MATERIAL AND METHODS

The present study was carried out in the department of Obstetrics and Gynaecology and department of Pathology & Microbiology, Maharani Laxmi Bai Medical College and Hospital, Jhansi in a period of twelve months.

Selection of cases: The study group comprised of patients who were forty years or above in age, attending out patient department of Obstetrics & Gynaecology and those who were hospitalized for dilatation and curettage and other operations like hysterectomy, Fothergill repair, cases of prolapse with or without history of spotting, menorrhagia, polymenorrhoea and bleeding per vaginum off and on.

CLINICAL EXAMINATION:

History: Detailed history of the present complaints was taken along with the duration of complaints. Obstetrical history under the headings of gravida, parity, and the year of last delivery noted. The menstrual history was taken under the headings of cycle which included the number of days the flow lasted and the duration of the menstrual cycle. The amount of flow and the date of the last menstrual period was also noted.
Fig. 1: Endometrial aspiration cannula and syringe.
GENERAL EXAMINATION:

Thorough general examination was done with special attention as regards to general condition, pulse, blood pressure, anaemia, jaundice and weight of the patient.

PER VAGINUM EXAMINATION: was done to know the size and consistency of the uterus, the condition of the tubes and ovaries and any evidence of pelvic inflammation. Any type of bleeding was also noted.

PER SPECULUM EXAMINATION: was done to see condition of cervix and see whether the bleeding was from the os or from some other area in the vagina.

METHODS OF COLLECTION OF MATERIAL:

A uterine cannula made of steel and a 20 cc (Fig. I) glass syringe were used for aspiration from the endometrial cavity and an endometrial curette having serrations at the proximal end and a wire inserted through the distal end was used for taking the biopsy from the endometrial cavity.

PREPARATION OF THE PATIENT:

A written consent of the patient was taken. She was asked to evacuate her bladder. Cases who were unable to evacuate the bladder, were catheterized.
No sedation was given to the patient before doing endometrial aspiration. Before doing endometrial biopsy, 10 mg of diazepam was given intramuscularly thirty minutes before the procedure.

Patients were made to lie down in lithotomy position.

**TECHNIQUE OF ENDOMETRIAL ASPIRATION:**

The vulva was painted with antiseptic solution and vagina was cleaned by antiseptic lotion. The part was draped by a cut sheet. Per vaginum examination was done to confirm the previous findings. Posterior vaginal wall was retracted by Sim's speculum. The anterior vaginal wall was retracted by anterior vaginal wall retractor. Volsellum was used to hold the anterior lip of cervix. A uterine sound was passed to know the length of uterus and the patency of the cervical os was determined. Endometrial aspiration cannula was passed into the uterine cavity. Negative pressure was created by a 20 ml glass syringe and negative pressure was maintained till the tip of the aspiration cannula had reached just beyond the level of the internal os and endometrial aspiration was done.

A drop of the aspirated material was placed on a slide and a smear was made. These slides were immediately fixed in a glass bottle containing equal amounts of ether with 80% alcohol.
An endometrial biopsy curette was introduced into the uterine cavity and curettage was done. The curettings obtained were placed in a bottle containing formal-saline.

**ENDOMETRIAL ASPIRATION SMEAR SLIDE**

Requirements for staining:

1. 80 per cent alcohol.
2. 70 per cent alcohol.
3. 50 per cent alcohol.
4. Distilled water.
5. Harris haematoxylin.
6. 25 per cent aqueous hydrochloric acid.
7. Running tap water.
8. 95 per cent alcohol.
10. EA50 stain.
11. Absolute alcohol.
12. Xylol alcohol.
13. DFX Mountant.

**STAINING AND MOUNTING OF THE SMEAR**

The fixed smears were processed and stained according to Papanicolou staining method. (Smolka and Goost, 1965).
The technique was as follows:

The smear was dipped in

- 80 per cent alcohol for half a minute
- 70 " " " "
- 50 " " " "
- Distilled water " "
- Harris haematoxylin for three minutes for nuclear staining.
- Distilled water half a minute
- 25% acqueous hydrochloric acid six dips.
- Running water six dips.
- Distilled water half a minute.
- Rinse in 50% alcohol
- Rinse in 70% alcohol
- Rinse in 80% alcohol
- Rinse in 95% alcohol.
- Orange G6 two minutes.
- 95% alcohol half a minute ) separate containers.
- 95% alcohol half a minute
- EA50 (Eosin Azure) one and a half minute.
- 95% alcohol half a minute ) separate containers.
- " " half a minute
- Absolute alcohol half a minute
- Xylol and alcohol in equal parts half a minute
- Mount in DPX.
TECHNIQUE OF ENDOMETRIAL BIOPSY:

An endometrial biopsy curette was introduced and curettage of the uterine cavity was done. The curettings obtained were placed in a bottle containing formal saline preservative.

The tissue was then processed in autotechnicon and sections were cut of five micron thickness. These sections were placed on slides which were stained by haematoxylin and eosin staining. Then the slides were viewed.

STEPS IN STAINING AND MOUNTING OF PARAFFIN SECTIONS:

(Culling, 1963).

I. Removal of wax:

   Xylol : 1-2 minutes.

II. Hydration:

   Absolute alcohol : 1 minute
   90 per cent alcohol : 1 minute

Staining:

   Haematoxylin : 30-40 minutes (section turns pink).

   Wash in distilled water : 10 minutes (section turns blue).

