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MORPHOLOGICAL PATTERNS OF ENDOMETRIUM IN INFERTILE WOMAN-A PROSPECTIVE STUDY

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ABSTRACT

Background and Objective: Infertility is a global health problem because 8-12% of all couples worldwide, around 80 million are infertile. Endometrium is the most sensitive indicator of ovarian function. Endometrial biopsy with routine hematoxylin and eosin staining is an important investigation in the Indian Scenario. Therefore the present study has undertaken to investigate the morphological patterns of endometrium in infertile women. **Materials and Methods**: This study was carried out in the Department of Pathology, Bangalore Medical College after the ethical clearance from the institutional ethics committee. The study included 90 cases with complaints of infertility (primary / secondary). The premenstrual D & C was done to obtain endometrial biopsy. The endometrial tissue was fixed; processed, sectioned and performed Hematoxylin & Eosin stain for morphology of endometrium, dating of endometrium and Ziehl-Neelsen (ZN) stain for acid fast bacilli was also performed. In this descriptive analysis, values are mentioned as percentages. **Result**: In the present study, we also observed 2 cases of Tuberculosis (TB), 5 cases exhibited cystic hyperplasia and only a single case of endometrial polyp was seen. But, the percentage of cases of secretory phase was more. Simple cystic hyperplasia was seen in 3 cases of primary and 2 cases of secondary infertility. **Conclusion**: In the present study we concluded that the anovulatory endometrium, simple cystic hyperplasia and luteal phase defect formed the major etiological basis of many cases of infertility and the tuberculosis endometritis and endometrial polyp formed the minor group of etiology.

Key words: Infertility, Endometrial Biopsy, Luteal phase defect, Tuberculosis endometritis, Endometrial hyperplasia.

INTRODUCTION

The ability to reproduce and to perpetuate the species is one of the most remarkable features of living system. Reproduction is the basic expectation of life and fertility in our culture stands for reproductive growth and continuity of species. Infertility is defined as inability of a couple to achieve conception after one year of unprotected coitus[1]. Primary infertility is failure to conceive at all whereas secondary infertility is failure to conceive after having borne a child or abortion. The problem of infertility has been with us as long as the recorded history of mankind. Infertility is a global health problem because 8-12% of all couples worldwide, around 80 million are infertile. In India because of population explosion, problem of infertility is sidelined. Around 10-15% of couples are infertile in India**.** This problem is compounded by the trend towards delayed child bearing to achieve socio-economic, educational and professional goals. Infertility is a life crisis, in some communities; it is attributed to witchcraft, punishment from god or angry of ancestors and even for compulsory suicide.

The purpose of investigating an infertile couple is to assess their chance of achieving a pregnancy and to identify the factors amenable for treatment. The causes of infertility may be due to male factor, female factor or both. Female infertility may occur due to disturbances involving any part of genital system or part of central nervous system that control the ovaries hormonally. Of these intrinsic and functional abnormalities of the endometrium contribute significantly[2].

The surface of the endometrium is lined with a single columnar lining epithelium overlaying the thick lamina propria. The epithelium extends down into the connective tissue of the lamina propria and forms long, tubular uterine glands. In the proliferative phase, the uterine glands usually are straight in the superficial portion of the endometrium, but they may exhibit branching in the deeper regions near the myometrium. As a result numerous uterine glands are seen in cross-section[3].

During the menstrual cycle, the endometrium exhibits morphological changes that directly correlate with ovarian function. The cyclic changes in a nonpregnant uterus are divided into three distinct phases: the proliferative (follicular) phase, the secretory (luteal) phase and the menstrual phase. During the proliferative phase of the cycle and under the influence of ovarian estrogen, the stratum functionalis increases in thickness, and the glands elongate and follow a straight course to the surface. The lamina propria in the upper regions of the endometrium is cellular and resembles mesenchymal tissue. The connective tissue in the basalis layer is more compact and appears darker. The endometrium continues to develop during the proliferative phase because of the increasing levels of estrogen secreted by the developing ovarian follicles.

