THE CARAMEL TEST AND ITS SIGNIFICANCE IN DETECTION OF OVULATION WITH OTHER PARAMETERS

by

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Many laboratory methods for detection and diagnosis of ovulation have been devised. Still this important basic phenomenon remains retrospective and numerous approaches have been realised to attest to its probable occurrence. The absolute confirmation of ovulation, though of profound biological significance has to be done by use of indirect means for want of simple direct one to establish such temporal relationship. The obvious significance in the detection and timing of ovulation is in the management of a case of infertile couple and greater importance for applying to the problem of fertility control. Cervical mucus displays cyclical activity which is maximal at ovulation to facilitate migration, capacitation and the storage of spermatozoa in the cervical crypts. Many analysis, although useful, cannot routinely monitor the fertility period and establish probable ovulation dates. The caramel test is the simplest and cheapest, which can be done in the rural area. It involves heating of cervical mucus and changing of the white colour to light brown (caramel) that is more significant in relation to the ovulatory phenomenon (Campos da Paz 1974). The present study was undertaken to assess reliability of caramel test in detection of ovulation in comparison to fern test and endometrial biopsy.

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Accepted for publication on 9-4-1980.

Material and Methods

The material for this study was collected from 100 women attending the Gynaecological Out Patient Department of Government Medical College Hospital, Nagpur during the year 1975-76 and 158 cases, both sterile and fertile on oral contraceptives from Gynaecological Out Patient Department of Kasturba Hospital attached to M.G. Institute of Medical Sciences, Sevagram, during the years 1976 and 1979. This group was selected for the reason that such patients are extremely co-operative to all measures directed towards the diagnosis and treatment of their symptoms.

General diseases, inflammatory disease of the uterine adenexas and the presence of pathological vaginal discharge were excluded by careful examination. At first visit to the hospital, a thorough personal and menstrual history was taken and in cases of secondary sterility, an enquiry

was made into the previous obstetrical history also. A thorough vaginal examination was then made and findings noted.

After cleaning the ectocervix the cervical mucus was withdrawn with the help of a tuberculin syringe. The mucus was then expelled on two glass slides and was allowed to dry in air for 5 to 10 minutes and then examined under low power of microscope for arborization. The ferning reaction was then graded (Isreal 1972). The other slide was longitudinally heated on an alcohol burner for two minutes. X-ray viewing glass was used for the chromatic phenomenon and one of the three, white, light caramel, or dark caramel was observed. The endometrial biopsy was obtained by dilatation and curettage, and the specimen was subjected for histological examination. The datas were then compiled and analysis was made.

Observations

The present study was carried out in 1548 samples of cervical mucus with fern test around 10th day, 13th to 16th days and 24th day of menstrual cycle as assessed by endometrial biopsy from 210 cases of biphasic, 32 cases of monophasic menstrual cycle with 16 cases on oral contraceptive.

In biphasic cycles, out of 210 samples collected on 10th day, brown appearance of cervical mucus with atypical fern pattern was observed in 208 samples. Endometrial biopsy showed proliferative phase in all the cases. The white appearance of cervical mucus was always associated with typical fern pattern and secretory phase of the endometrial biopsy about 13th to 14th day of the cycle, thereafter the cervical mucus showed a change over of colour from white to light brown around 15th day. From 16th day onwards the brown colour of cervical mucus was associated with atypical fern pattern like pre-ovulatory phase with secretory endometrium (Table I).

In anovulatory cycles the white colour of cervical mucus was always associated with typical fern pattern and proliferative and swiss-cheese appearance of endometrium throughout the cycle from 13th day onwards. However, on 10th day of the cycle, the brown appearance of cervical mucus was found to be associated with atypical fern pattern like biphasic (Table II).

TABLE I

Correlation of Caramel Test with Ferning and Endometrial Biopsy in Ovulatory Cycles

Days	Caramel Test			Fern		Endometrium		
	Brown	Light brown	White	Typical	Atypical	PP	SP	Mixed
10th	208	—	2	2	208	210	—	—
13th	—	—	210	208 (99.04)	2 (0.96)	—	210	—
14th	—	—	210	208	2	—	210	—
15th	—	208	2	208	2	—	210	—
16th	208	—	2	2	208	—	210	—
24th	208	—	2	2	208	—	210	—

PP : Prolferative phase
SP : Secretory Phase

TABLE II

Correlation of Caramel Test, Ferning and Endometrial Biopsy in Anovulation Cycles

Days	Caramel Test			Fern		Endometrium		
	Brown	Light brown	White	Typical	Atypical	PP	SP	X Swiss-cheese appear-ance
10th	32	—	—	—	3	29	—	3
13th	—	—	32	32	—	29	—	3
14th	—	—	32	32	—	29	—	3
15th	—	—	32	32	—	29	—	3
16th	—	—	32	32	—	29	—	3
24th	—	—	32	32	—	29	—	3

PP: Proliferative phase SP: Secretory phase

The Cervical mucus of fertile patients on oral contraceptives showed the brown colour of the cervical mucus with atypical fern pattern throughout the menstrual cycle. Early secretory activity was observed in all the cases from 10th day and atrophy of glands with decidual reaction was seen on 24th day of the cycle (Table III).

The results of caramel test, fern pattern and endometrial biopsy in different group of cases on various days of the menstrual cycle are summarised briefly in Table IV.

