

Prognostic value of in vitro sperm penetration into hormonally standardized human cervical mucus

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To analyze the prognostic value of the sperm cervical mucus penetration test (SCMPT), fresh semen samples of 99 male patients under infertility investigation were exposed to capillary tubes filled with freshly obtained cervical mucus (CM) of the patients' wives (WCM), fertile donors (DCM), and bovine CM (BCM). The quality of the human CM was standardized by oral administration of estrogens. The overall pregnancy rate after 6 months was 17.2% (17/99), and was significantly different in couples with poor and good SCMPT with WCM (1/44, 2.3% versus 16/55, 29%; $P < 0.001$) in a prospective study. Human CM was superior to BCM as a penetration medium in providing more information about sperm function. The results suggest that in vitro sperm penetration testing with hormonally standardized CM of female partners adds an important dimension to sperm analysis with regard to fertility prognosis. *Fertil Steril* 51:317, 1989

The evaluation of the ability of spermatozoa to penetrate cervical mucus (CM) provides some information about sperm function.¹ Although the usefulness of postcoital testing (PCT) as a screening procedure in infertility investigation remains unquestioned, the results are frequently not conclusive.^{2,3} Furthermore, the parameters of semen recorded by microscopic clinical analysis do not totally reflect male fertility potential.⁴ Additional objective, quantitative data can be obtained by in vitro capillary tube tests,⁵ particularly in cases of questionable PCT.⁶

The object of the present investigation was to assess whether CM of patients' wives, obtained after a standardized oral treatment with estrogens, is a valuable means for the sperm penetration assay

during infertility investigation. In couples with long-standing infertility, the penetration of spermatozoa into human CM was compared with the more readily available bovine cervical mucus (BCM) penetration test. Results were analyzed with regard to sperm functional capacity and fertility prognosis.

MATERIALS AND METHODS

In a prospective study, spermatozoa from males from 99 infertile couples were tested by the in vitro sperm-cervical mucus penetration test (SCMPT).⁵ Three different penetration media were compared: cervical mucus of patients' wives (WCM), cervical mucus of fertile donors (DCM), and bovine cervical mucus (BCM).

The mean duration of infertility within the study population was 5.2 years, with a range of 1 to 18 years. The mean age of the women was 29.6 years (21 to 40 years), and of their husbands, 32.6 years (23 to 50 years). Couples with clinical symptoms of infection of the lower genital tract were not included in the study. The female partners were checked for tubal patency and uterine factors by

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hysterosalpingography and/or laparoscopy. Free tubal patency (both sides) was found in 61%, some adhesions in 34%, and severe adhesions (but at least 1 patent tube) in 5%, a normal configuration of the uterus in 94% of women. Extensive hormonal analyses for pituitary, ovarian, thyroid, and adrenal function had been carried out in all women, and treatment performed whenever necessary. Sperm count and motility had been examined repeatedly; normal sperm count ($>40 \times 10^6/\text{ml}$) was found in 65%, oligozoospermia ($<20 \times 10^6/\text{ml}$) in 15%. Normal progressive motility ($>40\%$) was found in 63% of male patients, and marked asthenozoospermia ($<20\%$) in 15%. Of the samples, 69% showed adequate sperm morphology ($>60\%$ normal forms).⁶ The PCT was performed as a screening test at the beginning of the infertility investigation.^{7,8} Some disorders of sperm-mucus interaction were suspected in 31% of couples (<7 highly motile spermatozoa per high power field [HPF] seen in preovulatory CM 8 to 12 hours after intercourse). When there were no spermatozoa found in CM, a vaginal pool sample was examined to ensure that semen actually had been deposited in the vagina.

Performance of in Vitro SCMPT

Couples were seen in the infertility unit between the 9th and 13th day of the women's menstrual cycle, after a 5-day period of sexual abstinence before the in vitro SCMPT.

In order to standardize the mucus quality and to gain independence from the cycle function, oral treatment with estrogens (80 μg of ethinylestradiol per day for 7 days) was administered to the female patients as well as to the female donors before mucus collection. Medications with potentially negative effects on the rheologic characteristics of the mucus (e.g., clomiphene citrate) were stopped the previous cycle.

Semen was obtained at the hospital by masturbation into sterile glass jars. Sperm analysis was performed according to World Health Organization (WHO) criteria,⁶ and consisted of a determination of ejaculate volume, liquefaction time, sperm count, motility assessment, differential morphology, pH, viability, fructose concentration, and number of round cells, and was done by a single trained technician.

