

## **Original Research Article**

### **A Study of Chloride Levels in Cervical Mucosa and Its Correlation with Transvaginal Sonography**

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**Abstract:** Ovarian function can be evaluated by various procedures. Among various constituents of cervical mucus, Sodium Chloride (NaCl) is the major electrolyte responsible for positive fern test at the time of ovulation. The Aim was to evaluate variations in sodium chloride level in cervical mucus and establish a relationship between peak chloride level in cervical mucus by ovulatory & ovulation by premenstrual transvaginal sonography in both infertile and fertile patients. The Study was conducted upon 100 women of reproductive age group with no obvious gynaecological or obstetric disease. There were two groups group I of 50 infertile women and group II of 50 fertile women. History, physical-systemic-gynecological examinations, blood-urine-semen tests, Kahn's test and VDRL test were carried out before enrolment. Each patient was called thrice for TVS and cervical mucus examination. In Results In preovulatory phase, chloride level of all patients were  $\leq 0.5\%$ . In periovulatory phase, 40% of both cases showed 0.7%, 6% showed 0.9% in infertile whereas 20% showed 0.9% chloride level in fertile cases. In postovulatory phase, chloride level of all patients were  $\leq 0.5\%$ . According to endometrial pattern, 72% infertile and 92% fertile cases showed proliferative endometrium in concordance with follicular size of  $> 16$  mm. 68% infertile and 92% fertile cases showed secretory endometrium which was taken as confirmative of ovulation. For chloride estimation, chloride level estimation detected ovulation in only 72% infertile cases & all fertile cases while cycle was ovulatory in 68% infertile cases & 92% fertile cases according to TVS. In Discussion the Chloride levels in ovulatory cycle were found to be  $< 0.5\%$  in 96% of cases during preovulatory phase,  $\geq 0.5\%$  in 72% infertile and all of fertile cases during periovulatory phase,  $< 0.5\%$  in 88% of infertile and all of fertile cases whereas  $> 0.5\%$  in 12% of infertile cases during postovulatory phase. Chloride level estimation of cervical mucus is 96% accurate in infertile and 92% accurate in fertile cases compared to TVS.

**Keywords:** Transvaginal Sonography, Sodium chloride, Ovulation, Cervical mucosa, Infertile, Fertile.

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#### **INTRODUCTION**

Ovulation is a process resulting from hypothalamus, anterior pituitary and ovaries. The ovarian hormone influences the hypothalamo-pituitary axis to induce a FSH and LH surge, responsible for ovulation. Various procedures available for evaluation of ovarian function are vaginal cytology, basal body temperature recordings, premenstrual endometrial biopsy, steroid excretion estimation, LH surge test and ultrasonography for evidence of follicular rupture at time of ovulation [1]. Cyclic variations in the physiochemical properties of cervical mucus have been found from last few years. Various mucus studies are used in the diagnosis of various gynaecological disorders. At the time of ovulation, cervical mucus becomes copious, clear and watery. Peak of this mucus and peak of estrogen secretion occurs at same time [2].

Cervical mucus is a more sensitive indicator of estrogen activity [3]. Predictive method used for natural family planning is Billing's ovulation method in which development of changing mucus pattern has been considered [4].

Appearance of mucus has been correlated with the increasing estrogen level generated by the developing follicle. Mucus causes maximum wetness when luteinizing hormone is at maximum level. It has been considered that ovulation occurs an hour before to 48 hours after the peak symptom. Therefore, all the mucus wet days including peak symptom plus 72 hours following it are considered as fertile days of woman. Reason for infertility due to ovulatory failure has been found in 15-25% of infertile female population [5].

Sodium Chloride (NaCl) is the major electrolyte component among the various constituents of cervical mucus [6]. At peak concentration of cervical mucus, NaCl can be responsible for positive fern test at the time of ovulation. NaCl coincides with the peak of fern like crystallization of mucus on glass slide [7]. Crystallization requires presence of mucin like substances as well as NaCl in high concentration [8].

On the basis of above considerations, study has been made to evaluate variations in chloride levels in preovulatory, ovulatory and premenstrual phase of the menstrual cycle and its utility in prediction and detection of ovulation.

**MATERIALS AND METHODS**

Study was conducted upon 100 women attending the outpatient department of Obstetrics and Gynaecology, G.R. Medical College & Kamla Raja Hospital, Gwalior, from September 2004 to March 2005.

