VALUE OF CYCLIC HORMONAL ASSESSMENT BY VAGINAL CYTOLOGY IN INFERTILE WOMEN

by

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Infertility associated with anovulation is a well recognised entity but recently disturbed ovulation, early or delayed, accompanied with the luteal phase defect has been considered pathological as it leads to sterility or early miscarriages. (Gillam, 1955; Grant, *et al*, 1959; Jones and Purmand, 1962; Israel, 1967, and Arrata, and Iffy, 1971). Thus, detection as well as dating the ovulation is essential for infertility work up.

In the present study, the attention is focussed on detecting as well as timing the ovulation in 60 infertile women, after eliminating other factors leading to infertility.

Material & Method

To begin with 64 normally menstruating infertile women having primary sterility free from any clinically evident systemic disease, endocrinal disorder or pelvic pathology were investigated. Their ages were ranging from 20 to 38 years. The semen analysis of the husband was done. When subnormal semen or azoospermia was detected, it was confirmed by several repeated tests from carefully collected specimens after minimum of 5 days abstinence.

The ovarian function was evaluated by vaginal cytology and endometrial biopsy. Vaginal cytology was used for dating the ovulation and premenstrual endometrial biopsy for evidence of progesterone activity. From upper 1/3rd of lateral vaginal wall, smears were taken daily from 8th to 24th day, with Ayre's spatula, after treating the vagina with Chloramphenicol pessaries on 5th and 6th day as Rauscher (1960) considers treatment with appropriate antibiotics prior to taking the smears, a necessary prerequisite for a cinically valuable diagnosis. The smears were fixed in either alchohol and stained by a modified Papanicolaou method. The daily smears were interpreted within few hours and K.I. value was plotted graphically. The onset of the preovulatory phase which was definite smear pattern, was closely watched. The preovulatory phase which usually lasts from 2 to 4 days is characterised by progressive decrease in the number of leucocytes and histiocytes and increasing number of superficial squamous cells with thin wafer-like transparent cytoplasm which tend to lie flat and singly. At the end of the preovulatory phase many superficial cells, full of turgor, were seen in the clear background of the smear. This smear pattern is seen just prior to ovulation. At the time of ovulation the first change observed was loss of turgor and folding of the cells followed by clumping and crowding. The fall in K.I. was noticeable only after 24 to 48 hours. The placard formation of intermediate cells indicative of the progesterone activity (de Neef, 1965) was observed. Later on

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the leukocytes, Doderlein bacilli, histiocytes and cytolysis increased, the smear looked dirtier. and the superficial cells decreased in number.

On 24th day of the menstrual cycle, endometrial biopsy was taken from the lateral wall of fundal area during laparoscopy procedure. Laparoscopy helped in determining the tubal patency and in ruling out pelvic pathology. Two cases of tuberculous abdomen and 2 cases of blocked tubes seen at laparoscopy were excluded.

At the end of the cycle the onset of menstrual bleeding was noted. The duration of the luteal phase was calculated from the date of ovulation and the onset of subsequent menses. The deficient luteal phase was diagnosed if the duration of the luteal phase was less than 10 days or showing poor steroidogenesis or both. The poor steroidogenesis arising from the defective corpus luteum function was diagnosed when histological dating and the menstrual dating by the onset of menses, did not agree within 2 days. Ovulation day is theoretically assigned as day 14 while the onset of menses is designated theoretically as day 28, irrespective of the date of the previous menstrual period. (Jones, 1968). Histologically, the endometrium is dated according to the criteria described by Noyes, Hertig and Rock (1950), and all the 3 dates derived by ovulation, onset of menses and histology of endometrial biopsy should agree within 2 days. All histology slides were reviewed with the pathologist after the patient had completed her menstrual cycle and her complete K.I. curve was plotted. As mentioned above if the histological dating and the menstrual dating by onset of menses did not agree within 2 days a luteal defect was suspected. The diagnosis of deficient luteal phase was confirmed by repeat cytology and endometrial biopsies on the same days in the same manner in the next cycle. The women with defective corpus luteum were advised the proper treatment of replacement therapy.

Those women having anovulatory cycles with adequate estrogen reflected in their vaginal smears and endometrial biopsy, were advised "Clomid therapy".

In patients where azoospermia was the only responsible factor, artificial insemination with donor's semen was done on the day of the ovulation, timed from 2 cycles. In the cases of oligospermia husband's semen was used for insemination.

Results

Of the total 60 women studied, 40 had ovulatory cycles with adequate luteal phase ranging from 12-18 days (Fig. 1). Four had inadequately functioning corpus luteum (Figs. 2, 3, 4, 5) and 16 had anovulatory cycles. (Fig. 6).