Discussion

The present study has shown a good correlation between fern pattern of cervical mucus with secretory-endometrium in 99.04 per cent of cases in all the three phases of the ovulatory cycle. Similarly Engineer *et al* (1968) and Sarin (1971) has reported the correlation of cervical mucus in 99.1 per cent and 84.5 per cent respectively with endometrial biopsy. The remaining 2 cases of biphasic ovulatory cycle appear to be hormonally imbalanced. When the result of fern pattern

TABLE III

Correlation of Caramel Test, Ferning and Endometrial Biopsy in Patients on Oral Contraceptives

Days	Caramel Test			Fern		Endometrium		
	Brown	Light brown	White	Typical	Atypical	PP	SP	Dilata-tion cystic
10th	16	—	—	—	16	—	16	—
13th	16	—	—	—	16	—	16	—
14th	16	—	—	—	16	—	16	—
15th	16	—	—	—	16	—	16	—
16th	16	—	—	—	16	—	16	—
24th	16	—	—	—	16	All of the cases showed atrophy glands with decidua like reaction		

TABLE IV

Comparison of Studies on Caramel Test, Fern Pattern of Cervical Mucus and Endometrial Biopsy During Menstrual Cycle

Days	10	13	14	15	16	24
Ovulatory cycle						
Fern' test	Atypical	Typical	Typical	Typical	Atypical	Negative
Caramel test	Brown	White	White	Light brown	Brown	Brown
Endometrial biopsy	PP	SP	SP	SP	SP	SP
An Ovulatory Cycle						
Fern Test	Atypical	Typical	Typical	Typical	Typical	Typical
Caramel test	Brown	White	White	White	White	White
Endometrial biopsy	PP	PP	PP	PP	PP	PP
Oral Contra-ceptive						
Fern Test	Atypical	Atypical	Atyvical	Atypical	Atypical	Atypical
Caramel Test	Brown	Brown	Brown	Brown	Brown	Brown
Endometrial biopsy	SP	SP	SP	SP	SP	SP

PP: Proliferative phase
SP: Secretory phase

was compared with caramel test a good correlation in 100.00 per cent of cases was obtained between them. In the similar way the change of white colour of the cervical mucus to light brown or caramel on the day of ovulation was obtained in 99.04 per cent cases with secretory phase of endometrium and thereafter the light brown colour of the cervical mucus became dark brown and persisted with secretory phase till the onset of next cycle. Campos da Paz (1974) in his study has also reported a complete correlation between caramel test, fern pattern and endometrial biopsy on various days of the menstrual cycle. Though endometrial biopsy has profound significance in confirmation of ovulation but the specificity of the test in relation to particular date does not hold good, whereas caramel test can indicate the particular day of ovulation in a fertile woman; thus it appears to be superior to almost all the best available test at present for detection of ovulation.

Hence it is apparent from the present study that the white appearance of cervical mucus and a typical fern pattern is related to estrogenic stimulation and turning of the white to light brown or caramel in fertile period, the most earliest sign seen on cervical mucus may reflect follicular rupture, corpus luteum formation and fall in plasma estrogen levels. A positive caramel test following the white colour strongly suggest ovulation.

The features in anovulatory cycles were different from ovulatory cycles. In anovulatory cycles typical fern pattern was correlated in 100.00 per cent of cases with endometrial biopsy. The recent study was in complete agreement with Engineer *et al* (1968), who reported 100.00 per cent correlation between these

two parameters. The caramel test has shown a persistant white colour of cervical mucus with typical fern pattern and proliferative and swiss-cheese pattern of endometrial biopsy in 100.00 per cent of cases from 13th day onwards till the onset of next cycle. Campoz da Paz (1974) has reported a complete correlation between caramel test, fern pattern and endometrial biopsy in anovulatory cycle and stated that while colour with typical fern pattern though specifically not related to ovulatory phenomenon but certainly reflect estrogenic stimulatien.

The third group of cases showed still different picture from the other two groups. An early secretory activity with marked vacuolation of stromal cells and inhibition of proliferative phase was seen in all the cases from 10th day. The brown colour of cervical mucus was correlated with atypical fern pattern and endometrial biopsy in 100.00 per cent of cases. A similar type of result was also found in the series of Campos da Paz (1974). The early occurrence of secretory activity reflects both estrogen and progestrone action. Thus persistence of brown colour of cervical mucus with atypical fern pattern throughout the menstrual cycle reflects the deficiency of estrogen peak due to administration of combined estrogen and progesterone preparation.

So from the above discussion it can be concluded that caramel test runs parallel with endometrial biopsy hence this simple test can be adopted by couples to avoid the fertile period for family planning and also in the diagnosis and treatment of infertility.

Summary and Conclusions

A total of 1548 samples of cervical mucus from 258 cases of sterility and fertility were studied for caramel test and fern pattern with endometrial biopsy and the best correlation was obtained between them in all the group of cases. The caramel test is cyclical and correlates with fern pattern and endometrial biopsy. The light brown or caramel colour seen, following the white specimen of the fertile period, suggest some relation to ovulation.

The present study strongly suggest further work to ascertain the reliability of caramel test for detection of ovulation.

Acknowledgements

The authors are grateful to Dr. C. L. Jhaveri, M.D., F.A.C.S., F.C.P.S,. F.I.C.S., Consulting Gynaecologist for his guidance and supply of information from Campos da Paz to complete the present study.

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