Brazilian Journal of Medical and Biological Research (2001) 34: 767-770
ISSN 0100-879X Short Communication

In situ variation of cervical mucus pH during exposure to atmospheric air

C.H.M. Correa, A.L.G. Mattos and A.N. Ferrari

Fundação Universitária de Endocrinologia e Fertilidade, Porto Alegre, RS, Brasil

Correspondence
A.N. Ferrari
Fundação Universitária de Endocrinologia e Fertilidade
Rua Dr. Alcides Cruz, 101
90630-160 Porto Alegre, RS
Brasil

Received January 19, 2000
Accepted March 27, 2001

Abstract

The objective of the present study was to determine if exposure of cervical mucus to air during specular examination could modify mucus pH. Detection of changes is justified because of their possible interference with sperm-mucus interaction, since an acidic pH is unfavorable to sperm penetration and is associated with infertility due to the cervical factor. Twenty women with good quality mucus were evaluated. pH measurements of ecto- and endocervical mucus were made *in situ* using a glass electrode after 0-, 5- and 10-min exposure to air. There was a progressive alkalinization of mucus pH. Mean values of ectocervical mucus pH were 6.91, 7.16 and 7.27, while mean values of endocervical mucus pH were 7.09, 7.34 and 7.46 at 0, 5 and 10 min, respectively. Significant differences were found between the mean values obtained at 0 and 5 min, and at 0 and 10 min ($P<0.05$), whereas the differences in mean values at 5 and 10 min were not significant at either site. We conclude that 5 to 10 min of exposure to atmospheric air affects cervical mucus pH in a significant way. Since tests used to evaluate sperm-mucus interaction generally have not considered this possibility, we suggest that they should be performed immediately after mucus collection in order to avoid misinterpretation of the results.

Key words
• Cervical mucus pH
• Infertility
• Postcoital test

Abnormalities of the cervix and its secretion are reported to be responsible for infertility in approximately 5 to 10% of women (1). Evaluation of sperm-cervical mucus interaction is important in infertility investigation. The traditional method for the evaluation of sperm-cervical mucus interaction *in vivo* is the postcoital test (PCT), but *in vitro* cervical mucus-sperm penetration tests are also used (2,3).

Sometimes, negative or abnormal PCT are attributed to immunological problems in particular. Anyway, recent studies have shown that the incidence of immunological factors in these cases is low, and that 80% of them can be reversed by means of a bicarbonate douche (4). The improvement observed with mucus alkalinization suggests that the low mucus pH (5) causes the poor sperm-mucus interaction. In addition, it was reported that some cases of negative PCT (absence of progressive sperm) or absent PCT (absence of sperm) involving ovulatory mucus and normal sperm became normal during the performance of *in vitro* cervical mucus-sperm penetration tests (6,7). It was suspected that this alteration occurred due to the variation of mucus pH after exposure to air.

Previous studies have reported pH variation of mucus exposed to air (8,9), but those

studies were done *in vitro*, i.e., after mucus had been aspirated. The aim of this study was to determine if the exposure of cervical mucus to atmospheric air during specular examination could modify mucus pH *in situ*.

Twenty women attending this service during one year and who voluntarily agreed to take part in the study were evaluated. In all cases the women were of reproductive age, had regular cycles and presented good quality periovulatory cervical mucus. No pathological microorganisms were found on vaginal Gram-stained smears. Patients having organic or anatomic cervical changes, as well as patients using drugs that could change cervical mucus were excluded.

In order to collect the mucus for evaluation, the patient was examined in the gynecological position, the uterine cervix was exposed with a sterile, non-lubricated speculum and the ectocervix was cleaned of excess debris with a sterile cotton swab. Cervical mucus was obtained from the endocervix by gentle aspiration with a sterile tuberculin syringe (without a needle) daily from the 8th or 9th day of the cycle until the ideal score, described below, was reached.

To evaluate the quality of cervical mucus, the following parameters were taken into consideration: volume (0 = absent; 1 = 0.1 ml; 2 = 0.2 ml; 3 = 0.3 ml), spinnbarkeit (0 = 1 cm; 1 = 1-4 cm; 2 = 5-8 cm; 3 = 9 cm), ferning (1 = atypical fern formation; 2 = primary or secondary stems; 3 = tertiary or quaternary stems) and color upon overheating (slide uniformly heated over an alcohol burner for 1 min): A = dark brown, indicating the absence of crystallization or initial crystallization stages; B = light brown, indicating atypical crystallization; C = clear, indicating typical crystallization with tertiary or quaternary stems. The result is reported as the approximate percentage of each color in the sample (10).

Each item, except overheating, was scored from 0 to 3, with 3 being the optimal condition for each item. A score ≥6 indicated preovulatory mucus receptive to sperm penetration, a score <6 was associated with unfavorable cervical secretion, and a score <3 represented hostile mucus (2). Overheated mucus showed color variations from totally light brown to totally clear during the periovulatory phase.

