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HISTOPATHOLOGICAL STUDY OF ENDOMETRIUM IN INFERTILITY AT TERTIARY CARE CENTRE JAIPUR (RAJASTHAN)

Pathology

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ABSTRACT

Introduction: Infertility has become a medical as well as a social problem. Infertility refers to inability to achieve conception even after one year of unprotected coitus by a couple. In India, there is an estimated 10.2 million couples of infertility.

Aims:

1. To study the histomorphological patterns of endometrium among women with infertility.
2. To study the association between histomorphological patterns of endometrium among women with primary and secondary infertility.
3. To study and compare the grading of glycogen deficiency with Periodic Acid Schiff (PAS) staining in primary and secondary infertility cases.

Methods: In this we included 170 endometrial biopsies from cases of primary and secondary infertility submitted for histopathology in the department of pathology, SMS Medical College, Jaipur, Rajasthan.

Results: In this study total 170 infertile cases were studied, out of these 131 (77.05%) cases were of primary infertility and 39 (22.95%) cases were of secondary infertility. Maximum numbers of cases were observed in proliferative phase (anovulatory endometrium) in 110 (64.71%) cases.

Conclusion: Histomorphological study of the endometrium is one of the most reliable, safe, important and cheaper diagnostic tool in cases of primary and secondary infertility.

KEYWORDS

Infertility, Endometrium, Glycogen content

INTRODUCTION: -

Infertility has become a medical as well as a social problem. This can cause intense agony and trauma to the infertile couple specially women who are blamed for infertility.

Infertility refers to inability to achieve conception even after one year of unprotected coitus by a couple.[1] It is a global health problem and affects 80 million people (8-10% of couples) worldwide. In India, there is an estimated 10.2 million couples suffering from infertility.[2] Primary infertility is failure to conceive at all, whereas secondary infertility is failure to conceive after having borne a child or abortion.

Infertility has been attributed to male factors 25%, female factors 58%, and unexplained in 17% couples.[3] Female infertility may occur due to disturbances involving any part of genital system or parts of the central nervous system that control the ovaries hormonally. 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.

Normal endometrium is divided into two layers, the functionalis (stratum spongiosum) and the basalis (stratum basale). The decidua functionalis is the superficial two thirds of the endometrium, which proliferates under hormonal stimulation. The decidua basalis is the deepest region of endometrium. It does not undergo significant monthly proliferation but instead is a source of endometrial regeneration after each menses. The basalis adjoins the myometrium, serving to regenerate the functionalis and surface epithelium following shedding during menses. In the secretory phase, the endometrium also shows a stratum compactum.

The cyclical changes in a non-pregnant uterus are divided into 3 phases-The proliferative phase, secretory phase and menstrual phase.The length of normal cycle varies from woman to woman but the average length varies from 21 to 40 days.

The proliferative (follicular or preovulatory) phase is characterized by growth of glands, stroma, and vessels that is influenced by estradiol produced mainly by granulosa cells in the ovarian follicles. Following ovulation, the secretory (luteal or postovulatory) phase reflects the effect of the combined production of progesterone and estradiol by luteinized granulosa and theca cells of the corpus luteum. The regular sequence of morphologic changes determined by the fluctuating levels of ovarian steroid hormones forms the basis for histologic dating.Proliferative phase is variable.Growth of endometrium is the main characteristic of the proliferative phase.The pseudo stratification of the nuclei and the presence of mitotic activity in the glands and stroma are two constant features of the proliferative phase.

The secretory phase is constant in the normal cycle lasting 14 days from the time of ovulation to the onset of menstruation.The histologic features of secretory activity begin on the 16th day and persist until the onset of menstruation. Unlike in the proliferative phase, the changes in the glands and stroma are relatively discrete, varying sharply from one day to the next, thus permitting accurate dating.

