

Original Research Article

A 3 years study of vaginal hormonal cytology at tertiary hospital


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Abstract

Background: Vaginal cytology is an efficient inexpensive and a rapid method for identifying diseases including establishing the hormonal condition of the patient. The Evaluation of the endocrinologic status of the female patient by means of the study of vaginal cells is actually one of the earliest diagnostic applications of clinical cytology. Though the vaginal cytologic characteristics of gynecologic patients have been studied throughout the world this subjected has received little attention. Vaginal cytology is useful for assessing ovarian function from puberty throughout the reproductive years, menopause and senium. Also it is used to estimate time of ovulation, to determine ovarian dysfunction, to assess placental function or dysfunction in obstetrics, to assist in selecting hormonal therapy and to follow hormonal treatment results.

Aim and objectives: To ascertain the effective utilization of cost effective test of vaginal hormonal cytology as a supplement for costly biochemical hormonal estimation. A prospective study was undertaken with the following objectives: To study the normal hormonal patterns in females of different age groups and to study abnormal hormonal patterns in high risk pregnancies and in different clinical situations.

Materials and methods: A prospective, hospital based study was done on vaginal smears obtained from One hundred and Sixty (160) patients, attending Gynecology and Obstetrics Out-patient department and few In-patients at Gandhi hospital, Secunderabad during the period of August 2004 to October 2006. The study group included randomly selected female patients of reproductive age groups (18 years to 35 years.); with normal menstrual cycles; normal perimenopausal (36 years to 45 years.) and postmenopausal (above 45 years.) women, pregnant patients in the 1st, 2nd, 3rd trimesters

and 1st stage of labour as well as post partum patients. The study group also included patients with primary infertility, menorrhagia, secondary oligomenorrhoea (scanty menstruation), threatened abortions, inevitable abortions, pre mature rupture of membranes, post maturity and patients with neoplastic lesion i.e. ovarian tumor. The material collected for the vaginal smear dried very rapidly during collection. Hence fixation was done within 30 sec. of spreading. The fixatives used were – 95% Ethanol or 80% Isopropyl Alcohol. Other fixatives used were ether / 95% alcohols; 100% methanol; Rapid fixative as cytospray; 2 to 10% carbowax in 95% ethanol (for mailing unstained smears). The stains used were Papanicolaou's stain and Giemsa stain.

Results: Vaginal hormonal cytology is a useful diagnostic aid in the female reproductive process. It may also provide a key to more effective conception control, as well as improvement in the treatment of menstrual disorders, anovulation, and other underlying disorders. In the present Indian scenario with lack of adequate clinical history available to the pathologist, in a rural setup as well the urban conditions; vaginal hormonal study is still a low cost diagnostic and prognostic test procedure. Hence the present study emphasizes the utility of vaginal smear study even today compared to the costly biochemical parameters available in the market which is a financial burden to poor patients.

Conclusion: Vaginal smear collected between 4-11 days and 12-16 days of the normal menstrual cycle showed scattered intermediate and superficial cells. Vaginal smears collected between 17-28 days of normal menstrual cycle showed, predominately intermediate cells with folding and crowding. The KPI peak is between 12-16 days. This is extreme midzone shift, with clusters of navicular cells in all the three trimesters, of pregnancy. First stage of labour could be suspected with identification of increase in the number of superficial cells, with marked decrease in the intermediate cell values.

Key words

Vaginal hormonal cytology, Tertiary care hospital.

Introduction

The Evaluation of the endocrinologic status of the female patient by means of the study of vaginal cells is actually one of the earliest diagnostic application. The vaginal cytologic characteristics of gynecologic patients have been studied throughout the world. The degree of maturation of the vaginal squamous epithelium is hormone dependent, and therefore, the quantitative relationship of squamous cells of varying degrees of maturity in a vaginal smear may serve as an index of the hormonal status of the female [1-3].

Vaginal cytology is an efficient inexpensive and a rapid method for identifying diseases including establishing the hormonal condition of the patient. For the successful application of cytology for evaluation, precise technique of taking smears, meticulous preparation of smears and ability to recognize the morphological variations are essential [4, 5].

Vaginal cytology is useful for assessing ovarian function from puberty throughout the reproductive years, menopause and senium. Also it is used to estimate time of ovulation, to determine ovarian dysfunction, to assess placental function or dysfunction in obstetrics, to assist in selecting hormonal therapy and to follow hormonal treatment results. The vaginal smear is supposed to represent the ultimate effect of all pregnancy hormones on the vaginal epithelium which is very sensitive hormone receptor. The increasing levels of hormones after conception bring about changes in the vaginal epithelium. The cytological pattern of vaginal smears changes suddenly at the end of pregnancy due to drop in the hormone levels [6-9].

Vaginal smears can be of value in assessing the diagnosis and prognosis of both early and late pregnancy. The diagnosis of pregnancy can be made by the vaginal smear method alone as earlier as the 3rd week.

