Repeated mid-cycle tests of in-vitro sperm penetration (Kremer tests) in healthy women during three menstrual cycles

B. Jonsson, B.-M. Landgren and P. Eneroth

Department of Obstetrics and Gynaecology, Karolinska sjukhuset, Box 60 500, S-104 01 Stockholm and 1 Unit for Applied Biochemistry, Clinical Research Centre, Huddinge sjukhus, S-141 86 Huddinge, Sweden

The outcome of Kremer testing has been evaluated daily, during the mid-cycle, in 15 healthy women over three menstrual cycles, using a permanent and a variable normal, male donor. There was a significant correlation (r = 0.539; t = 6.33; P < 0.001, n = 100; Spearman rank correlation with correction for ties) between the results obtained with the two men. The best Kremer test results were seen on the day of the LH peak and the two preceding days. No correlation was present between serum oestradiol levels and Kremer test results except on the day before the LH surge. Enough cervical mucus for Kremer testing was obtained when serum oestradiol levels were a mean of 693 pmol/l (range 214–2140). Variations in Kremer test results obtained in one woman's menstrual cycle were of the same magnitude as the difference between women. Four women had a favourable mid-cycle Kremer test result in all three cycles studied, six women had a favourable mid-cycle Kremer test result during two of their three cycles and five women only during one of the three cycles studied. The outcome of the Kremer test was not directly correlated to the quality of the cervical mucus as evaluated by the scoring system used by WHO. It is suggested that the Kremer test, performed with two male donors daily during the mid-cycle in three menstrual cycles, affords results that will allow reliable prediction of conceptional potential.

Key words: Kremer test/cervical mucus/menstrual cycles

Introduction

A sperm penetration test in cervical mucus in vitro (e.g. Kremer test) supposedly forms a useful tool in the investigation of the infertile couple (Kremer, 1965; Ulstein, 1972).

We have recently described the use of semen donors in Kremer tests performed with cervical mucus and ejaculates from infertile couples. The outcome of the test demonstrated that normal spermatozoa from healthy donor males differed in their capacity to penetrate the cervical mucus of the same woman (Jonsson et al., 1986).

Our previous study also raised the question of how much the quality of cervical mucus varies between days at mid-cycle and if the results obtained during one menstrual cycle are representative of other cycles in the same woman.

In order to investigate these methodological problems involved in the performance and interpretation of Kremer tests, the present study was undertaken.

Materials and methods

Clinical material

Fifteen healthy women volunteered for the study. Their mean age was 31.7 years (range 21–40 years). All had a history of normal regular menstrual cycles of 24–35 days. They had all been pregnant 1–4 times and had delivered 1–3 children. The couples used condoms for contraception during the study. They had not used steroid contraceptives or an IUD for at least 3 months preceding the study.

Eleven men volunteered as sperm donors. They were all healthy with proven fertility. Their mean age was 38 years with a range of 30–42 years. Before the ejaculates were used for Kremer testing in the study, a routine morphological sperm analysis (Belsey, 1980) was performed at each test occasion. The sperm concentration was at least 50 X 10⁶/ml and at least 70% of the sperm had good progressive motility.

Plan of study

The women attended the clinic daily from cycle day 8 until the estimated time of ovulation. Cervical mucus was collected into baby-feeding tubes (containing ~0.2 ml). The volume, Spinnbarkeit, Fernstest, viscosity and the number of cells were measured using the screening system recommended by WHO (Belsey, 1980).

The cervical mucus was then sucked into microcapillaries, sealed by clay and immediately frozen at −20°C until used for Kremer testing. Blood samples were withdrawn from an antecubital vein at the time of the cervical mucus sampling. The serum was stored at −20°C until analysed.

Laboratory methods

Commercial radioimmunoassay kits purchased from Radioimmunoassay Systems Laboratory (Los Angeles, CA, USA) were used for oestradiol and progesterone measurements. LH and FSH were assayed using kits from Diagnostic Products Inc., Los Angeles, CA). The Kremer tests (Kremer, 1965, 1968) were performed with thawed cervical mucus samples (stored at −20°C) and fresh spermatozoa within 4 h after ejaculation, using a modified method (Jonsson et al., 1986).