Dating of the first half of the secretory phase is based primarily on glandular changes whereas dating of the second half is based mainly on stromal alterations. The morphologic changes of the secretory phase begin 36 to 48 hours after ovulation. The first diagnostic evidence of ovulation, however, is the presence of abundant subnuclear glycogen vacuoles in the undulating, tortuous glands.

Menstrual endometrium shows glandular and stromal breakdown that rapidly affects all the functionalis by the end of day 28. Menstrual phase shows fibrin thrombi in small vessels, condensed and collapsed stroma, and necrotic debris. With this necrosis, a true neutrophilic infiltrate becomes a part of the physiologic process.

Infertility is either anovulatory (absence of ovulation) or ovulatory (with normal ovulation). Secretory phase of endometrium in premenstrual biopsies indicates that the ovulation has occurred and therefore the cause of infertility is other than anovulation. Whereas, proliferative phase in premenstrual biopsies indicate anovulation and this is a major cause of infertility.

For causes of infertility a wide range of investigations can be done, ranging from hormone assay to hysteroscopy, laparoscopy and biopsy.[4]

Endometrial biopsy remains the gold standard investigation to diagnose infertility. It is an important, safe and cheaper diagnostic tool in case of primary and secondary infertility. It is a sensitive indicator as it reflects the ovarian function. Dating the endometrium is possible by its histological examination. It is often used clinically to assess hormonal status or document ovulation. Dating of endometrium is done by Noyes criteria.[5]

Histopathological examination can diagnose other pathology which is associated with infertility, such as tuberculosis, endometritis and hyperplasia etc.

In luteal phase deficiency there is inadequate growth and function of the corpus luteum. As a result, there are inadequate secretory changes in the endometrium which hinder implantation. Diagnosis of luteal phase defect was made by using Jones's criteria (1976). According to him luteal phase defect is defined as lag of more than two days in histological development of endometrium compared to the day of the cycle.

Unopposed estrogenic stimulation of the endometrium causes endometrial hyperplasia characterized by an increase in gland to stroma ratio, irregularities in the gland shape and variation in gland size. In latest WHO classification 2014 differentiates between 2 categories of endometrial hyperplasia.[6]

1. Hyperplasia without atypia
2. Atypical hyperplasia / endometroid intraepithelial neoplasia.

In hyperplasia without atypia the glandular epithelium and stroma resembles proliferative endometrium.The diagnosis of atypical hyperplasia is based on the presence of nuclear atypia.

In tubercular endometritis the granulomatous response is variable. The findings may be diffuse or focal stromal infiltration of lymphocytes and plasma cells with involvement and distortion of the glands or classical tubercular granulomas characterized by focal collection of epitheloid cells, few langhans' type of giant cells surrounded by a cuff of lymphocytes. Often the granulomas are non-necrotizing.

In chronic non-specific endometritis endometrial inflammation is non-specific and mixed inflammatory infiltrate is composed of plasma cells, lymphocytes and neutrophils and eosinophil infrequently.

Unexplained infertility is defined as the absence of conception despite 12 months of unprotected intercourse, not explained by anovulation, poor sperm quality, tubal pathology or any known cause of infertility.[7]

Glycogen content in endometrium

Glycogen is believed to be the direct source of nutrients for the early conceptus from the time it enters the uterine cavity to the time it is actively supported by maternal blood stream. It is one of the pathological factors of sterility in poor quality endometrium that leads to death of ovum before or after implantation.The implantation depends upon the receptive condition of the endometrium, which is determined by glycogen. Mckay et al (1956) have established a definite cyclic pattern for most of the metabolic constituents in normal Endometrium. Glycogen is present in traces in the proliferative phase and increase progressively during the secretory phase. The deficiency of Glycogen content in Endometrium is called "Glycopenia Uteri".[8,9] The analysis of investigating the infertile couple is to assess their chance of achieving a pregnancy and to identify the factors amenable to treatment.

