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Morphometric Evaluation of Endometrial Blood Vessels and Its Clinico-pathological Relation in Patients with Dysfunctional Uterine Bleeding.

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Abstract:
Introduction
Disturbances of menstrual bleeding are a medical and social problem. The term dysfunctional uterine bleeding (DUB)should be reserved for those patients in whom not only the pelvic examination is normal but in whom there is no other demonstrable extragenital cause for bleeding. The present study was designed to analyze the various histopathologic patterns of endometrium with respect to the number as well as morphology of endometrial blood vessel in DUB patients using common stains.
Aims:
To analyze the various histopathological patterns of endometrium with respect to the number as well as morphology of endometrial blood vessel in Dysfunctional Uterine Bleeding patients using common stains.
Methods and Material:
Over a period of 3 years, we evaluated the histopathological appearance of endometrium with special emphasis on endometrial blood vessels in 180 patients in the reproductive age group with DUB. The specimens were obtained either by dilatation and curettage or hysterectomy. The stained tissue sections were used for endometrial dating, pattern analysis and evaluation of various characteristics of endometrial blood vessels.. Appropriate control was taken.. For statistical analysis and comparison of means student unpaired "t"-test was used
Results:
*Broadly the two groups were identified. **A)** Non secretory type of endometrium with 75 cases **B)** Secretory type of endometrium with 105 cases. Commonest pathology of **Group A** was Simple hyperplasia where as that of **Group B** was Irregular ripening and both showed significant vascular changes in terms of mean vascular density, congestion and dilatation. Insignificant vascular changes were seen in proliferative and secretory endometrium in group A and B respectively.*
Conclusions:
The observed altered vascular morphology in the different endometrial pattern found may be the underlying pathological mechanism for DUB.
***Key-words**: Dysfunctional Uterine Bleeding, Endometrial blood vessels, Morphometry, DUB.*

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I. Introduction

Disturbances of menstrual bleeding are a medical and social problem. Menorrhagia is a common gynaecological problem in women of reproductive age, accounting for as much as 20% of outpatient clinic visits.[1] The disorder may not only confer iron deficiency anaemia and require hysterectomy, but also causes considerable social discomfort and reduces quality of life. Although commonly associated with fibroid and carcinoma, approximately 50% of menorrhagia patients present without evidence of any uterine pathology, suggesting a defect in the cellular process and regulatory mechanism of menstruation. [1,2] The term dysfunctional uterine bleeding (DUB)should be reserved for those patients in whom not only the pelvic examination is normal but in whom there is no other demonstrable extragenital cause for bleeding. [2,3] The present study was designed to analyze the various histopathologic patterns of endometrium with respect to the number as well as morphology of endometrial blood vessel in DUB patients using common stains.

II. Material and Methods

The present study spanned over a period of 3 years and was conducted in the Department of Obstetrics and Gynaecology and Department of Pathology and included 180 cases. All those married patients in reproductive age group who were not pregnant, not using any intrauterine contraceptive device and not taking any medication and who presented with irregular /intermittent/prolonged/heavy/decreased menstrual bleeding were included in the study group. Any pelvic pathology and bleeding or coagulation disorder were ruled out first. Twenty young to middle age patients who presented with documented lesions like uterine/ cervical fibroid, uterine prolapsed and cervical lesion etc. were included in the control group. The endometrial specimens were obtained either by dilatation or curettage (D&C) or hysterectomy and were examined after staining them with haematoxylin and eosin (H&E) and with inclusion of special stains like reticulin, Masson's trichrome and Periodic acid schiff's stain wherever required were used for dating of the endometrium, pattern analysis and evaluation of various characteristics in different endometrial patterns. On the basis of architecture of glands, lining epithelium of glands, nuclear and cytoplasmic features, stromal features and any other superadded hormonal changes of eostrogen and progesterone, the endometrium was categorized into two types:- 1) Non secretory type of endometrium:- This included proliferative phase, disordered proliferative endometrium, simple hyperplasia and complex hyperplasia. 2) secretory type of endometrium:- This group comprised of secretory phase, Luteal phase insufficiency (irregular ripening, irregular shedding etc.) and pill endometrium.