Comparisons between Kremer test results obtained with frozen mucus (−20°C) and mucus kept at +2 to +4°C for 1–5 days were performed.
Results

Cycle length
The cycle length of the 45 cycles studied in 15 women varied from 21 to 35 days with a mean of 28 days. Figure 1 shows the mean and range of the three cycles studied for each individual woman.

Hormone levels
The time relationship of the oestradiol peak to the LH peak is shown in Table I. The oestradiol surge occurred 2 days prior to the LH peak in three cycles, in 16 cycles on the day before the LH peak and in 16 cycles on the day of the LH peak. In 10 cycles the LH and oestradiol surges could not be identified. All cycles were ovulatory as characterized by Landgren et al. (1980).

Cervical mucus
The number of days when sufficient cervical mucus could be withdrawn for Kremer tests varied between the three cycles in each woman. The effect of storage on the quality of cervical mucus was studied in five women. Eight capillaries were obtained from each woman on each occasion. Four of these were stored for 5 days at +2 to +3°C. The others were stored at −20°C for 5 days. All capillaries were tested against one fresh semen sample. The mean score was seven for both the frozen and the refrigerated mucus samples, thus confirming a previous report (Ulstein, 1972). Freeze-stored mucus was subsequently used.

Each daily cervical mucus sample was tested against two donor males. One of these was used against all women (Dp), whereas the identity of the other donor male varied (Dv). The agreement in Kremer test results between the two donors was significant when all cycle days were calculated (r = 0.539; t = 6.33; P < 0.001; n = 100; Spearman rank correlation with correction for ties). But the results differed markedly on a few test occasions. Thus, when the outcome of the Kremer test during the 45 cycles was evaluated, Dp was found to yield a favourable (7—9 test points) Kremer test result and Dv a bad result (0—3 test points) in 12 cycles, whereas in three cycles a favourable score was obtained with ejaculates from Dv and a bad one with Dp. Since there might be a compatibility factor involved in the outcome of the Kremer test, we decided to select the best result obtained when evaluating the functional status of an individual cervical mucus sample.

The outcome of the sperm penetration test, related to the day of the LH peak, is presented in Table II. When the mean score for each of the three menstrual cycles for the entire group of women was used and the time interval LH−5 to LH−3 was compared with the time interval LH−2 to LH−0, a significant
There was no difference in the outcome of the Kremer test (\(P = 0.006, n = 15\), one-way sign test). There was no difference in the outcome of the Kremer test between cycle days LH—2, LH—1 and the day of the LH peak (Table III; Freedman's rank test). The mean oestradiol level on the first day allowing withdrawal of cervical mucus for Kremer testing was 693 pmol/l (range 214–2140).

A correlation between the results of the Kremer test and the serum oestradiol levels was found only on day LH—1 (\(P < 0.001, n = 33\); Spearman rank correlation with correction for ties).

The relationship between the clinical evaluation of the quality of the mucus and the Kremer test results is shown in Figure 2. The clinical evaluation gave a favourable score in each of the three cycles during at least one day at mid-cycle (LH—0 to LH—2) in 13 of the 15 women (87%). In the remaining two women, this favourable outcome was seen in two of the three cycles studied. This was not in agreement with the Kremer test results. Five of 15 women showed a favourable mid-cycle Kremer score (7–9 points) during only one of their three cycles. Six women had a favourable mid-cycle Kremer test result during two of their three cycles and four women during all three cycles.

Favourable results of the Kremer test (7–9 points) during at least one mid-cycle day (LH—2 to LH—0) were observed in 60, 66 and 71% of the women during each of the three respective menstrual cycles. The average number of days with a favourable Kremer test (7–9 points) was 1.1 days during the first cycle and 1.2 and 1.6 days during the second and third cycles respectively. The longest period with favourable results was 7 days in one cycle (Figure 3) and in the other two cycles this woman had an excellent outcome of the Kremer test over 5 and 6 days respectively. A poor result (<3 test points) during at least one mid-cycle day was seen in 50, 73 and 90% of all women in their three respective cycles. The average number of days during the LH—2 to LH—0 interval with an unfavourable test result (<3 points) was 1 day.