To predict the date of labour vaginal cytology is a simple and inexpensive parameter, as compared to estimation of urinary total oestrogen, ultrasound studies and amniotic fluid analysis.

Exfoliate vaginal cytology can reveal hormonal imbalance present in some of complications of pregnancy and thus serve as a guide to treatment and prognosis. Vaginal smears could be employed as an accurate diagnostic and prognostic aid in abortion.

Vaginal cytology is a valuable investigation in patients with threatened abortion. An abnormal smear may be used as an indication to commence therapy, which is thought to prevent abortion. A hormonal upset could be a primary cause for occurrence of abortion, or it could be secondary to fetal death due to other causes [10-13].

The haphazard use of hormones in pregnancy can be avoided and a more scientific approach to hormone lack obtained by examination of vaginal smear.

Aim and objectives

Aim

To ascertain the effective utilization of cost effective test of vaginal hormonal cytology as a supplement for costly biochemical hormonal estimation.

A prospective study has been undertaken with the following objectives:

- To study the normal hormonal patterns in females of different age groups
- To study abnormal hormonal patterns in high risk pregnancies and in different clinical situations.

Materials and methods

A prospective, hospital based study was done on vaginal smears obtained from One hundred and Sixty (160) patients, attending Gynecology and Obstetrics Out-patient department and few In-patients at Gandhi hospital, Secendurabad during the period of August 2004 to October 2006.

The present study emphasizes the role of vaginal hormonal cytology in recognizing the normal and abnormal cytohoromonal patterns. The study group included randomly selected female patients of reproductive age groups (18 years to 35 years.); with normal menstrual cycles; normal perimenopausal (36 years to 45 years.) and postmenopausal (above 45 years.) women, pregnant patients in the 1st, 2nd, 3rd trimesters and 1st stage of labour as well as post-partum patients. The study group also included patients with primary infertility, menorrhagia, secondary oligomenorrhoea (scanty menstruation), threatened abortions, inevitable abortions, premature rupture of membranes, spost maturity and patients with neoplastic lesion i.e. ovarian tumor.

A case proforma was prepared for each patient and all the subjects selected for this study, were studied in detail with particular reference to features mentioned in the proforma.

After careful clinical examination; prior instructions; and consent of the patient, vaginal per-speculum examination was done, and smears were made from upper part of the lateral vaginal wall. All these smears were immediately fixed in 95% ethylalcohol and were later stained by routine papanicolu on staining method using EA-36 and OG-6 cytoplasmic stains. In 100 cases extra smears were taken and stained with Geimsa stain.

Vaginal smear sampling instruments

- Ayre's spatula.
- Cusco's /Sims speculum.
- Glass slides.
- Fixative.

Sampling Technique

- Patient must be in dorsolithotomy position.
- No lubricant /antiseptic /saline must be used; as it may result in acellular smear.
- Mucus or any other discharge from the cervix must be gently removed by a gauze pad.

- The Sims /Cusco's speculum is inserted and the cervix positioned; Perspeculum findings of the cervix and vagina are noted.
- The Ayre's spatula is inserted into the vagina and gentle scraping of the upper third of the lateral vaginal wall is done. The speculum is then removed and the material adherent to the spatula is smeared on to pre labeled clean glass slides. Both sides of the sampler should be spread swiftly on the slide on the same side as the label. The slides are immediately placed in the fixative.

Fixation

The material collected for the vaginal smear dries very rapidly during collection. Hence fixation must be within 30 sec. of spreading. The fixatives used were -95% Ethanol or 80% Isopropyl Alcohol.

Other fixatives used were ether / 95% alcohols; 100% methanol; Rapid fixative as cytospray; 2 to 10% carbowax in 95% ethanol (for mailing unstained smears).

Staining

The stains used were Papanicolaou's stain and Giemsa stain.

Results

The present study deals with cyto hormonal evaluation of the vaginal smears for assessment of normal pattern in different age groups and to identify the abnormal patterns. One hundred and sixty patients were subjected to vaginal hormonal cytology.

Satisfactory vaginal smears

Satisfactory vaginal smears were obtained in One hundred and forty five (145) cases (90.63%) and in the remaining fifteen (15) cases (9.37%) vaginal smears were unsatisfactory for hormonal evaluation. Ratio of satisfactory to unsatisfactory preparations was 9.6:1.

Vaginal Hormonal Patterns

Normal cyto hormonal pattern was studied in One hundred and thirty five (135) patients, which included women having normal menses, normal pregnancy, postpartum group, perimenopausal women and postmenopausal period. Abnormal cyto hormonal pattern was studied in twenty five (25) patients, which included cases of primary infertility, menorrhagia, secondary oligomenorrhoea (scanty menstruation), threatened abortion, inevitable abortions, incomplete abortions, premature rupture of membrane, post maturity and neoplastic lesion i.e. ovarian tumor.