The overall vascularity of the endometrium was judged by counting the average number of blood vessels in 10 high power fields (HPF) and compared with control. Also the number of blood vessels showing vascular dilatation and congestion in 10 high power field (HPF) were counted. Thin walled capillaries and arterioles including spiral arterioles were included in the counting. Dilated vascular sinuses were excluded. The statistical test used was student's unpaired t-test. P values more than 0.05 was considered non significant (NS) and the values < 0.05 and <0.001 was considered significant (S) and highly significant (HS) respectively.

III. Observations

The study included a total 180 cases of married women in the reproductive age group who were diagnosed to have DUB clinically. Maximum number of patients, 107 cases (59.4%), suffering from dysfunctional uterine bleeding were found to be in the fifth decade, followed by 49 cases (27.2%) in fourth decade. Least number of patients were observed in 21 to 30 years age group.

The presenting complaints were menorrhagia, polymenorrhagia, menometrorrhagia, hypomenorrhoea, oligomenorrhoea and amenorrhoea in decreasing order of frequency. Majority of the DUB (38.9%) presented with menorrhagia followed by polymenorrhagia (21.2%). The least number of cases (1.1%) presented with amenorrhoea.

Table I shows various endometrial histopathological patterns of those seen in DUB cases .Of the total 180 cases studies, secretory pattern predominated over the non secretory pattern. Of those 105 cases constituted secretory pattern while 75 cases showed non secretory endometrial pattern. Irregular ripening (27.8%) (Figure I) constituted the majority cases in non-secretory endometrial pattern. Secretory phase (18.9%) of endometrium (Figure II) came in the second place while it was revealed that pill effect(Figure III) on endometrium (5.6%) constituted the least number of cases in this group. Disordered proliferative endometrium/ Simple hyperplasia (20.5%) (Figure IV) was the chief cause of DUB in patients with non secretory endometrial pattern, followed by complex hyperplasia(Figure V) which accounted for 11.8% of cases. Proliferative type of endometrium (9.4%)(Figure VI) was the least common type of endometrial pattern in non secretory endometrium.

A control group of 20 patients consisted of patients in reproductive age group who presented with other diseases like non hormone producing ovarian tumors, uterine diseases like serosal and intramural leiomyoma and cervical lesions with regular cycles and no abnormal bleeding and with histopathological diagnosis of either proliferative phase or secretory phase of endometrium.

The mean vascular density, average number of blood vessels showing congestion and dilatation was calculated and the results are shown in Table II and III in control as well as study group and the values were compared and significance was calculated as shown in Table IV.

In the non secretory group the average number of blood vessels/10HPF (mean vascular density) was insignificant in proliferative, simple hyperplasia and complex hyperplasia without atypia while it was significantly increased in complex hyperplasia with atypia. In the secretory group it was insignificant ($p>0.05$) in secretory pattern but significantly increased in irregular ripening ($p<0.05$). The increase in the number of blood vessels was highly significant ($p<0.001$) in irregular shedding and pill endometrium when compared to the control group .

In the present study of the total, as much as 42.5% of the cases (non secretory endometrium- 3% and secretory endometrium- 39.5%) showed a significant increase in the mean vascular density, while 57.2% showed no significant increase in the mean vascular density. The average number of blood vessels which

showed significant dilatation in this study was seen in majority of cases i.e. 71.7% (non secretory endometrium- 32.2% and secretory endometrium- 39.1%) compared to 28.3% cases who did not exhibit a significant number of dilated vessels. Regarding congestion of blood vessels, it was revealed that majority of cases (70.7%) (Non secretory endometrial pattern- 32.2% and secretory endometrial pattern 38.5%) showed significant increase in the number of blood vessels exhibiting congestion, whereas 29.3% of cases could not reveal any significant congestion