The secretory (luteal) phase of the menstrual cycle is initiated after ovulation of the mature follicle. The additional changes in the endometrium result from the influence of both estrogen and progesterone secreted by the functioning corpus luteum. As a result, the functionalis layer and the basalis layer of the endometrium become thicker as a result of increased glandular secretion and edema in the lamina propria. The epithelium of the glands undergo hypertrophy (enlarge) because of increased accumulation of the secretory product. The glands also become highly coiled (tortuous), and their lumen becomes dilated and nutritive secretory material that is rich in carbohydrates (glycogen). The coiled arteries continue to extend into the upper portion of the endometrium (functionalis layer) and become prominent because of their thicker walls.

If fertilization of the ovum & implantation of the embryo doesn't occur, the uterus enters the menstrual phase. During the menstrual phase, the endometrium in the functionalis layer degenerates and is shed. The shed endometrium contains fragments of disintegrated stroma, blood clots, and uterine glands. Some of the intact uterine glands are filled with blood. In the deeper layers of the endometrium the basalis layer, the bases of the uterine glands remain intact during the shedding of functionalis layer and the menstrual flow. The endometrial stroma of most of the functionalis layer contains aggregations of erythrocytes that have been extruded from the torn and disintegrating blood vessels. In addition the endometrial stroma exhibits the infiltration of lymphocytes and neutrophils. Dating of the endometrium by its histological appearance is helpful clinically to assess hormonal status, document ovulation, determine causes of endometrial bleeding and infertility[4]. It is impossible to precisely date the endometrium in the proliferative phase since the duration may vary between 10-20 days even under physiological conditions. Therefore, the proliferative phase is divided into three stages: early, mid & late proliferative phases. In contrast, the secretory phase can be fairly precisely related to the day of the cycle.

Though Luteal phase defect is a controversial entity, evaluation of the LPD and treatment is a wide spread practice. LPD is characterized by a defect in which the endometrium either is not exposed to enough progesterone or does not respond properly to the progesterone that is produced. As a result the endometrium does not undergo the changes necessary to allow implantation to occur. However one must add that many investigators have failed to find any correlation between luteal phase defects and fecundity in infertile women.

Tuberculosis endometritis is generally associated with a tuberculosis salphingitis. Very rarely it could be due to a hematogenous spread of a pulmonary focus. Histologically the extent of the inflammation may vary profoundly. The findings may be diffuse or focal stromal infiltration of lymphocytes and plasma cells with involvement and destruction of the glands, or classical tubercular granulomas characterized by focal collections of epithelioid cells, a few Langhan's type of giant cells surrounded by a cuff of lymphocytes. Histological picture is suspicious TB if the focal mononuclear infiltrate, inflammatory cell in lumina of glands, dense stroma & foci of necrosis or calcification is seen.

Endometrial hyperplasia represents biologic and morphologic changes ranging from an exaggerated physiologic state to a carcinoma –in situ. Basically they evolve in a proliferative endometrium resulting from unopposed, prolonged estrogen stimulation. Histologically in simple cystic (without atypia) there are enlarged cystically dilated glands with variable size and shape with an increased gland: stroma ratio. In complex adenomatous (without atypia) there is papillary infoldings and budding and crowding of glands. In simple cystic and complex adenomatous with atypia there is cytological atypia represented by enlarged nuclei of variable size and shape and loss of polarity with an increase in nucleo-cytoplasmic ratio, prominent nucleoli, irregular clumping of chromatin and parachromatin clearing. Progression to cancer increases with the degree of atypia[5].

Endometrium is the most sensitive indicator of ovarian function. The premenstrual endometrial biopsy is useful in identifying anovulatory cycles, luteal phase defects and infections specially tuberculosis and also in confirming ovulation. Endometrial biopsy with routine hematoxylin and eosin staining is an important investigation because; endometrial biopsy can be practiced in developing countries like India, where complex expensive immunological and hormone assay procedures are not easily available and/or affordable[3]. Therefore the present study has undertaken to investigate the morphological patterns of endometrium in infertile women.

