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Histopathological Study of Endometrium in Infertile Women and Its Correlation with Hormonal Status

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Abstract: *Cataract seems to be an ageing process. The aim of this study to find out the various morphometric and histopathologcial changes present in human cataractous lenses. There are many precipitating factors such as diabetes,hypertension,exposure to sunlight etc The observational prospective interventional cross-sectional type of study was carried out in 200 extracted cataractous lenses in the Department of Ophthalmology, Maharani Laxmi Bai Medical College Jhansi, during the period of 22 month duration (Feb 2018 to Nov. 2019). Detailed history, statistical data, morphometric study and histological picture of the selected cases were carried out. Of the cases observed, 49 were males and 51 were females. The age group ranged from 40 to 84 years. Nuclear, cortical and posterior subcapsular cataract was seen in 120, 55 and 54 respectivelyIn some cases more than one type of cataract was present. The etiology of cataract in 154 was due to senility; in 16 it was due to diabetes; in 14 it was due to hypertension; in 2 it was due to diabetes and hypertension and in 10 of them it was due to prolonged exposure to sunlight,and in 4 it was prolong systemic drugs administration. The size of the extracted cataractous lens ranged from 5 to 9 mm in diameter and 3 to 5 mm in thickness and the colour ranged from pale grey present in 36, yellow grey in 50, yellow in 62, amber yellow in 48 and dark brown in 4 cases. These lenses of differnt etiology were processed for H & E stain and they showed different histological pictures. Most of the extracted cataractous lens showed homogenous appearance with loss of concentric lamination in 120 cases (60%).*

Key words: *Human cataract lens, Morphometry, Diabetes, Senility, Hypertension, Histology.*

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I. Introduction

According to World Health Organization (WHO) "Infertility" is 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 (and there is no other reason, such as breastfeeding or postpartum amenorrhea). Infertility may be caused by infection in the man or woman, but often there is no obvious underlying cause[1]. Most of the time, infertility is some degree of subfertility in which 1 in 7 couples need specialist help to conceive. Subfertility can be either primary or secondary. Primary subfertility is a delay for a couple who have had no previous pregnancies and secondary subfertility is a delay for a couple who have conceived previously, although the pregnancy may not have been successful for example, miscarriage and ectopic pregnancy[2]. The chance to conceive depends on the length of sexual exposure, frequency of coitus and couple's age. The normal, young aged couples have a 25% chance to conceive after 1 month of unprotected intercourse, 70% of the couple's conceive by 6 months, and 90% of the couples have a probability to conceive by 1 year. Only 5% of the couples will conceive after one and a half year or two years[3,4]. The most common known causes of infertility are spermatozoal defects, ovulatory disorders and tubal disease. But the biggest group is due to "unexplained infertility". This is when a couple fails to conceive after about 18 months of regular intercourse, and no cause is found. The World Health Organisation believes that between 60 million to 80 million couples in the world are infertile. Between 2 to 10% of couples are unable to conceive a child by natural means, and a further 10 to 25% are unable to have a second or subsequent child.

Primary infertility: It is defined as the absence of a live birth for women who desire a child and have been in a union for at least 12 months, during which they have not used any contraceptives. The World Health Organization also adds that women whose pregnancy spontaneously miscarries or whose pregnancy results in a still born child without ever having had a live birth, would present with primarily infertility.[5]

Secondary infertility: It is defined as the absence of a live birth for women who desire a child and have been in a union for at least 12 months since their last live birth, during which they did not use any contraceptives.[5]

Causes of Female infertility: According to the Center of Disease Control (CDC, 2013)[6] the causes of female infertility can be divided into three broad categories including defective ovulation, transport and implantation.

