SIMPLE TESTS FOR DETECTION OF OVULATION DURING LACTATIONAL AMENORRHEA

by

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Several demographic studies have demonstrated that breast feeding affords some degree of protection against pregnancy (Berman *et al*, 1975; Bonte *et al*, 1976; Jain *et al*, 1970; Martin *et al*, 1964; Chen *et al*, 1974; Population Report, 1975). Search for objective indices that might indicate return of ovulation during lactation had received a fresh impetus in recent years because if such indices were available, contraceptive measures could be initiated at the appropriate time. Endometrial biopsy has been found to be a very useful index and has been used extensively in the past to obtain data on occurrence of ovulation during different period of lactation and ovulation rate prior to return of menstruation (Sharman, 1951; El-Minawi and Foda, 1971; Udesky, 1950; Thompkins, 1943; Perez *et al*, 1972). But it is an invasive technique and this trauma cannot be repeatedly inflicted on women. Basal body temperature chart (BBT)—a simple non-invasive method has been reported to be useful (Perez *et al*, 1972; Cronin, 1968) for detection of ovulation during lactation but, maintenance of B.B.T. chart is not practicable in developing countries where literacy rate is low. Vaginal cytology and cervical mucus arborisation (CMA) have been shown to be good indicators of hormonal activity during menstrual cycle (Moghissi *et al*, 1972) and have been extensively used in infertility follow up. So far there had been only one investigation where serial estimation of cervical mucus arborisation and vaginal cytology has been undertaken in lactational amenorrhea (Perez *et al*, 1972). In this investigation two other parameters endometrial biopsy and basal body temperature chart were also studied and the authors have not clearly stated the parameters based on which they had diagnosed return of fertility. The main emphasis of the paper was on return of fertility during lactation and the paper did not contain details of changes in vaginal cytology or cervical mucus arborisation during lactation or how dependable these two indices were when compared with endometrial biopsy and B.B.T. chart as indicators of return of fertility. An attempt was therefore made to investigate changes in vaginal cytology and cervical mucus arborisation during lactation and evaluate the usefulness of these simple parameters for detection of ovulation during lactational amenorrhea.

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Material and Methods

One thousand nine hundred and twelve

urban women belonging to the low income group of population, who had delivered in Erskine hospital, Madurai, were investigated prospectively. They were requested to attend the family planning clinic once a month. During each visit clinical examination findings on the state of vagina, cervix and uterus were recorded. Serial vaginal cytology and cervical mucus arborisation test were done once a month. Endometrial biopsy was done in a subsample during different periods of lactational amenorrhea. All these women were requested to report on the first day of the first period after lactational amenorrhea for endometrial biopsy, so that occurrence of ovulation during lactational amenorrhea can be calculated. Whenever endometrial biopsy was done length of uterine cavity was also measured.

Although 1912 women were initially registered for the study, the follow up rate was quite poor. Majority of women came at irregular intervals throughout the subsequent 18 months, so that ample data on cytology and cervical mucus changes during different periods of lactation before and after resumption of menstruation was obtained. However, complete set of serial monthly vaginal smears and cervical mucus arborisation data until return of menstruation was obtained in only 208 women. Endometrial biopsy during various periods of lactational amenorrhea was done in 402 women and in 192 women endometrial biopsy was obtained on the first day of period following lactational amenorrhea. Reliability of cytology and CMA test as indicators of return of ovulation and menstruation was evaluated against endometrial biopsy and menstrual data.