MATERIALS AND METHODS

The present prospective study was carried out in the Department of Pathology, Bangalore Medical College, Bangalore after the ethical clearance from the institutional ethics committee. The study included 90 cases with complaints of infertility (primary / secondary) who were referred to Pathology Department, from Gynecology Departments of Vani Vilas Hospital, Bowring & Lady Curzon Hospital and other hospitals attached to Bangalore Medical College. The patients who failed to conceive after one year of unprotected coitus following marriage were investigated as cases of primary infertility and the patients who failed to conceive after having achieved a previous conception were investigated as cases of secondary infertility.

The patients with complaints of infertility (Primary / Secondary) are included in the present study whereas, those with complaints of infertility within one year of marriage, non co-operative patients and Male factors causing infertility are seen (Semen analysis – abnormal) are excluded from the study.

Before examining the case, their husband's semen analysis was done routinely to rule out the male factors causing infertility. The case was included for the study only if it was a normal semen analysis. Detailed clinical history in the form of menstrual cycle, Last menstrual period (LMP), age at marriage and obstetric history (in secondary infertility) were obtained. The procedure of Dilatation & Curettage (D&C) was explained and informed consent was taken from the entire patient. The premenstrual D & C was done to obtain endometrial biopsy. D & C was done any time in cases of Amenorrhea and prolonged bleeding. The endometrial tissue was fixed in 10% formalin for 24 hours and routinely processed. 5-6 micron sections were cut and performed Hematoxylin & Eosin stain for morphology of endometrium & dating of endometrium (Noye's criteria used) [6] and Ziehl-Neelsen (ZN) stain for acid fast bacilli in all suspected cases.

The present prospective study was a descriptive study and the values are mentioned in percentages.

RESULTS

The various morphological patterns of endometrium in infertility patients belonging to secretory phase, proliferative phase and hyperplasia were documented in the present study. In a total of 90 endometrial biopsies studied, 74 (82.2%) cases belonged to primary infertility and the remaining 16 (17.8%) cases to secondary infertility (Table-1). In present study a total of 90 cases were studied. Out of which 74 cases were primary infertility and 16 cases were secondary infertility. Majority of cases belonged to primary infertility. All secondary infertility cases have a previous history of conception.

Table 1: The different type of Infertility patients. n=90

Type of infertility	Number	Percentage
Primary	74	82.2
Secondary	16	17.8
Total	90	100.0
Inference	Majority of cases are of primary infertility.	

Data pertaining to age distribution in different infertile patients were given in Table-2. The youngest patient seen was 18 years old & eldest was 38 years in primary infertility cases with an average age of 24.2 years. The youngest patient seen was 25 years old & eldest was 36 years in secondary infertility cases with an average age of 29.75 years. In primary infertility group maximum cases 43 (58.2%) belonged to the age group of 21-25 years. In secondary infertility group maximum cases 11 (68.7%) belonged to the age group of 26-30 years.

Table 2: Age distribution among primary and secondary infertility patients. n=90

Age in years	Primary		Secondary	
	Number	Percentage	Number	Percentage
18-20	7	9.4	-	-
21-25	43	58.2	1	6.3
26-30	19	25.7	11	68.7
31-35	4	5.4	3	18.7
36-40	1	1.3	1	6.3
Total	74	100.0	16	100.0
Inference	Majority of primary infertility patients were in the age group of 21-25 years and that of secondary were in 26-30 years.			

The inflammatory conditions like non specific endometritis and TB endometritis were also included. In the present study, we observed the percentage of cases of secretory phase were maximum (Table-3). Among secretory phase there were 6 cases (11.7%) of early secretory phase, 17 cases of mid secretory phase (33.3%) and 28 cases of late secretory phase (55%).
Proliferative (anovulatory) endometrium was seen in 25 (27.8%) cases of primary infertility & 4 (25%) cases of secondary infertility. Proliferative endometrium (anovulatory cycle) is the major cause of infertility (Table-4). The secretory phase endometrium in premenstrual biopsy indicates that the cycle is ovulatory and indirectly rules out all the cause of infertility related to ovulation.

