their chance of achieving a pregnancy and to identify the factors amenable to treatment [2]. It also yields valuable supplementary information about the utero-ovarian endocrine relation of the particular woman [3]. Endometrial biopsies are obtained for a number of reasons that include abnormal uterine bleeding in certain age groups, incomplete abortions, or suspected neoplasia and the endometrium may be sampled prior to certain procedures to treat infertility to determine the phase of the cycle to guide further tests or treatments [4].

Study done by author Pradhan *et al.* [2] and Asuzu *et al.* [4] showed infertility as the most common indication for endometrial biopsy and secretory phase endometrium as the commonest morphologic pattern encountered. Lesions like endometrial polyps were found among other causes to be the most common structural cause of abnormal uterine bleeding. These findings were of particular importance in patients being considered for *in-vitro* fertilization.

Almost all functional disturbances involved in infertility result in morphological changes in the endometrium since hormone levels fluctuate depending upon various biorhythms, the histological examination of the endometrial biopsy is the most reliable parameter for evaluating the cause of infertility [3].

Embryo implantation is considered as the last barrier in assisted reproductive technology. Inadequate uterine endometrial receptivity is responsible for approximately two-thirds of implantation failures. Hence intensive research work has been performed to understand the physiology, regulation, and the clinical assessments of the uterine receptivity to improve the success rate of *in-vitro* fertilization and embryo transfer. In this regards the cervix is considered as the 1st indirect site to assess uterine receptivity thus emphasizing pap smear examination in cases of infertility [5].

The present study is carried out to study the morphological variations in endometrial biopsies done in patients with infertility. Secondly to study cervico-vaginal pap smear changes in infertility cases.

Material and Methods

The study is a prospective cross-sectional study carried out during the period of June 2018 to July 2020. Married women visiting infertility clinic, who have undergone endometrial biopsy as an infertility evaluation were included in the study. Informed consent for the procedure was taken. Among the biopsies only premenstrual endometrial biopsy specimens were included in the study. Inadequate biopsy samples were excluded from the study.

The ethical clearance for the study was obtained number SDMCDSIECNO 2021/Medical/ Patholoy/ PG/09. The endometrial specimen once received in histopathology laboratory was processed under standard operating procedures of the laboratory. Formalin fixed paraffin embedded sections of 5-micron thickness was taken. Haematoxylin and Eosin stains were used for staining the slides, special stains like Ziehl Neelsen stain were used for tuberculosis in indicated cases. Clinical details like age, abnormal uterine functioning symptoms and first-time hormonal assay done during infertility evaluation was recorded and this information was obtained from hospital medical records and laboratory information system. Tuberculosis PCR (TB-PCR) done on biopsies were noted. Cervico-vaginal pap smear of these cases was processed under liquid-based cytology and reported using Bethesda system for cervical cytology. The histopathological findings and pap smear findings were statistically analyzed as descriptive analysis using mean and standard deviation using SPSS version 20.4.

Results

A total of 650 endometrial biopsies were done during study period and among 650, 66 females underwent endometrial biopsy for infertility which accounts 10% of total biopsies done for infertility evaluation in the hospital. Amongst 66 cases of infertility, 42 (64%) were of primary infertility, 24 (36%) cases were of secondary infertility. The 64 (42%) cases of patient were in the age range of 30-40 years. The youngest patient was 20 year old and the oldest was 49 year. The mean age was 35.6 year (Fig. 1).

The histomorphological findings of the endometrium were secretory endometrium as most common finding which accounted for 32 (41.5%) cases. Most of the cases of secretory endometrium were seen in a range of 30-40 years. The next common histopathological finding was proliferative endometrium, accounts for 16 (20%) cases. This was closely followed by progesterone (external hormone therapy) induced endometrium which accounted for 13 (16%) cases. Of the 16 cases of proliferative endometrium seen in this