Normal cyto hormonal patterns

Normal menstrual cycle

Vaginal hormonal cytology was done in thirty five (35) subjects having previous normal menstrual cycles. In twenty five (25) of these, in the age group (15 years - 46 years) vaginal smears were obtained only on single day of the menstrual period. Three (3) of these smears were unsatisfactory for hormonal evaluation. In the remaining 22 cases vaginal smears were studied between 4 -11 days 10 cases; between 12 - 16 days in 7 cases; and between 17-28 days in the remaining 5 cases.

Vaginal smears in ten (10) cases collected between 4-11 days, showed varying number of scattered intermediate and superficial cells. Background showed occasional leucocytes and a few lactobacilli. Parabasal cells were not seen. Cytological indices in these cases showed maturation index =0/40-80/20-60; mean maturation value 73.3+6.15; karyopyknotic index 38.5%+12.5; eosinophilic index 18.8%+6.3; superficial cell index 38.5%+12.50 and mean folded cell index 20.5%+15.50.

Vaginal smears of seven (7) cases collected between 12-16 days, also showed varying number of scattered intermediate and superficial cells. Background was clean and there were occasional lactobacilli. Parabasal cells were not seen. Cytological indices in these cases showed maturation index =0/30-80/20-70; mean

maturation value 78.9%+8.99; karyopyknotic index 52.9%+18.9; eosinophilic index 22.4%+8.9; superficial cell index 47.9%+18.90 and mean folded cell index 10.1%+24.80.

Vaginal smears of 5 cases collected between 17-28 days showed predominantly scattered and crowded intermediate cells. These intermediate cells showed folding and envelop effect. Background showed good number of lactobacilli. Parabasal cells were not seen. Cytological indices in these cases showed maturation index=0/60-90/10-40; mean maturation value 64.2+4.83; karyopyknotic index 18.7%+9.91; eosinophilic index 7.0%+2.61; superficial cell index 18.7%+9.91; folded cell index 53.7%+7.90; crowded cell index 31.2%+6.94.

Although these single smears were of little value, they showed high maturation value, KPI; EI; and SCI between 12-16 days (ovulation time). These single smears showed predominately intermediate cells with increased folded cell index and crowded cell index between 17-28 days (Secretory Phase).

In 10 women aged between 21-34 yrs, vaginal hormonal cytology was done on three days, between 4-11 days, between 12-16; and between 17-28 days in the same menstrual cycle.

The cytological indices in these 10 cases showed shift to right, mean maturation value 92.1+6.62; karyopyknotic index 82.1%+7.72; eosinophilic index; 40.18%+6.14; superficial cell index 82.1%+7.72; and folded cell index 5.6%+1.57; between 12-16 days (ovulation time). While in the period of 20-28 days (secretory phase) there was mid-zone shift, mean maturation value 63.4+6.24; karyopyknotic index 17.6%+8.12; eosinophilic index 7.41%+3.14; superficial cell index 17.6%+8.12; folded cell index 34.8%+8.09; and crowded cell index 33.2%+7.81. P value for 4-11 days (proliferative period) to 12-16 days (ovulation time) was 0.032 and the P

value for 12-16 days (ovulation time) to 17-28 days (secretory phase) was 0.0043.

Normal Pregnancy

Vaginal hormonal cytology was done in 65 pregnant women in the age group of 18-34 years, who were clinically normal. In four (4) of these cases smears were unsatisfactory for hormonal evaluation.

In Ten (10) cases vaginal hormonal cytology was done only in first trimester (10-12 weeks), which included four (4) cases of primigravida, three (3) cases of second gravida, two (2) cases of third gravida, and one (1) case of fifth gravida. Vaginal smears in these cases showed predominantly intermediate cells, which showed folding and envelop effect and were seen in clusters.

In 20 cases vaginal hormonal cytology was done only in second trimester (16-20 weeks), which included ten (10) cases of primigravida, six (6) cases of second gravida, and four (4) cases of third gravida. Vaginal smears in these cases showed predominantly intermediate cells, which showed folding and envelop effect and were seen in clusters. Few cyanophilic superficial cells and occasional eosinophilic superficial cells were seen. Background showed many lactobacilli. Navicular cells found 74.68%+8.63% of the total epithelial cells. Cytological indices shown a midzone shift.

In twenty five (25) cases vaginal hormonal cytology was done only in third trimester (34-36 weeks), which included ten (10) cases of primigravida, eight (8) cases of second gravida, five (5) case of third gravida, and two (2) cases of fourth gravida. Vaginal smears in these cases also showed similar features as in second trimester vaginal smears with mid zone shift of cytological indices.

Postpartum Period

Vaginal hormonal cytology was done in the post partum period 18 women who had clinically established normal pregnancies. In three (3)

cases vaginal smears were unsatisfactory for hormonal evaluation, others showed predominantly parabasal cells with few intermediate cells occasional superficial cells. Background showed red cells, leukocytes and cell debris.

