MATERIAL AND METHOD
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The present study was carried out in the Department of Obstetrics and Gynaecology and Department of Pathology, Maharani Laxmi Bai Medical College and Hospital, Jhansi in a study period from 1st June 1981 to 30 April 1982.

A- SELECTION OF CASES :

The study group comprised of cases attending out patient department of Obstetrics and Gynaecology. These cases were further evaluated under following headings :-

History :

All the patients were less than 32 years of age with normal fertility and cycle length of 28±2 days. After detail history and thorough clinical examination cases were selected. History included complete interrogation of patient, Obstetrical history and full account of menstrual history. Patient was asked for any complaints.
Examination of patient:

(i) General examination:

Thorough general examination was done with special attention as regards to blood pressure and weight of patient.

(ii) Systemic examination:

Brief systemic examination of cardiovascular system, respiratory system, central nervous and of abdomen was done.

(iii) Local examination:

In it a perspeculum, per vaginum and bimanual examination was done.

Per speculum examination:

It was done to inspect cervix and vaginal wall.

Per vaginum examination:

The vagina was examined by palpation.

The direction and texture of cervix was determined.
**Bimanual Examination:**

The uterus and its appendages were examined bimanually to determine the position and the size of uterus. The ovaries were examined to determine any enlargement.

**B- Dosage Schedule:**

After registration, the patient was asked to take first tablet of centchroman (30 mg) on the first day of ensuing menses. Day of which first tablet was taken, was termed as the pill day of patient. The patient was advised to take one pill on that day every week. The patient was also advised to take one extra tablet on first day of each subsequent menses in addition to weekly tablet. If menses day and weekly pill day were same, no additional tablet was taken.

**C- Collection of Materials:**

Hormonal assessment was determined by vaginal cytology and cervical mucus study for pH, spinnbarknit and fern test on 8th, 14th and 22nd day of cycle of one pretreatment cycle, three treatment cycle and one post treatment cycle.
Endometrial biopsy was to be done if cycle delays more than 45 days.

VAGINAL CYTOLOGY:

Smear was studied for superficial cell, intermediate cells and parabasal cells.

Superficial cells are large, delicate, polyhedral cells with sharply defined cell borders. The cytoplasm is light, transparent without structure and may be eosinophilic or cyanophilic. Nucleus is pyknotic.

Intermediate cells are medium sized cells with extreme variation in size. The cytoplasm in most of the cells is cyanophilic. The nuclei are large and vesicular.

Parabasal cells are rounded cells with cyanophilic stain. The nucleus is large, rounded with distinct structure.

Vaginal cytology was taken to determine maturation index and karyopyknotic index.
Maturation index:

This cytohormonal evaluation expresses the level of cellular maturation attained at time of exfoliation. A differential count of superficial cells, intermediate cells and parabasal cells was performed. A count of two hundred cells had made in different fields and number of these cells were recorded. This was expressed in following way for hundred cells as:

\[ M.I. = \frac{\text{Parabasal cells/Intermediate cells}}{\text{Superficial cells}}. \]

Karyopyknotic index:

Percentage of cells with nuclear pyknosis is referred as karyopyknotic index. Two hundred superficial and intermediate squamous cells were counted and those with pyknotic nucleus were expressed as the percentage of total cells.

Smear taking and fixation:

- Smear was taken before any gynaecological examination.

- Patient was put in lithotomy position.

- Simis speculum was applied after retracting labia majora with left hand.
- After holding the speculum with left hand, index finger of right hand was passed in the vagina and discharge was collected over finger from lateral vaginal wall.

- Smear was prepared from discharge by spreading evenly on a clean and fix labelled slide.

- Smear was immediately transferred in a mixture of equal parts of 95 percent alcohol and ether. Fixation of smear was completed after 10 minutes.

**Staining**

It was done according to Papanicolaou staining method (M. Smolka and H.J. Goost, 1965).

The technique was as following:

The smear was dipped in:
- 80 percent alcohol for half minute.
- 70 percent alcohol for half minute.
- 50 percent alcohol for half minute.
- Distilled water for half minute.
- Harris Haematoxylin for 3 minutes for nuclear staining.
- Distilled water for half minute.
- 25 percent aqueous hydrochloric acid (6 dips).
- Running water for 6 minute.
- Distilled water for half minute.
- Rinse by 50 percent alcohol.
- Rinse by 70 percent alcohol.
- Rinse by 80 percent alcohol.
- Rinse by 95 percent alcohol.
- Orange G-6 for 2 minutes.
- 95 percent alcohol half minute separate
- 95 percent alcohol half minute container's.
- EA 50 for 1½ minutes.
  (Resin Amure)
  - 95 percent alcohol for ½ minute separate
  - 95 percent alcohol for ½ minute containers.
  - 95 percent alcohol for ½ minute
  - Absolute alcohol for ½ minutes.
  - Xylool alcohol (in equal parts for ½ minute.
  - Xylool for ½ minute.
  - Mount in DPX.

Composition of papanicolaou stain:
Harris's haematoxylin:

- Haematoxylin 1.0 g
- Alcohol 95 percent 10.0 ml
- Aluminium or Ammonium Sulphate 20.0 g
- Distilled water 200.0 ml
- Yellow mercuric oxide 0.5 g
- Glacial acetic acid 8.0 ml

Orange - G6:

Orange - G, 0.5 percent solution in
95 percent alcohol 100.0 ml
Phosphotungstic acid 0.015 g

Papanicolaou's polychrome stain, BA 50:

Light green (Yellowish) 0.375 g
Bismarck brown 0.4 g
Rosin, yellowish 2.5 g
Distilled water 50.0 g
96 percent pure alcohol 609.0 g
Pure methanol 160.0 g
Phosphotungstic acid 1.7 g,
dissolved in 5.0 ml of 50
percent ethanol lithium.
Carbonate solution saturated  0.5 ml
Glacial acetic acid  1.0 ml

DPX: It is prepared by B.D.H. Laboratories (Glaxo).

Cervical mucous examination:

It was done for pH estimation, spinbarkeit test and for fern test.

Collection of cervical mucous:

Patient was kept in lithotomy position.
Sim's speculum was applied after retracting labia minora with left hand.

Anterior vaginal wall was retracted with help of anterior vaginal wall retractor and anterior lip of cervix was caugthetid by volsellum.

External OS was cleaned with help of cotton swab.

Mucous was taken as high as possible by sucking into pipette.

pH estimation:

It was done by putting a drop of
cervical mucus over pH indicator paper and colour change in the indicator paper was noted and matched against standard.

**Spinabarkheit test:**

It was done by putting the cervical mucus on one slide and covering it with other slide. Both slides were separated and distance in centimeter upto which the two slides could be separated without breaking of mucus was noted.

**Fern test:**

Mucus collected by pipette was spread on a glass slide and a thick smear was made and was allowed to dry for 20 minutes. Glass slide was prepared by washing in the distilled water. It was examined under low power.

**Endometrial biopsy:**

Tissue was taken out with the help of endometrial biopsy curette and was fixed in 40 percent solution of formal saline. This fixed tissue was processed in autotechnique.
Sections were cut with the help of microtome at 5 micron thickness.

Staining was done by routine haematoxylin eosin stain (D.F.A. Culling, Handbook of Histopathological techniques, Second Edition 1963, 204). Mounting was done in D.P.X.