AIM AND OBJECTIVES: -

1. To study the histomorphological patterns of endometrium among women with infertility.
2. To study the association between histomorphological patterns of endometrium among women with primary and secondary infertility.
3. To study and compare the grading of glycogen deficiency with Periodic Acid Schiff(PAS) staining in primary and secondary infertility cases.

MATERIAL AND METHODS: -

The present study was carried out in the department of pathology, SMS Medical College, Jaipur. The study included 170 cases of primary and secondary infertility in reproductive age group. Detailed clinical history from each patient was recorded on a performa and details regarding age, menstrual cycle, last menstrual period (LMP), age at marriage and obstetric history were obtained. Endometrial biopsies / curettage received in 10% formalin and processed routinely. Paraffin embedded block were made, thin 3-4 mm sections were cut and stained with Haematoxylin and eosin stain and histomorphological features were studied. Special stains like Ziehl – Neelsen stain for acid fast bacilli was done whenever required. PAS stain was also done to demonstrated glycogen.

RESULTS AND OBERVATION: -

The present study comprised of total 170 endometrial biopsies for evaluation of primary and secondary infertility.

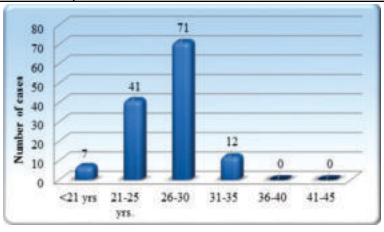
Table 1: Distribution of cases according to type of infertility (n= 170)

Type of infertility	No.	Percentage
Primary	131	77.05
Secondary	39	22.95
Total	170	100%

In our present study a total of 170 infertile patients in reproductive age group were taken for endometrial biopsy study. Out of these 170 total infertile patients, 131(77.05%) cases were of primary infertility and 39 (22.95%) cases were of secondary infertility.

Table 2: Age Distribution among primary infertility cases (n=131)

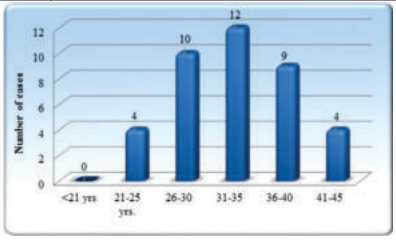
Age Group	Type of infertility			
	Primary			
	No.	%	Mean	SD
<21 yrs	7	5.34	20.00	2.00
21-25 yrs.	41	31.30	23.73	1.36
26-30	71	54.19	27.62	1.51
31-35	12	9.16	32.58	1.16
36-40	-	-	-	-
41-45	-	-	-	-
Total	131	100%	-	-
p value	p<0.001 (S)			



The table showing age distribution among primary infertility cases where most of the patients were from age group 26-30 years contributing 71 (54.19%) cases of total 131 cases of primary infertility. Youngest patient in our study from primary infertility group was of 20 years and eldest was of 35 years of age.

Table 3: Age Distribution among secondary infertility cases (n=39)

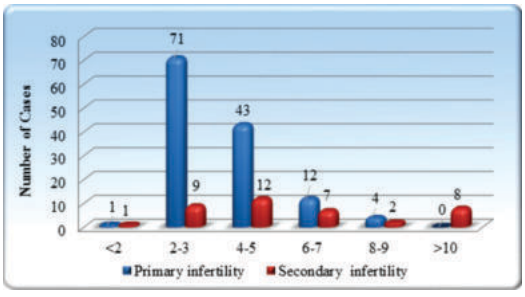
Age Group	Type of infertility			
	Secondary			
	No.	%	Mean	SD
<21 yrs	-	-	-	-
21-25 yrs.	4	10.26	24.00	1.15
26-30	10	25.64	27.08	1.00
31-35	12	30.77	33.31	0.75
36-40	9	23.08	37.77	1.48
41-45	4	10.26	44.50	0.58
Total	39	100.00		
p value	p<0.001 (S)			



The table showing age distribution among secondary infertility cases where most of the patients were from age group 31-35 years contributing 12 (30.77%) cases. The youngest patient was 23 years old and oldest patient was 45 years in our study.