The pH of cervical mucus was determined when the cervical score was ≥6/9 and the percentage of clear color was 50% or more upon overheating. Patients were examined daily and when the cervical mucus score was close to the predetermined values, pH measurement was programmed for the following day. On this occasion, pH was first measured and mucus quality was then evaluated. If mucus had not reached the predetermined score, the result was discarded and a new measurement was programmed for the following day or following cycle.

For all measurements pH was determined with a glass microelectrode (Beckman® No. 39535), 24 cm long and with a diameter of 5 mm, connected to a pHmeter (Digimed®), regularly calibrated against standard buffer solutions (pH 4.0 and 7.0). After being used for a patient, the microelectrode was wiped with tissue paper, washed with deionized water and disinfected with a solution of benzalkonium chloride. Prior to reuse it was washed with deionized water, recalibrated and washed again.

The first pH measurement (time zero) was made immediately after placement of the vaginal speculum and removal of vaginal secretion. The microelectrode was placed in contact with ectocervical mucus exactly in front of the external cervical os, where the measurement was made. The microelectrode was then gently inserted into the cervical canal until it reached the internal os, where endocervical pH was measured. These measurements were recorded as time zero. The electrode was removed from the cervix, cleaned with tissue paper and washed with deionized water.

The vaginal speculum was kept open and

the same procedure was repeated after 5 and 10 min. After the measurements, the mucus was aspirated with a tuberculin syringe and evaluated according to the criteria previously described. At the time of pH measurement, the following scores were recorded: volume - score 2 for four patients (20%) and score 3 for sixteen patients (80%); spinnbarkeit - score 2 for eight patients (40%) and 3 for twelve patients (60%); ferning - tertiary or quaternary (score 3) in all samples.

Total cervical score was 7/9 for two patients (10%), 8/9 for eight patients (40%), and 9/9 for ten patients (50%).

Color at overheating - 90% of the patients (N = 18) showed a percentage of clear color equal to or higher than 50%, while 10% (N = 2) showed a lower percentage of clear color.

The present study demonstrated that the lowest ectocervical pH value at time zero was 6.33 and the highest 7.35, at 5 min the lowest value was 6.78 and the highest 7.62, at 10 min the lowest value was 6.85 and the highest 7.73. Only one subject showed a reduction in ectocervical pH between 0 and 5 min. Mean ectocervical pH was 6.91 ± 0.235 at time zero, 7.16 ± 0.215 at 5 min and 7.27 ± 0.220 at 10 min. The values recorded at 0 and 5 min were significantly different, as also were the values recorded at 0 and 10 min ($P<0.05$, ANOVA), but there was no significant difference between the mean values recorded at 5 and 10 min.

The lowest endocervical pH value at time zero was 6.77 and the highest 7.40, at 5 min the lowest value was 6.89 and the highest 7.54, at 10 min the lowest value was 7.03 and the highest 7.69. Only one subject showed a reduction in endocervical pH between 0 and 5 min. Mean endocervical pH was 7.09 ± 0.161 at time zero, 7.34 ± 0.159 at 5 min and 7.46 ± 0.164 at 10 min. The values recorded at 0 and 5 min were significantly different, as also were the values recorded at 0 and 10 min ($P<0.05$, ANOVA), but there was no significant difference between the values obtained at 5 and 10 min.

The mean ectocervical and endocervical pH values obtained at different times are shown in Table 1.

The clinical relevance of pH changes after mucus exposure to the air is affected in practically all tests currently used to evaluate sperm-mucus interaction because in all of them aspirated mucus already exposed to the air is used and does not express physiological conditions. If lack of sperm penetration is due to a low mucus pH (9,11-13), the penetration ability could not be preserved at the time of examination. In fact, some investigators have observed that, even with persistently negative PCT, *in vivo* penetration tests can be normal (6,7). A possible explanation could be the pH increase due to exposure to air when *in vitro* tests are performed. The contradictory results obtained in some PCT could also be explained by mucus pH changes at different times of interpretation. In addition, since pH values are different for each patient, there is no pattern for mucus alkalinization and subsequent correction at the time of test interpretation. Thus, PCT should be interpreted cautiously, since its results are obtained after cervical mucus has been aspirated and its pH is higher. This could mask the etiological factor, leading to a false diagnosis.

Ragni et al. (14) compared *in vivo* endocervical pH measurements first performed with paper and then with a microelectrode and observed that, although similar, the measurements made with an electrode were

Table 1. Mean ecto- and endocervical pH values in 20 subjects at each evaluation time.

Time (min)	Ectocervical pH	Endocervical pH
0	6.91 ± 0.235	7.09 ± 0.161
5	7.16 ± 0.215*	7.34 ± 0.159*
10	7.27 ± 0.220*	7.46 ± 0.164*

Data are reported as means ± SD. *P<0.05 compared to time zero (ANOVA).

slightly superior. According to the findings in the present study, a possible explanation for these differences would be the period of time between the two measurements, rather than the different procedures.

