

Original Research Article

Histopathological evaluation of endometrial glycogen deficiency in primary and secondary infertile women in Bangladesh: a cross-sectional study

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ABSTRACT

Background: Infertility is a major public health issue, affecting a significant proportion of couples worldwide. Endometrial receptivity, influenced by glycogen content, plays a crucial role for successful implantation. This study aimed to evaluate the glycogen content and histomorphological patterns of the endometrium in infertile women in Bangladesh, focusing on differences between primary and secondary infertility.

Methods: This cross-sectional observational study was conducted at BIRDEM General Hospital, Dhaka, from July 2019 to June 2021. A total of 110 endometrial samples from infertile women from both primary and secondary infertility were analyzed. Samples were collected during the secretory phase of the menstrual cycle and assessed by using Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) staining. Glycogen content was graded on a scale from '0' to '++++' and analyzed across different types of infertility and histomorphological patterns.

Results: The majority of participants were aged 26-30 years (43.64%), with primary infertility being more prevalent (63.64%) than secondary infertility (36.36%). Deficient glycogen content was observed in 71.43% of primary infertility cases, compared to 60.00% of secondary infertility cases. Normal glycogen levels were more frequent in the secretory phase, particularly the late secretory phase, with 36 cases showing '+++' and 5 cases showing '++++' grades. Variations in glycogen content were also noted across histomorphological patterns, with Luteal Phase Defect (LPD) cases displaying mixed glycogen levels.

Conclusion: The study underscores the importance of adequate glycogen content in endometrial receptivity and its association with infertility. The findings suggest that histopathological evaluation of glycogen levels, particularly using PAS staining, can be an effective diagnostic tool for assessing endometrial health in infertile women. Addressing glycogen deficiencies may improve reproductive outcomes, and further research is needed to understand the underlying mechanisms contributing to variability in glycogen content.

Keywords: Infertility, Endometrial glycogen, Histomorphology, Luteal phase defect, PAS Staining, Primary infertility, Secondary infertility

INTRODUCTION

Infertility is a significant global health issue, affecting an estimated 8-12% of couples worldwide, which translates

to approximately 80 million couples struggling with conception at any given time.¹ The World Health Organization defines infertility as the failure to achieve a clinical pregnancy after 12 months of regular, unprotected

sexual intercourse. It has far-reaching implications, including psychological distress, social stigma, and economic burdens, highlighting the need for effective diagnostic and treatment approaches.² This research focuses on female infertility, specifically the endometrium's role in successful conception. In Bangladesh, infertility affects 4% of couples, but there's limited research on specific endometrial factors contributing to this issue.³ The biological role of the endometrium in supporting pregnancy is complex. It prepares for potential embryo implantation during the menstrual cycle, thickening in response to estrogen during the proliferative phase and becoming more secretory under the influence of progesterone during the luteal phase. These hormonal changes create a nurturing environment that facilitates the attachment of a fertilized ovum.^{4,5}

Endometrial receptivity, marked by its ability to support embryo implantation, is dependent on the coordinated expression of various biochemical markers, including glycogen, which serves as a critical energy source during the early stages of embryo development.⁶ Glycogen is synthesized and stored in the endometrial glands, where it provides nutrients that support the developing embryo before placental connections are established.⁷

Progesterone plays a pivotal role in regulating glycogen synthesis during the secretory phase of the menstrual cycle. Research has shown that progesterone induces the production of enzymes involved in glycogen synthesis, ensuring that the endometrium is adequately prepared to support the embryo immediately after implantation.⁸

This aspect of endometrial function highlights why any disruption in glycogen production or storage can lead to poor endometrial receptivity, ultimately causing implantation failure and contributing to infertility.⁹ Studies show glycogen deficiency, also known as "glycopenic uteri," can lead to inadequate endometrial preparation and implantation failures, suggesting hormonal imbalances affecting glycogen synthesis could contribute to infertility.¹⁰ The role of endometrial glycogen in supporting embryo development has been supported by numerous studies across different populations. In a study conducted by Rajan et al, the researchers observed that large masses of glycogen (+++++) were present in only 14.9% of secretory endometrium samples from infertile women compared to 77.7% in fertile controls, indicating a clear link between glycogen content and fertility status.¹¹