Women of reproductive age group with no obvious gynaecological or obstetric disease were included in the study. Detailed obstetric and gynaecological history of each patient was recorded with particular emphasis on menstrual cycles and period of infertility. Patients were investigated for Hb%, blood sugar, urine routine and microscopy, semen analysis, Kahn's test and venereal disease research laboratory test. Only women with healthy looking cervixes and vagina were selected. Women with cervicitis, vaginitis, cervical erosion, previous electrocauterization of cervix and other such conditions were excluded from the study.

Women were divided into two groups. Group I: Consisting of 50 infertile women in age group of 18 to 35 years, having regular menses with no associated clinically detectable abnormality. Group II: Consisting of 50 women of 18 to 35 years, who had two or more conceptions and were without clinically detectable disease.

Patients were instructed not to have coitus 24 hours prior to the performance of mucus test, nor to use

any kind of lubricant or douching before the study of cervical mucus since semen, prostatic secretions and other chemicals are known to interfere with the study of chloride levels.

After recording the data of last menstrual period, each patient was called thrice. On each visit cervical mucus was studied for chloride concentration by test sheet method [9]. For collection of cervical mucus tuberculin syringe was used [10]. Test sheets were prepared by serially impregnating filter paper sheets with solutions of silver nitrate and potassium chromate. First visit was in immediate post menstrual period i.e. 2-3 days after stoppage of bleeding - on 6<sup>th</sup> to 9<sup>th</sup> day of cycle. Second visit was in ovulatory period i.e. 13<sup>th</sup> to 16<sup>th</sup> day of cycle and third in premenstrual phase. i.e. 20<sup>th</sup> to 25<sup>th</sup> day of cycle. During these visits, transvaginal sonography was done which included follicular monitoring & endometrial pattern study in second visit and assessment of ovulation (follicular size and presence of free fluid in pouch of Douglas) in third visit.

**RESULTS**

Out of total 100women, 50 cases were infertile patients and 50 cases were fertile controls. Distribution of cases and controls according to chloride levels in preovulatory phase between 6<sup>th</sup> to 9<sup>th</sup> day of cycle was found as per Table 1. Distribution of cases and controls according to chloride levels in periovulatory phase between 13<sup>th</sup> to 16<sup>th</sup> days of cycle was found as per Table 2.

Distribution of cases and controls according to chloride levels in postovulatory phase between 20<sup>th</sup> to 25<sup>th</sup> days of cycle was found as per Table 3. Distribution of cases according to follicular size as shown by TVS during ovulatory phase was found in following Figure 1.

Distribution of cases according to endometrial pattern as shown by TVS during ovulatory phase and post ovulatory phase were found as per Table 4 and Table 5. Accuracy of chloride estimation for detection of ovulation in infertile cases and fertile cases were found as per following Figure 2.

**Table 1: Distribution of cases and controls according to chloride levels in preovulatory phase (between 6<sup>th</sup> to 9<sup>th</sup> day of cycle)**

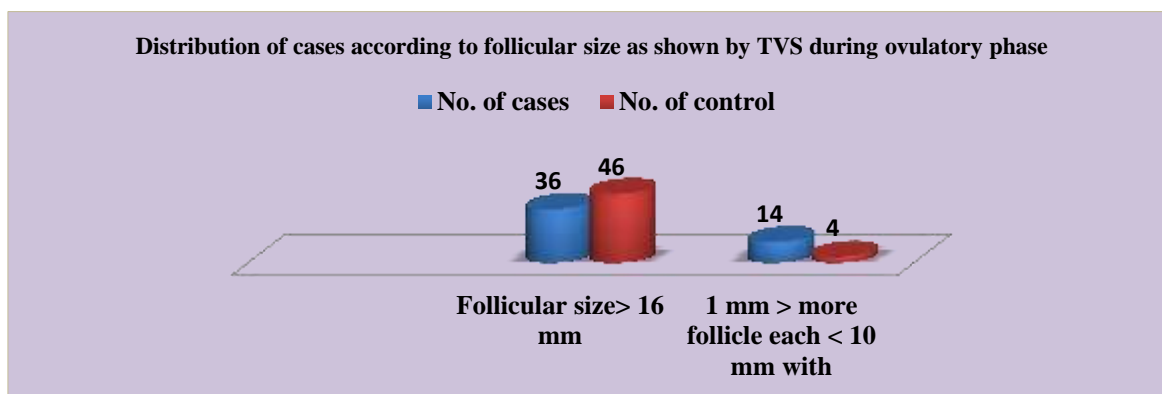
Sr. No.	Cases (Infertile cases)			Control (Fertile cases)		
	No. of cases	% of cases	Chloride level in %	No. of cases	% of cases	Chloride level in %
1	10	20	0.1	10	20	0.1
2	38	76	0.3	38	76	0.3
3	2	4	0.5	2	4	0.5
<b>Total</b>	<b>50</b>	<b>100</b>		<b>50</b>	<b>100</b>	