Defective Ovulation	**Endocrine disorders:** The dysfunction of hypothalamus and pituitary gland can lead to an excess amount of prolactin, this may prevent ovulation. Moreover, other endocrine glands including adrenals and thyroid may also delay ovulation. **Physical disorders**: Certain physical disorders such as obesity, anorexia nervosa, and excessive exercise may lead to overweight or malnutrition, and later the menstrual cycle, thus make the couple infertile. **Ovarian disorders**: Polycystic ovary syndrome (PCOS) can lead to infertility because of an increased amount of testosterone and LH. Low FSH levels also hinder the production of eggs from the ovarian follicles, and lead to form fluid-filled ovarian cysts that eventually cover the whole ovaries and prevent conception. **Endometriosis:** endometriosis can cause infertility with the growth of endometrial tissue in the Fallopian tubes or around the ovaries. Endometriosis is usually more common in women in their mid-twenties and older, especially when postponed childbirth has taken place[7]
Defective Transport	Defective transport of ovum or sperm **Ovum :** Defective transport of ovum may be occurs in Pelvic Inflammatory Disease (PID), gonorrhea, peritonitis, previous tubal surgery and Scar tissue after abdominal surgery. **Sperm:** Presence of psychosexual problem such as vaginismus, or dyspareunia may hinder fertilization and make the couple infertile. Defect in female part of cervix has also cause defect in sperm transport.
Defective Implantation	**Congenital anomaly and fibroids**: Congenital uterine anomaly such as bicornuate uterus and uterine fibroids near the fallopian tubes or cervix may alter implantation of the zygote and cause infertility.

II. Method and Material

This observational prospective interventional cross-sectional type of study was conducted at Department of Pathology in association with Department of Gynecology at MLB Medical College Jhansi, U.P, India, over a period of 22 month duration (Feb 2018 to Nov. 2019). Total 200 patients of age group between 20 - 45 years who were attending OPD in Gyanecology department and satisfied the eligibility criteria,were included in this study. Out of total, 100 infertile female for case group-A, and 100 fertile female for control group-B were selected**.** The main aim of the study was, to correlate the histopathology of endometrium and hormonal status in infertile women. The procedures followed were in accordance with the ethical standarded committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000. The necessary permission from the Ethical and Research Committee was obtained for the study.

Inclusion criteria: Control-100

- All included females had married
- Fertile women age between 20 to 45 years with informed written consent
- Who agreed to participate in the study
- No history of involuntary primary or secondary infertility
- Willingness to discontinue hormonal contraceptives for 1 month prior to and through the duration of the study
- At least 1 child delivered within 24 months prior to study entry
- Most recent pregnancy resulting in a live birth (no interim spontaneous abortions)
- Tubal ligation within 24 months of study entry is acceptable if all other criteria are met

Inclusion Criteria: Case-100

- Infertile women age between 20 to 45 years with informed written consent
- Who agreed to participate in the study
- History of primary or secondary infertility for a period of at least 12 months
- No history of tubal ligation and hormonal treatments in the month preceding study entry

Exclusion criteria:

- Patients unwilling to give a written consent and routine follow up protocols will be excluded from the study
- Females of age < 20 years and >45 years
- Who were received medication that could alter thyroid function test
- Who were suffering from diabetes mellitus (DM), hypertension (HTN) and other chronic illness.
- Who were with history of intrauterine contraceptive device (IUCD), pelvic inflammatory disease(PID) previous history of abortion, miscarriage, and D & C
- In this study the male causes of infertility were excluded from the study.

Collection of blood sample: Fasting 4 ml venous blood samples were collected from all participants in there early follicular phase of menstrual cycle i.e, between days 3rd to 5th in plane bulbs and tested for following

parameters-Serum LH (Reference range: 2.12-10.89 microIU/ml), Serum FSH (Reference range: 3.85-8.78 microIU/ml), Serum TSH (Reference range: 0.5-5 microIU/ml), Serum prolactin (Reference range 2-29 microIU/ml)

Collection of biopsies: Endometrial biopsy is the removal of a sample of endometrial tissue for pathologic or fertility evaluation. Endometrial biopsies of 200 females were collected from the department of Obstetrics and Gynecology, and samples were preserved in 10% formalin and were processed by paraffin tissue processing, sections of 5 microns thickness were cut and stained with Hematoxylin and Eosin (H&E). The stained sections were studied under the microscope in view of the menstrual cycle, date of last menstrual period (LMP) and date of dilatation and curettage (D&C). For dating of the endometrium, the criteria described by Dallenbach Hellweg were applied.

Statistical Analysis**:** Data will be analysed by the Statistical Package for the Social Sciences (SPSS for windows, version 25.0). Obtained results of case group were compared with control group for determination of difference of significance. P-value was calculated by using Student's unpaired t-test, the p-value < 0.05 were considered as significant.