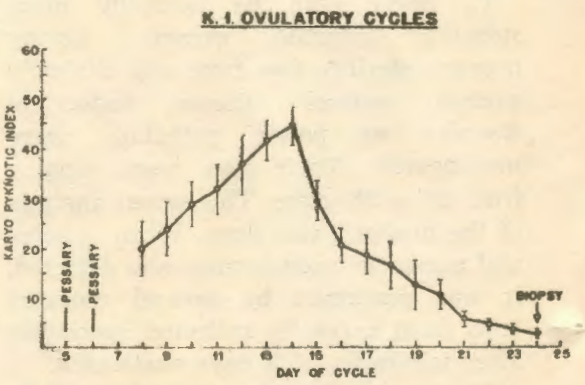

Fig. 1

Close correlation was noted between vaginal cytology and endometrial biopsy in 40 women with adequate luteal function, while vaginal smears did not show ovulation in 2 out of 4 cases with

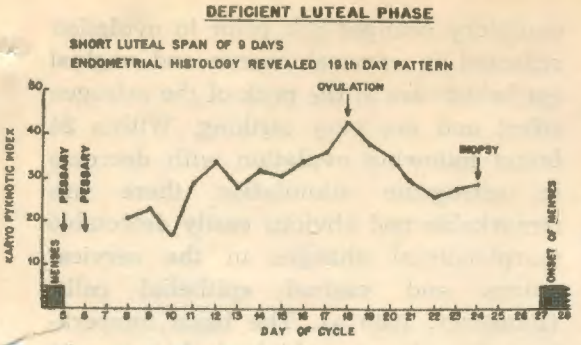

Fig. 2

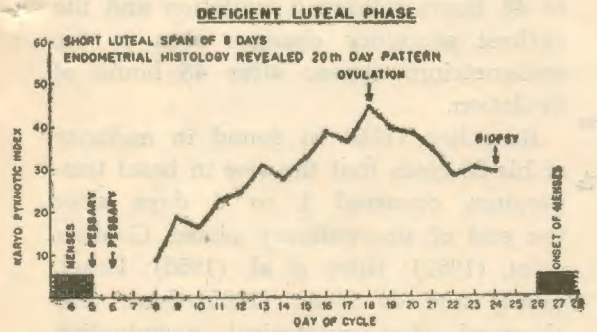

Fig. 3

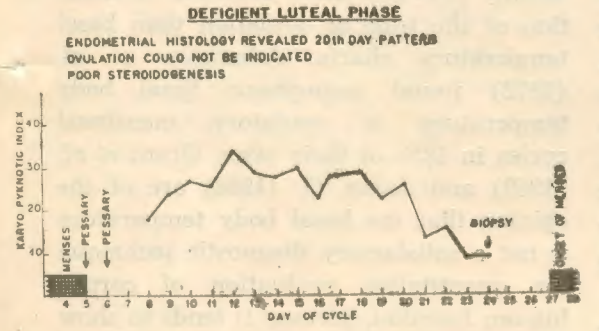

Fig. 4

defective corpus luteum. In 16 women with anovulatory cycles the plotted readings of K.I. showed an erratic curve.

The follow up of the 40 ovulating women is shown in Tables 1, 2 and 3.

TABLE III

The Results of Artificial Insemination using Husband's Semen

Successful	Unsuccessful	Under trial
3	1	3

Though clinically all the known factors were eliminated sterility could not be explained in 3 cases

Six of our cases were lost to follow up and 12, recently investigated couples, found normal, are being followed up.

Among 16 women who had anovulatory cycles, 4 were successfully treated with Clomid; 2 were 38 years of age and did not respond to Clomid therapy, 2 had azoospermic husbands, and the remaining 8 were lost to follow up.

Two of the 4 women with inadequate luteal phase were seen for the first time with incomplete abortion in second month of their first conception, and were found to have inadequate luteal function on subsequent investigations.

TABLE I

Analysis of 40 Ovulating Women

Azospermic husbands	Oligospermic husbands	Unexplained infertility	Lost to follow up	Recently investigated
12	7	3	6	12

TABLE II

The Results of Artificial Insemination with Donor's Semen

Successful	Unsuccesful	Under trial	Refused
4	5	1	2

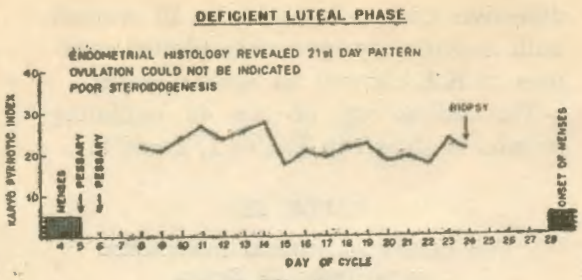

Fig. 5

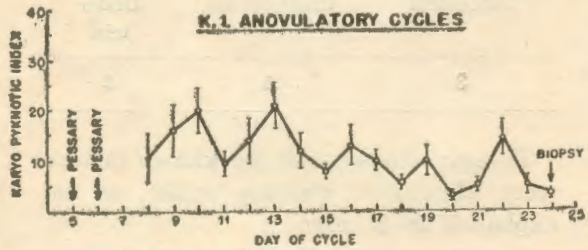

Fig. 6

Discussion

Definitive evidence of ovulation is pregnancy or the observation of a corpus luteum by endoscopy. Many of the indirect methods for detecting ovulation, such as hormonal estimation by bioassays or radio-immunoassays and histochemistry of the endometrium as well as directly visualising the ovaries for the presence of corpus luteum require highly specialised techniques which are costly and not available easily.