In female patients and donors with proven fertility, the cervix was exposed with an unlubricated speculum and the cervical score according to Insler et al.⁹ was assigned. The vagina and the exocervix

were cleaned of excess debris with a large sterile cotton swab. The cervical mucus was obtained by gentle aspiration from the endocervix by means of a special device (Aspigliaire, IMV, L'Aigle, France). The fresh CM was immediately aspirated into capillary tubes. Care was taken to prevent air bubbles from disrupting the mucus column. After the capillary tube was filled, it was sealed at one end with modeling clay that was pressed carefully into the capillary tube so that one drop of mucus protruded through the open end. This end was put into the semen reservoir of the penetration meter described by Kremer.⁵ To obtain the most reproducible data, specimen were used directly after liquefaction.

For the BCM penetration test, Penetrak (Serrono, Freiburg, FRG), a commercially available assay, was run in duplicate, at room temperature. The results were evaluated with low-power (LFP) magnification of a Zeiss light microscope (Zeiss, Oberkochen, FRG), and the duplicate values were averaged. In case the penetration depth was markedly different ($>10\text{ mm}$), testing was repeated with the same semen sample. The penetration meter was observed microscopically (LFP) after 2 hours incubation, and additionally after 6 hours at 37°C in a humidified atmosphere. The penetration parameters were evaluated by a single observer without knowledge of the other variables of sperm quality.

In the SCMPT, the following criteria were examined in addition to the migration distance (penetration depth of the most vanguard spermatozoon): the penetration density (number of penetrated spermatozoa), and the quality and duration of motility. Each parameter was graded from 0 to 3 and the results summarized in a score, which is shown in Table 1. In order to test the prognostic value of SCMPT with WCM and BCM for fertilizing capacity of spermatozoa, the cumulative score was designed before this prospective study was started. The median score value was determined to be the breakpoint between inadequate (group I) and adequate (group II) SCMPT.

Additionally, to evaluate the prognostic significance of sperm penetration testing with BCM, a penetration distance of $\geq 30\text{ mm}$ or less after 2 hours (instructions of the manufacturer) was determined to select patients with poor (A) and good (B) BCM test.

The pregnancy rate was determined 6 months after performance of SCMPT.

The capacity of spermatozoa to penetrate the three different media was compared. The variables

Table 1 Sperm Penetration Meter Score

Penetration parameter	0	1	2	3
Migration distance (mm)	<15	15–29	30–44	≥45
Sperm density (number/LPF ^a)	0–9	10–49	50–99	≥100
Motility grade ^b	Immotile	In situ motility “shaking”	Slow forward motility	Highly ^c propulsive motility

^a LPF, low power field (100×).^b Determined after 2 hours and after 6 hours.^c Of the majority of spermatozoa.

of penetration and sperm analysis were correlated and analyzed for discriminating ability with regard to subsequent fertility.

Statistical Analyses

A computer package program (SAS) was used to perform Spearman rank correlation, chi-square, and two-tailed Fisher's exact test, Wilcoxon, and Friedman tests.

RESULTS

Comparison of Penetration Media

The same therapeutic regimen was used before SCMPT for both female patients and female donors, and resulted in a cervical index of >10 in more than 80% of the women at the day of examination. There were no significant differences between the quality of CM of patients' wives and CM of fertile donors, as judged with the Insler score.

The motility grade after 2 and after 6 hours differed significantly in bovine and human CM ($P < 0.001$; Friedman test). Table 2 shows the correlation between penetration in WCM, DCM, and BCM (Spearman rank correlation).

The penetration distance of spermatozoa was markedly reduced in BCM compared with WCM or

DCM ($P < 0.001$), but a significant positive correlation was found ($P < 0.001$). A better correlation was found between both types of human CM ($r = 0.73$). Although there were differences for the number of penetrated spermatozoa, the sperm density, a closer correlation in BCM and WCM or DCM was found, which again was higher for wives' and donors' CM ($r = 0.89$).

Correlation of SCMPT with Sperm Analysis

The depth of penetration in all types of CM was correlated with parameters of routine sperm analysis. The penetration distance of spermatozoa into BCM correlated best with progressive motility after liquefaction of semen ($r = 0.56$), followed by viability ($r = 0.55$), morphology (% normal forms; $r = 0.53$), and sperm count ($r = 0.48$) after 2 hours, and was slightly higher after 6 hours observation and with regard to the sperm density.