III. Results

Table 1: Age wise Patients distribution in Case and Control Group

Age group of females	Case group (100)		Control group (100)	
	Numbers	Percentage	Numbers	Percentage
20-25 years	35	35%	47	47%
26-30 years	32	32%	28	28%
31-35 years	17	17%	12	12%
36-40 years	14	14%	08	08%
41-45 years	2	2%	05	05%
Total	100	100%	100	100%

In our study numbers of patients in the age group of 20-25 years, 35were in case and 47 were in control group, in the age group of 26-30 years 32 were in case and 28 were in control group, 17 were in case and 12 were in control group present in the age group 31-35, 14 in case and 08 in control group were present in 36-40 years of age group and in the age group of 41-45 years, 02 in case and 05 in control group were present.. Mean age of females in case and control groups was 28.68 ± 5.68 and 27.83 ± 6.46 respectively.

Table 2: Age group wise mean LH, FSH ,TSH and Prolactin values in Case and Control groups

Age group of females	Mean LH ±SD		Mean FSH ±SD		Mean TSH ±SD		Mean PRL±SD	
	Case	Control	Case	Control	Case	Control	Case	Control
20-25 years	7.83±2.86	6.53±1.80	6.79±2.48	6.06±1.29	3.33±1.78	3.78±0.97	23.43±8.52	15.22±6.37
26-30 years	7.77±2.68	6.61±1.82	6.71±2.50	5.95±1.29	3.31±1.78	3.80±0.96	23.15±8.26	15.22±6.20
31-35 years	7.71±2.39	6.57±1.81	6.71±2.41	6.12±1.27	3.36±1.84	3.76±1.02	22.51±7.40	15.34±6.32
36-40 years	7.51±2.76	6.54±1.78	7.07±2.51	6.02±1.28	3.09±1.64	3.75±0.97	23.21±9.15	15.35±6.3
41-45 years	3.12±0.88	6.59±1.84	7.5±0.45	6.02±1.31	2.2±1.34	3.93±0.92	25.6±13.73	15.24±6.34
Total	7.84 ± 2.85	6.63± 1.82	6.75 ± 2.50	6.01 ± 1.31	3.32 ±1.78	3.78 ±1.01	23.40±8.48	15.22±6.2

LH: Luteinising hormone, FSH: Follicle stimulating hormone, TSH: Thyoroid stimulating hormone, PRL:

Prolactin, SD: Standard deviation (All values measured in mIU/ml)

In this study, the mean value of LH in case group was 7.84± 2.85 mIU/ml and in control group 6.63±1.82 mIU/ml, the mean FSH value in case group was 6.75 ± 2.50 mIU/ml and in control group was 6.01±1.31 mIU/ml, mean TSH value of case group was 3.32 ±1.78 mIU/ml and control group was 3.78±1.01 mIU/ml and the mean Prolactin value of case and control groups was 23.40 ±8.48 mIU/ml and 15.22±6.2 mIU/ml respectively.

Table 3: Mean LH value in study

Groups	Mean LH value ±SD	P-Value
Case	7.84 ± 2.85	0.000212
Control	6.63± 1.82	

In our study, the mean LH Value in case and control groups was 7.84 ± 2.85 and 6.63 ± 1.82 mIU/ml respectively and the P value was 0.00212 (P <0.05) which was statistically significant.

Table 4: Mean FSH value in study

Groups	Mean FSH value ±SD	P-Value
Case	6.75 ± 2.50	0.003927
Control	6.01 ± 1.31	

In table 4,the mean Value of FSH in case and control groups was 6.75 ± 2.50 and 6.01 ±1.31 mIU/ml respectively and the P value less than 0.05 (P value 0.003927) which was statistically significant.

Table 5: Mean TSH value in study

Groups	Mean TSH value ±SD	P-Value
Case	3.32 ± 1.78	<.012852
Control	3.78 ± 1.01	

Mean TSH value of case and control groups was 3.32 ± 1.78 and 3.78 ± 1.01 mIU/ml respectively and the P value was <0.012852, which was statically significant (<0.05) in this study.

Table 6: Mean PRL value in study

Groups	Mean PRL value ±SD	P-Value
Case	23.40 ±8.48	<.00001
Control	15.22 ± 6.32	

The Mean PRL (Prolactin) value in case and control groups was 23.40 ± 8.48 and 15.22 ± 6.32 mIU/ml respectively and the P value was <0.00001calculated which was statistically significant.