study, one case, who was 36-year-old female was diagnosed as chronic granulomatous endometritis (tuberculosis etiology confirmed by TB PCR). The other findings were polyp, endometritis, products of concept and hyperplasia. Eight cases accounted for endometrial polyp (Fig. 1), five cases found in the age group of 20-30 years, 2 cases in age group 30-40 years and only 1 case of 42-year-old lady. Only four cases of endometritis were seen. Two of these were in the age group 20-30 years and other two is from 30-40 years age group. Of the 4 cases of endometritis seen in this study, one was a case of secondary infertility with tubal block seen in a 31year-old woman. Chronic endometritis was confirmed with CD138 immunohistochemistry positivity (Fig. 2). There were 4 cases of benign hyperplasia(simple hyperplasia without atypia, (Fig. 3) found in a women age group 30-40 years. 1 case of menstrual endometrium seen in a 41-yearold woman with secondary infertility and 1 case was of retained products of conception seen in 30year women (Fig. 4). TB PCR was performed on 22 cases as they were clinically suspected for tuberculosis and out of 22 cases, 21 cases were negative and 1 was positive for tuberculosis. Cervical PAP smear was done in 38 individuals out of 66 cases. Most of the cases were NILM (77%) in the women age group 30-40 years (71%). In those 5 cases were of bacterial vaginosis (17% of NILM) and 6 were candidiasis (21% of NILM). Six cases (16%) are of reactive inflammatory changes and rest 2 cases (5.5%) were unsatisfactory (Table 1).







HISTOPATHOLOGICAL DIAGNOSIS IN RELATION TO AGE

Figure 2: Bar diagram showing morphological changes in endometrial biopsy



Endometrium appears polypoidal

Irregular glands, thick walled blood vessels

Figure 3: Hyperplastic endometrial polyp



Few plasma cells and few decidualized cells



IHC: CD 138 Positive in plasma cells (Grade 1)

Figure 4: Secretory endometrium with chronic endometritis



Figure 5: Benign endometrial hyperplasia (Simple hyperplasia without atypia)

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Table 1: Age wise distribution in PAP interpretation										
PAP interpretation	Age wise	Percentage								
	20 - 30	30 - 40	40 - 50							
NILM	6	20	4	77						
Reactive changes (Inflammatory)	0	5	1	16						
Unsatisfactory	2	0	0	5.5						

Discussion

Human endometrium is the most favored site for the implantation of fertilized ovum. Present study evaluated the adequacy of endometrial development based on correlation of menstrual history with glandular and stromal morphology [2]. Nonovulatory cycles are quite common in cases of infertility as have been observed by different workers with variable frequency. The incidence of anovulatory cycle and abnormal endometrium was high in cases with irregular cycle [6].

Adequate follicular development and functionally efficient corpus luteum formation are the essential prerequisites for the preparation of good endometrial bed which is essential for successful implantation of blastocyst and continuation of pregnancy [1].

Interpretation of endometrial biopsy specimens requires a complete and accurate clinicalhistory, menstrual status, and the date of last menstrual period, along with history of exogenous hormones or drugs [7]. In our study of 66 cases of infertility, 64% were of primary infertility and 36% cases were of secondary infertility. The maximum numbers of patient were in the age range of 30-40 years (64%) irrespective of type of infertility. The youngest patient was 20 year old and the oldest was 49 year. Our findings correlated well with study done by Pradhan *et al.* [2] who observed 85% cases of primary infertility, Asuzu *et al.* [4] noticed 73% cases of as primary infertility, Nandedkar *et al.* [3] had 89% primary infertility and Kaur *et al.* [7] observed 77% primary infertility.

The secretory phase of endometrium in the premenstrual period is indicative of ovulation and thus it rules out anovulation as a cause of infertility but, luteal phase defect may be the cause of infertility in ovulatory cycles. The diagnosis of luteal phase defect is done by Jones criteria, which is defined as lag of more than two days in histological development of endometrium compared to the day of the cycle [8]. Some studies showed infertility as the most common indication for endometrial biopsy and secretory phase endometrium as the commonest morphologic pattern encountered [4].

To discuss certain common histopathological findings in endometrium biopsies are follows:

Secretory endometrium: biopsy tissues display subnuclear vacuolation of epithelium indicative of ovulation and thus it rules out anovulation as a causative factor of infertility. When the premenstrual endometrial biopsy shows normal secretory phase corresponding to the day of the cycle then it indicates that cause of infertility is not in the endometrium [9].

Proliferative endometrium: Anovulatory cycle is quite common in cases of infertility. Anovulatory cycle is due to functional disturbances in hypothalamus, pituitary, ovarian or endometrium. The endometrium may fail to respond because of lack of progesterone receptors [9]. Progestins or oral contraceptive induced changes: the endometrial response depends on duration of hormone exposure. The effects of progestins are placed into three general morphological patterns for better understanding of the entire spectrum of progestin induced changes. these patterns are [9].