Similarly, Gupta et al. found that 24.7% of secretory phase endometrial samples from infertile women showed glycogen deficiency, which was associated with inadequate endometrial preparation and subsequent implantation failures.¹² Glycogen is a crucial biochemical marker for endometrial health and successful pregnancy outcomes. However, there is a gap in localized research, especially in Bangladesh, where factors like environmental conditions, lifestyle, and genetic predispositions can influence reproductive health. A study

by Karthik et al showed significant glycogen depletion in 12% of infertile cases.¹³ However, such data are sparse in Bangladesh, and addressing this research gap can provide a clearer understanding of the prevalence and causes of endometrial glycogen deficiency among infertile women in the region. The diagnostic assessment of endometrial health typically involves a combination of imaging, hormonal assays, and histopathological evaluations, with endometrial biopsy being the gold standard for direct tissue assessment.¹⁴

Endometrial biopsy allows for detailed examination of tissue samples, enabling the identification of structural and biochemical markers that indicate endometrial receptivity. Among the diagnostic tools used to assess endometrial function, periodic acid Schiff (PAS) staining is particularly useful for detecting and grading glycogen content. PAS staining allows for the visualization of glycogen as magenta-colored granules, providing a clear indication of the adequacy of endometrial preparations.¹⁵ Studies have consistently demonstrated the reliability of PAS staining in clinical settings.

For example, Girish et al, highlighted that PAS staining is effective in evaluating glycogen content, and their research confirmed that reduced glycogen levels were associated with luteal phase defects, thus linking it directly to infertility.¹⁰ PAS staining is a useful diagnostic tool for assessing endometrial health and identifying infertility causes. However, there is a lack of data from Bangladesh, making it challenging to understand glycogen deficiency patterns among infertile women. This study aims to develop localized strategies for infertility.

METHODS

Study type

This was a cross-sectional observational study.

Study place

The study was conducted in the Department of Pathology, BIRDEM General Hospital, Dhaka.

Study duration

The study duration was from July 2019 to June 2021.

Inclusion criteria

The study included 110 endometrial curettage or biopsy samples from infertile women diagnosed with either primary or secondary infertility.

Exclusion criteria

Endometrial samples from patients suffering from conditions other than infertility were excluded.

Additionally, patients with irregular menstrual cycles were provided hormone therapy to regularize their cycles, as prescribed by the treating doctors of the Gynecology Special Unit and Care, Department of Gynecology and Obstetrics of BIRDEM.

Following the treatment, endometrial samples were collected between the 21st and 23rd days of the menstrual cycle. Comprehensive clinical histories were obtained for all participants, including details on the menstrual cycle, the last menstrual period (LMP), duration of infertility, and obstetric history for those with secondary infertility.

Upon collection, the endometrial samples were fixed in formalin and subjected to gross examination according to guidelines from standard surgical pathology textbooks. The tissues were then processed routinely, and paraffin blocks were prepared.

Sections of 3-4 microns thickness were cut from each biopsy, and these sections were stained with Hematoxylin and Eosin (H&E) for morphological examination. The stained sections were assessed for endometrial morphology, and histological dating was performed to diagnose Luteal Phase Defect (LPD). LPD was diagnosed based on the JONES criteria, which defines LPD as a delay of two or more days in the histologic development of the endometrium.

To evaluate glycogen content, additional sections (3-4 microns) were cut and stained using periodic acid schiff (PAS) and periodic acid schiff with diastase (PAS-D). PAS staining was employed to detect and grade the glycogen content in the endometrial tissues, while PAS-D staining served to confirm the presence of glycogen by digesting it, resulting in a negative reaction where glycogen was present. The glycogen content was assessed using the grading system established by Arzac and Blanchet, with contributions from subsequent studies by Desai et al, Maru et al and Pradhan et al.¹⁶

The grading scale was as, 0: Negative reaction (absence of glycogen). +: Very small granules. ++: Coarse granules. +++: Small masses. ++++: Large masses.

According to Arzac and Blanchet's system, the grades ranged from '0' to '++++'. A '0' grade indicated a negative reaction to PAS staining, suggesting the absence of glycogen (Figure 1). Grades '+' and '++' were characterized by very small and coarse granules, respectively, typically found in the proliferative phase, situated basally or around the nuclei of glandular epithelial cells (Figure 2).

Grades '+++' and '++++' indicated the presence of small and large masses, respectively. Small masses were predominantly found in the subnuclear and perinuclear zones during the early secretory phase, while in the late secretory phase, they appeared in the apical portions and the gland lumens (Figure 3). Large masses, typically

observed before menstruation, were associated with decidualized endometrium, reflecting advanced glycogen storage in preparation for possible implantation.