Perimenopausal Period

Vaginal hormonal cytology was done in five (5) perimenopausal women in the age group of 42-47 years. These were having prolonged irregular cycles and vaginal hormonal cytology was done on 40th day in two (2) cases; 45th day in two (2) cases and 60th day in one (1) case. These smears showed variable number of scattered intermediate and superficial cells with occasional folding of cytoplasm in the intermediate cells. Background was clean and there were a few lactobacilli. The cytological indices in these cases were maturation index = 0/40-60/40-60, and mean maturation value 82.0; karyopyknotic index 50.0%; eosinophilic index 8.5%; superficial cell index 50.0%; and mean folded cell index 9.5%.

Post Menopausal Period

Vaginal hormonal cytology was done in fifteen (15) clinically normal menopausal women in the age group of 45-65 years. Three (3) cases vaginal smears were unsatisfactory for hormonal evolution. All these cases showed oestrophy pattern. Vaginal smears were showing predominantly scattered intermediate cells, a few varying number of parabasal cells and occasional cyanophilic or eosinophilic superficial cells. Background was clean in nine (9) cases, in the remaining three (3) cases there were a few leukocytes, but cytolysis was not seen. A few lactobacilli were seen. Cytoplasmic vacuolation and increased cytoplasmic eosinophilia and parabasal and intermediate cells were not observed.

Cytologic indices studied in these cases showed maturation index = 0-30/70-90/0-10; mean maturation value 42.7+7.67; karyopyknotic index 6.37%+3.36; eosinophilic index 3.73%+2.16; mean superficial cell index 6.37%+3.36.

Abnormal Cytohormonal Patterns

Primary Infertility: Vaginal hormonal cytology was done in five (5) women, who were clinically diagnosed as having primary infertility problem, and they belonged to the age group of 21-26 years. In three (3) of these cases vaginal smear was collected only on single day of the menstrual cycle i.e. between 17-28 days. In the remaining two (2) cases, three vaginal smears were taken one each between 4-11 days; 12-16 days; and 17-28 days, on three different dates of the same menstruation cycle to know the cytohormonal pattern. These cytohormonal patterns were then compared with the normal cytohormonal pattern observed in this study.

Vaginal smears in these cases of primary infertility patients showed predominant superficial cells between 4-11 days of the cycle, and further shift to the right was observed between 12-16 days. These patients also showed further shift to the right in the 17-28 days of the cycle. Various cytological indices during these three phases of the cycle were compared and the changes were statically significant (P=0.34). In these cases intermediate cells showed occasional folding of the cytoplasm, and crowding of intermediate cell was not observed.

Cytohomonogram Showing the High Plateau Curve in Primary Infertility Cases, in Contrast to Normal Cycle

Menorrhagia

Vaginal hormonal cytology was done in seven (7) parous women in the age group of 35-40 years who had excessive menstrual blood flow. In two (2) cases vaginal smears were unsatisfactory for hormonal evaluation. Out of the remaining five (5) cases, vaginal smear was collected on single day i.e. on 25th day of the menstrual cycle in three cases and vaginal smears were taken on 9th, 18th and 25th day of the same menstrual cycle in two (2) cases. Vaginal smear taken on 9th day showed maturation index = 0/35-40/55-60; mean maturation value 76.2+2.6, karyopyknotic index 58%+7.4; eosinophilic index 9.8%+2.5; mean superficial cell index 64%+7.1; and folded index 10%+3.2. Vaginal

smears taken on 18th day showed maturation index=0/20-30/70-80 maturation value 82+3.4, karyopyknotic index 76%+6.2; eosinophilic index 28%+2.4; mean superficial index 77.2+2.6; and folded index 5.6%+1.6; and vaginal smear taken on 25th day showed maturation index=0/10-15/85-90; mean maturation value 86%+3.2, karyopyknotic index 86%+4.6; eosinophilic index 33.2%+2.8; superficial cell index 85.2%+2.6; and folded index 2.8%+1.2. These vaginal smears showed progressive increase in the number of superficial cells during the course of the menstrual cycle. Crowding of intermediate cells was not seen. Background showed a few lactobacilli. The changes in mean cytological indices in these patients suggested possibility of high plateau curve pattern indicating an anovulatory cycle.

Mean Cytological Indices at the Different Phases of menstrual cycles in menorrhagia Cases

Oligomenorrhoea (Scanty Menstruation)

Vaginal hormonal cytology was done in two cases having scanty menstruation and secondary infertility.

Case No 1

Vaginal smear in 30 years old women showed almost similar distribution of intermediate and superficial cells in smears taken on 7th day, 16th day and 26th day. On 7th day maturation index=0/60/40; maturation value 64, karyopyknotic index 40%; eosinophilic index 6%; superficial cell index 40%; and folded index 12%, On 16th day maturation index=0/70/30; maturation value 62, karyopyknotic index 30%; eosinophilic index 5%; superficial cell index 30%; and folded index 14%, Vaginal smear taken on 26th day maturation index=0/75/25; maturation value 63, karyopyknotic index 25%; eosinophilic index 25% and folded index 13%. In all these smears crowding of intermediate cell was not observed. Background showed a few lactobacilli.