Table-3: Percentage of cases showing different phases of menstruation and different types of hyperplasia. n =90.

Type of endometrium	Number of cases	Percentage
Secretory phase	51	56.7
Proliferative phase (anovulatory cycle)	29	32.3
TB endometrium	2	2.2
Simple cystic hyperplasia	5	5.5
Endometrial polyp	1	1.1
Non specific endometritis	2	2.2
Total	90	100

Table-4: Morphological patterns of endometrium in primary & secondary infertility.
n =90.

Type of endometrium	Primary infertility		Secondary infertility	
	No. of cases	Percentage	No. of cases	Percentage
Secretory phase	42	46.7	9	56.2
Proliferative phase	25	27.8	4	25.0
TB endometritis	1	1.4	1	6.3
Simple cystic hyperplasia	3	4.0	2	12.5
Polyp	1	1.4	-	-
Non specific endometritis	2	2.7	-	-
Total	74	100	16	100

The dating of endometrium was done for diagnosis of luteal phase defect as per Noye's criteria[5].The diagnosis of LPD was made as directed by JONES criteria which considers LPD as a lag by two or more than two days in the histological development of the endometrium (Table-5). We have taken more than two days as the criteria for LPD as normal women can also have an occasional abnormal cycle which lags by 2 days and we found that the majority of cases coincided with LMP.

Table-5: Luteal phase defect in both primary and secondary infertility cases.
n =90.

Morphology of endometrium	Number of cases	Percentage
Coincides with LMP	41	80
Lag by 3-4 days	3	6
Lag by 5-6 days	5	10
Lag by 7 or >7 days	2	4
Total	51	100

In the present study there were 2 cases of Tuberculosis (TB). ZN stain was done for AFB in those 2 cases and another 3 cases clinically and histological suspected cases of TB. But, we found that all cases were negative for acid fast bacilli. We also observed, out of 90 cases, 5 cases exhibited cystic hyperplasia and only a single case of endometrial polyp was seen. Simple cystic hyperplasia was seen in 3 cases of primary and 2 cases of secondary infertility patients.
Figure:1-8 demonstrates high power section of endometrium showing cystically dilated glands, gland in gland appearance of anovulatory cycle, day 17 of menstrual cycle but the biopsy was done on 25th day of menstrual cycle, peak secretion day 21 but, the biopsy was done on 22nd day of menstrual cycle, day 26 of menstrual cycle but, the section was taken on day 27, granuloma and giant cells in the endometrium, endometrium showing langhan's giant cell and section of endometrium showing simple cystic hyperplasia respectively.

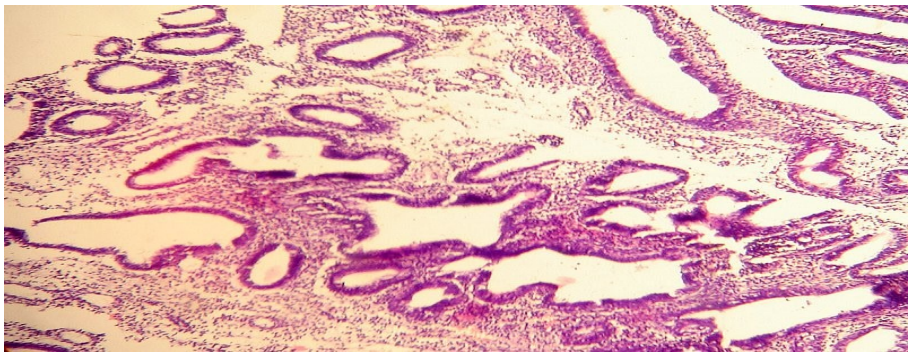

Fig-1: Section of endometrium showing cystically dilated glands in high power objective.