Observations

Vaginal cytology

Two weeks after delivery, vaginal smear showed numerous RBC, polymorphs and histiocytes. Vaginal cells were predominantly parabasal. By one month superficial and intermediate cells started appearing. By 6 weeks, cellular exudate consisting of RBC, histiocytes and polymorphs had almost disappeared. The number of parabasal cells had decreased and superficial and intermediate cells increased. Twelve weeks after delivery, vaginal smears consisted of predominantly superficial and intermediate cells, irrespective of whether or not the woman was menstruating (Table I). This pattern remained essentially

TABLE I

Vaginal Cytology in Lactational Amenorrhea

	Duration in weeks					
	2	4	6	8-12	13-24	>24
No. of cases	1912	1086	606	404	368	208
% of smears with R.B.C. and W.B.C. predominating	100.0	70.5	20.6	2.2	0.0	0.0
% smear containing predominantly parabasal cells	100.0	90.4	20.3	10.6	0.0	0.0
% smears containing predominantly superficial and intermediate cells	0.0	9.6	79.7	89.4	100.0	100.0

unaltered during the subsequent months irrespective of menstrual status of the women.

Cervical Mucus Arborisation

Since cervical mucus arborisation (CMA) could be evaluated only when all traces of bleeding has disappeared, CMA studies could be undertaken only 6 weeks after delivery. Women whose vaginal smears showed RBC were excluded from CMA evaluation.

Cervical mucus very seldom showed evidence of arborisation 6 weeks after delivery and when present CMA was atypical. By 12 weeks, a majority of lactating women (72.5%) showed delicate tracery atypical arborisation. By 24 weeks, cervical mucus showed evidence of arborisation in all cases. The degree of arborisation varied from delicate tracery pattern (+) (48.5%) to almost typical (+++) (10.6%). A typical arborisation pattern was by far more common (35.5%). By 24 weeks however majority of women showed (++) arborisation (61.0%) irrespective of their menstrual status (Table II).

TABLE II

Cervical Mucus Arborisation During Lactational Amenorrhea

	Duration of lactational amenorrhea in weeks			
	6	8-12	13-24	>24
No. of cases	462	404	368	208
% showing no arborisation	80.1	15.1	5.4	—
% +	15.2	72.5	48.5	15.9
% ++	4.7	8.7	35.5	61.0
% +++	—	3.7	10.6	23.1

Endometrial Biopsy

Endometrial biopsy was done during various periods of lactational amenorrhea, from 6 weeks to 2 years after delivery in 402 women. Endometrial biopsy was attempted in 25 women between 6 and 12 weeks pospartum. No endometrial tissue was obtained in 4 women. Biopsies done between 6 and 8 weeks after delivery revealed sheets of decidual cells, dense inflammatory cellular exudate, and sparse simple endometrial glands. Biopsies done between 9 and 11 weeks showed varying degrees of proliferation of endometrial glands, decidual cells and inflammatory exudate had disappeared in most of the cases. Endometrial biopsy was attempted in 377 women from 3 months to 24 months after delivery. Adequate tissue was obtained only in 185 women. Among these

TABLE III

Endometrial Pattern in Lactational Amenorrhea

	Duration of lactational amenorrhoea (in months)						
	<3	3-5	6-8	9-11	12-23	>24	Total
No endometrium obtained	4	95	53	45	8	1	205
Scanty	4	9	10	3	1	0	27
Proliferative	7	21	84	14	9	4	139
Endometritis	9	1	0	0	0	0	10
Mild cystic change	0	1	3	4	2	1	11
Secretory	1	2	3	2	1	0	9
Total	25	129	153	68	21	6	402

biopsies, only 8 (4.1%) showed secretory endometrium and the rest varying degrees of proliferation. Occasionally small cystic dilatation of glands were seen (Table III).

One hundred and four endometrial biopsies were obtained on the first day of the first period after delivery. Of these, biopsy material was inadequate for evaluation in 6, and 12 showed advanced necrosis and haemorrhage. Thus only 86 biopsies could be properly evaluated, of them 23 (26.8%) showed secretory change. The rest showed endometrial glands in varying degrees of proliferation. Further classification of the endometrial biopsy pattern depending upon duration of lactational amenorrhea to study the incidence of ovulation at different periods of lactational amenorrhea was not attempted because of the small number of biopsies.