Case No 2

Vaginal smear in 34 years old woman also showed, almost similar distribution of intermediate and superficial cells in smear taken on 6th day, 16th day and 28th day. On 6th day maturation index=0/65/35; maturation value 64.2, karyopyknotic index 35%; eosinophilic index 6%; superficial cell index 35%; and folded index 12%. On 16th day maturation index=0/60/40; maturation value 66, karyopyknotic index 40%; eosinophilic index 8%; superficial cell index 40%; and folded index 12%. On 28th day maturation index=0/60/40; maturation value 64.8, karyopyknotic index 40%; eosinophilic index 6%; superficial cell index 40%; and folded index 10%. In all these smears, crowding of intermediate cells was not observed. Background showed a few lactobacilli.

Cytohormonogram was made in both these cases by plotting KPI values against the days of menstrual cycle. Both these patients showed 'hypo-ovarian' curve.

Threatened abortion

Vaginal hormonal cytology was done in three (3) cases who were clinically diagnosed as threatened abortion. These patients were in the age group of 18-24 years and the period of amenorrhoea ranged between 8-20 weeks.

Vaginal smears in these patients showed maturation index of 0/80-98/2-20; with maturation value of 51+3.61, karyopyknotic index 8.33%+5.3; eosinophilic index 3%+2.65; superficial cell index 8.33%+5.3; folded cell index 86.66+2.9; and mean crowded cell index of 75%+5.0. Mean navicular cell count in these four cases was 72.42%+6.28. Background showed a few lactobacilli and minimal cytolysis.

In the remaining one case vaginal hormonal cytology showed progesterone deficiency pattern. She was 20 year old primigravida with amenorrhoea of 12 weeks with vaginal hormonal cytology showing maturation index of 0/60/40 and maturation value of 62, karyopyknotic index 40%, eosinophilic index 8%, superficial cell index 40%, folded cell index 30%, crowded cell

index 10% and navicular cell count was 20%. Background showed a few lactobacilli and neutrophils.

Inevitable abortion

Vaginal hormonal cytology was done in two (2) pregnant woman aged 20 and 24 years with clinical diagnosis of inevitable abortion.

Case No1

Vaginal smear from 20 year old primigravida with amenorrhoea of 10 weeks, showed maturation index of 0/70/30, maturation value of 58, karyopyknotic index of 30%, eosinophilic index of 7%, superficial cell index of 30%, folded cell index of 20%, crowded cell index of 6%, navicular cell count of 18%, intermediate cells were less crowded. background showed few isolated endometrial cells RBC neutrophils and a few lactobacilli.

Case No 2

Vaginal smear from 24 year old pregnant woman of secondgravida with amenorrhoea of 20 weeks, showed maturation index of 0/75/25, maturation value of 56, karyopyknotic index of 25%, eosinophilic index of 6%, superficial cell index of 25%, folded cell index of 18%, crowded cell index of 6%, navicular cell count of 16%, intermediate cell were less crowded. Background showed RBC neutrophilis and few lactobacilli

Incomplete abortion

Vaginal smears were collected from two (2) cases with clinical diagnosis of incomplete abortion.

Case No 1

Vaginal smear from 18 years old woman of primigravida, with amenorrhoe of 10 weeks, showed maturation index of 0/70/30, maturation value of 58, karyopyknotic index of 30%, eosinophilic index of 7%, superficial cell index of 30%, folded cell index of 20%, crowded cell index of 6%, navicular cell count of 18%, Background showed RBC 's neutrophilis , trophoblastic cells, decidual cells and few lactobacilli.

Case No 2

Vaginal smear from 22 years old pregnant woman of secondgravida with amenorrhoe of 12 weeks, showed maturation index of 0/65/35, maturation value of 55, karyopyknotic index of 35%, eosinophilic index of 8%, superficial cell index of 35%, folded cell index of 18%, crowded cell index of 5%, navicular cell count of 16%, Background showed RBC's neutrophilis, decidual cells and few lactobacilli.

Premature rupture of membranes

Vaginal smear were taken from two (2) pregnant women with premature rupture of membranes

Case No 1

Vaginal smear from 20 year old pregnant woman of secondgravida, with amenorrhoe of 34 weeks, showed maturation index of 0/75/25, maturation value of 60, karyopyknotic index of 25%, eosinophilic index of 8%, superficial cell index of 25%, folded cell index of 60%, crowded cell index of 35%, Background showed few neutrophilis , lactobacilli and degenerating squamous cells (possibly from foetal skin).

Case No 2

Vaginal smear from 28 year old pregnant woman of third gavida, with amenorrhoe of 36 weeks, showed maturation index of 0/60/40, maturation value of 62, karyopyknotic index of 40%, eosinophilic index of 8%, superficial cell index of 40%, folded cell index of 55%, crowded cell index of 30%, Background showed few neutrophilis, lactobacilli and lanugo hairs.

Post maturity

Vaginal hormonal cytology was done in two (2) pregnant women clinically diagnosed as post dated pregnancy.