Decidual changes: abundant tissue on curettage with many glands predominantly inactive and stroma appears decidualized with lymphoid infiltrate and vascular ectasia seen.

Secretory changes: moderate amount of tissue with mild tortuous glands lined by columnar cells and plump stromal cells and vascular ectasia.

Inactive changes: sparse tissue with small inactive glands and variable amount of stroma.

Endometritis: It is a persistent inflammation of the endometrial mucosa often caused by the pathogens ascending into uterine cavity. Common infection includes *Chlamydia* mycoplasma/ ureaplasma, *Entercoccus faecalis, Escherichia coli* and *Streptococcus agalactiae*. Chronic endometritis has also been frequently identified during the investigation and workup for infertility, recurrent miscarriages and *in-vitro* fertilization embryo transfer with implantation failure. Endometrial inflammation often is non-specific and rarely has morphologic features that indicate definite etiology. Plasma cells are the critical histologic feature for the diagnosis of endometritis. The morphologic features of non-specific endometritis and plasma cell infiltrate along with lymphocytes, lymphoid aggregates and variable presence of neutrophils in surface epithelium and glands. There are reactive stromal changes and altered gland development and breakdown also noted [10-13].

Polyps: Endometrial polyp appear to originate from localized overgrowth of the basalis with glands and stroma participating in the lesion [14]. They have been implicated as a possible cause of infertility, either by physically interfering with blastocyst implantation or altering the development of secretary phase endometrium, making it less receptive to the implanting embryo [15-18]. Polyps are variable in size, single or multiple, sessile or pedunculated. The glands and stroma of endometrial polyps show diverse histologic patterns hence for practical purpose polyps are divided into benign polyps which include proliferative/hyperplastic polyp, atrophic polyp, functional polyp, mixed endometrial and endocervical polyp, adenomyomatous polyp and atypical polypoidal adenomyoma. The histologic features include large tissue fragments, polypoidal shape with three side's surface epithelia. The stroma is dense has thick-walled arteries. The Glands showing more irregularity, tortuosity and dilatation. These glands are out of phase of menstrual cycle or show hyperplastic changes [19-20].

Hyperplasia: WHO classification subdivided hyperplasia into four categories according to their nuclear alterations and degree of architectural crowding defined by the extend of back-to-back glandular crowding. They are simple hyperplasia with or without atypia and complex hyperplasia with or without atypia. The morphological features hyperplasia without atypia includes nuclear pseudostratification, uniform chromatin distribution, small to indistinct nucleoli and variable amount of mitotic figure. Glands are irregular branching, infolding and outpouching noted these glands are haphazardly spaced in abundant stroma. Morphological features of atypical hyperplasia include nuclear stratification with loss of nuclear polarity. Enlarged irregular nucleus and coarsening of chromatin creating vesiculate nucleus and prominent nucleoli. glands show overcrowding, and closely packed in stroma with highly irregular outlines [21-24].

The various endometrial histopathological patterns in the different Indian studies have been compared in the Table 2. In the current study, secretory phase was seen in 41 % of infertility cases. Secretory phase reported by Ahmed *et al.* [25] (40%) which are nearly similar with our study. Pradhan *et al.* [2], Sharma *et al.* [1], Abbasi *et al.* [6] and Desai *et al.* [24] reported secretory endometrium in 64%, 66%, 64%, 63% respectively, was higher than in our study and Kaur *et al.* [7] and Asuzu *et al.* [4] reported less number of secretory phase (8% and 16%) compared to our study. Twenty percent of our patients showed proliferative endometrium and 5% cases were chronic enodmetritis. This observation is in concordance with results obtained by Sharma *et al.* [1], Desai *et al.* [24], Pradhan *et al.* [2] and Abbasi *et al.* [6], who all had 3-4% of cases of endomteritis and 15-18% cases of endometrium

Endometrial polyps were seen in 9% of our patients in concordance with study done by Asuzu *et al.* [4]. In the current study, benign hyperplasia of endometrium was seen in 5% of cases. Similar

in proliferative phase.