Table 7: Endometrial Biopsy in Case groups

Endometrial Biopsy		Number of Cases		Percentage
Proliferative phase		05		05%
Secretory phase	Early secretary phase	77	17	17%
	Mid secretary phase		33	33%
	Late secretary phase		27	27%
Inflammatory endometrium (endometritis)		09		09%
Endometrial hyperplasia		06		06%
Disordered proliferative endometrium		03		03%

In the case group, endometrial biopsy of maximum number of infertile females showed secretary phase (77%) out of which (33%) were in mid secretary phase followed by late secretary phase (27%), early secretary phase (17%), endometritis (9%), endometrial hyperplasia (6%), ,proliferative phase (5%) and disordered proliferative endometrium (3%) was found in minimum number of cases.

Table 8: Endometrial Biopsy Vs Mean LH, FSH, PRL and TSH value in Case Group

Endometrial Biopsy	Mean LH ±SD	Mean FSH ±SD	Mean TSH ±SD	Mean PRL ±SD
Proliferative phase	7.88 ± 2.63	6.77± 2.47	3.33 ± 1.82	22.49 ±7.33
Early secretary phase	8.08 ± 2.07	6.68 ±2.49	3.44 ± 1.87	22.26± 6.79
Mid secretary phase	7.91 ± 2.19	6.79 ± 2.46	3.36 ± 1.80	21.90 ± 6.95
Late secretary phase	7.68 ± 2.66	6.72± 2.44	3.35 ± 1.78	23.39±8.60
Inflammatory endometrium (endometritis)	8.22 ± 1.78	6.97 ± 2.45	3.21 ± 1.92	21.35± 7.28
Endometrial hyperplasia	7.18 ± 3.30	7.24 ± 2.21	3.05 ± 1.33	24.52± 9.31
Disordered proliferative endometrium	8.94 ± 2.01	5.03 ± 1.98	3.86 ± 1.91	24.38±4.22

In table 8, it is clearly understood that high levels of LH and FSH affect the endometrial biopsy pattern. And high values of PRL and TSH affect the secretary phase of endometrial biopsy. But endometrial hyperplasia and endometritis can occur under normal hormonal status.

Table 9: Endometrial Biopsy Vs Mean LH, FSH, PRL and TSH values in Control Groups.

Endometrial Biopsy	Mean LH ±SD	Mean FSH ±SD	Mean TSH ±SD	Mean PRL ±SD
Late secretory	6.63± 1.82	6.01 ± 1.31	3.78 ± 1.01	15.22 ± 6.32

In Table 9, in endometrial biopsies, all females of the control group were present in late secretory phase. The mean LH,FSH,TSH and Prolactin were 6.63±1.82,6.01±1.31,3.78±1.01,15.22±6.32 mIU/ml respectively.

Table 10: Secretary phase of endometrial biopsy

Group	Mean LH ±SD		Mean FSH ±SD		Mean TSH ±SD		Mean PRL ±SD	
Case (75)	7.68±2.66	P value 0.000693	6.72±2.44	P value 0.007217	.35±1.78	P value 0.032726	23.39±8.60	P value < 0.00001
Control (100)	6.63±1.82		6.01±1.31		3.78±1.01		15.22±6.32	

Secretary phase of endometrial biopsy is divided into 3 classes' i.e early, mid and late secretary phases. Basically these phases of endometrium are affected by TSH and PRL hormone, which was statistically significant in this study.

