

Human sperm chemotaxis: both the oocyte and its surrounding cumulus cells secrete sperm chemoattractants

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BACKGROUND: Human sperm chemotaxis to pre-ovulatory follicular fluid is well established *in vitro*. However, it is not known whether the female's oocyte–cumulus complex secretes sperm chemoattractants subsequent to ovulation (for enabling sperm chemotaxis within the Fallopian tube) and, if so, which of these cell types—the oocyte or the cumulus oophorus—is the physiological origin of the secreted chemoattractant. **METHODS:** By employing a directionality-based chemotaxis assay, we examined whether media conditioned with either individual, mature (metaphase II) human oocytes or the surrounding cumulus cells attract human sperm by chemotaxis. **RESULTS:** We observed sperm chemotaxis to each of these media, suggesting that both the oocyte and the cumulus cells secrete sperm chemoattractants. **CONCLUSIONS:** These observations suggest that sperm chemoattractants are secreted not only prior to ovulation within the follicle, as earlier studies have demonstrated, but also after oocyte maturation outside the follicle, and that there are two chemoattractant origins: the mature oocyte and the surrounding cumulus cells.

Key words: chemotaxis/cumulus cells/oocyte-conditioned medium/ovum/sperm chemoattractant

Introduction

The occurrence of mammalian sperm chemotaxis to follicular fluid has been demonstrated *in vitro* primarily in humans (Ralt *et al.*, 1994), mice (Oliveira *et al.*, 1999) and rabbits (Fabro *et al.*, 2002) (for reviews, see Eisenbach, 1999, 2004). In all these species, only a small fraction of the sperm population—the fraction of capacitated cells—is chemotactically responsive (Cohen-Dayag *et al.*, 1994, 1995; Oliveira *et al.*, 1999; Fabro *et al.*, 2002) (for a review on capacitated sperm, see Jaiswal and Eisenbach, 2002). Recently, Sun *et al.* (2003) demonstrated that human and rabbit sperm chemotactically respond, similarly well, to human, rabbit and bovine follicular factors. This indicated lack of chemotaxis-related species specificity between these mammalian species, suggesting that at least some of the mammalian sperm chemoattractants, which originate in the female genital tract, are common or very similar (Sun *et al.*, 2003). Their identities are not known.

Although the ability of human follicular fluids to attract sperm by chemotaxis is highly correlated with oocyte fertilizability (Ralt *et al.*, 1991), it is unlikely that follicular fluid *per se* is involved in sperm chemotaxis *in vivo* for two main reasons. First, at ovulation, only a very small fraction (~1% or less in pigs) of follicular fluid is transported into the

oviduct along with the oocyte–cumulus complex (OCC) (Hansen *et al.*, 1991; Brussow *et al.*, 1998; Hunter *et al.*, 1999). Second, if sperm chemotaxis is essential for fertilization, the chemoattractant gradient is expected to be maintained for as long as the oocyte survives and can be fertilized [in humans, ~24 h post-ovulation (Harper, 1982)]. This requires a continuous supply of chemoattractant, not a single event, as is the limited supply of follicular fluid at ovulation. Taken together, it is, therefore, more reasonable that the OCC secretes a sperm chemoattractant(s) subsequent to ovulation, when it resides at the fertilization site within the oviduct. However, it is not known whether the female's OCC secretes sperm chemoattractants subsequent to ovulation and, if so, which of these cell types—the oocyte or the cumulus oophorus—is the physiological origin of the secreted chemoattractant. Here we addressed these questions *in vitro*, using human gametes and cumulus cells.

Materials and methods

The protocol of this study has been approved by the Barzilai Medical Center Ethic Committee on the use of Human Subjects in Medical Research in accordance with the Helsinki Declaration and by the Israeli Ministry of Health.