All data, including patient histories, morphological findings, and glycogen content gradings, were recorded systematically using a predesigned data collection sheet. Statistical analysis was performed to determine the distribution and correlation of glycogen content among different phases and to assess its relationship with infertility.

Ethical considerations were strictly followed throughout the study, ensuring informed consent was obtained from all participants, and patient confidentiality was maintained.

RESULTS

The age distribution of the participants (n=110) showed that most were aged 26-30 years (43.64%), followed by 31-35 years (28.18%). Participants aged 21-25 years made up 19.09%, while those aged 36-40 years were 7.27%. Only 1.82% were over 40 years old (Table 1).

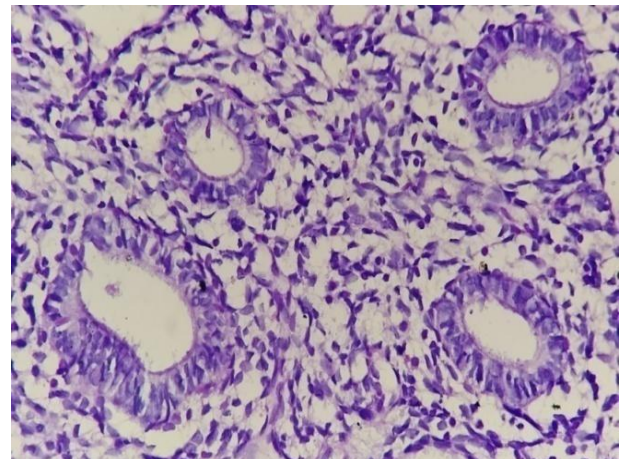


Figure 1: '0' (no reaction) after PAS staining; 40X.

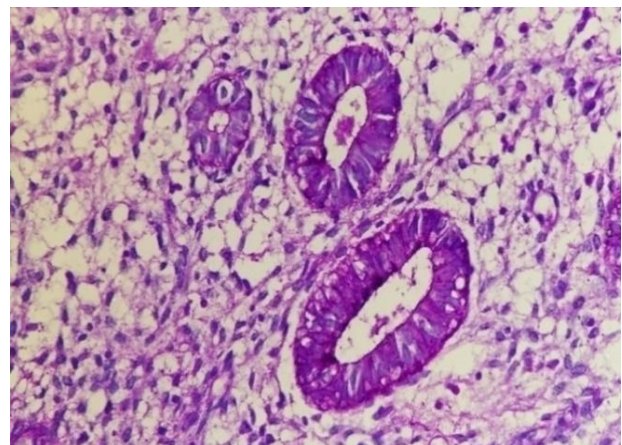


Figure 2: '++' (coarse granules) surrounding lumen are found in proliferative phase; 40X.

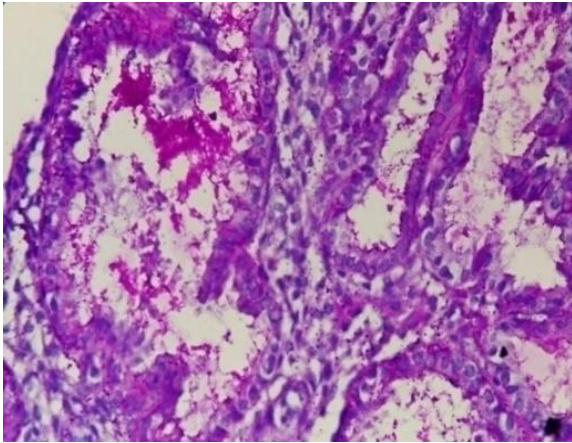


Figure 3: '+++ (Small masses) in the lumen of gland in LSP; 40X.

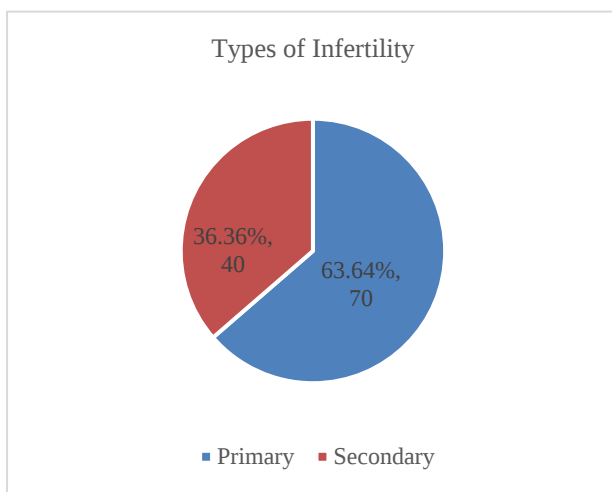


Figure 4: Types of infertility among the participants (n=110).