Case No1

Vaginal smears taken from primigravida aged 23 years with amenorrhoea of 42 weeks, showed maturation index of 0/90/10, maturation value of 45, karyopyknotic index of 10%, eosinophilic index of 2%, superficial cell index of 10%, folded cell index of 82%, crowded

cell index of 76%, navicular cell count of 72%. There was a slight increase in the superficial cell count and intermediate cells were also seen in groups. In this case though pregnancy was postdated, vaginal hormonal cytology was indicating normal pregnancy pattern.

Case No2

Vaginal smears taken from primigravida aged 25 years who has completed 42 weeks of amenorrhoea, showed maturation index of 10/85/15, maturation value of 40, karyopyknotic index of 15%, eosinophilic index of 4%, superficial cell index of 15%, folded cell index of 60%, crowded cell index of 50%, navicular cell count of 60%. There was a slight increase in the superficial cell count with appearance of parabasal cells.

Neoplastic lesion

Vaginal hormonal cytology was done in two (2) cases with clinical diagnosis of benign ovarian tumour.

Case No 1

Vaginal smear was taken from a 35 year old woman having right sided simple serous cystadenoma of the ovary and normal menstrual cycles. The smear was taken on 22nd day of the cycle and it showed, intermediate cells and a few superficial cells. Cytological indices showed maturation index of 0/70/30, maturation value of 61.5, karyopyknotic index 10%, eosinophilic index 2%, superficial cell index 20%, folded cell index 82%, crowded cell index 76% and navicular cell count of 72%. karyopyknotic index 30%, eosinophilic index 5%, superficial cell index 30%, folded cell index 22%, crowded cell index 12%. Background showed a few lactobacilli. Cytohormonal features were consistent with normal pattern on 22nd day of the cycle.

Histopathological examination of the ovarian tumour proved to be simple serous cystadenoma of the right ovary.

Case No 2

Vaginal hormonal cytology was done in a 25 years old woman having right-sided mucinous cystadenoma of the ovary and normal menstrual cycle. Vaginal smear was taken on 25th day of the cycle, and it showed intermediate cells and a few superficial cells, cytological indices showed maturation index 0/70/30, with maturation value of 60, karyopyknotic index 30%, eosinophilic index 8%, superficial cells index of 30%, folded cell index of 25% and crowded cell index of 19%. Background showed a few lactobacilli. Cytohormonal features were consistent with normal pattern on 25th day of the cycle.

Histopathological examination of the ovarian tumour done later proved it to be mucinous cystadenoma of the right ovary (**Photo – 1 to 8**).

Photo – 1: Vaginal smear showing predominantly superficial cells on 14th day of the normal cycle.

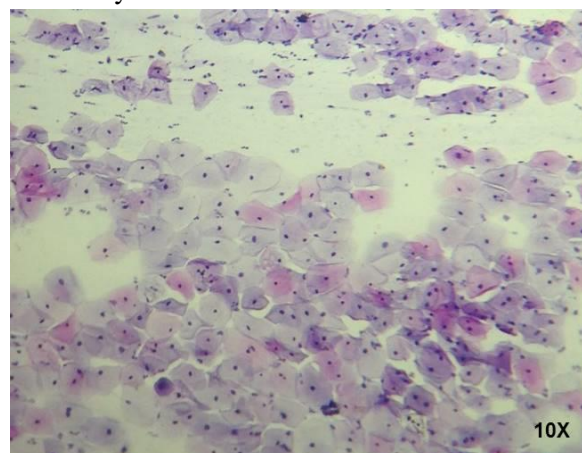


Photo – 2: Vaginal smear showing crowded and folded intermediate cells on 26th day of the normal cycle.

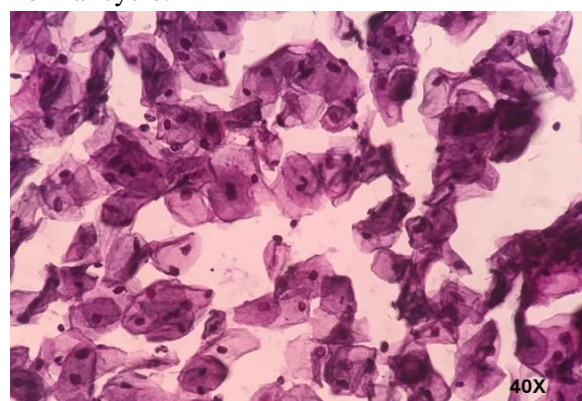


Photo – 3: Vaginal smear showing extreme mid zone shift with many navicular cells in pregnancy (Low power view).