Discussion

The present study provides data on changes in vaginal cytology and CMA during various periods of lactation. Apparently a gradual return of ovarian activity occurs during the first 3 months following delivery as indicated by gradual replacement of parabasal cells by intermediate and superficial cells and appearance of cervical mucus arborisation. However, once the circulating endogenous steroid levels have reached sufficient levels to cause maturation of vaginal cells and cervical mucus arborisation, vaginal cytology and CMA ceased to be sensitive indicators of hormonal activity and remained unaltered by subsequent changes in hormonal profile. Cytologist use the subtle alteration in the ratio of superficial and intermediate cells and variations in CMA which occurs during ovulation as the indicators of ovulation in infertility investigations. In order to detect this kind of variations vaginal smears and cervical mucus should be tested at least twice a week. Even when smear taking once a month was attempted we had regular follow up in only 10% of women. It is obvious therefore, that repeated examination twice a week for periods upto 1 year is not practicable in healthy parous lactating women. The subtle changes in the ratio of superficial to intermediate cells cannot be identified if smears are taken once a month. Thus it is not possible to obtain reliable information on ovulation during lactational amenorrhea using vaginal cytology as a parameter.

It was disappointing to note that neither CMA nor vaginal cytology was useful in the prediction of return of ovulation during lactational amenorrhea. Beyond 3 months after delivery, cytology showed a normoestrinic smear consisting of superficial and intermediate cells, irrespective of menstrual or lactational status. Cervical mucus also showed arborisation, though the degree varied in different individuals. Clinical examination in women during lactational amenorrhea often revealed a small cervix almost flush with the vaginal vault and a hyperinvoluted small mobile uterus even when vaginal cytology was normoestrinic and cervical mucus showed arborisation. Attempts at obtaining endometrial biopsy were successful only in about half of these women. Apparently, the threshold of sensitivity of uterus, vagina and cervix to the circulating steroids variey markedly. What was sufficient to cause normal maturation of vaginal epithelium and cervical mucus arborisation was insufficient to cause return of uterine size to normal or regeneration of endometrium. Thus, serial vaginal smear and cervical mucus studies

are not of much use in predicting return of menstruation and ovulation.

The pattern of endometrial changes observed in the present study is similar to that reported by earlier workers (Sharman, 1951; El-Minawi and Foda, 1971). El-Minawi and Foda (1971) reported that reparative endometritis is the most common finding in endometrial biopsies obtained within 3 months after delivery. Occurrance of occasional cystic changes in endometrial glands was also reported by El-Minawi and Foda (1971). Reported incidence of secretory endometrium in randomly done endometrial biopsies during lactational amenorrhea showed considerable variation from 1.5% (Udesky, 1950) to 6% (Tompkins, 1943). In the present study, 4.1% of random biopsies showed secretory endometrium—similar to the 3.9% secretory change reported by El-Minawi and Foda (1971). The appearance of proliferative endometrium during lactational amenorrhea did indicate an impending return of menstruation but the trauma of biopsy cannot be repeatedly inflicted for the purpose of detecting impending return of menstruation. The fact that the soft, thin walled, hyper-involuted uterus during lactational amenorrhea is extremely vulnerable for perforation also precludes wider use of this procedure.

Among biopsies done on the first day of period 26.8% showed secretory change, indicating that ovulation preceedes the onset of menstruation in about a fourth of women. Incidence of ovulation reported here is similar to that reported by Cronin (1968) using BBT chart as indication of ovulation. This escape ovulation during the period of amenorrhea accounts for the reported 7-10% conception rate during lactational amenorrhea.

Data presented here show that vaginal cytology and cervical mucus arborisation test are not useful to predict return of ovulation during lactational amenorrhea for two reasons.

(a) threshold of sensitivity of the uterus, vagina and the cervix to circulating steroids varied markedly so that these tests had very little predictive value as far as return of ovulation was concerned.