The histomorphological analysis of the participants (n=110) revealed that the most common pattern was the secretory phase, observed in 47.27% of cases. Within this group, 25.45% were in the late secretory phase, 3.64% in the early secretory phase, and 18.18% had luteal phase defect (LPD). The proliferative phase was identified in 37.27% of participants. Other findings included endometrial hyperplasia in 10.00% of cases, endometrial polyps in 2.73%, tuberculous endometritis in 1.82%, and non-specific endometritis in 0.91% (Table 2).

Figure 4 illustrates the distribution of infertility types among the participants (n=110). The majority of the cases were classified as primary infertility, accounting for 63.64% (70 participants), while 36.36% (40 participants) had secondary infertility.

The grading of glycogen content in different types of infertility (n=110) presented in Table 3 showed a higher prevalence of deficient glycogen levels among women with primary infertility (71.43%) compared to those with secondary infertility (60.00%). Within the primary

infertility group, 4.29% had a grade of '0' (negative reaction), 45.71% showed '+' (very small granules), and 21.43% had '++' (coarse granules). In contrast, none of the secondary infertility cases had a '0' grade, 32.50% showed '+', and 27.50% had '++'.

Normal glycogen levels were observed in 28.57% of primary infertility cases and 40.00% of secondary infertility cases. For normal grades, 27.14% of primary infertility cases had '+++ (small masses), compared to 30.00% in the secondary infertility group. Additionally, 1.43% of primary infertility cases had '++++ (large masses), whereas 10.00% of secondary infertility cases reached this grade. The p values for differences in deficient ('0' to '++') and normal ('+++ and '++++') glycogen levels between the two groups were 0.269 and 0.066, respectively, indicating no statistically significant difference. Table 4 presents the distribution of glycogen content across different histomorphological patterns of the endometrium (n=110). In the proliferative phase, the majority of samples showed deficient glycogen content, with 3 cases graded as '0' (negative reaction), 30 cases graded as '+' (very small granules), and 8 cases as '++' (coarse granules). No cases in the proliferative phase had normal glycogen grades ('+++ or '++++'). In the secretory phase, there was a higher presence of normal glycogen levels, with 36 cases showing '+++ (small masses) and 5 cases displaying '++++ (large masses).

Within the subcategories, the early secretory phase had 1 case graded as '++', with 3 cases displaying '++++'. Among other histomorphological patterns, endometrial hyperplasia had 6 cases at '+' and 5 at '++', while endometrial polyps predominantly showed deficient glycogen with 3 cases at '+'.

Table 1: Age distribution of the participants (n=110).

Age (in years)	Frequency	%
21-25	21	19.09
26-30	48	43.64
31-35	31	28.18
36-40	8	7.27
>40	2	1.82

Table 2: Histomorphological distribution of the participants (n=110).

Histomorphological pattern	Frequency	%
Proliferative phase	41	37.27
Secretory phase	52	47.27
Early secretory phase	4	3.64
Late secretory phase	28	25.45
LPD	20	18.18
Endometrial hyperplasia	11	10.00
Endometrial polyp	3	2.73
Tuberculous endometritis	2	1.82
Non-specific endometritis	1	0.91

Table 3: Grading of glycogen content in different types of infertility (n=110).

Glycogen content grade (PAS staining)	Primary infertility (n=70)		Secondary infertility (n=40)		P value
	N	%	N	%	
Deficient Glycogen	50	71.43%	24	60.00%	0.269
0	3	4.29%	0	0.00%	
+	32	45.71%	13	32.50%	
++	15	21.43%	11	27.50%	
Normal glycogen	20	28.57%	16	40.00%	0.066
+++	19	27.14%	12	30.00%	
++++	1	1.43%	4	10.00%	

Table 4: Glycogen content in different morphological patterns of endometrium (N=110).