IV. Discussion

On histopathology in the present study, a variety of endometrial patterns were seen in cases of dysfunctional uterine bleeding and on blood vessels morphometry various vascular alterations were observed. The most common endometrial pattern seen was irregular ripening followed by disordered proliferation/ simple hyperplasia and least common was irregular shredding. A significant change in vascular morphology in form of increase in number of blood vessels, significant dilatation and congestion was seen in simple hyperplasia, complex hyperplasia with or without atypia, irregular ripening, irregular shedding, and pill endometrium as also observed by Roger et al, Abdulafia et al, Morgan et al, Livingstone and Fraser , Nyaha et al and Makhija et al. [4,5,6,7,8,9] accounting for the menstrual abnormalities like menorrhagia and polymenorrhoea. This may partly explain the underlying mechanism in the above mentioned dysfunctional uterine bleeding.

However we did not find any significant vasodilatation and congestion in secretory phase in this present study. Similar to our findings, Rees et al, Hourian et al, Ryan et al and Shaw et al. [10,11,12,13] also showed that there was no difference in vascular morphology across the different phases of menstrual cycle. However Makhija et al. [9] observed that there is significant dilatation and congestion even in secretory phase of endometrium. It has been observed that ovulatory dysfunctional uterine bleeding was predominantly associated with decreased endometrial vasoconstriction and defective vascular plug formation, leading to excessive bleeding. [7, 8,9] This could explain the abnormal bleeding encountered in secretory phase.

In anovulatory bleeding, unopposed estrogen leads to excessive endometrial proliferation and hyperplasia. Large thin walled tortuous and fragile superficial blood vessel could lead to increased blood loss as also suggested by Livingstone and Fraser. [7] However the menorrhagia may also be due to the local effects of prostaglandin (PGE2 and PGI2), low levels of PGF2α, Nitric oxide (both secreted from vascular endothelium) and reduces levels of endothelin-1 secreted from vascular endothelium and increased tissue plasminogen activator and subsequent increased fibrinolytic activity as suggested by the study conducted by Livingstone and Fraser [7] and Shaw [8]. The study conducted by Mints et al. [14] concluded that the up regulation of VEGF-A and its receptors for e.g. VEGFR-1 and VEGFR-2 in capillaries which enhance the proliferation of vascular endothelial cells, augments vascular permeability and induce fenestration in capillaries and venules could be reason for menorrhagia in such patients. Thus various biochemical and molecular factors play an important role in menorrhagia apart from vascular morphological changes in different endometrial patterns as discussed above.

Thus it was concluded that the observed altered vascular morphology in different endometrial patterns described above may be the underlying pathological mechanism for dysfunctional uterine bleeding. Since proliferative and secretory pattern did not show any significant alteration in vascular morphology with regard to mean vascular density, dilatation and congestion, menorrhagia in these cases may be due to some local molecular mechanism like effects of prostaglandins (PGF2α and PGE2) prostacyclins (PGI2), nitric oxide, reduced levels of endothelin-1 and up regulation of VEGF – A and its receptors e.g. VEGFR-1 and VEGFR-2 which require further studies at molecular level.

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Table I- Various histologial patterns of endometrium

Endometrial pattern	Number of cases	Percentage
NONSECRETORY TYPE	**75**	41.6%
1. Proliferative phase	17	9.4%
2. Disordered proliferative endometrium/ simple hyperplasia	37	20.5%
3. Complex hyperplasia	21	11.7%
SECRETORY TYPE	**105**	58.4%
1. Secretory phase	34	18.9%
2. Irregular ripening	50	27.8%
3. Irregular shedding	11	6.1%
4. Pill effect	10	5.6%

Table II – Analysis of microvascular density, dilatation, congestion in control group.