Only a weak correlation was found for the penetration distance of spermatozoa in human CM (WCM as well as DCM) and progressive motility ($r = 0.29$), sperm count ($r = 0.44$), morphology ($r = 0.37$), and viability ($r = 0.34$) after 2 hours (WCM). These correlations were not better after a shorter (30 minutes) or longer observation time and for the number of penetrated spermatozoa. No significant correlation was found for the variables of penetration with sperm volume, pH, fructose concentration, and number of round cells.

Prognostic Value of SCMPT

The results of SCMPT in hormonally standardized CM of patients' wives were analysed with regard to subsequent pregnancy. The overall pregnancy rate after 6 months was 17.2% (17/99).

Penetration parameters were summarized by means of the SCMPT score. The median value had been defined to be the breakpoint. Group I (inadequate SCMPT) consisted of 44 couples (44%; score 0 to 6), and group II (adequate SCMPT) of 55 cou-

Table 2 Correlation of Sperm Penetration into Bovine and Human Cervical Mucus

SCMPT		Correlation coefficient (r) ^a		
		BCM-WCM	BCM-DCM	WCM-DCM
Penetration distance	I ^b	0.458	0.445	0.727
	II ^c	0.399	0.316	0.741
Sperm density	I ^b	0.783	0.868	0.892
	II ^c	0.619	0.708	0.866

^a $P < 0.0001$ for all correlation coefficients.^b I, after 2 hours.^c II, after 6 hours.

Table 3 Results of Sperm Cervical Mucus Penetration Test into Wives' Cervical Mucus with Regard to Subsequent Pregnancy Rate (6 Months)

SCMPT	SCMPT poor ^a	SCMPT good ^b
Pregnancies (number)	1/44	16/55
Percent	2.3 ^c	29 ^c

^a Score 0–6.

^b Score ≥ 7 .

^c $P < 0.001$

ples (56%; score ≥ 7). Patients of both groups did not differ in female or male age, duration of infertility, and cervical index. Other infertility factors (uterine, tubal, ovarian) were equally distributed. Patients were comparable for endocrine parameters of pituitary, thyroid, and adrenal function. There was no difference in subsequent specific treatment.

Whereas there was only one pregnancy in group I with poor results of SCMPT (2.3%), 16 couples of group II with good results of SCMPT achieved a pregnancy (29.1%) within the first 6 months after penetration testing ($P < 0.001$). These findings, indicating the prognostic value of human CM as medium for the in vitro SCMPT, are presented in Table 3 (Fisher's two-tailed exact test).

There was one couple of group I with a subsequent pregnancy. In spite of excellent sperm count ($>60 \times 10^6/\text{ml}$) and motility ($>60\%$ forward progression) and a positive PCT, a SCMPT score of 0 was evaluated. However, the cervical index was only 5, and spermatozoa showed adequate penetration of donor's CM.

In the following 6 months, there were three additional pregnancies in group I (1 couple with score 0 and 2 with 6) and five additional pregnancies in group II; two couples of group II were lost for follow-up (pregnancy rate after 12 months: 9.1% versus 39.6%).

Subsequent fertility also was prospectively compared in patients with semen samples with poor or good penetration of BCM (less than 30 mm [A] and ≥ 30 mm [B]). No significant difference in pregnancy rates was found (A: 9/62 [15%]; B: 8/37 [22%]; $P > 0.05$).

When the above-mentioned cumulative SCMPT score was applied to BCM testing of spermatozoa, there was no significant difference, either. The pregnancy rate in case of inadequate SCMPT (I) with BCM was 13% (6/47), and 21% (11/52) when SCMPT with bovine CM was adequate (II) ($P > 0.05$).

Comparing the group of patients with normal and reduced sperm count ($<40 \times 10^6/\text{ml}$) or motility ($<40\%$ forward progression) with regard to subsequent fertility, no significant differences were found.

However, when the SCMPT results with human CM were analyzed separately in the group of patients with reduced sperm counts or motility, SCMPT proved to be of prognostic value. Pregnancy rate within the following 6 months was significantly higher in cases of adequate SCMPT ($P < 0.01$; Fisher's two-tailed exact test). Even in couples with normal sperm quality of the male, markedly more pregnancies were achieved when penetration of WCM was good (II).

This additional information to sperm analysis was not obtained when BCM was used as penetration medium.

Analysis of Variables of Penetration and Sperm Analysis

The different penetration parameters analyzed (Wilcoxon tests) with respect to subsequent fertility are shown in Table 4.

