- ii. LH also promotes the formation of the Corpus Luteum.
- V. Corpus Luteum secretes luteal hormone mainly progesterone in addition to a little amount of oestrogen.
  - a. Progesterone dominates and leads to: i. Suppression of Gonadotropin Releasing Hormone, which inhibits the release of LH,
    - ii. It stimulates the proliferation of the endometrium in the uterus.
  - **b. Oestrogen** at its peak secretion, inhibits the release of FSH by suppressing GnRH.
  - **c.** The hormone Relaxin facilitates the parturition action on the uterine tissues.
- VI. In the event of fertilization of the ovum, the corpus luteum takes up the function of a temporary endocrine gland and maintains pregnancy in the first 3 months (that is, the first trimester of pregnancy). The Corpus Luteum itself is maintained by the hormone Human Chorionic Gonadotrophin (HCG), which is produced by the developing syncytiotrophoblasts of the placenta in the uterus.

After the first trimester (that is, the first 12 weeks of gestation) the placenta completely takes over the function of the corpus luteum to carry on pregnancy until term.

### OR

### VII. In the absence of pregnancy

- a. There is atrophy of the Corpus Luteum leading to withdrawal of the hormones oestrogen and progesterone, which causes menstruation (withdrawal bleeding from the uterus).
- b. The lack of oestrogen and progesterone at the time of the menstruation, stimulates the GnRH and triggers the release of FSH and then LH from the pituitary, and thus reinitiates the "Menstrual Cycle". This cycle is repeated every month during the reproductive period, interrupted by pregnancy, and ceases at menopause.

The ovarian medulla contains large blood vessels, lymphatics and nerves in loose connective tissue stroma. It also contains interstitial cells, which secrete oestrogen and a few hilus cells, which secrete androgen.

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luteal phase of the menstrual cycle and in the different trimesters of pregnancy, it can be judged by the following indices (as shown in diagram 4.5 and 4.6)

- i. CCI Crowded Cell Index
- ii. FCI Folded Cell Index
- iii. Navicular Cell Count and Index
  - **1. Crowded Cell Index:** This index represents the relation of the mature squamous cells, lying in clusters of four or more in comparison to clusters of three or less crowded mature cells (Table 4.1). This index is cumbersome to assess and is often similar to folded cell index.
  - **2. The Folded Cell Index:** This index represents the relation of all folded mature, squamous cells to flat squamous (mature) cells.
  - **3. Navicular Cell Count:** Papanicolaou originally described these as



Collection of more than 3 in a group

Folded edges

Collection of less than 3 in one group

Flat cells

Diagram. 4.5: Crowded cell index (CCI)

Diagram. 4.6: Folded cell index (FCI)

navicular cell of pregnancy (1925). These are modified intermediate cells with heavy glycogen deposit in the cytoplasm. These cells have a delicate cytoplasm, thickened cell borders and small oval eccentrically placed nuclei. They exhibit a pronounced tendency to cluster formation. In the Papanicolaou stain these appear as pale blue or green; thesecells increase with the predominance of progesterone, in the luteal phase of the menstrual cycle and in pregnancy.

According to Papanicolaou there is no striking difference between the navicular cells occurring in normal pregnancy and those seen innonpregnant women in he luteal phase of the menstrual cycle. But Wachtel and Terzano (1968) have mentioned in their observations that navicuar cells in a pregnant woman seem to be smaller and to have a much heavier looking membrane, while those found during the luteal phase of the menstrual cycle are larger and more navicular in shape. However Shamim, while working on a project on Hormonal Cytology in Pregnancy (for her MD thesis, 1974-75), found a characteristic navicular shape or typical boat shaped navicular cell (Microphotograph 4.1) in



**Microphotograph 4.1:** Boat shaped Navicular Cell in Urinary Sediment Smear

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childhood, when the slow regeneration and the lack of protective action by the large squamous cells along with the absence of lactobacillus, makes the vaginal mucosa, fragile and liable to infections. This smear pattern continues till puberty, when endogenous oestrogen production starts.

### vi. Pre-Puberty and Puberty (9-15 years)

Vaginal hormonal cytology study in these phaseshas been given very little attention in English medical literature. **Sonek (1967)** was the first one to study the prepubertal as well as pubertal vaginal cytology. He found only one detailed study of the cytodiagnosis of the female child in a paper (in French language) by DeBrux and Delsace (1958), after search of world literature.

Sonek in 1967 has extensively studied the Prepuberty period from 9 to 12 years and described it in great detail; in addition, he has described a special technique for the collection of smears from the fragile vagina of childhood as shown in Diagram 6.3.

A glass pipette was used, through which a small cotton applicator was introduced into the vagina, so that it did not make contact with the vulva or the ectocervix and directly reached the lateral vaginal wall. Speculum should not be employed in children.