Diagnosis	Number of cases (n=20)	Average number of blood vessels/10 HPF (Mean Vascular Density)	Average number of dilated blood vessels/10 HPF	Average number of congested blood vessels/10 HPF
Nonsecretory endometrium				
Proliferative phase	7	3.0 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
Secretory endometrium				
Early secretory	5	3.9 ± 0.1	0.7 ± 0.1	0.3 ± 0.07
Mid secretory	3	4 ± 0.1	0.9 ± 0.2	0.6 ± 0.07
Late secretory	5	4.1 ± 0.1	1.2 ± 0.1	0.9 ± 0.07

Table 3: Analysis of microvascular density, dilatation, congestion in study group.

Diagnosis	Number of cases	Average number of blood vessels/10HPF	Average number of dilated vessels/10HPF	Average number of congested blood vessels/10HPF
Non secretory type of endometrium				
1) Proliferative	17	3.4±0.4	0.7±0.2	0.9±0.07
2) Disordered proliferative endometrium/ simple hyperplasia	37	3.9±0.8	0.9±0.1	1.2±0.5
3) Complex Hyperplasia without atypia	16	5.0±0.4	2.7±0.2	1.0±0.1
4) Complex hyperplasia with atypia	05	6.9±0.6	3.4±0.4	1.4±0.1
Secretory type of endometrium				
5) Secretory	34	4.7±0.1	1.1±0.1	0.8±0.4
6) Irregular ripening	50	4.8±0.9	3.3±0.2	1.0±0.1
7) Irregular shedding	11	5.6±0.1	2.6±0.2	1.3±0.7
8) Pill endometrium	10	6.5±0.2	3.1±0.2	1.6±0.2
TOTAL	180			

Figure I: Irregular ripening of endometrium—photomicrograph shows increase in mean vascular density and prominent dilatation and congestion in blood vessels (H& E, 400 X)

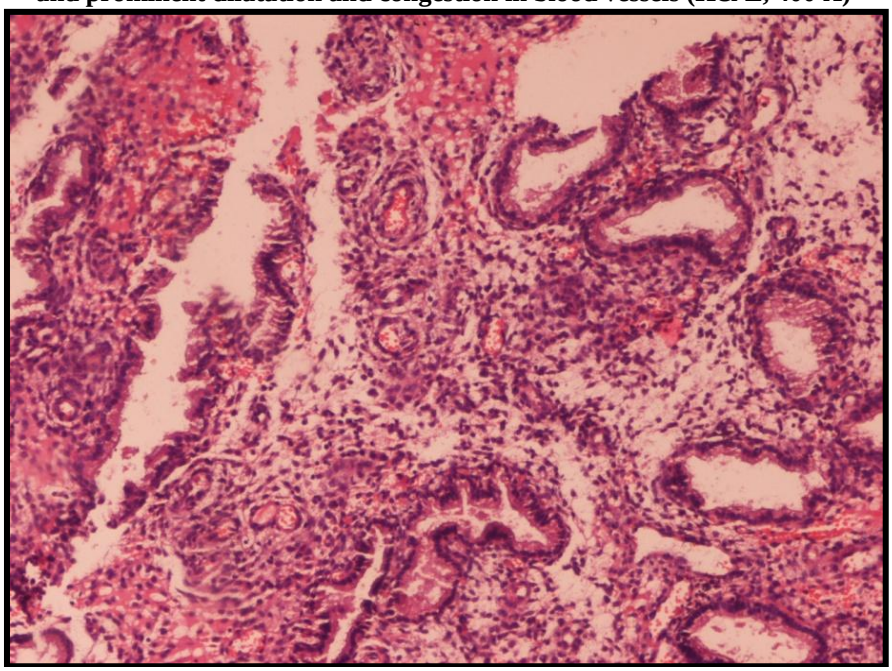


Figure II: Late secretory endometrium—photomicrograph showing well developed spiral arterioles (H&E 400X).