In most cases of infertility due to cervical factor, the treatment is homologous artificial insemination (HAI). Intrauterine insemination is most frequently used (15). Although this is a low risk procedure, it is expensive, requiring sperm preparation in the laboratory and usually controlled ovarian stimulation and ultrasonography, generally restricting the treatment to specialized centers (16). Cervical factor cases due to low cervical mucus pH may be treated by means of a vaginal douche with sodium bicarbonate. This procedure is extremely simple and inexpensive and shows satisfactory results. Several studies have shown a significant increase in PCT and in conception rates with the use of a vaginal douche with bicarbonate in previously selected couples (4,17-19).

The authors suggest that greater attention should be paid to pH in the investigation of infertility and emphasize the need to perform pH measurements immediately after speculum placement in order to avoid mucus pH changes. Future research is needed to evaluate the exact period of time when cervical mucus pH begins to change and, consequently, the correct moment to perform sperm penetration tests in order to reproduce physiological conditions. We also recommend to check cervical pH before proceeding to HAI or evaluating other potential causes of poor sperm-mucus interaction, and to consider bicarbonate douche treatment if the pH is low.

References

1. Sims JA & Gibbons WE (1996). Treatment of human infertility: the cervical and uterine factors. In: Adashi EY, Rock JA & Rosenwaks Z (Editors), *Reproductive Endocrinology, Surgery, and Technology*. Lippincott-Raven, New York, 2142-2169.
2. World Health Organization (1992). *WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction*. 3rd edn. Cambridge University Press, Cambridge, UK.
3. Moghissi KS (1993). Diagnosis and classification of disturbed sperm cervical mucus interaction. In: Insler V & Lunenfeld B (Editors), *Infertility: Male and Female*. Churchill Livingstone, New York, 299-314.
4. Ferrari AN & Mattos ALG (1996). El uso del bicarbonato de sodio en el test postcoital. *Revista Latino Americana de Esterilidad y Fertilidad*, 10: 33-36.
5. OImsted SS, Dubin NH, Cone RA & Moench TR (2000). The rate at which human sperm are immobilized and killed by mild acidity. *Fertility and Sterility*, 73: 687-693.
6. Jonsson B, Eneroth P, Landgren BM & Wikborn C (1986). Evaluation of *in vitro* sperm penetration testing of 176 infertile couples with the use of ejaculates and cervical mucus from donors. *Fertility and Sterility*, 45: 353-356.
7. Overstreet JW (1986). Evaluation of sperm-cervical mucus interaction. *Fertility and Sterility*, 45: 324-326.
8. Breckenridge MAB, Pederson DP & Pommerenke WT (1950). A pH study of human cervical secretions. *Fertility and Sterility*, 1: 427-434.
9. Kroeks MVAM & Kremer J (1977). The pH in the lower third of the genital tract. In: Insler V & Bettendorf G (Editors), *The Uterine Cervix in Reproduction*. Georg Thieme, Stuttgart, 109-118.
10. Ferrari AN (1978). Detecção da ovulação pelas mudanças de cor do muco cervical. *Revista da Associação Médica Brasileira*, 24: 8-14.
11. Zavos PM & Cohen MR (1980). The pH of cervical mucus and the postcoital test. *Fertility and Sterility*, 34: 234-238.
12. Peek JC & Matthews CD (1986). The pH of cervical mucus, quality of semen, and outcome of the postcoital test. *Clinical Reproduction and Fertility*, 4: 217-225.
13. Eggert-Kruse W, Köhler A, Rohr G & Runnebaum B (1993). The pH as an important determinant of sperm-mucus interaction. *Fertility and Sterility*, 59: 617-628.
14. Ragni G, Ruspa M, Bestetti O, De Lauretis L, Olivares D & Wyssling H (1984). Measurement of pH in the lower female genital tract during the periovulatory period: comparison of the electrometric and colorimetric procedures. *Acta Europaea Fertilitatis*, 15: 377-380.
15. Nuojua-Huttunen S, Tomas C, Bloigu R, Tuomivaara L & Martikainen H (1999). Intrauterine insemination treatment in subfertility: an analysis of factors affecting outcome. *Human Reproduction*, 14: 698-703.
16. Philips Z, Bazarra-Llorens M & Posnett J (2000). Evaluation of the relative cost-effectiveness of treatments for infertility in the UK. *Human Reproduction*, 15: 95-106.
17. Ansari AH, Gould K & Ansari VM (1980). Sodium bicarbonate douching for improvement of the postcoital test. *Fertility and Sterility*, 33: 608-612.
18. Jenkins JM, Anthony FW, Purdie B, Gilbert D, Noon R & Masson GM (1989). Acidic endocervical mucus: a potential cause of subfertility. *Contemporary Review of Obstetrics and Gynaecology*, 1: 273-278.
19. Everhardt E, Dony JMJ, Jansen H, Lemmens WAJG & Doesburg WH (1990). Improvement of cervical mucus viscoelasticity and sperm penetration with sodium bicarbonate douching. *Human Reproduction*, 5: 133-137.