Chemicals and solutions

All chemicals were obtained from Sigma Chemical Company (USA), unless a different company is specified. The medium used was Biggers–Whitten–Whittingham (BWW) medium: 95 mmol/l NaCl, 4.8 mmol/l KCl, 1.3 mmol/l CaCl₂, 1.2 mmol/l MgSO₄, 1.2 mmol/l KH₂PO₄, 25 mmol/l NaHCO₃, 20 mmol/l sodium lactate, 5 mmol/l glucose, and 0.25 mmol/l sodium pyruvate, pH 7.4 (Biggers *et al.*, 1971), supplemented with HEPES (50 mmol/l, pH 7.4) and bovine serum albumin (fraction V; 3 mg/ml). Standard P-1 medium (Irvine Scientific, USA) was supplemented with 10% Serum Substitute Supplement (SSS; Irvine Scientific).

Sperm

Human semen samples were obtained from two healthy donors after 3 days of sexual abstinence. Informed consent was obtained from each donor. Semen samples with normal sperm density, motility and morphology [according to WHO guidelines (World Health Organization, 1993)] were allowed to liquefy for 30–60 min at room temperature. Human sperm were separated from the seminal plasma by the migration–sedimentation technique (Hauser *et al.*, 1992), which avoids the centrifugation stress. Briefly, the inner conical tube of the separation device and the bottom part of the larger surrounding tube were filled with 900 µl of BWW medium. Semen (200 µl) was gently added to the bottom of the larger tube. The device was incubated under an atmosphere of 5% CO₂ at 37°C for 20 min, after which the motile sperm were collected from the inner tube. Following this procedure, the sperm concentration was adjusted to 5 × 10⁶ cells/ml. Every sperm sample was analysed for the percentage of motile cells using a Makler counting chamber (Sefi Medical Instruments Ltd, Israel) and a computerized sperm analysis software program (Hobson Tracking System Ltd, UK). The sperm suspensions were incubated under an atmosphere of 5% CO₂ at 37°C for 2 h to obtain capacitated sperm (Cohen-Dayag *et al.*, 1995).

Oocytes

Oocytes were retrieved from three randomly selected women undergoing transvaginal oocyte aspiration for IVF. The women were treated with GnRH analogue and gonadotrophin for ovarian stimulation. Oocyte retrieval was performed 34 h after hCG injection. The oocytes of each of the first two women were incubated together for the preparation of conditioned media. Those of the third woman were individually incubated. Although the results obtained with the media of the three women were similar, we included in this study only the separated, well-defined oocytes retrieved from the third woman. A total of 16 oocytes were retrieved from her, of which six were successfully fertilized. The media used in this study were conditioned with these six oocytes (oocyte, cumulus, or both).

Oocyte–cumulus-conditioned media

All OCC (oocytes surrounded by cumulus cells), retrieved as described above, were sequentially washed twice in P-1 medium (supplemented with 10% SSS) at a ratio of 1:100 (vol:vol), thus diluting 10⁴-fold any potential chemical contaminant (e.g. follicular fluid) from the original solution. Subsequent to this wash, individual OCC were incubated each in 100 µl P-1 medium for 2 h at 37°C under an atmosphere of 5% CO₂. The individual OCC were then removed and the remaining media were separately aspirated, centrifuged for 5 min at 800 g to remove cumulus cells and debris, and then frozen and kept at –20°C. Prior to being used, these conditioned media were thawed and centrifuged for 10 min at 3000 g to remove debris.

Cumulus-conditioned media

Cumulus-conditioned media were obtained by two procedures. Cumulus-conditioned medium #1 was prepared by cutting individual cumulus cells from the OCC, employing two 23-Gauge syringe needles. Subsequent to oocyte removal, the sheared cumulus cells were incubated at 37°C in 100 µl fresh P-1 medium (containing 10% SSS) for 2 h, followed by 5 min centrifugation at 800 g to remove the cumulus cells. Each individual supernatant was immediately frozen at –20°C. Cumulus-conditioned medium #2 was prepared by adding 100 µl P-1 medium to the pellet of cumulus cells resulting from the 800 g centrifugation step (without further incubation) and then frozen. Prior to being used, each of the conditioned media was thawed and centrifuged for 10 min at 3000 g to remove cells and debris. Due to being frozen and thawed, the cumulus cells in the second preparation were probably lysed. Therefore, cumulus-conditioned medium #2 probably contained mainly a lysate of cumulus cells.