**Table 2: Distribution of cases according to chloride levels in periovulatory phase (between 13<sup>th</sup> to 16<sup>th</sup> day of cycle)**

Sr. No.	Cases (Infertile cases)			Control (Fertile cases)		
	No. of cases	% of cases	Chloride level in %	No. of cases	% of cases	Chloride level in %
1	1	2	0.1	-	-	0.1
2	13	26	0.3	-	-	0.3
3	13	26	0.5	20	40	0.5
4	20	40	0.7	20	40	0.7
5	3	6	0.9	10	20	0.9
<b>Total</b>	<b>50</b>	<b>100</b>		<b>50</b>	<b>100</b>	

**Table 3: Distribution of cases and controls according to chloride levels in postovulatory phase (between 20<sup>th</sup> to 25<sup>th</sup> day of cycle)**

Sr. No.	Cases (Infertile cases)			Control (Fertile cases)		
	No. of cases	% of cases	Chloride level in %	No. of cases	% of cases	Chloride level in %
1	6	12	0.1	10	20	0.1
2	38	76	0.3	40	80	0.3
3	6	12	0.5	-	-	0.5
<b>Total</b>	<b>50</b>	<b>100</b>		<b>50</b>	<b>100</b>	



**Fig 1: Distribution of cases according to follicular size as shown by TVS during ovulatory phase**

**Table 4: Distribution of cases according to endometrial pattern as shown by TVS during ovulatory phase**

Sr. No.	Endometrial pattern	Cases (Infertile cases)		Control (Fertile cases)	
		No. of cases	% age	No. of control	% age
1.	Proliferative Endometrium	36	72	46	92
2.	Thin/proliferative/endometrium not corresponding to the phase of cycle	14	28	4	8
<b>Total</b>		<b>50</b>	<b>100</b>	<b>50</b>	<b>100</b>

**Table 5: Distribution of cases and control according to endometrial pattern as shown by TVS during post ovulatory phase**

Sr. No.	Endometrial pattern	Cases (Infertile cases)		Control (Fertile cases)	
		No. of cases	%age	No. of cases	%age
1	Secretory endometrium	34	68	46	92
2	Proliferative endometrium	12	24	4	8
3	Endometrial hyperplasia	4	8	-	-
<b>Total</b>		<b>50</b>	<b>100</b>	<b>50</b>	<b>100</b>