Discussion

In the present study, the influence of the male factor was explored by comparing the results of one healthy male with those obtained with other healthy males. The outcome demonstrates that the results of an individual test can be affected by the individual male, as we have previously demonstrated (Jonsson et al., 1986). This is why we decided to select the best results with an individual cervical secretion sample and two healthy semen donors.

The relationship between the serum oestradiol and LH peaks indicates that the estimation of the mid-cycle day was correct in most cycles. The uncertainty can be estimated to be ~1 day and might have influenced our findings. Thus, similar Kremer test results were obtained on the day of the LH peak and days LH—1 and LH—2. The results are similar to those of Kerin et al. (1976), who obtained optimal sperm migration on the day preceding the LH peak and the day of the LH peak in 13 women.

Our results, as well as those of Kerin et al. (1976), are in agreement with data on the probability of conception during various days. The probability was reported to be highest on the 2 days preceding ovulation followed by the day of ovulation and 3 days before ovulation (Dixon et al., 1980).

Carlberg et al. (1969), in a study of six women, reported that sperm penetration during the mid-cycle increased gradually in the mucus from three women and sharply in three other women. These latter authors could relate the daily changes in sperm penetration to the decrease in acidic acid content of the mucus and to increasing amounts of free oestradiol in urine. In the present investigation, a serum oestradiol peak was not found necessary for a favourable sperm penetration of cervical mucus in individual subjects. Except for day LH—1, there was no direct correlation between the oestradiol levels and the outcome of the Kremer test. However, the correlation on this particular day was pronounced and may also be biologically significant (see Dixon et al., 1980).

In the present study, the formation of cervical mucus (enabling Kremer testing) corresponded to an average oestradiol level in serum of 693 pmol/l (range 214–2140), but it is difficult to define a threshold level needed to support the formation of cervical mucus. Although there was no remarkable individual or group differences in serum oestradiol levels between the three cycles, marked changes in sperm penetration could be noticed. This indicates that the functional properties of cervical mucus may be regulated by other hormonal influences.

A significant correlation between conception rates and the results of in-vitro sperm penetration has been reported (Ulstein, 1973). If Kremer tests are used as a predictor for conception,
it is necessary to know the fluctuations in the test outcome between cycles in normal fertile couples. In the present study, the variations in Kremer test results between menstrual cycles within each woman appeared to be of the same magnitude as between the women. Thus, even among a group of previously fertile, healthy women, the results of the Kremer test vary greatly during one menstrual cycle and between cycles. This makes it difficult to predict fertility in women suffering from infertility if only a few Kremer tests are carried out. Our data indicate that three mid-cycle tests should be carried out during three menstrual cycles. A normal finding is then at least one favourable Kremer test.

Our results seem to indicate that four women (27%) would have been able to conceive during each cycle and six women in two out of three cycles. Four of these six women might have conceived during a cycle selected at random. Of the five women with only one favourable cycle result, two might have become pregnant during a cycle selected at random.

This calculation indicates that 10 of the 15 women (66%) might have been able to conceive during one menstrual cycle. According to Tietze et al. (1950), 30% of couples conceive within 1 month and close to 60% within 6 months. Since we used the best test outcome of the results obtained with two male donors and were also able to perform daily testing, which may not reflect the frequency of coitus by the couple who wish to conceive, this is perhaps why the conception rate has been overestimated. It is, therefore, not surprising that our estimate of conceptional rates is higher than that of Tietze et al. (1950). Tietze et al.’s results, as well as our Kremer test data, nevertheless indicate that there are limitations in a healthy couple’s possibility of accomplishing pregnancy.

The data from the WHO scoring seem to indicate that the female potential for conception is much higher (14.5 out of 15 or 97%) than indicated by the Kremer test. However, the former procedure does not take the male factor into account, which probably explains the calculated difference between the two tests.

References


Received on November 9, 1988; accepted on April 27, 1989