In routine practice, the cotton applicator can be used directly to prepare a smear without the glass pipette but an experienced cytopathologist is required to differentiate the contamination caused through the ectocervix and the vulva and discard it from the results of the hormonal assessment. Further the difference between the proliferative states of the epithelia found in the upper part of vagina when compared to the lower portion, does not play a significant role in the assessment of the cytologic pattern in smears from children. These smears are fixed in isopropyl alcohol and stained by Shorrs (1941). Evaluation of the smears is carried out by the method of Schmitt (1953) with some modifications.



Preparation technique of specimen in female children (Sonek 1967)

Diagram 6.3: Technique of collection of specimen

### Prepubertal Phase: (age 9-12 years)

The period three to four years prior to the onset of Menstruation at Menarche is known as the Pre-Pubertal period.

The vaginal smear patterns studied by Sonek (1967) showed that the increase in the proliferation of cells was not continuously progressive, hence most smears were of mixed cell type. Further, there was irregularity of cellular proliferation, as characterized by the variation of cell size among the same type of cells. Superficial cells were sometimes found as giant cells with binucleation. During this period, the duration of the menstrual cycle were either prolonged or decreased. and were not regular as that observed in the normal cycle. Very few cases of stabilized cyclic changes were found in female children between 9 and 12 years.

**Puberty: (age 12–15 years)** The pattern studied by Sonek (1967) from serial vaginal smears in puberty was as follows:

- A. Ovulatory cycles A few cases (5.9%)
- B. Anovulatory cycles 90.6% (rest Indistinct)

The hormonal cytology of the girls with anovulatory cycle was of the following types:

1. Normo – oestrogenic\* 44.1%

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as a temporary endocrine gland during the first trimester of pregnancy. It provides additional oestrogen and progesterone required for the maintenance of the fertilized Approximately 6 days after ovum. fertilization, implantation of the fertilized ova is complete and the developing syncytiotrophoblast of the placenta in the uterus complement the ovarian production of oestrogen and progesterone. Along with these steroids, the trophoblast commences production of chorionic gonadotrophins in the first month of pregnancy (Zender, 1959). Thus it may be assumed that the pregnancy preserving function of the corpus luteum beyond the first month of pregnancy has been overestimated.

Von Haam (1961) has mentioned that experimentally the progesterone difference between the arterial and venous placental blood in the different trimesters of pregnancy was measured via isotopic progesterone markers. In his series he has reported a progesterone production per day of 25 to 50 milligrams during the first half of pregnancy and up to 280 milligrams during the second half. The excretion of oestrogen during pregnancy also increases about 100 times as compared to that seen in the normal cycle. Brown (1956) found that the oestrogens produced by the placenta increased from 1 milligram per day in the tenth week of pregnancy to 50 to 100 milligrams per day at the end of pregnancy.

The classification of the vaginal cytology of pregnancy as proposed by Pundal (1958) was accepted by most workers in this field.

## Hormono-Cytology of Pregnancy was classified into:

- 1. Acytolytic
- 2. Cytolytic

#### Acytolytic Pregnancy Smear Pattern

1. Cytology of the first trimester of pregnancy (upto 3 months or 12 weeks) is labeled as corpus luteum phase.

- 2. Cytology of the last two trimesters of pregnancy is labeled as placental phase.
  - a. Placental phase extends from 12 weeks up to the last two weeks before term. This is also labelled as Preterm phase, that is up to 38 weeks of pregnancy.
  - b. Cytology of pregnancy in the last 2 weeks (38 to 40 weeks) is labelled as "at term" phase (38–40 weeks).
    - Kamnitzer et al (1959) opined that the classification could be expanded to comprise the entire vaginal "gravido – puerperal" cycle. Therefore they suggested the addition of one more phase after "at term" (c)
  - c. Cytology at the beginning of Labour or near Term

## Corpus Luteum phase—the first 12 weeks of gestation

The corpus luteum phase of the menstrual cycle after conception in the last 2 weeks of the last menstrual cycle continues as the corpus luteum phase of pregnancy, which persist until 12 week of gestation. The trophoblast of the developing placenta also aids gestation by enhancing progesterone production, from the first month onwards. The predominant hormone of pregnancy is progesterone which on account of its dual action, synergistic as well as antagonistic, allows the proliferation up to the intermediate layer and reverses the mature squamous epithelium to the intermediate cell layer, respectively. The excessive proliferation of intermediate layer leads to the following characteristic changes in the smear pattern in this phase.

# Hormonal Cytology Pattern in the first trimester of pregnancy: Microphotograph 3

- 1. Heavy exfoliation of the intermediate cells with placard formation
- 2. Intermediate cells are cyanophilic, shining large flat polygonal cells with smooth