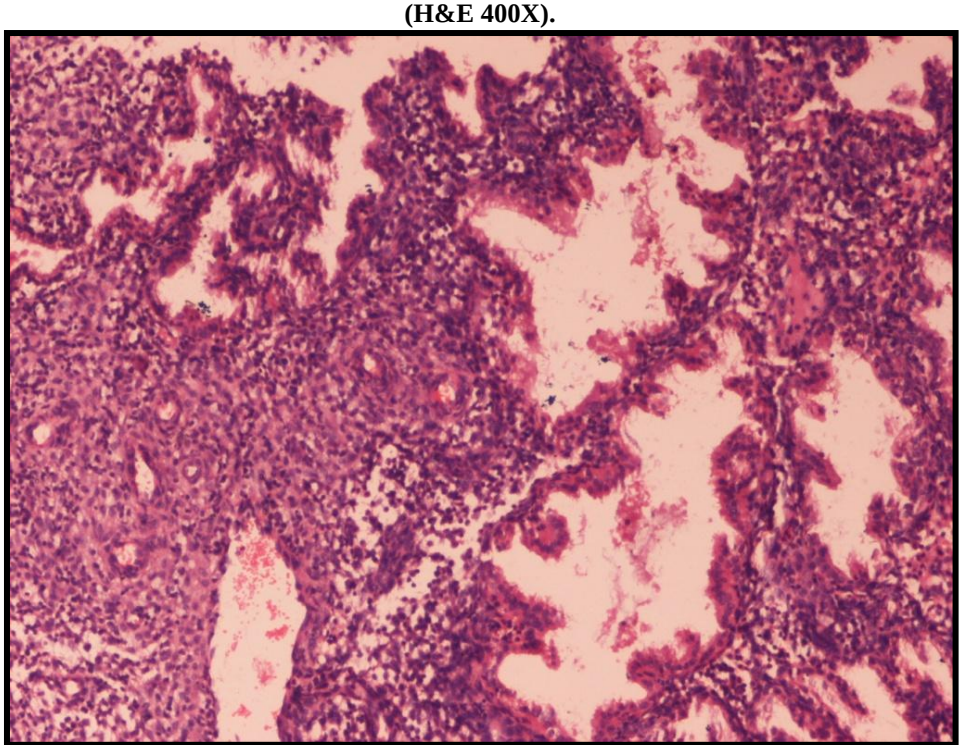
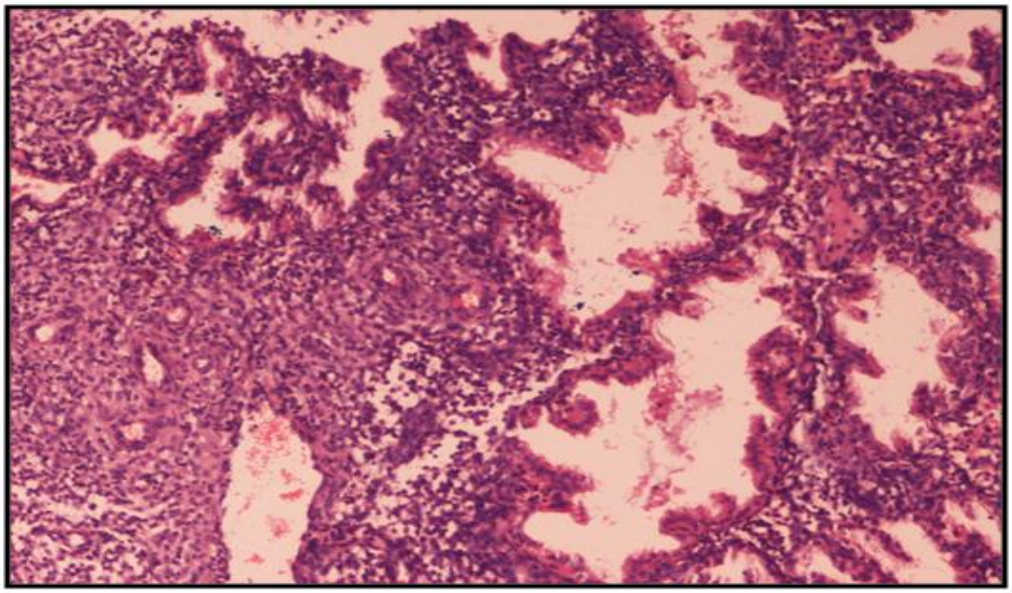