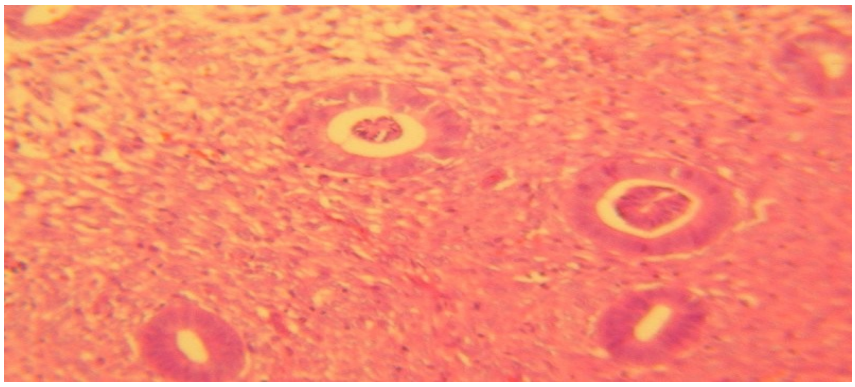

Fig-2: H &E high power section of endometrium showing gland in gland appearance of anovulatory cycle.

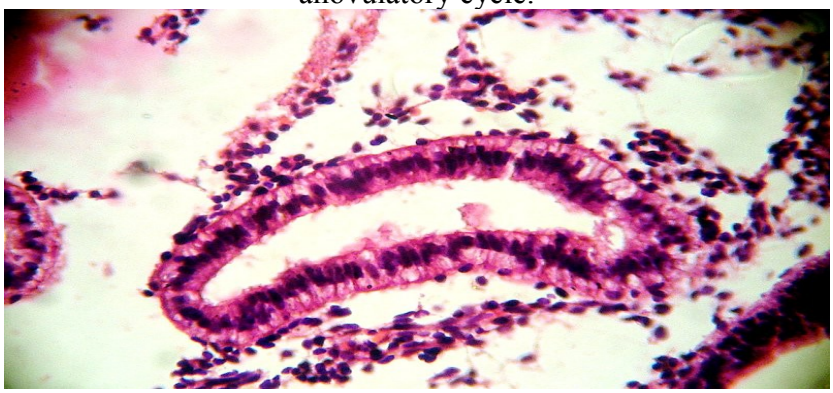

Fig-3: H&E high power section of endometrium showing day 17 but the biopsy was done on 25th day of menstrual cycle.

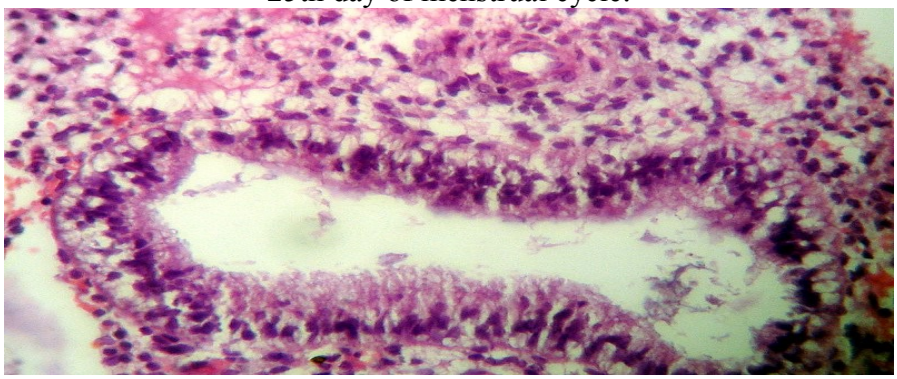

Fig-4: H&E high power section of endometrium showing peak secretion day 21 but, the biopsy was done on 22nd day of menstrual cycle.