Whereas only weak statistically significant differences were found for the penetration distance into BCM ($P < 0.05$), this parameter was markedly different in WCM ($P < 0.02$). Spermatozoa of all patients who later caused a pregnancy penetrated DCM. As the majority (84%) reached the end of the capillary after 2 hours incubation time (grade 3, >45 mm), there was no significant discrimination. Semen samples of patients who later achieved a pregnancy offered significantly higher sperm numbers and a better motility grade in human CM ($P < 0.01$), but not in bovine CM as penetration medium.

Furthermore, classical parameters of semen quality were analyzed with regard to subsequent pregnancy after 6 months (Table 5). Between fertile and infertile male patients, sperm analysis revealed no significant differences with regard to sperm volume, sperm count, percentage of propulsive motility, morphology (% normal forms), viability, fructose concentration, pH, and number of round cells, as well as total sperm count and total number of propulsive motile spermatozoa.

DISCUSSION

There is increasing concern that microscopic semen analysis alone may not be a sufficient criterion by which to judge male fertility potential. Semen

Table 4 Sperm-Cervical Mucus Penetration Test and Subsequent Pregnancy

Parameter		Pregnant	Not pregnant	Total	P
n (%)		17 (17%)	82 (83%)	99 (100%)	
Penetration distance (mm)	BCM	28 (9–55)	21 (0–55)	23 (0–55)	<0.05
	WCM	55 (0–55)	45 (0–55)	54 (0–55)	<0.02
	DCM	55 (36–55)	55 (0–55)	55 (0–55)	NS ^a
Sperm density (n/LPF) ^b	BCM	180 (20–300)	120 (0–300)	140 (0–300)	NS
	WCM	160 (0–300)	60 (0–300)	60 (0–300)	<0.03
	DCM	220 (30–300)	70 (0–300)	80 (0–300)	<0.01
Quality of motility ^c (motility grade 0 + 1) ^d	BCM	24% (71%)	39% (66%)	37% (66%)	NS (NS)
	WCM	12% (12%)	21% (37%)	19% (41%)	<0.05 (<0.01)
	DCM	12% (18%)	20% (48%)	18% (42%)	<0.05 (<0.02)

^a NS, not significant.^b LPF, low power field.^c Frequency percentage after 2 hours (after 6 hours).^d See Table 1.

analysis, although basic in infertility workup, may not directly simulate any of the events in the conception process, and does not predict subsequent fertility except in the extremes of oligozoospermia and azoospermia. There is a great deal of variation in semen characteristics among repeated specimens from the same man.^{1,4}

The cervix is a major barrier regulating sperm transport to the site of fertilization, but poor results of PCT are not necessarily indicative of an immunologic cause of infertility.^{10,11} Postcoital testing is not a consistent predictor of pregnancy.^{1,7} In vitro capillary tube tests provide more objective, quantitative, and reproducible data for evaluation of penetration ability.^{5,12} The SCMPT is convenient for regular laboratory use, and is much less expensive than, for example, laparoscopic sperm recovery, the hamster ovum penetration assay or other tests to assess the fertilizing capacity of spermatozoa. The results of the present investigation indicate that SCMPT adds an important dimension to sperm analysis in evaluating the functional properties of spermatozoa. In vitro SCMPT with WCM, obtained under standardized hormonal con-

ditions, was found to be of prognostic value for a subsequent pregnancy.

Several media have been used as a substitute for human CM for capillary tube tests, e.g., synthetic media like polyacryl, hen's egg white, and BCM.^{13,14} Bovine cervical mucus is in several properties similar to human CM: human spermatozoa penetrate BCM at nearly the same rate as human CM, BCM shows the same ferning pattern as human CM and has similar structural properties in laser-scattering spectroscopy; it can be frozen and stored without alteration of its viscoelastic properties.^{13,15} There may be difficulties in collecting human CM, e.g., dependence on the cycle function, limitation of amount of mucus per collection, or differences due to colonization with microorganisms, whereas BCM is more readily obtained.

However, microbial colonization was not found to markedly influence sperm-mucus interaction in vivo and in vitro in a large series of asymptomatic infertile couples.⁸

Successful sperm penetration varies according to the time of the menstrual cycle.^{7,16} The stimulating effect of estrogens on CM production generally is

Table 5 Parameter of Semen Analysis with Regard to Subsequent Pregnancy

Parameter ^a n (%)	Pregnant 17 (17%)	Not pregnant 82 (83%)	Total 99 (100%)	P
Sperm volume (ml)	3.3 (1.4–7.5)	3.2 (1–8.7)	3.2 (1–8.7)	NS ^b
Sperm count (millions/ml)	52 (23–76)	42 (1–91)	44 (1–91)	NS
Propulsive motility (%)	50 (25–70)	40 (10–60)	40 (10–70)	NS
Morphology (%)	60 (55–69)	60 (40–69)	60 (40–69)	NS
Viability (%)	70 (60–80)	65 (50–80)	67 (50–80)	NS
Total count (millions)	176 (75–369)	137 (2–498)	140 (2–498)	NS
Total prop. motile sperm.	88 (20–184)	62 (5–249)	64 (5–249)	NS