Oocyte-conditioned media

Oocyte-conditioned media were obtained from mature metaphase II stage oocytes (existence of the first polar body) by two procedures. In one procedure, the stripped oocyte, which had been mechanically separated from the cumulus cells in the procedure described above for cumulus-conditioned medium #1, was treated with hyaluronidase (40 IU; for <30 s) in order to remove any remaining cumulus cells. The resulting fully stripped oocyte was washed five times in P-1 medium (containing 10% SSS) and then transferred into 100 µl fresh P-1 medium (containing 10% SSS) for 2 h incubation at 37°C. Following this treatment the oocyte was removed for ICSI and the remaining medium was defined as oocyte-conditioned medium #1. In the other procedure, the oocyte, following ICSI, was incubated overnight at 37°C in 100 µl P-1 medium (containing 10% SSS). This medium, following oocyte removal, was defined as oocyte-conditioned medium #2. Both oocyte-conditioned media were frozen at –20°C. Prior to the experiment, the media were thawed and centrifuged for 10 min at 3000 g to remove debris.

Chemotaxis assay

Chemotaxis assays were performed, as described earlier (Fabro *et al.*, 2002), at room temperature in a Zigmond chemotaxis chamber consisting of two parallel, rectangular wells separated by a wall and closed with a coverslip (Zigmond, 1977). The main advantage of this chamber is that the chemoattractant gradient in it is linear and, consequently, the swimming of the cells relative to the direction of the gradient can be easily analysed (Fabro *et al.*, 2002). Unless indicated otherwise, one well contained sperm in BWW and the other well contained either a conditioned medium diluted in BWW or, as a control, BWW only. Following sealing of the chamber, the cells in it were allowed to equilibrate for 10 min, and then the movement of sperm on top of the partition wall, in the middle of the field between the two wells, was video-recorded for 5 min. The tracks made by the sperm were subsequently analysed relative to the direction of the chemical gradient (defined as the X-axis) by our motion analysis system. As described in detail elsewhere (Fabro *et al.*, 2002), we analysed three directionality-based parameters. One parameter was the mean net distance travelled along the chemoattractant gradient (i.e. the distance made along the X-axis, $\bar{\Delta X}$). Another parameter was the percentage of cells whose net distance of swimming is towards the chemoattractant well (cells making trajectories with positive projection on the X-axis, i.e. with projection in the direction of the gradient; tracks with $\Delta X > 0$). The third parameter was the percentage of cells travelling a longer

distance in the direction of the chemoattractant gradient (X -axis) than in a gradient-less direction, perpendicular to the former (Y -axis; i.e. cells making trajectories whose projection on the X -axis is positive and longer than the projection on the Y -axis; $\Delta X/|\Delta Y| > 1$, $|\Delta Y|$ being the absolute value of the distance in the Y direction). In the case of random movement, the expected values are $\sim 0 \mu\text{m}$ for $\overline{\Delta X}$, $\sim 50\%$ for the percentage of cells with $\Delta X > 0$, and $\sim 25\%$ for the percentage of cells with $\Delta X/|\Delta Y| > 1$ (Fabro *et al.*, 2002). In practice, these values (in control experiments with no chemoattractant) may be somewhat higher (e.g. $\sim 30\%$ instead of 25% for the percentage of cells with $\Delta X/|\Delta Y| > 1$) probably due to the fact that sperm were added to only one of the wells of the Zigmond chamber, resulting in asymmetric distribution (see Discussion).

Analysis of the sperm kinetic parameters

The analysis was carried out by the motion analysis system (each time for 250 cells) during the last 5 min of each recording. The measured kinetic parameters were: VCL, curvilinear velocity (the time-average velocity of the sperm head along its actual trajectory); VSL, straight line velocity (also termed progressive velocity; the time-average velocity of the sperm head along a straight line from its first position to its last position); LIN, percentage linearity (the ratio $\text{VSL}/\text{VCL} \times 100$); and STR, percentage straightness (the ratio between the straight line from the first point on the smoothed path to the last point on this path and the total distance along the smoothed path, multiplied by 100) (Davis and Siemers, 1995; Mortimer, 1997).