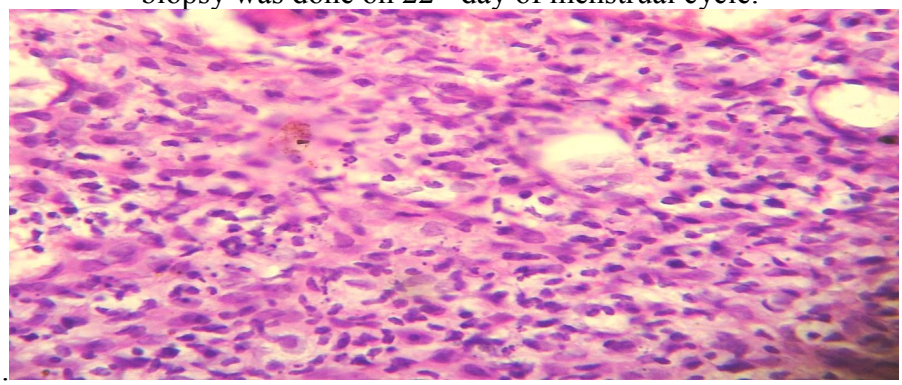

Fig-5: H &E high power section of endometrium showing day 26 of menstrual cycle but, the section was taken on day 27.

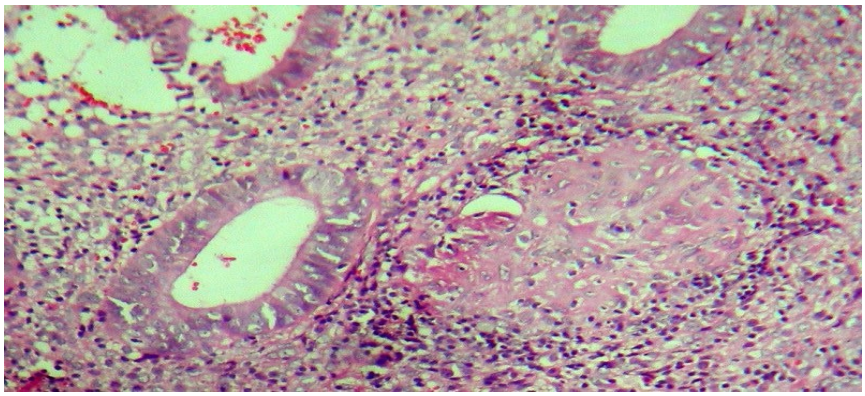

Fig-6: H &E high power section of endometrium showing granuloma and giant cells in the endometrium

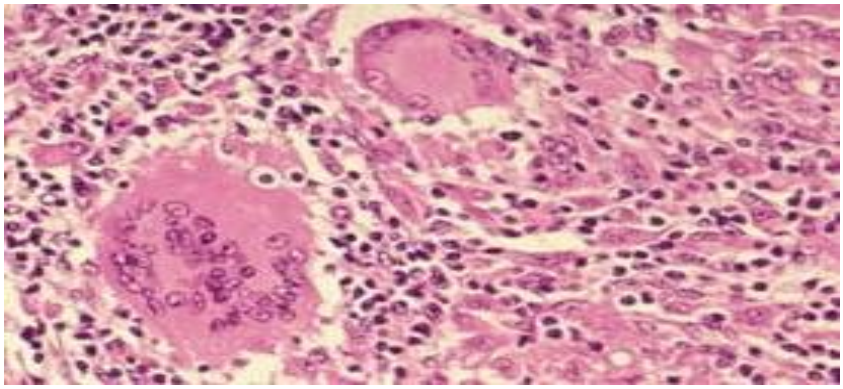

Fig-7. H &E high power section of endometrium showing langhan's giant cell.

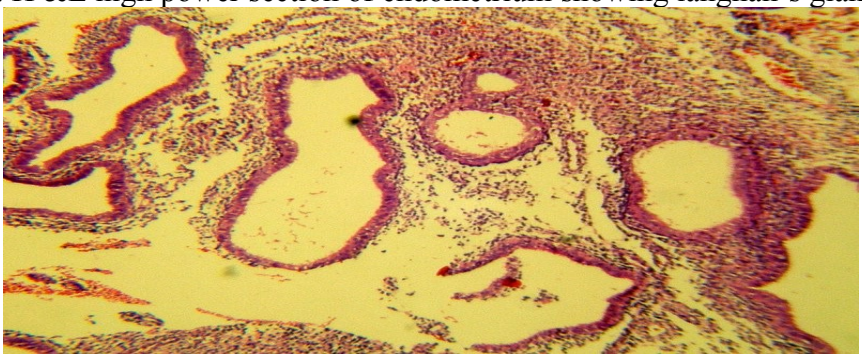

Fig-8. H &E high power section of endometrium showing simple cystic hyperplasia.