Table 4: Duration of infertility in primary and secondary infertility groups

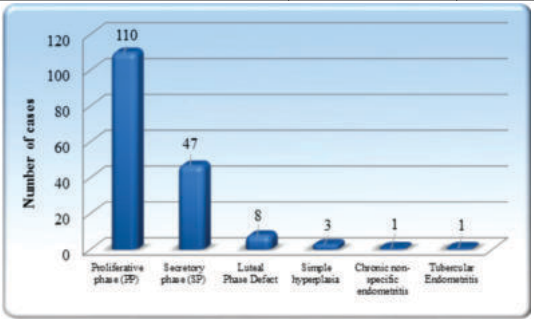
Duration (years)	Primary infertility		Secondary infertility	
	No. of Cases	Percentage	No. of Cases	Percentage
<2	1	0.76	1	2.56
2-3	71	54.24	9	23.08
4-5	43	32.82	12	30.77
6-7	12	9.16	7	17.95
8-9	4	3.05	2	5.13
>10	0	0.00	8	20.51
Total	131	100.00	39	100.00



In this study, among primary infertility group, 1 (0.76%) patient presented within 1-2 years. 71 (54.24%) patients presented between 2-3 years & 43 (32.82%) patients presented between 4-5 years after marriage. Among secondary infertility group 1 (2.56%) patient presented within 1-2 years, 43 (23.08%) patients presented between 2-3 years, 22 (30.77%) patients presented between 4-5 years, and 7 (17.95%) patients presented between 6-7 years after their last conception. Thus in both groups (79.41%) cases presented for endometrial biopsy within 2-5 years after their marriage in case of primary infertility or after their last conception in case of secondary infertility.

Table 5: Morphological pattern of endometrium in primary infertility and secondary infertility cases (n=170)

Histomorphological pattern	Number of Cases	Percentage
Proliferative phase (PP)	110	64.71
Secretory phase (SP)	47	27.65
Luteal Phase Defect	8	4.70
Simple hyperplasia	3	1.76
Chronic non-specific endometritis	1	0.59
Tubercular Endometritis	1	0.59
Total	170	100.00

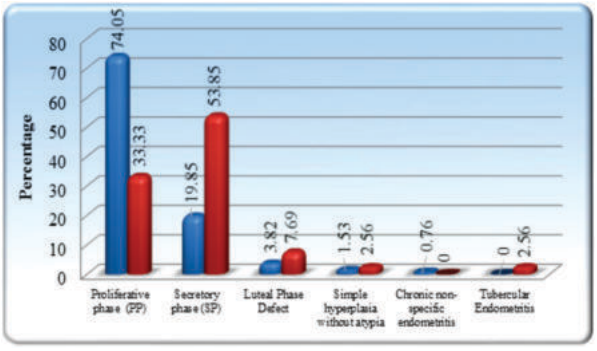


In the present study, we observed maximum number of cases in Proliferative (anovulatory) endometrium in 110 (64.71%) patients. This shows proliferative (anovulatory) endometrium is the major cause of infertility in our study. Disordered proliferative endometrium was seen in 5 (2.94%) cases. 3 cases were seen in primary infertility and 2 cases were seen in secondary infertility.

Secretory endometrium in 47(27.65%) cases, followed by luteal phase defect in 8 (4.70%) cases. Simple hyperplasia was seen in 3(1.76%) cases and 1 case each of chronic non-specific endometritis and tubercularendometritis was seen.