Figure III: Pill endometrium exhibiting combined estrogen progesterone pill effect. Small sized sparse glands situated in a pseudodecidualized stroma. Mean vascular density is increased in this field (H&E 400X).



Figur IV: Disordered proliferative endometrium -- photomicrograph shows variable sized glands, some with mild cystic dilatation, present in a compact to loose stroma (H&E 100X).

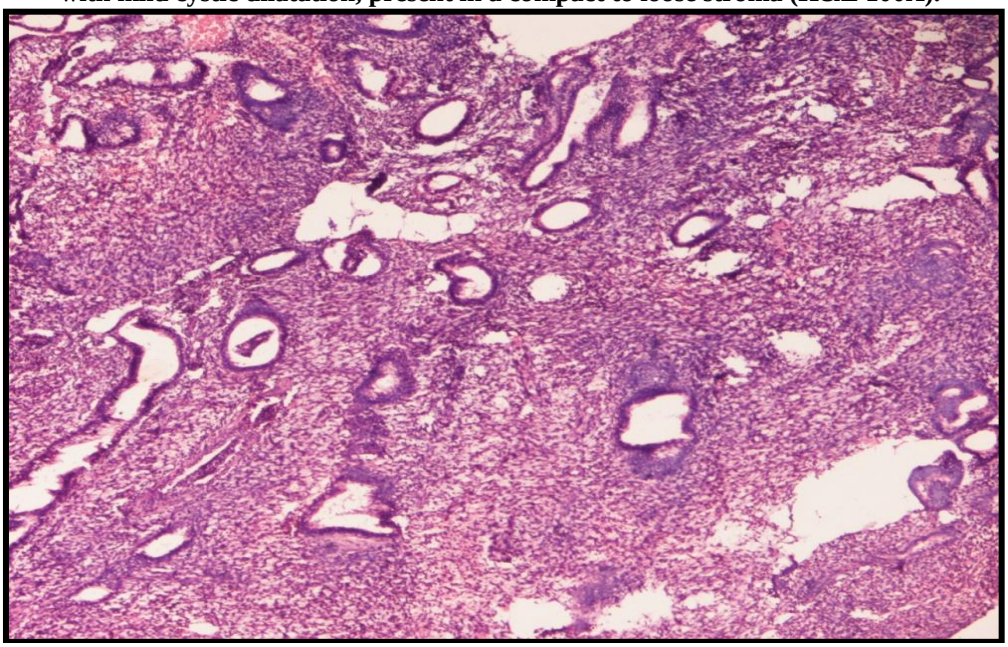


Figure V: Complex hyperplasia with atypia—high power view of the same slide showing marked increase in mean vascular density (H&E 400X).