IV. Discussion

In our study, the maximum number of infertile female were in the age range of 20-30 years 67. Also in the control group, maximum numbers of fertile female were in the age range of 20-30 years 75. And minimum number of patients in case 02 and control 05 group were found in 36-45 years of age group. Mean age in case and control group was 28.68 ± 5.68 and 27.83 ± 6.46 respectively. Similar findings were also found in Pakistan in a study done by Kafeel et al.[8], where he found mean age was 29 years, and age ranged from 21-37 years. The mean age of infertility in Nigerian women was 31.7 years[9] Whereas, in Algeria women presented in more older age. Mean age was 33 years and age ranged from 23- 43 years[10] The reason may be that overall women literacy and employment rate is higher in Algeria[11,12] In our study, the mean value of LH, FSH, TSH and Prolactin in case group was 7.84± 2.85 mIU/ml, 6.75 ± 2.50 mIU/ml, 3.32 ± 1.78 mIU/ml and 23.40 ± 8.48 mIU/ml and in control group was 6.63 ± 1.82 mIU/ml, 6.01± 1.31 mIU/ml, 3.78 ±1.01 mIU/ml and 15.22 ± 6.2 mIU/ml respectively The study showed that the increased levels of FSH, LH and prolactin were found in infertile group when compared with the control group. These findings are in agreement with Ban et al., (2013) and Aroma et al., (2014)[13,14] Scott MG et al., (1989) and Choudhury et al., (1995) which reported that the elevated levels of prolactin hormone are very common in infertile women as compared to fertile women[15,16] Aroma et al., (2014) emphasized that increased FSH, LH and Prolactin levels are significantly associated with infertile women[16] The present study also showed hyperprolactinemia as the cause of infertility in women. Similarly, increased levels of prolactin have also been reported by Parijatham and Saikumar (2014), Goswami et al., (2009) and Kumkum et al., (2006)[17-19] A serum level below the normal range limits for FSH (3.85-8.78 μIU/ml) and LH (2.12-10.89 μIU/ml) indicates that the factors causing infertility could be defects in the pituitary gland, GnRH or the hypothalamus[20]. On the other hand, FSH and LH values above the normal limits suggest a problem arising from other components of the reproductive system, probably a defect in the negative feedback regulation mechanism in the hypothalamus by estrogen and progesterone[21] Cases where FSH and LH serum levels are within the normal limits suggest that the cause of infertility is due to other factors such as damage to the ovary, viral infection, chemotherapy, drugs and other hormonal abnormalities with estrogen and progesterone that are responsible for development of the endometrium and also unexplained infertility could be a factor.[22]

Thyroid hormones have profound effects on reproduction and pregnancy. Thyroid dysfunction is implicated in a broad spectrum of reproductive disorders, ranging from abnormal sexual development to menstrual irregularities and infertility. Hypothyroidism is associated with increased production of TRH, which stimulates pituitary to secrete TSH and PRL. In this study, the majority of infertile (79%) as well as fertile (100%) women were euthyroid. However, the distribution of thyroid dysfunction in the study group was somehow different – hypothyroidism was more prevalent in the infertile group (17%) then hyperthyroidism (4%)**.** Our findings correlates with the study by Goswami Binita et al (2009)[23], in their study they investigated 160 women with primary infertility and 80 fertile women with similar age and socioeconomic status were enrolled as the controls. The association between thyroid dysfunction and levels of serum prolactin, LH and FSH as their menstrual status were reviewed. They found most of the control (86%) and infertile women (87%) were euthyroid. Prevalence of Hypothyroidism was seen in 8% of the infertile subjects whereas in the control group it was found to be 5%. Hyperthyroidism was found in 5% of the infertile patients. Elahi et al (2007)[24], in their study of infertile (140) and fertile women (152), also found most of the infertile women (89.3%), & control women (93.4%), were euthyroid. The incidence of hypothyroidism (6.4%) was slightly higher as compared to hyperthyroidism (4.3%). Hyperprolactinemia adversely affects fertility potential by impairing GnRH pulsatility and thereby ovarian function. In this study the prevalence of hyperprolactemia induced infertility was 10%.

In our study secretory phase was present in 77 % study cases. Among the secretory phase in case group, mid secretory phase was most frequent (33% cases), followed by late secretory phase (27%) and early secretory phase (17%). Emokpae M. A.et al[25] study showed maximum cases were of mid secretory phase (28%)

followed by late secretory phase (14.5%) and early secretory phase (25.5%). Our findings varies with the findings of Kafeel et al[8] where he found early secretory phase (37.5% cases) was most common followed by mid secretory phase (14.1% cases) and late secretory phase (5.0% cases). Other studies conducted in India and Pakistan, found secretory phase was the most common cause of infertility followed by proliferative phase.[26,8,27] Endometritis is known to cause infertility either by disturbing the cyclic endometrial rhythm or as a result of the accompanying tubal inflammation and secondary anatomical abnormalities which are always present. Endometritis and endometrial hyperplasia was found in 9% and 6% cases respectively in our study. Our finding was consistent with the finding of Emokpae M. A. et al[25] with endometritis and endometrial hyperplasia, 8.4% and 5.1% respectively. In Girish et al[28] it was 5.5 % and 4.4% respectively, also found higher percentage (14.1%) and (4.13%) in Pakistan[8]. In this study proliferative phase was present in 5% cases in study group.This finding was similar to the finding of Emokpae M. A. et al[25], and varies with the finding of Zawar MP[27] proliferative phase (50.0%). Kaur P et al[26] with 23%. Disordered proliferative endometrium present in minimum number (3%) cases in study group. In our study all females of control group had late secretory phase in endometrial biopsies.