Table 6: Comparison of morphological pattern of endometrium in primary infertility and secondary infertility cases

Histomorphological pattern	Type of infertility			
	Primary		Secondary	
	Number of Cases	Percentage	Number of Cases	Percentage
Proliferative phase (PP)	97	74.05	13	33.33
Secretory phase (SP)	26	19.85	21	53.85
Luteal Phase Defect	5	3.82	3	7.69
Simple hyperplasia without atypia	2	1.53	1	2.56
Chronic non-specific endometritis	1	0.76	0	0.00
Tubercular Endometritis	0	0.00	1	2.56
Total	131	100.00	39	100.00



In present study we observed, 97 (74.05%) cases of proliferative endometrium in primary infertility group and 13 (33.33%) cases in secondary infertility group.Secretory endometrium was seen in 26 (19.85%) cases of primary infertility group and 21 (53.85%) cases of secondary infertility group.Luteal phase defect was seen in 5 (3.82%) cases of primary infertility group and 3 (7.69%) cases of secondary infertility group.Simple hyperplasia withoutatypia was seen in 2 (1.53%) of primary infertility group and 1 (0.76%) case of secondary infertility group.Chronic non-specific endometritis was seen in 1 (0.76%) case of primary infertility group while granulomatous endometritis was seen in 1 (2.56%) case of secondary infertility group.

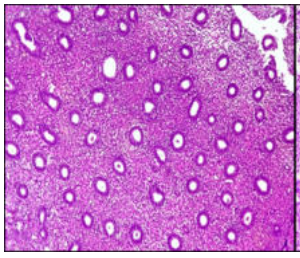
The glycogen content was graded as follows (Arzac and Blanchet 1948)

- 0-Negative reaction
- Mild (+)- small amount of glycogen seen in early and mid-proliferative phase. Distribution is perinuclear & very small granules seen.
- Moderate (++) – In early secretory phase glycogen particles are initially infranuclear and later supranuclear. Coarse granules seen.
- Heavy (+++) – In mid and late secretory phase shows large amount of glycogen in lumen of glands. Small masses seen.
- Intense (++++) – Predecidual cells and stroma also shows large masses of glycogen.

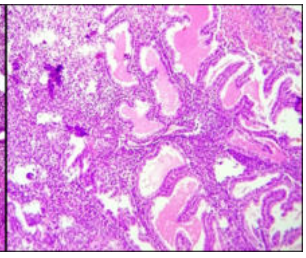
In our study, among primary infertility group, out of total 96 cases of proliferative endometrium, 75 (78.12%) cases showed absence of glycogen content and 21 (21.87%) cases showed mild glycogen positivity. Out of 26 cases of secretory endometrium, 20 (76.92%) cases showed glycogen content. Out of 20 cases, 1 case showed moderate (++), 3 cases showed heavy (+++), and 16 cases showed Intense (++++) positivity.Out of 5 cases of luteal phase defect only 2 (40%) cases showed moderate to heavy glycogen content. All cases of chronic non-specific endometritis, simple hyperplasia without atypia and tubercular endometritis were glycogen deficient.

In our study among secondary infertility group, out of total 13 cases of proliferative endometrium 11 (84.61%) cases showed absence of glycogen and only 2 (15.38%) cases showed mild(+) glycogen content. Out of 21 cases of secretory endometrium, 14 (66.66%) cases showed glycogen content. Out of 14 cases, 1 case showed mild (+), other 1 case showed moderate (++), 2 cases heavy (+++) and 10 cases showed intense (++++) positivity.Out of 3 cases of luteal phase defect, only 1 (33.33%) case showed moderate (++) glycogen content. All cases of chronic non-specific endometritis, simple hyperplasia without atypia and tubercular endometritis were glycogen deficient.