Of the many methods advocated for the detection of ovulation, four are currently outstanding as clinically proved and applicable; (1) The progressive maturation of the epithelial cells in the daily vaginal smears followed by a progestational smear; (2) increased amount and fluidity of the cervical mucus followed by a decreased amount of mucus and absence of the fern formation; (3) the biphasic basal temperature chart; (4) secretory changes observed in the endometrium. The maximum degree of preovulatory changes just prior to ovulation reflected in cervical mucus and vaginal epithelium are at the peak of the estrogen effect and are very striking. Within 24 hours following ovulation with decrease in estrogenic stimulation there are remarkable and obvious easily detectable morpholoigcal changes in the cervical mucus and vaginal epithelial cells. (Rauscher, 1960 a). The basal temperature chart becomes biphasic between 24 to 48 hours following ovulation and the earliest secretory changes seen in the endometrium appear after 48 hours of ovulation.

Rauscher (1960 b) found in majority of his 68 cases that the rise in basal temperature occurred 1 to 6 days after the end of preovulatory phase. Goldhar *et al,* (1952); Riley *et al,* (1955); Israel, (1967) and de Neef, (1965) have also observed that cytological examination usually provides more accurate information of the time of ovulation than basal temperature charts. Johansson *et al,* (1972) found monophasic basal body temperature in ovulatory menstrual cycles in 12% of their cases. Grant *et al,* (1959) and Jones, G. (1968) are of the opinion that the basal body temperature is not a satisfactory diagnostic technique for quantitative evaluation of corpus luteum function, because it tends to show all or none response.

Moreover, basal body temperature chart to be meaningful in detecting ovulation, it should be charted daily and accurately after taking special precautions, which is very difficult in our class of patients.

The cervical mucus is an unreliable indicator of ovulation in the presence of hormonal deficiencies or endocervical infection (Kleegman and Kaufman 1966) and also as the arborization test becomes

negative only some days after ovulation. (Zondak, 1957; Israel, 1967).

The vaginal smear method is the most satisfactory technique for dating ovulation because of its sharp end point and its simplicity. (Rakoff, 1960; Rauscher, 1960; Bickembach, and Soost, 1960; Moggian G, 1960). Hence, the vaginal cytology was utilized for timing the ovulation in the present study.

Though the endometrial biopsy has its limitation, in not timing the ovulation, it is an extremely useful evidence of progesterone activity, Jones (1949) reported that endometrial biopsy was the most satisfactory diagnostic method for detection of the luteal defect in comparison to the basal body temperature. The urinary pregnanediol determination, is only a research tool as one reading is not enough to diagnose defective corpus luteum and it is very expensive.

In the present infertility work up, the vaginal cytology and the endometrial biopsy in addition to detecting the ovulation helped in defining the adequacy of the luteal phase.

Arrata and Iffy (1971) have substantiated the belief that ovulation can occur at other time than usual midcycle, early or late and may result in an increased incidence of pregnancy anomalies.

The luteal phase defect is associated with repeated early abortions, wherein because of lack of progesterone, the correlation between proper tubal transport of the ovum, uterine motility to ensure proper localization of implantation and development of the endometrium for a proper nidation site, do not exist.

Various authors (Gillam, 1955; and Moszkowski, E. *et al*, 1962) have reported incidence of defective luteal phase between 3 to 10%. In this series similar incidence of 6.6% was noted.

Often the dating of ovulation, in patients having defective corpus luteum is difficult. Therefore, the diagnosis of poor steroidogenesis is achieved by comparing the histological dating and menstrual dating.

The male factor is responsible partly or wholly in 30 to 40% of infertile couples. (Speroff *et al.* 1973; Masani, 1973) which correlates with 32% incidence in the present study. This factor is responsible for sterility in fair number of couples who could be helped with the insemination programme on pin pointing the ovulation with the help of vaginal cytology. Behrman (1968) also finds vaginal smears more dependable in his insemination programme. If a single insemination per cycle is to be done, the timing must be much more precise, as the life of the ovum is 12 hours. Oligospermic husband is benefited on being advised the correct day of copulation after timing the ovulation and abstain from the same, for few days prior to ovulation. Ideally the deposition of the spermatozoa should occur just prior to the ovulation so that pregnancy is more likely to occur since spermatozoa would reach the fallopian tubes before the ovum does; this was followed in our insemination therapy done at the peak of preovulatory phase.

Conclusions

1. Anomalous ovulation occurring at odd times and producing pathological ova are responsible for sterility and pregnancy wastage. They are required to be detected during infertility work up.

2. The timing of the ovulation is also very essential for insemination to result in successful pregnancy.

3. The inadequate luteal phase can be diagnosed from the short luteal span or poor progesterone production, both of

which can be studied by timing the ovulation with the help of vaginal cytology and dating the endometrial histology pattern.

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