In this study, 6% patients had hypergonadotrophic hypogonadism ,including 1% cases of endometrial hyperplasia and 5% cases with proliferative phase on their endometrial biopsies, an indication that failure to conceive may lie in the ovaries. 1% of the patients had hypogonadotrophic hypogonadism and showed endometrial hyperplasia in endometrial biopsies which indicate that there may be dysfunction of the hypothalamus or the pituitary which are unable to secrete adequate gonadotropins to stimulate the ovary . In our study 83% in case group were present with normal level of LH,FSH and Prolactin, out of which 70% endometrium biopsies showed secretory phase, 6% showed endometritis, 4% with endometrial hyperplasia and 3% showed disordered proliferative endometrium. Prolactinemia were present in 10 % of cases out of which 7% showed secretory endometrium and 3% showed endometritis in endometrial biopsies. In study by Emokpae et al[25] noted 4.7% infertile cases had hypergonadotropic –hypogonadism,in there endometrial biopsies showing proliferative phase and endometrial hyperplasia, 3.45% with hypogonadotropic-hypogonadism, in endometrial biopsies showing endometrial hyperplasia, 1.1% with hypergonadotropic –hypergonadism in endometrial biopsies showing secretory endometrium, 8.54% cases with hyperprolactinimia, in there endometrial biopsies showing secretory endometrium and endometritis, and maximum number of cases present with normal hormonal status and in endometrial biopsies showing secretory endometrium, endometritis, endometrial hyperplasia. In our study all females of the control group had normal hormonal status.

V. Conclusion

Infertility have drastic effects on couple's lives, hence it is important to improve their reproductive health issue. Histopathological study of endometrium i.e, endometrial biopsies can be an effective screening test in infertility. Hormonal disturbances if present in the infertile females are reflected in the endometrium in the form of anovulatory cycle, inadequate proliferative/ secretory phase, endometrial hyperplasia along with intrinsic abnormalities like endometritis. Limitation of this study: The enodmetrial biopsy procedure is a lengthy and difficult to perform without expert. Time of blood collection for hormonal evaluation was the also main limitation concern in this study. Confidential issue: In Indian tradition it was also a big challenge to explain the test subject to keep their issues secrets.

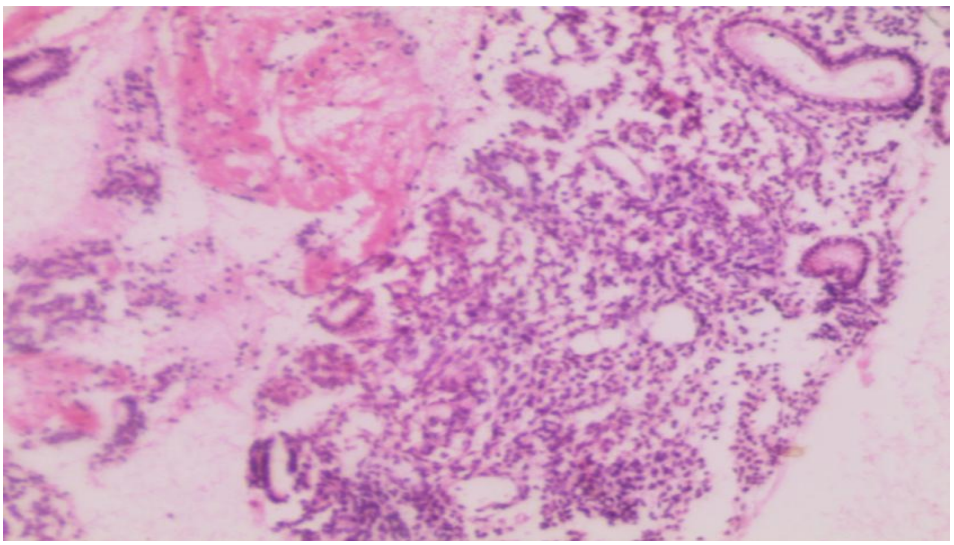

Figure 1: Late secretary phase of endometrium 10X (H&E Stain).