DISCUSSION

The magnitude of the problem of infertility varies from place to place, country to country and also the concern of infertile couple. Many couple does not approach medical profession because of superstition and myths. Females attending the infertility clinics are more than the males because of general belief that females are at fault always. Human endometrium is important in nidation of young fertilized ovum[7]. The endometrial biopsy is an essential step in the investigation of infertile women. Many authors have concluded that endometrial biopsy is safe reproducible and adequate means of providing histological evidence of normal endometrial development[8.9.10. 11].

Detection of ovulation and assessment of corpus luteum function are basic goals for evaluation of the infertile woman. They are usually accomplished by accurate histological study of endometrial biopsy specimen obtained in the late secretory phase[12]. A normal pregnancy is of course impossible without successful implantation of fertilized ovum. Implantation is the embedding of the blastocyst in the endometrial stroma.

Endometrial biopsy is the oldest and most important tool in the investigation of infertile female as endometrial histology is sensitive indicator of ovarian function. In India endometrial biopsy study for infertility is a routine investigation since many decades. Venugopal Shetty (1959), studied 102 consecutive unselected cases of sub fertility and reported 74 cases showing secretory endometrium, 25 cases proliferative endometrium and 3 cases had inadequate material. He also studied the glycogen and alkaline phosphatase content of the endometrium and stated that the biochemical changes run parallel to the histological changes that we commonly observe in the endometrium[13].

According to Ross GT et al., 15-20% of all infertile women have anatomical or functional disturbances. Indirect confirmation of ovulation was obtained by use of Basal Body Temperature charts in earlier days[14]. BBT began to rise simultaneously with the LH surge. A significant rise did not occur until 2 days after the LH surge. This coincided with the rise of serum progesterone to a mean level of over 4 ng/ml[15].

Georg Sillo-Seidl (1970) analyzed the premenstrual endometrial biopsy of 1000 sterile women. Of 1000 women 191 became pregnant either directly as the therapeutic result of uterine intervention; or indirectly, after hormonal or operative treatment as indicated by histological result, (e.g., polyps, tumors). Histology showed 56% of them having normal secretory endometrium and rest showed other findings like anovulatory cycles, polyps, carcinoma and etc[16]. Ganguly et al (1972) states that endometrial biopsy is still considered the most important investigation in cases of infertility as it provides an opportunity to examine the target tissue for estrogen and progesterone hormones. Besides evaluating the occurrence of ovulation it also furnishes with information of some other pathological lesions which may lead to infertility[17].

According to Najma Abbasi et al (1976), 500 cases of infertility in which the male partner was having normal seminogram. Premenstrual biopsy was done and finding were as follows 447 (89%) females with primary infertility and 53(11%) with secondary infertility. The age of the cases varied from 16-42 years. Maximum women belonged to the age group of 21-25 years. Around 80% had normal menses and 20% had irregular menses. Histopathological study of endometrium revealed proliferative phase in 23%, secretory phase in 71%, tuberculosis endometrium in 4% and others 2%. Thus the authors feel that the endometrial biopsy has a great role in cases of infertility as it helps to give the information about the occurrence of ovulation, regular ripening of the endometrium and other abnormal endometrial reaction due to hormonal imbalance. This is the only method to label the diagnosis of endometrial tuberculosis in an apparently healthy female[18].

CONCLUSION

In the present study we concluded that the anovulatory endometrium, simple cystic hyperplasia and luteal phase defect formed the major etiological basis of many cases of infertility. The endometritis, tuberculosis endometritis and endometrial polyp formed the minor group of etiology.

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