Statistical analysis

All statistical analyses used the InStat 3 software package (Graph Pad Software, USA). Each experiment included a control (BWW medium) and a number of different dilutions of the test conditioned media. In total, we repeated each dilution and control for four to eight times (5144–10794 tracks). The mean values of $\overline{\Delta X}$ and the mean values of the percentages of cells with $\Delta X > 0$ or $\Delta X/|\Delta Y| > 1$ [each percentage was transformed using arcsin square root transformation (Winer *et al.*, 1991, p. 356)] of the different dilutions were compared with the mean values of the respective control, using one-way ANOVA followed, when appropriate, by Dunnett treatment versus control procedure (Winer *et al.*, 1991, p. 169).

Results

Sperm chemotaxis to oocyte-cumulus-conditioned medium

To determine whether the female's OCC secretes sperm chemoattractants, we examined the ability of oocyte-cumulus-conditioned media to attract human sperm by chemotaxis. We employed the directionality-based assay used in recent studies (Oliveira *et al.*, 1999; Fabro *et al.*, 2002; Bahat *et al.*, 2003; Sun *et al.*, 2003) and described in Materials and methods. As elaborated in detail elsewhere (Fabro *et al.*, 2002), this assay is independent of the sperm's speed and pattern of movement, and it therefore measures chemotaxis only. Each of the three measured directionality-based parameters yielded a significantly higher response than the BWW control at dilutions of $1:10^3$ and $1:10^4$ (Figure 1). In accordance with the expected bell-shaped dependence of a chemotactic response on the chemoattractant concentration (Adler, 1973; Ralt *et al.*, 1994), no response was observed at higher and lower dilutions of the conditioned media, i.e. the values

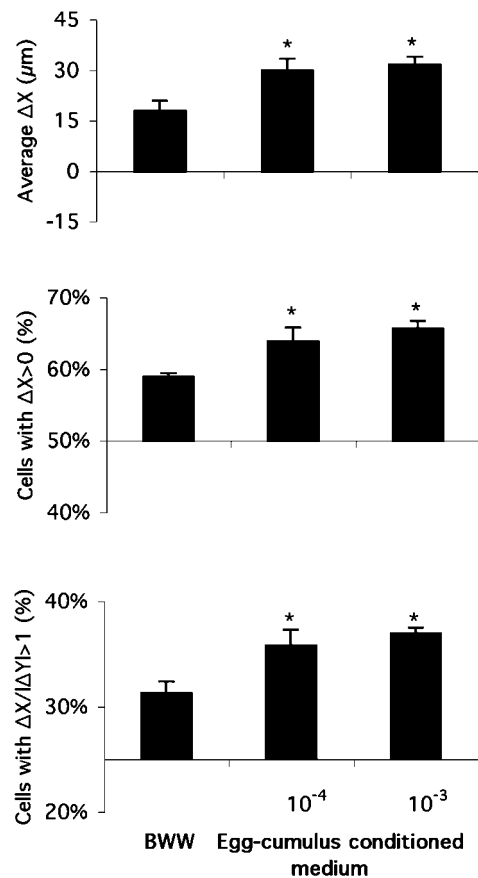


Figure 1. Human sperm chemotaxis to a medium conditioned with the oocyte-cumulus complex and diluted with Biggers-Whitten-Whittingham (BWW) medium. The parameters indicated on the Y -axis are defined in Materials and methods. The results, shown with respect to the expected values in the case of random movement, are averages + SEM of four experiments, with two or three microscope fields in each experiment. An asterisk above the columns indicates a statistically significant difference between the column and the BWW column ($P < 0.05$), determined as described in Materials and methods. The total numbers of sperm analysed for chemotaxis at each dilution of the oocyte-cumulus-conditioned medium and at the BWW control varied between 5144 and 5639.

of the three parameters were not significantly different from the values of the negative control (BWW control; data not shown). As in previous studies of human sperm chemotaxis (Cohen-Dayag *et al.*, 1994; Ralt *et al.*, 1994; Cohen-Dayag *et al.*, 1995; Eisenbach and Tur-Kaspa, 1999; Jaiswal *et al.*, 1999), the fraction of chemotactically responsive sperm was relatively small ($\sim 8\%$). The fraction of responsive cells was similarly small in a positive-control experiment with bourgeonal (Spehr *et al.*, 2003) as the chemoattractant, the peak response being at $1-10 \text{ nmol/l}$ (data not shown). The motility of the sperm seen on the bridge between the wells was not significantly affected by the oocyte-cumulus-conditioned medium, as judged by the sperm kinetic parameters (Table I). These results suggest that the OCC secretes sperm chemoattractants. Since the oocyte-cumulus-conditioned medium may contain secretions from the oocyte, the cumulus cells, or both of them, we also examined conditioned media of each of them alone.