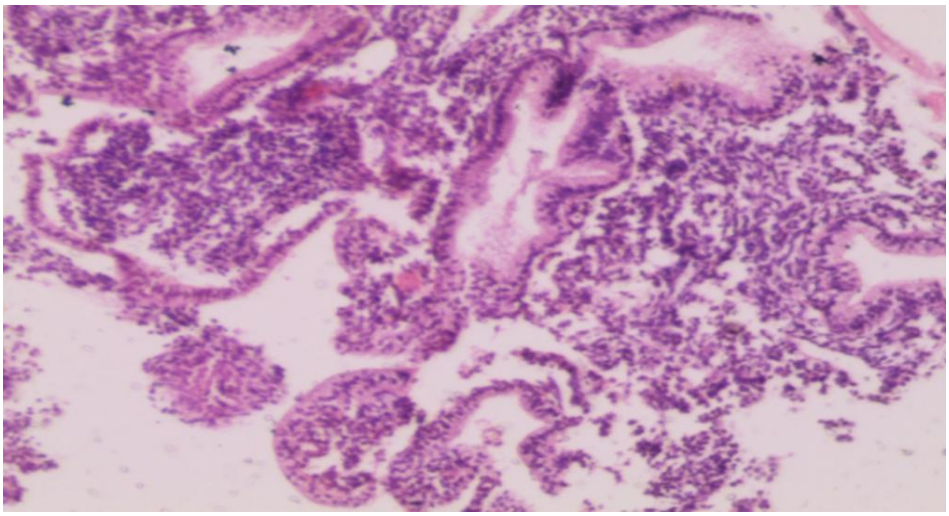

Figure 2: Mid secretary phase of endometrium 10X (H&E Stain).

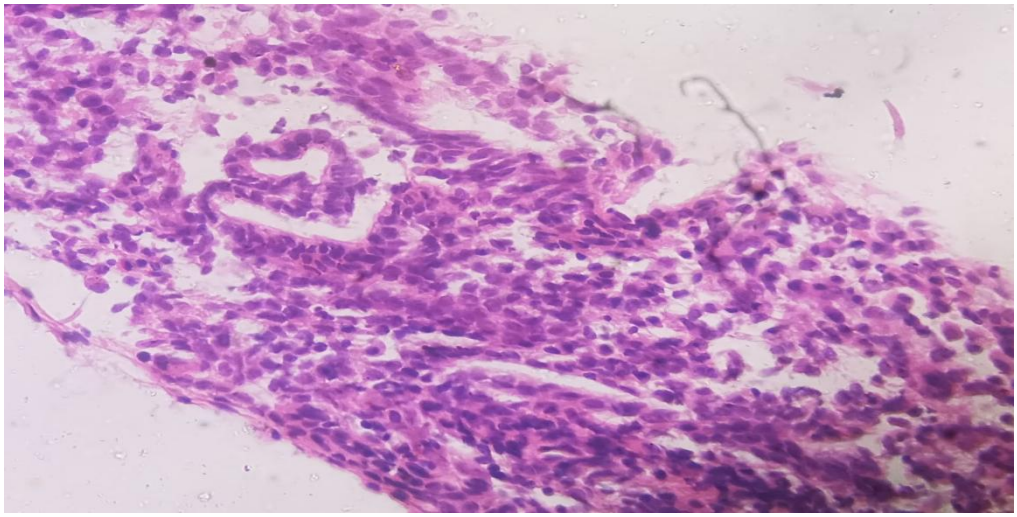

Figure 3: Chronic endometritis of endometrium 40X (H&E Stain).