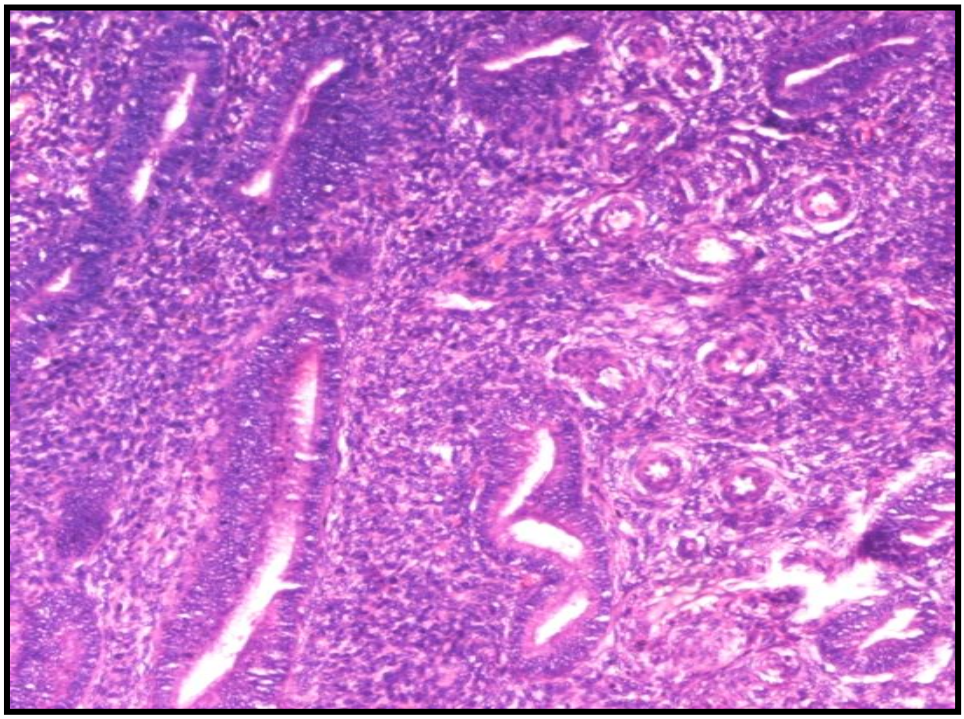
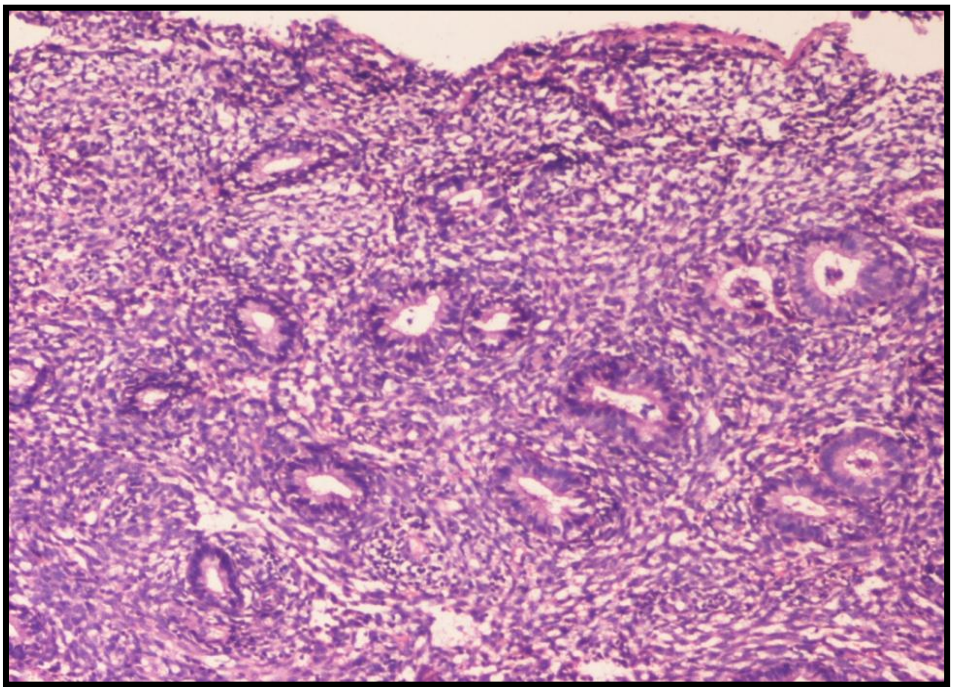


Figure VI: Proliferative phase --section shows small caliber glands situated in compact to loose stroma. Mean vascular density is not prominent in this phase (H&E 400X).



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