

SERIAL COLPO-CYTOLOGY IN 130 INFERTILE WOMEN WITH OVULATORY CYCLES*

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Colpo-Cytology and cervical mucus studies have always had a definite place in the management of infertility cases. In the near future too, they will continue to play a significant role and give valuable guidance to the gynaecologists practising in the developing countries.

Although recent advances in biochemistry have made hormone assays freely available for endocrine studies, they are at present and will continue to be out of reach of the hospital class of patients we treat. At least in them, cytology and mucus studies will serve as the screening procedure and will enable one to select cases which may require sophisticated hormone assays.

Two hundred and thirty cases of infertility, in whom routine investigations were normal were referred in the last 6 years (1971-1976) to the Cytology Clinic

of Cama and Albles Hospitals for serial cytology. Out of these 130 women had ovulatory cycles and 100 had anovulatory cycles.

Material and Methods

Detailed history and clinical examination and results of investigations done were tabulated in all cases. Cases with lower genital tract infections were treated before including in the studies. Lateral vaginal wall scrapes were taken every 3rd day after the cessation of menstrual period. Around ovulation time, smears were taken daily. Cervical mucus studies were done concurrently. In selected cases, endometrial biopsies were performed. Coitus was advised in all on the day of ovulation. In 30 cases, 50 inseminations were performed with husband's semen at about the day of ovulation.

Cytology smears were fixed in equal parts of alcohol and ether and stained by Papanicolaou stain (Shorr staining was not used). Interpretation of smears was done taking into account the following criteria:

1. Percentage of different types of epithelial cells.

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2. The aspect of cells, whether flat or folded.

3. Mode of exfoliation, whether singly, in clusters or in placards.

4. The number of leucocytes and Doderlein's bacilli and the degree of cytolysis.

5. Presence of RBC's.

6. Karyopyknotic Index—counts of 300 cells and above.

In our Laboratory, we divide the menstrual cycle into 7 phases. This division fundamentally coincides with that of De Allende (1950), Arronet and Turnbull (1957) and Pundel (quoted by De Neef 1965).

Smears are rarely taken in Phase I. The next 3 phases show a progressively estrogen dominant smear. After ovulation there is a neutralisation of this effect and smears show characteristic progesterone effect.

This is demonstrated in photographs number 1, 2 and 3.

Analysis of Cases

Out of 130 cases, there were 104 with primary sterility and 26 with secondary sterility.

Table I gives the age groups of the patients studied. Majority of the patients were between the ages of 20 to 30.

TABLE I
Age Groups of 130 Patients

Total Cases	Less than 20 years	21-25 Years	26-30 Years	31-35 Years	More than 36 Years
130	17	60	29	18	6

Phase I — Menstrual phase
Phase II — Post-menstrual phase
Phase III — Preovulatory phase
Phase IV — Ovulatory phase
Phase V — Postovulatory phase

Table II gives the duration of sterility in the cases under investigation. Cases are seen late as many are referred for this investigation as a last resort when all other investigations are normal.

TABLE II
Duration of Sterility—Total Patients 130

Total Cases	2-5 Years	6-10 Years	11-15 Years	More than 15 Years
130	64	42	18	6

Phase VI — Luteal phase
Phase VII — Premenstrual phase.

Generally, Phase I corresponds to the menstrual phase. Phases II and III correspond to the proliferative phase and phases V to VII correspond to the secretory phase of the histologic terminology of the endometrium.

Menstrual History

One hundred and seven patients had normal menstrual cycles. There were 23 cases in whom cycle length varied from 30 to 54 days.

Day of Ovulation: Day of Ovulation is charted in Figure I.

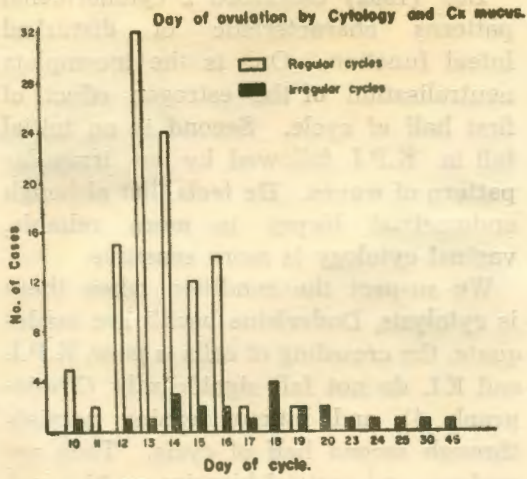


Diagram I

Chart showing day of ovulation in women with regular and irregular cycles.