^a Median (range).^b NS, not significant.

accepted. Therefore, in this study, the attempt was made to standardize mucus quality by obligatory pretreatment with ethinylestradiol and by performing the test on certain fixed days of the menstrual cycle. The oral administration of estrogens provided a uniform rich and high quality cervical mucus in sufficient quantities, and allowed a thorough examination of sperm function as the only variable.

The rate of side effects was low (<4%). The dosage of ethinylestradiol was tolerated in the majority of women and had to be reduced because of sickness in three patients to 40 µg/day and in 1 to 20 µg/day.

In order to avoid false negative results of SC-MPT, testing should be repeated when the quality of CM is poor or a cervicitis is suspected.

The results of the present investigation suggest that the mucus barrier filters those spermatozoa with a higher energy and stronger fertilizing capacity. Maybe the penetrated spermatozoa represent the selected "healthier" group. Inability to penetrate the barrier may be due to morphologic abnormalities, lower energy potential, enzymatic defects, or differences in motility pattern.¹⁷ It has been reported that migration distance decreased as a result of freezing much more than did sperm motility.¹⁸ The motility grade of the penetrated spermatozoa in wives' CM, particularly after an observation time of 6 hours, was a highly discriminating factor for subsequent fertility, which might indicate that SC-MPT is a consistent predictor of sperm function.

The results of BCM penetration testing have been clearly correlated with fertility by Alexander.¹³ The correlation of BCM penetration and sperm progressive motility, morphology, and count in this study is consistent with the reports of others.¹⁹ However, additional information, in particular with regard to patients with reduced sperm count and motility, was obtained when WCM was used.

Although there were some differences in BCM, the capacity of spermatozoa to penetrate human CM only proved to be of prognostic value for a subsequent pregnancy. When several parameters of successful mucus penetration were taken into consideration, a better discrimination with clinical relevance was achieved. In this study, two groups of couples were determined prospectively with inadequate and adequate SC-MPT to analyze the value of sperm-mucus penetration tests in infertility investigation. A highly significant difference of the

pregnancy rate between both groups was found (2% versus 29%, $P < 0.001$). This information for fertility prognosis was not obtained when BCM was used as penetration medium.

A variety of factors affect sperm penetration.^{20,21} Although penetration and duration of sperm motility in mucus were found to be more closely associated with fertility than other semen variables, in vitro SC-MPTs do not differentiate between the separate functional characteristics of the spermatozoa and the mucus. When cross-matching is applied, additional information can be obtained about the male or female factor responsible for deficient sperm-mucus interaction. Crossed SC-MPT permits detection of rare specific CM/husband's sperm incompatibility or more common CM abnormalities and male subfertility. The results of a previous investigation indicate that the success of the sperm-CM interaction appears to depend more on the functional competence of the spermatozoa than on the quality of the CM itself when the hormonal influence is standardized.

When BCM or DCM is used, no information about the immunologic situation of the infertile couple in question and disorders of sperm-mucus interaction due to local antigen-antibody reaction can be obtained. The weaker correlation of percent progressive motility and penetration of WCM compared with BCM indicates that penetration of wives' cervical mucus measures another property of sperm quality not analyzed by routine sperm analysis and penetration tests with nonhuman material. For example, antibodies can adhere to spermatozoa and reduce their ability to penetrate mucus.^{22,23} Antibodies of the IgA class are responsible for the "shaking phenomenon" when spermatozoa come in contact with human CM.²⁴ The correlation of local antibody status and SC-MPT outcome will be evaluated in a current study.

Penetration tests, for example with bovine CM, may be useful as a screening test, but the use of CM of patients' wives is clearly superior to other penetration media in evaluating the specific immunologic situation of the couple in question. Results of SC-MPT might help to select patients with longstanding infertility for treatment with intrauterine homologous insemination or in vitro fertilization.

Although the lack of penetration does not exclude a subsequent pregnancy, the presented data support the view that penetration of human CM is a good indicator of sperm function. With regard to fertility prognosis, in vitro SC-MPT with hormonally standardized CM of female partners can be

recommended as an essential part of infertility investigation.

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