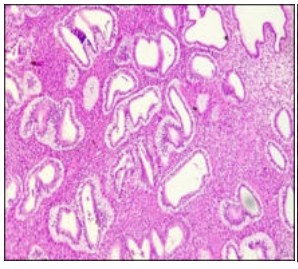
PHOTOGRAPH



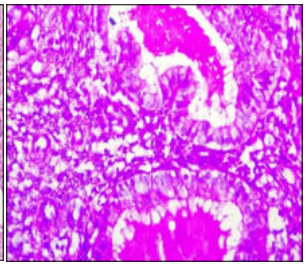
Proliferative phase (H&E, 100X)- Endometrium showing tubular glands regularly spaced in abundant stroma (anovulatory cycle).



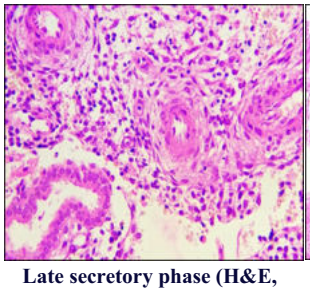
Mid secretory phase (H&E,100X) showing glands distended with secretions and stroma shows edema.



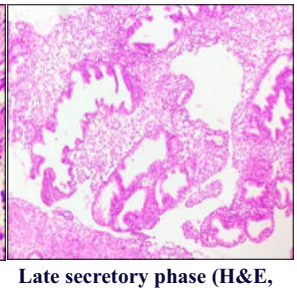
Early secretory phase (H&E, 100X) showing regular distribution of subnuclear vacuoles in glandular epithelium.



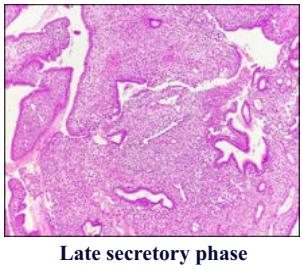
Mid secretory phase (PAS, 400x) showing glycogen in lumen of the gland



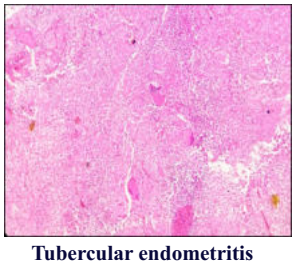
Late secretory phase (H&E, 100x) showing predecidual change around spiral arteries with intervening zones showing edema. The gland is tortuous and show secretory exhaustion



Late secretory phase (H&E, 100x) showing irregular serrated glands with predecidual change in stroma



Late secretory phase (H&E,100X) showing sheets of predecidualized stromal cells with granular lymphocytes and tortuous glands.



Tubercular endometritis showing necrotizing cell granuloma and langhans giant cells (H&E, 100X)

DISCUSSION: -

In our study, cases of primary infertility were 131 (77.05%) which were higher than secondary infertility cases which were 39 (22.95%). The percentage of primary and secondary infertility cases in our study were almost similar to the study done by Sharma et al[10] (2016) who found 74% cases of primary infertility and 26% of cases secondary infertility and Mori et al[11] (2020) found 74% cases of primary infertility and 26% cases of secondary infertility. Similarly higher incidence of primary infertility was seen in studies done by Silbina et al[12](2017), Achalkar et al[13](2018), and Preethi et al[14] (2019) who found 92.40%, 86.5% and 82.7% cases of primary infertility respectively.

In our study, among primary infertility group majority of the women, 71 (54.19%) cases were in the age group of 26 to 30 years comparable to the study done by Achalkar et al (2018) who found 46.8% cases in this age group. The mean age of primary infertility was 25.98 years in our study. The youngest patient in primary infertility was 20 years while the oldest patient was 35 years in our study.

This study shows maximum cases of secondary infertility in age group of 31 to 35 years (30.77%), similar to study done by Silbina et al[12] (2017) and Achalkar et al[13] (2018) who found maximum 50% and 48.1% cases in this age group respectively.In comparison to our study the number of maximum cases of secondary infertility was in lower age group of 21-30 years in study done by Sharma et al[10] (2016) who found 26% cases.In our study numbers of maximum cases among primary infertility and secondary infertility were in higher age group compared to above studies because of late marriages in urban population and higher education.