Histomorphological pattern	Glycogen content grade				
	Deficient			Normal	
	0	+	++	+++	++++
Proliferative phase	3	30	8	-	-
Secretory phase	16			36	
Early secretory phase	-	-	1	3	-
Late secretory phase	-	-	7	16	5
LPD	-	3	5	12	-
Endometrial hyperplasia	-	6	5	-	-
Endometrial polyp	-	3	-	-	-
Tuberculous endometritis	-	2	-	-	-
Non-specific endometritis	-	1	-	-	-

DISCUSSION

The present study provides a comprehensive analysis of endometrial glycogen content and histomorphological patterns among infertile women in Bangladesh, focusing on primary and secondary infertility. The findings indicate that most participants were in the age group of 26-30 years, similar to previous studies showing a higher prevalence of infertility in this demographic due to factors such as career prioritization, delayed marriages, and lifestyle changes. These trends were consistent with data from Al-Turki et al. who observed similar age distributions in Saudi Arabia, reinforcing that infertility primarily affects women in their reproductive prime.¹¹

Additionally, other studies have found comparable age prevalence among infertile populations, supporting the significance of early diagnosis and intervention in younger age groups to improve outcomes.¹⁸ Histomorphological evaluation revealed that the secretory phase was the most common pattern observed among participants, accounting for 47.27% of cases. This included early and late secretory phases, along with a significant proportion of Luteal Phase Defects (LPD). These results align with findings by Girish et al., who also identified a prevalence of LPD associated with glycogen deficiency, highlighting that insufficient glycogen levels during the secretory phase can impair endometrial preparation, thus contributing to infertility.¹⁰ Additionally, Gupta et al. corroborated these observations, showing that while the secretory phase typically

demonstrates normal glycogen accumulation ('+++ to '++++'), deficiencies during this phase are a marker of compromised endometrial receptivity.¹¹ The study found that primary infertility was more prevalent (63.64%) compared to secondary infertility (36.36%), which is consistent with findings by Rajan et al who reported higher rates of primary infertility associated with endometrial abnormalities, including glycogen depletion.⁹

However, studies by Shivappa et al suggested a slightly higher prevalence of glycopenic endometrium among secondary infertility cases, highlighting the variability across populations and emphasizing the need for localized studies to understand the etiology in specific demographics.¹⁹ The study found that primary infertility cases (71.43%) have more deficient glycogen content than secondary infertility (60.00%), suggesting lower levels may affect endometrial receptivity, a finding consistent with previous research on primary infertility.¹⁰ The distribution of normal glycogen levels further supports this assertion, as normal glycogen content was observed more frequently in secondary infertility cases (40.00%) compared to primary infertility (28.57%), a pattern similarly reported by Karthik et al., reinforcing that while both groups are affected, the extent of glycogen depletion varies.¹² Comparative analysis of glycogen content across histomorphological patterns in this study revealed that the secretory phase showed higher normal glycogen levels ('+++ and '++++'), especially in the late secretory phase, aligning with Gupta et al. and Shivappa et al who reported

that glycogen is a key nutrient for the embryo during early gestation, and its adequate presence is essential for successful implantation.^{11,19} The findings related to LPD cases, which had mixed glycogen content, further highlight the complexities of endometrial receptivity. While some cases showed deficient glycogen, others presented normal levels ('+++'), suggesting that LPD may involve multifactorial causes beyond glycogen depletion alone. This is supported by observations from Girish et al., who found that LPD often correlates with hormonal imbalances affecting glycogen synthesis, leading to variable presentations.¹⁰

Studies like those by Rajan et al and Shivappa et al also noted similar patterns, where LPD was linked to inconsistent glycogen content, highlighting the need for comprehensive histological and biochemical evaluations to diagnose and treat this condition effectively.^{9,19} The presence of other endometrial conditions, such as hyperplasia and endometritis, albeit less frequent, emphasizes the broader spectrum of endometrial pathologies contributing to infertility. Gupta et al highlighted that conditions like hyperplasia could complicate normal endometrial function, further disrupting glycogen metabolism and impairing implantation.¹¹

Similarly, studies by Puente et al found that conditions such as endometrial hyperplasia and tuberculous endometritis, although rare, still significantly affect fertility by altering endometrial receptivity.²⁰ This study emphasizes the importance of adequate glycogen content in the endometrial environment for successful implantation. It suggests targeted diagnostic strategies, such as glycogen evaluation using histochemical staining methods, to improve fertility outcomes. Future research should explore localized patterns and biochemical mechanisms for effective therapeutic interventions, especially in under-researched regions like Bangladesh.^{9,10} Further investigations into the role of hormonal therapies, as well as localized studies focusing on genetic and environmental factors affecting glycogen metabolism, would be essential to enhance treatment strategies and address infertility effectively.

The limitations of the study were that the study was conducted in a single hospital with a small sample size. So, the results may not represent the whole community.