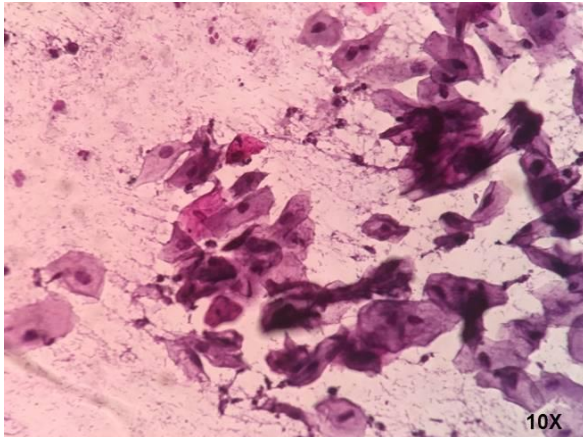


Photo – 6: Mid trimester vaginal smear showing folded intermediate cells with neutrophils in the background.

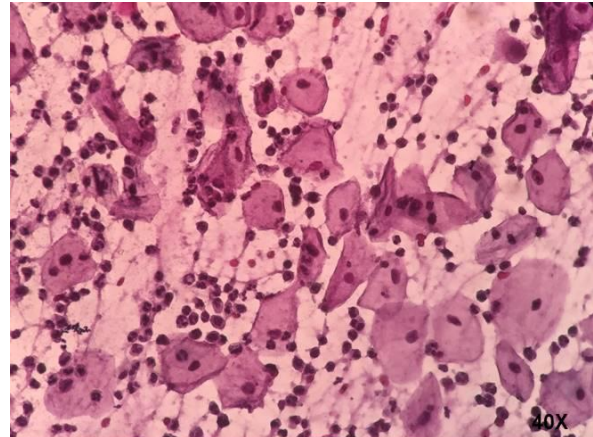


Photo – 4: Vaginal smear showing extreme mid zone shift with many navicular cells in pregnancy (High power view).

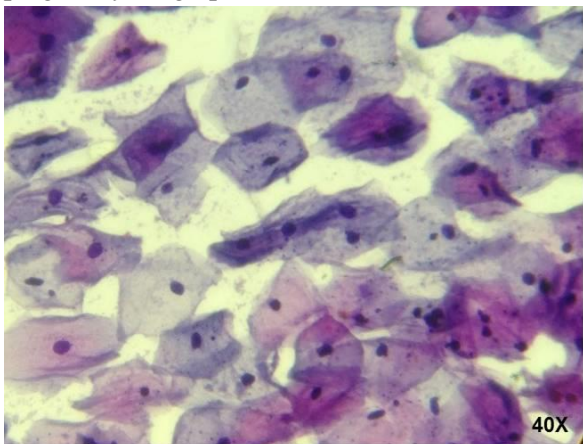


Photo – 7: Post-menopausal smear showing predominantly parabasal cells with few neutrophils in the background.

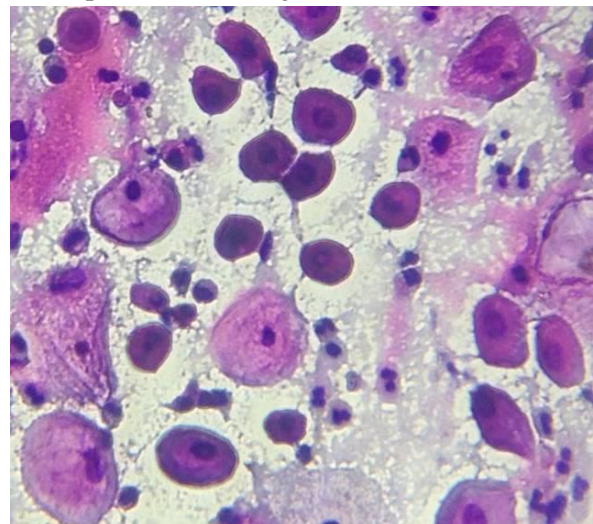


Photo – 5: Vaginal smear showing predominantly folded intermediate cells with many lactobacilli in the background.

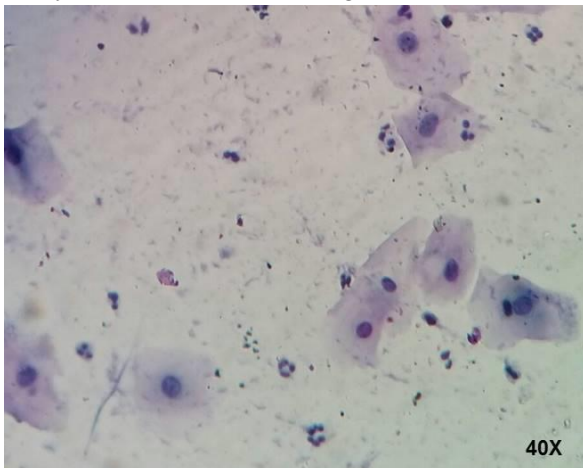
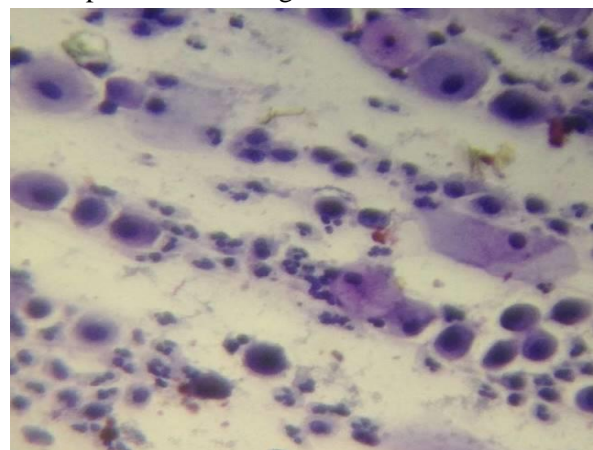


Photo – 8: Post-menopausal smear showing parabasal and intermediate cells with few neutrophils in the background.