In our study maximum cases in primary infertility, 71 (54.24%) patients came within 2-3 years duration of infertility almost similar to studies done by CJ Girish et al[15] (2012) and Achalkar et al[13] (2017) who found maximum cases 47.30% and 46.8% cases respectively in 2-3 years age group.

In comparison to our study duration of 4-6 years in maximum cases (41.39%) was higher in study done by Nandedkar et al[16] (2015).In our study most of the patients of primary infertility presented with in few years after marriage nowadays because of increasing awareness and accessibility of treatment facility.

In our study maximum among secondary infertility group maximum patients 12 (30.77%) came for endometrial biopsy within 4-5 years after last conception similar to the study done by CJ Girish et al[15] (2012) who observed most of secondary infertility patient (37.5%) came within 4-5 years duration of infertility.

Table7: Comparison of Histopathological findings in infertility with other studies (in percentage)

	Manjunath et al[17] 2011 (%)	Nandedkar et al[16] 2015 (%)	Silbina et al[12] 2017 (%)	M Ahmed et al[4] 2018 (%)	Preethi et al[14] 2019 (%)	Present study (%)
Proliferative (anovulatory) endometrium	32.3	15.75	22.8	41.33	48.52	64.71
Secretory phase	56.7	67.52	63.29	40.30	35.86	27.65
Simple hyperplasia	5.5	1.10	2.53	10.72	9.70	1.76
Luteal phase defect (LPD)	20	9.52	8.86	-	-	4.70
Tubercularendometritis	2.2	1.63	2.53	0.51	2.10	0.59

In our study out of total 170 cases of primary and secondary infertility proliferative endometrium (anovulatory) was seen in maximum 110 (64.71%) cases. In comparison to our study lower incidence of proliferative endometrium was seen in studies done by Nandedkar et al (2015)and Silbina et al(2017) who found proliferative endometrium in 15.75% and 22.8% cases respectively.

Table 8: Comparison of glycogen deficiency with other studies

S. No.	Studies	Glycogen depletion in endometrium
1	Gupta et al[18] (2013)	24.7%
2.	Sharma et al[10] (2016)	28.13%
3.	Pradhan et al[19] (2017)	28.88%
4.	Achalkar et al[12] (2018)	28.13%
5.	Desai et al[20] (2019)	32.08%
6.	Present study	32.72%

In our study, among primary and secondary infertility groups out of 110 cases of anovulatory endometrium 87 (79.09%) cases showed absence of glycogen and 23 (20.90%) cases showed mild glycogen content. Glycogen depletion was noticed in 32.72% cases of secretory endometrium of both primary and secondary infertility cases. Out of total 5 cases of luteal phase defect from primary infertility group glycogen deficiency was found in 3 (60%) cases in this group. Out of total 3 cases of luteal phase defect from secondary infertility group

glycogen deficiency was found in 2 (66.66%) cases.For the proper implantation and development of fertilized ovum, adequate amount of glycogen should be present in the glandular secretion. As glycogen provides nourishment to blastocyst in the early stages of gestation. Glycogen deficiency leads to improper implantation leading to death of ovum causing infertility. Hence it can be concluded that glycogen deficiency was more common in anovulatory cycles and luteal phase defect cases of both primary and secondary infertility cases leading to infertility.

CONCLUSION:-

In a developing country like India, where complex expensive immunological and hormonal assay procedures are not easily available or affordable endometrial biopsy is a valuable investigation for infertility. Proper correlation, clinical data and dating of endometrium helps to diagnose functional abnormalities of the endometrium as well as intrinsic abnormalities such as tuberculosis, most of whom are otherwise asymptomatic patient of infertility.

Endometrial biopsy is an economic tool for measurement of endometrial glycogen content and gives significant information about ovulation and luteal phase deficiency. Thus histomorphological study of the endometrium is one of the most reliable, safe, important and cheaper diagnostic tool in cases of primary and secondary infertility.

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