Day of ovulation was predicted accurately in all cases at least in one of the cycles studied. This was done by cytology and cervical mucus only. B.B.T. charts were not suitable for this class of patients.

In women with regular cycles, a variation from day 10 to day 19 was observed, although the majority ovulated on 13th or 14th day.

Day of ovulation in women with irregular cycles is more important. Marked variation was noticed in these 23 cases. Here either the follicular phase or the luteal phase or both were prolonged and the ovulation varied from day 10 to day 45.

Although cyto-hormonal assays are not as reliable as radio-immuno assays, they do indicate a pattern of hormone production. We were able to discern certain patterns.

Variation in K.I. in Follicular Phase

The peak values of K.I. in the first half of menstrual cycles vary significant-

ly as reported by most authors. In our series, they varied from 25 to 75.

Peak Values of K.I. at the time of ovulation quoted by different authors is as followed:

de Allende & Orias, 1950	45-90%
Arronet & Turnbull, 1957	60.90%
Wachtel & Plasier, 1954	30-50%
Rakoff, 1966	30-80%
Weid C.L., 1968	35-85%
Pundel, 1952	10-90%
Present Series, 1977	25-75%

K.P.I. Charts

Daily K.P.I. was charted graphically. Figure 2 gives the K.P.I. charts of 2

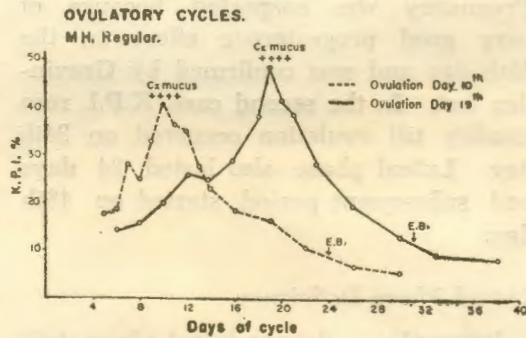


Diagram II

K.P.I. charts of 2 women with regular cycles.

women who had regular cycles. In one the K.P.I. tends to rise rapidly from day 5 and reaches a peak on day 10. She ovulated a little early in the cycle on the 10th day and the subsequent luteal phase was normal.

In the 2nd case, the graph is essentially normal although ovulation occurred on the 19th day. Both these women conceived during this study.

Figure 3 gives the K.P.I. charts of women with irregular cycles. These charts are more important. The first cases is from a patient whose cycle length was normally 6-8 weeks. In the

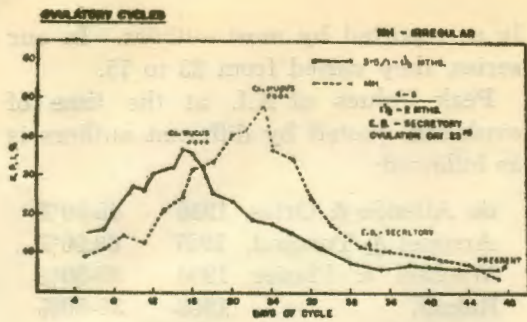


Diagram III

K.P.I. charts of 2 women with irregular cycles.

cycle under study, we were able to pinpoint ovulation on the 10th day and she was advised coitus accordingly. The luteal phase seemed prolonged as usual. Pregnancy was suspected because of very good progesterone effect on the 45th day and was confirmed by Gravidindex test. In the second case, K.P.I. rose steadily till ovulation occurred on 24th day. Luteal phase also lasted 24 days and subsequent period started on 48th day.

Luteal Phase Deficiency

Jones (1972) defines luteal phase deficiency as inadequate progesterone production by the corpus luteum and feels that a significant number of unexplained infertility is due to this cause.