   Acid alcohol for few seconds washed in distilled water (section blue again).
One per cent eosin 2-4 minutes
Wash in distilled water 3-4 minutes.

**Dehydration:**

90 per cent alcohol 10-15 seconds
Absolute alcohol 10-15 seconds
Absolute alcohol 30 seconds
Clearing xylol 15 seconds
Xylol few seconds.

**Mounting:** was done with DPX and section covered with coverslip.

**INTERPRETATION OF CYTOLOGY SLIDES:**

The slides reveal outstanding cellular characteristics of various types of endometrial cells. Two main types of cells are recognised - epithelial and stromal cells. Endometrial epithelial cells present in aggregates or clumps. These cells are small and columnar to cuboidal in shape, with scanty finely vacuolated cyanophilic cytoplasm and eccentrically placed nucleus having fine uniform chromatin.

Endometrial stromal cells can be derived from spongiosa or compact layer of functional endometrium. They may be shed in groups or singly. This cell is small, round or irregular and showing variation in size. Cytoplasm is cyanophilic and vacuolated. Nucleus is eccentric round and has prominent granular chromatin pattern.
The cellular diagnosis of proliferative endometrium is made by presence of an active chromatin nuclear pattern and absence of cytoplasmic vacuolization in endometrial epithelial cells.

Diagnosis of secretory endometrium by cell study depends on comparatively less activity of nuclei and some evidence of vacuolization of cytoplasm in endometrial epithelial cells.

Diagnosis of cystic and stromal hyperplasia is based on presence of large numbers of hyperchromatic endometrial stromal cells usually clumped or in extensive sheets. Cellular smears of adenomatous hyperplasia tend to have atypical clusters or groups of endometrial epithelial cells and may be difficult to differentiate from cells noted in cases of endometrial polyps.

Malignant cells from endometrium (adenocarcinoma) may desquamate in groups or less frequently as isolated cells. The malignant endometrial cell is larger and round, oval or columnar. The nucleus is round oval showing pleomorphism or deformed and pushed aside by cytoplasmic vacuolation. The chromatin is irregularly distributed and hyperchromasia is usually moderate.

Nucleolar hypertrophy is a fairly constant characteristic. The cytoplasmic borders are usually indistinct. When intact the cytoplasm is pale and
cyanophilic and exhibits one or several large vacuoles or it may be foamy due to numerous microvacuoles.

**Interpretation:**

**Proliferative phase:** The stroma is dense and compact. The glands are tall, rounded and lining is of tall columnar epithelial cells. There is no secretion in glands. The blood vessels are cork screw shaped.

**Secretory phase - Early:** A subnuclear vacuole appears and this gradually moves towards periphery of cell and is then extended into the lumen of the gland. The stroma is loose, the blood vessels are dilated.

**Late secretory phase:** The glands are filled with secretion. The luminal borders are frayed. The stroma is loose and shows decidual reaction. There is infiltration by polymorphs and eosinophils.

**ENDOMETRIAL HYPERPLASIAS:**

Silverberg classified the hyperplasias into four categories which in order of increasing severity are called simple, cystic, adenomatous and atypical.

**SIMPLE HYPERPLASIA:** There is increased thickness of endometrium, increased crowding of glands and evidence of oestrogenic activity. The glands of simple hyperplasia are generally small, round and regular and fail to show
cellular atypia. The glands are generally crowded and the morphology of the proliferative phase is universally encountered.

**Cystic Hyperplasia**: Coexistence of large cystically dilated glands and small round glands in a voluminous endometrial curettage specimen. Glands are round in shape neither angularity nor cellular atypia is encountered. There is stratified epithelium with proliferative activity seen in cystic hyperplasia.

**Adenomatous Hyperplasia**: Above findings plus angularity and abnormal shapes of the hyperplastic endometrial glands. A typical finding is the presence of small buds projecting from larger often cystically dilated glands. Amount of endometrium obtained at curettage is more voluminous in adenomatous hyperplasia than in the less severe forms.

**Atypical Hyperplasia**: The crowding and variations in size and shape of glands described in the previous patterns persist along with the presence of foci of cytologic atypia. There are regions of dysplasia with nuclei piled up with their axes pointing in different directions, anisonucleosis with large, gigantic and bizarre nuclei existing next to nuclei of more normal size, nuclear hyperchromasias and pleomorphism and exfoliation of atypical cells and amorphous eosinophilic cell debris into enlarged gland lumina. Presence of a striking degree of cytoplasmic
eosinophilia of glandular cells. These eosinophilic cells show some degree of cytologic atypia.

ENDOMETRIAL CARCINOMA

Endometrial adenocarcinomas are usually glandular in pattern with a smaller per cent being papillary. Increasing anaplasia of the tumour is manifested by a tendency to grow in solid nests or sheets with less formation of glands or papillae.

Grade I adenocarcinomas are composed entirely of tumour growing in a glandular or papillary pattern. Grade II tumours have an intermediate pattern of growth, there is moderate differentiation of tumour cells manifesting predominantly glandular elements, but with a mixture of solid growth pattern. Grade III tumours comprise of poorly differentiated lesions growing predominantly in solid sheets of cells.

Carcinoma in situ is used to denote a small focus of tumour in an otherwise benign curetting. There is tufting and infolding into gland lumen. The cells in each instance are tall and pale and even with hyperplasia there may be some evidence of secretory activity (Novak & Woodruff, 1974).