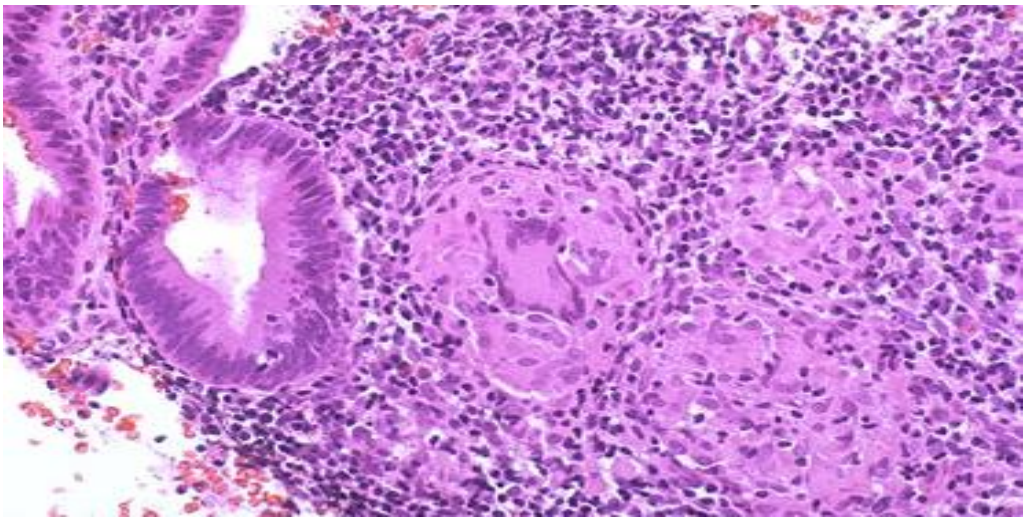

Figure 4: Granulomatous endometritis of endometrium 40X (H&E Stain).

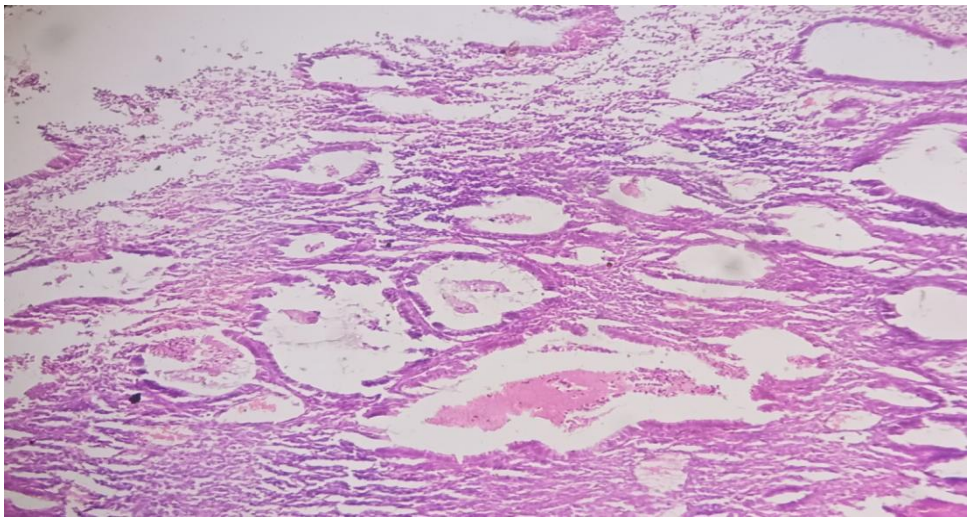

Figure 5 : Disordered proliferative endometrium 10x (H&E Stain).

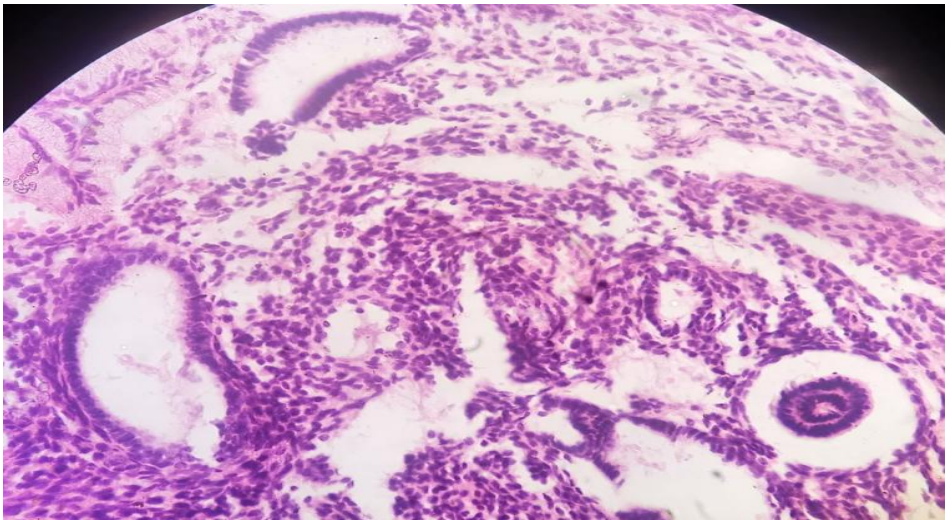

Figure 6: Proliferative phase of endometrium 10X (H&E Stain).

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