(b) practical difficulties in getting parous normal lactating women to attend the clinic frequently for these tests. Endometrial biopsy apart from being a traumatic, invasive procedure gives information only after ovulation has occurred. Thus none of these three tests have practical value in prediction of return of ovulation during lactational amenorrhoea.

Demographic data both from India (Prema *et al*, 1979; Prema and Philips, 1980) and from elsewhere—show that less than 10% of women conceive during lactational amenorrhea. Until reliable simple tests for prediction of return of ovulation during lactation become available it may be justifiable to use return of menstruation as the guideline for initiation of contraceptive measures. In countries with liberalised abortion laws, unwanted pregnancies occurring during lactational amenorrhea could be terminated and concurrent contraceptive measures might be initiated at the time of termination of pregnancy.

Summary

Search for simple objective indices which indicate return of ovulation during lactation received a fresh impetus in recent years, because if available—these indices may be of help in initiating contraceptive measures at the appropriate time. A prospective study of 1912 women was undertaken to evaluate the usefulness of two simple parameters vaginal cytology and cervical mucus arborisation in pre-

dicting return of ovulation and menstruation during lactational amenorrhea. Data from this prospective study showed that between 6-12 weeks after delivery the number of parabasal and basal cells in vaginal smears showed a progressive decline. After 12 weeks vaginal smear consisted predominantly of superficial and intermediate cells, irrespective of menstrual or lactational status. Cervical mucus started to show a typical arborisation by 12 weeks after delivery. Arborisation progressively increased with duration of lactation upto 24 weeks, after which it remained unaltered irrespective of lactational or menstrual status. It was also noted that the threshold of sensitivity of endometrium, uterus, cervix and vagina to circulating steroid varied markedly, so that cervical mucus arborisation and vaginal cytology were not of much use in predicting return of fertility. Though endometrial biopsy did give some indication, the trauma of repeated biopsy could not be inflicted on these women. Endometrial biopsy showed that ovulation occurred prior to the first period in 26.8% of lactating women. Until such time a simple reliable test which could indicate return of ovulation during lactation is available, resumption of menstruation after lactational amenorrhea may be the most reliable indicator for the need for initiating contraceptive measures during lactation.

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References

1. Breast feeding—aid to infant health and fertility control. Population Report Series J. No. 4, 1975.
2. Berman, M. L., Hanson, K. and Hellman, I. L. Am. J. Obstet. Gynec. 114: 524, 1975.
3. Bonte, M., Balan, J. and Van, H.: J. Bio. Social Sciences. 1: 97, 1969.
4. Chen, L. C., Ahamad, S., Gesche, M. and Mosley, M.: Pop. Studies. 28: 277, 1974.
5. Cronin, T. J.: Lancet. 2: 422, 1968.
6. El-Minawi, M. F. and Foda, M. S.: Am. J. Obstet. Gynec. 111: 17, 1971.
7. Jain, A., Hsu, T. C., Friedman, C. and Chang, M. C.: Demography. 7: 255, 1970.
8. Martin, W. J., Morley, D., Woodland, M.: J. Trop. Paediat. 10: 82, 1964.
9. Moghissi, K. S., Syner, F. N., Evans, T. N.: Am. J. Obstet. Gynec. 114: 405, 1972.
10. Perez, A., Vela, P., Masnick, G. S. and Potter, R. G.: Am. J. Obstet. Gynec. 114: 1041, 1972.
11. Prema, K., Naidu, A. N. and Neela Kumari, S.: Am. J. Clinic Nutr. 32: 1298, 1979.
12. Prema, K. and Philips, F. S.: Ind. J. Med. Res. (in press).
13. Sharman, A.: Fertil. Steril. 2: 371, 1951.
14. Tompkins, P.: Am. J. Obstet. Gynec 45: 48, 1943.
15. Udesky, I.: Am. J. Obstet. Gynec. 59: 843, 1950.