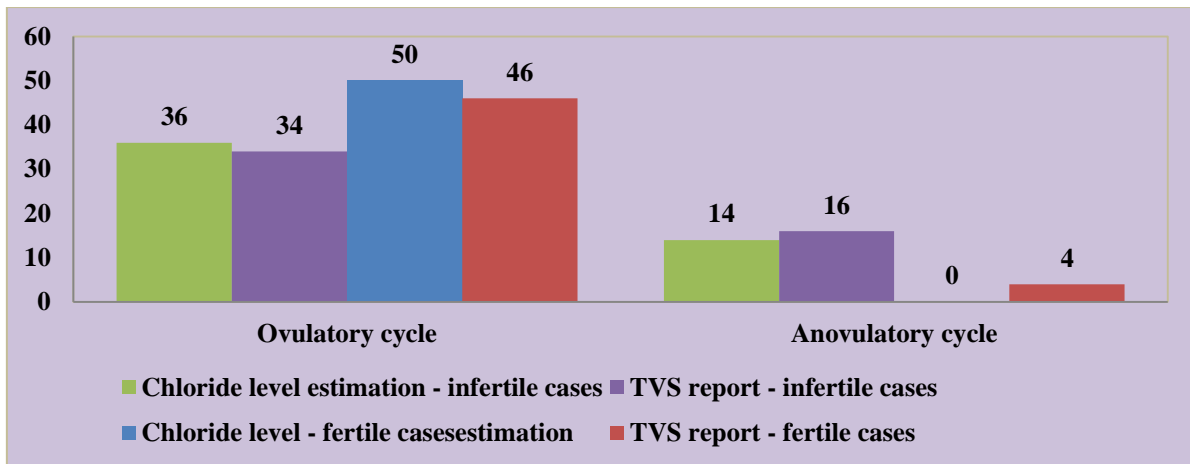


Fig 2: Accuracy of chloride estimation for detection of ovulation in infertile and fertile cases

## DISCUSSION

Chloride levels in ovulatory cycle were found to be  $<0.5\%$  in 96% of cases between 6<sup>th</sup>-9<sup>th</sup> day of cycle,  $\geq 0.5\%$  in 72% infertile and all of fertile cases between 13<sup>th</sup>-16<sup>th</sup> day of cycle,  $<0.5\%$  in 88% of infertile and all of fertile cases whereas  $>0.5\%$  in 12% of infertile cases between 20<sup>th</sup>-25<sup>th</sup> day of cycle. This difference in chloride levels from preovulatory, periovulatory to postovulatory phase was found to be statistically significant. These observations were in correlation with those of McSweeney *et al.*; who found change in spot intensity with a drop below 0.5% in the chloride concentration in post ovulatory phase of the cycle [9]. Hardy *et al.*; found that positive chloride spot disappear in post ovulatory phase of the cycle [11]. Also, Singh and Boss and Mathur *et al.*; found fall in chloride levels in post-ovulatory phase of ovulatory cycles [12, 13].

According to endometrial pattern, in ovulation phase, 72% infertile cases showed proliferative endometrium in concordance with follicular size of  $> 16$  mm which is about to rupture whereas 92% of control cases showed the same. 28% infertile cases showed thin endometrium or endometrium out of phase having no dominant follicle preclusive of anovulatory cycles and only 8% showed the same in fertile group. In post ovulation phase, 68% infertile cases showed secretory endometrium which was taken as confirmative of ovulation and 92% in fertile cases showed the same. According to these results, 68% infertile cases had ovulatory cycle while 24% infertile cases showed proliferative phase whereas only 8% fertile cases showed proliferative endometrium which showed endometrial hyperplasia. Similarly, it was found by Parson *et al.* that 7-8% of women are anovulatory and anovulatory cycles were seen 3-6 times often in infertile women [14]. Moreover, as per Strowitzki T *et al.*; endometrium is definitely a fertility-determining factor [15].

Ovulation was detected in 72% of infertile and all of fertile cases by estimating chloride levels of cervical mucus. Taking ovulation & pre menstrual sonography as the standard parameter for detection of ovulation, ovulation was confirmed in 68% of infertile and 92% of fertile cases. Results of chloride level estimation of cervical mucus were thus found to be 96% accurate in infertile and 92% accurate in fertile cases. Fallacy rate was statistically insignificant ( $P < 0.5$ ). However, it was noted that chloride level estimation was false positive in only 4% cases. Thus it is possible to locate the approximate day of ovulation by cervical mucus study and cases of sterility can be advised coitus on the appropriate days. Also the time for artificial insemination can be detected easily. Similar types of result were also found by Guida M *et al.* that urinary LH level determination yielded a 100% correlation with the simultaneous ultrasonographic diagnosis of ovulation. Mucus secretions and characteristics yielded a 48.3% correlation when simultaneously evaluated with ovulation. Beta-glucuronidase levels yielded a 27.7% correlation. The salivary ferning test had a 36.8% ovulation-detection rate at the day of ovulation, but 58.7% of results were uninterpretable. Body temperature measurements yielded a 30.4% correlation with the simultaneous ultrasonographic diagnosis of ovulation [16].

## CONCLUSION

It has been concluded that chloride level estimation in the cervical mucus to detect ovulation offers a reliable and sought method in the treatment of infertility in developing country like India. Testing of the cervical mucus for chloride by "Spot Test Paper" can be used as means of appraising ovarian hormone activity which is simple, fairly reliable, easy to interpret, more economical, more rapid and can be done readily by the patient herself or in O.P.D.

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