CONCLUSION

The present study highlights the critical role of endometrial glycogen content and histomorphological patterns in understanding infertility among women in Bangladesh. The findings indicate that primary infertility is more prevalent than secondary infertility, with a significant proportion of cases showing deficient glycogen levels, particularly during the secretory phase. The analysis also underscores the importance of adequate glycogen content for optimal endometrial receptivity, with

variations in glycogen distribution linked to different types of infertility and phases of the menstrual cycle.

These insights suggest that histopathological evaluation, including PAS staining for glycogen assessment, should be integrated into routine diagnostic protocols for infertility management. By addressing the observed glycogen deficiencies, particularly through targeted hormonal therapies, it may be possible to improve reproductive outcomes for women affected by infertility. Further research, particularly localized studies, is needed to explore the underlying causes of glycogen variability and to develop more effective, region-specific treatment strategies.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Human Reproduction*. 2007;22(6):1506–12.
2. Organization WH. International Statistical Classification of Diseases and Related Health Problems: Alphabetical index. World Health Organization. 2004: 824.
3. Magdum M, Chowdhury MAT, Begum N, Riya S. Types of Infertility and Its Risk Factors among Infertile Women: A Prospective Study in Dhaka City. *J Biosci and Med*. 2022;10(4):158–68.
4. Lessey BA. The role of the endometrium during embryo implantation. *Hum Reprod*. 2000;15(6):39–50.
5. Sandra O. Hormonal control of implantation. *Annales d'Endocrinol*. 2016;77(2):63–6.
6. Revel A. Defective endometrial receptivity. *Fert and Ster*. 2012;97(5):1028–32.
7. Ishihara S, Taketani Y, Mizuno M. Stimulatory action of progesterone on the synthesis of glycogen in primary cell culture of human endometrium. *Asia-Oceania J of Obstetrics and Gynaecol*. 2004;14(1):117–22.
8. Flannery CA, Choe GH, Cooke KM, Fleming AG, Radford CC, Kodaman PH, et al. Insulin Regulates Glycogen Synthesis in Human Endometrial Glands Through Increased GYS2. *The J Clin Endocrinol & Metabol*. 2018;103(8):2843–50.
9. Rajan A, Parijatham BO, Srivastava V. A Correlative Study of Endometrial Glycogen Content in Female Infertility. *Journal of Pharma Res Int*. 2021;22:97–109.
10. Girish C, Kotur N, Nagarajappa A, Manjunath M. A correlative study of endometrial glycogen content and other contributory factors on female infertility. *Int J Biomed Adv Res*. 2012;3(1):45-8.

11. Gupta A, Mathur SK, Gupta A. Co-orelation of histological dating and glycogen content by histochemical stain during various phases of menstrual cycle in primary infertility. *Open J of Pathol*. 2013;3(2):65-8.
12. Lr K, R RPS, D S, Shetageri SN. A study on glycogen content of endometrial glands in infertile women. *J Pathol of Nepal*. 2021;11(2):1796–801.
13. McCluggage WG. My approach to the interpretation of endometrial biopsies and curettings. *J Clin Pathol*. 2006;59(8):801–12.
14. Mathew SM. A comparison of pipelle endometrial biopsy with dilatation and curettage for evaluation of endometrial pathology in abnormal uterine bleeding. *J of Evid based Med and Healthcare*. 2020;7(21):1–5.
15. Zawar MP, Deshpande NM, Gadgil PA, Mahanta AA. Histopathological study of endometrium in infertility. *Indian J Pathol Microbiol*. 2003;46(4):630–3.
16. Desai KN, Maru AM. Histopathological study of endometrium in cases of infertility in tertiary care hospital. *Int J Clin Diagn Pathol*. 2019;2(1):29–32.
17. Al-Turki HA. Prevalence of primary and secondary infertility from tertiary center in eastern Saudi Arabia. *Middle East Fertility Society J*. 2015;20(4):237–40.
18. Samarakoon S, Rajapaksa L, Seneviratne HR. Prevalence of primary and secondary infertility in the Colombo District. *Ceylon J Med Sci*. 2007;45(2):4584.
19. Shivappa P, Isaacs R, Mandrelle K, Goyal S. Glycopenic endometrium in infertility. *International Journal of Research in Medical Sciences*. 2022;10(8):1663–7.
20. Puente E, Alonso L, Laganà AS, Ghezzi F, Casarin J, Carugno J. Chronic Endometritis: Old Problem, Novel Insights and Future Challenges. *Int J of Fertility & Ster*. 2019;13(4):250.

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