Most authorities agree that serum hormone levels are less valuable in the diagnosis of this condition than properly dated endometrial biopsies. Although Jones does not agree that cytology and cervical mucus help in its diagnosis, Pundel (1962) stated that it is possible to utilize the vaginal smear as a qualitative test of luteal function during the menstrual cycle by an experienced cytologist provided he studies lateral vaginal wall scrapes taken every 3rd day and in the absence of infection.

Luz (1962) described 2 cytohormonal patterns characteristic of disturbed luteal function. One is the incomplete neutralisation of the estrogen effect of first half of cycle. Second is an initial fall in K.P.I. followed by an irregular pattern of waves. He feels that although endometrial biopsy is more reliable, vaginal cytology is more sensitive.

We suspect the condition when there is cytolysis, Doderleins bacilli are inadequate, the crowding of cells is poor, K.P.I. and E.I. do not fall significantly (Photograph 4) and mucus ferning persists through second half of cycle. Then we perform endometrial biopsies on 21st and 28th day and date it accurately. We have come across 6 cases.

The incidence of luteal phase deficiency is as follows:

Israel and Mazer	1963	3.5%
Gilliam	1965	10.7%
Botella Lluisa	1958	10.0%
Ganguly <i>et al</i>	1972	.95%
Present series	1977	4.6%

The suggested treatment is with small doses of intramuscular progesterone or with progesterone suppositories. The use of synthetic progestin should be avoided as they may further depress the corpus luteal function.

Diagnostic Accuracy of the Tests Used

We were able to compare the accuracy of cytology, cervical mucus and endometrial biopsy. Out of 130 cases, 124 were ovulatory in all the cycles studied, whereas 6 showed anovulation in at least one of the cycles. Vaginal cytology was found to be the most accurate as out of 130 cases 128 were ovulatory and 2 were found to be anovulatory giving an accuracy of 98.4%.

Endometrial biopsies were correct in 116 cases, whereas in 14 cases results

were inconclusive giving an accuracy of 89.6%.

However, it is suggested that all the tests be used concurrently and results evaluated together to get the best diagnostic accuracy.

Other Factors

During this study, certain other contributory factors were discovered. Ten patients had cervical factors. Six had stenosis due to cervicitis which was treated surgically and 4 had absent mucorrhoea treated hormonally. Five patients had small uteri and we felt that prognosis was poor. Seven patients had husbands with poor semen quality and we felt that only artificial insemination with donor semen would help. Most patients had multiple factors and were problem cases of infertility.

Pregnancy Rate

The overall pregnancy rate within 1 year of completion of studies was 13.9. However, after a subsequent laparoscopy, some cases were found to have tubal disease and the corrected pregnancy rate was 15.5%.

Howard Jones has reported a pregnancy rate of about 10% in these so called normal infertile couple group.

Discussion

Many workers like Forman (1956), Marcus and Marcus (1963) and Cohen (1956) feel that cervical mucus reflects hormonal activity more accurately than vaginal cytology. On the other hand, Moggian (1960), Behrman (1960) and Wachtel (1954) feel that the vaginal smear is the most precise and easy way to diagnose ovulation. Pundel reports that cytological findings when correlated with cervical mucus studies, yield maximum accuracy.

From the studies carried out, we feel that vaginal cytology is the most accurate parameter in the diagnosis of the time of ovulation. Despite the inconvenience to the patient and tediousness of daily observations, the results are clinically rewarding.

Study of cervical mucus for evidence of ovulation is also a useful aid. However, when we evaluate the merits and demerits of both methods, we feel cytology is more useful. Moreover, in case where cervical factor is suspected and the cervix is at fault, vaginal cytology becomes the only available method to detect the time of ovulation.

Currently, when hormone assays either radio-immuno or chemical are not freely available in our country there is only serial vaginal cytology which offers a lot of information to the practicing gynaecologist. We therefore feel that this important study should not be denied to the infertile patients who in our experience have co-operated most willingly in this rather tedious and prolonged investigation.

Acknowledgement

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See Figs. on Art Paper II