Discussion

In spite of the various non-invasive techniques of knowing the hormonal status and identifying the hormonal imbalances, non-invasive hormonal status estimations by vaginal smear examination is most sought after for its low cost status. Prompt determination of the patients hormonal status is a key both to the detection of the underlying cause and to its proper management. Clinical utilization of hormonal cytology has been expanding as a logical result of its immense value to the patients-20. In this study, various normal and abnormal hormonal patterns recognized by vaginal hormonal cytology have been evaluated [14-18].

In this study, cytological indices used are maturation index maturation value, karyopyknotic index, eosinophilic index, superficial cell index, folded cell index, crowded cell index, and mean intermediate cell value. Many authors consider, maturation index as a more informative index. The maturation value is not suggested as a means of reporting cyto hormonal findings to the clinician. It is, however, a working mechanism useful for communication among cytologist. Karyopyknotic index is useful in recognizing

ovulatory cycles, but parabasal cells were not evaluated in this index, eosinophilic index most often altered by artifacts. The eosinophilic index, if determined without assessing the KPI or MI, is of limited value. Superficial cell index has limited value, as cytoplasmic tinctoral characteristics are not ascertained. Folded cell index assesses the tendency of the cells towards folding. Mature squamous cells that are folded are usually less mature than are flat cells that have lost the tendency to fold. Crowded cell index is relatively difficult to assess because cell clusters often contain so many cells that, they do not lend themselves to accurate counting. Mean intermediate cell values are useful in assessing the course of pregnancy. Similar problems in assessing the cytological indices have been described in the literature [19, 20].

In majority of the patients (90.63%) satisfactory vaginal smears were obtained, for vaginal hormonal cytology, and the ratio of satisfactory to unsatisfactory preparations was 9.6:1. There is varying incidence of this ratio in the different studies (**Table - 1**). The higher incidence of satisfactory preparation in this study, probably indicates the efficacy of the procedure.

Table – 1: Ratio of satisfactory to unsatisfactory preparations of vaginal hormonal cytology in different studies.

Authors	Satisfactory (No. of cases)	Unsatisfactory (No. of cases)	Ratio of satisfactory to unsatisfactory
John of Leeton (1963)	185	21	8.80:1
McLennan and McLennan, et al. (1971)	1454	1000	1.45:1
Efstratiades, et al. (1983)	703	431	1.63:1
Present study	174	16	10.9:1

Conclusion

The salient observations noted in this prospective study on vaginal cytology are

- The isopropyl alcohol fixative used is a satisfactory fixative.
- The yield of material is satisfactory in the samples (90%).
- Vaginal smear collected between 4-11 days and 12-16 days of the normal menstrual cycle showed scattered intermediate and superficial cells.
- Vaginal smears collected between 17-28 days of normal menstrual cycle showed, predominately intermediate cells with folding and crowding.

- The KPI peak is between 12-16 days.
- This is extreme midzone shift, with clusters of navicular cells in all the three trimesters, of pregnancy.
- First stage of labour could be suspected with identification of increase in the number of superficial cells, with marked decrease in the intermediate cell values.
- Immediate postpartum period can be judged by identifying parabasal cells and few intermediate cells, MI was 60-65/30-35/0-5, thus making the study most valuable in the rural background of this country where in the clinical history is of meager help.
- Vaginal smears in postmenopausal period showed predominantly oestrophy pattern MI was 0-30/70-90/0-10.
- Anovulatory cycles can be suspected in cases of primary infertility by high plateau curve on cytohormonogram
- Severe ovarian disturbances causing secondary infertility showed hypoovarian curve on cytohormonogram
- While threatened abortion cases can be identified by progesterone deficiency pattern, inevitable abortion cases showed high KPI (30%) and few isolated endometrial cells
- Ovarian tumors did not reflect any changes in hormonal patterns.
- Vaginal hormonal cytology is a useful diagnostic in the female reproductive process. It may also provide a key to more effective conception control, as well as improvement in the treatment of menstrual disorders, anovulation, and other underlying disorders.
- In the present Indian scenario with lack of adequate clinical history available to the pathologist, in a rural setup as well the urban conditions; vaginal hormonal study is still a low cost diagnostic and prognostic test procedure. Hence the present study emphasizes the utility of the vaginal smear study even today

compared to the costly biochemical parameters, which are a financial burden to patients.

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