Various Studies	Histopathological patterns								
	Secretary endometrium	Proliferative endometrium	Endometritis	Bengign hyperplasia	polyp	Product of concepts	Progestin induced changes/pill endometrium		
Pradhan et al. [2]	64	29	3	3	-	-	-		
Abbasi <i>et al</i> . [6]	66	30	4	-	-	-	-		
Sharma <i>et al</i> . [1]	64	28	2	6	-	-	-		
Asuzu <i>et al.</i> [4]	8	9	4	9	9	60	-		
Desai <i>et al.</i> [24]	63	28	4	5	-	-	-		
Kaur <i>et al.</i> [7]	17	69	3	-	-	12	-		
Ahmed et al. [25]	40	41	2	10	-	-	-		
Present study	32	16	4	4	8	1	13		

 Table 2: Comparison of various endometrial histopathological patterns in the different Indian studies

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low incidences (5%) have been obtained in studies done by Desai *et al.* [24]. A comparable study by Asuzu *et al.* [4] and Ahmed *et al.* [25] reported benign hyperplasia (9% and 10%) was slightly higher than in our study. Identification of endometrial hyperplasia is important as it is thought to be precursor of endometrial carcinoma. Out of the 66 cases, 1.2% was diagnosed as products of conception. Higher percentages (60%) were seen in studies by Asuzu *et al.* [4], 16% of our patients presenting with AUB showed pill endometrium on histopathology.

Granulomatous inflammation of the endometrium is infrequent and often caused by mycobacterium tuberculosis and infection usually indicates systemic disease. The granulomatous response is variable, often the granulomas are non-necrotising and surrounding stroma can show lymphocytic infiltration. Acid fast stains rarely demonstrate characteristic bacilli and thus culture of fresh tissue or RT-PCR of paraffin embedded tissue may be needed to establish the diagnosis. other infection of granulomatous inflammation includes cryptococcosis, coccidioidomycosis, blastomycosis and CMV [9].

In our study, tuberculous endometritis was found in 1.51% of cases of primary infertility on histological basis. In tubercular lesions, discrete granulomas consisting of epithelioid cells, Langhans giant cells, lymphocytes and few plasma cells without central caseation were observed in histosections. The corresponding results of tubercular endometritis found by Desai *et al.* [24], Schaefer *et al.* [26], Gupta *et al.* [27], Sareen *et al.* [28], Sabharwal *et al.* [29], Shastrabudhe *et al.* [30] were 3.5%, 5.1%, 8.7%, 2%, 1.34% and 2.6%, respectively, which correlated with our study (Table 2). A low

prevalence of tuberculous endometritis and endometrial carcinoma was also noted in similar studies [24, 26-30]. TB-PCR done is 22 cases (clinically suspected) out of 66 cases. Out of 22 cases 21 is negative and 1 is positive.

Cervicitis is an inflammation of the cervix and can be an acute or chronic condition and if untreated it may lead to endometritis and infertility [31]. Pelvic Inflammatory Disease (PID) and infections of female reproductive system that is often caused by bacteria resulting from a common Sexually Transmitted Infection (STI), such as gonorrhea or Chlamydia also may cause infertility. Chronic infections in the cervix can also reduce the amount or quality of cervical mucus, the sticky or slippery substance that collects on the cervix and in the vagina. Reduced amount or quality of cervical mucus can make it difficult for women to get pregnant [32-33].

In the study done by Hamont et al., abnormal cytology (i.e., Borderline Nuclear Changes: BNC) was observed significantly more often in women eligible for IVF treatment (the cases) as compared with the women in the population-based screening program for cervical cancer (the controls) (6.1 and 3.9%, respectively) (chi-square test, p < 0.02) [34]. Present study has noticed 77% cervical smears to be negative for intraepithelial lesions with no squamous or endocervical abnormalities. Predominantly cases showed signs of cervicitis or shift in flora *i.e.*, bacterial vaginitis as shown in Table 1. Study done by Quasim et al. [5] entitled "assessment of cervical cytomorphological changes in infertile women undergoing IVF/ICSI" shows significant correlation between positive cervicitis and negative pregnancy test (P value = 0.04) thus highlighting the inflammatory findings in cervix which could be associated cause for infertility.

Conclusion

Present study concludes that secretary phase was predominant finding yet other pathological causes identified were endometritis, hyperplasia, polyp and hormone changes which can be addressed in infertility. Cervical smear examination by screening method also helps in identifying the associated causes for infertility which can be managed immediately. Thus, endometrial biopsy plays a significant role in diagnosing uterine pathological conditions, hence present study recommends for endometrial biopsy in preliminary evaluation of infertility cases.

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