Table I. Kinetic parameters of sperm in gradients of conditioned media in a Zigmond chamber

| Tested medium | Dilution | VCL ($\mu\text{m/s}$) | VSL ($\mu\text{m/s}$) | STR (%) | LIN (%) |
|-----------------------------------|------------------|----------------------------|----------------------------|------------|------------|
| Oocyte-cumulus-conditioned medium | Control | 87 \pm 2 | 29 \pm 2 | 73 \pm 3 | 34 \pm 2 |
| | 10 ⁻³ | 86 \pm 2 | 30 \pm 1 | 77 \pm 3 | 36 \pm 1 |
| | 10 ⁻⁴ | 90 \pm 2 | 29 \pm 2 | 75 \pm 2 | 34 \pm 2 |
| Cumulus-conditioned medium #1 | Control | 90 \pm 1 | 31 \pm 1 | 73 \pm 1 | 36 \pm 1 |
| | 10 ⁻³ | 85 \pm 2 | 32 \pm 1 | 80 \pm 1 | 36 \pm 2 |
| | 10 ⁻⁴ | 87 \pm 2 | 31 \pm 1 | 77 \pm 2 | 37 \pm 2 |
| Cumulus-conditioned medium #2 | Control | 88 \pm 3 | 32 \pm 1 | 76 \pm 2 | 37 \pm 1 |
| | 10 ⁻³ | 88 \pm 2 | 32 \pm 1 | 78 \pm 2 | 35 \pm 2 |
| | 10 ⁻⁴ | 87 \pm 3 | 32 \pm 1 | 80 \pm 1 | 38 \pm 1 |
| Oocyte-conditioned medium #1 | Control | 90 \pm 2 | 30 \pm 2 | 73 \pm 2 | 34 \pm 1 |
| | 10 ⁻³ | 88 \pm 3 | 31 \pm 1 | 80 \pm 2 | 37 \pm 2 |
| | 10 ⁻⁴ | 85 \pm 3 | 32 \pm 1 | 82 \pm 1 | 38 \pm 2 |
| Oocyte-conditioned medium #2 | Control | 88 \pm 2 | 32 \pm 1 | 75 \pm 1 | 37 \pm 0 |
| | 10 ⁻³ | 87 \pm 2 | 31 \pm 1 | 79 \pm 2 | 37 \pm 1 |
| | 10 ⁻⁴ | 87 \pm 2 | 31 \pm 1 | 79 \pm 2 | 37 \pm 1 |

The values are the mean \pm SEM of three to eight experiments. VCL = curvilinear velocity; VSL = straight line velocity; STR = percentage straightness; LIN = percentage linearity (see Materials and methods for full definitions).

Sperm chemotaxis to cumulus-conditioned medium

Each of the cumulus-conditioned media chemoattracted sperm (Figure 2), suggesting that the cumulus cells secrete a chemoattractant(s). In a control experiment for the chemotactic responsiveness of the P-1 medium, present in all conditioned media, we found that it was chemotactically inert in the dilutions that we used (data not shown). The motility of the sperm was not affected by the cumulus-conditioned medium (Table I).

Sperm chemotaxis to oocyte-conditioned medium

Each of the types of oocyte-conditioned media that we used yielded at 1:10³ and 1:10⁴ dilutions a significantly higher response in the chemotaxis assay than the BWW control (Figure 3), indicating that a mature oocyte secretes sperm chemoattractants even when it is not surrounded by the cumulus cells. The observation that both oocyte-conditioned media yielded similar results even though medium #1 had been incubated with the oocyte for 2 h and medium #2 (following ICSI) had been incubated overnight, suggests that the chemoattractant secretion from the oocyte declines with the oocyte's age or after sperm entry. Here, too, the motility of the sperm was not affected by the oocyte-conditioned medium (Table I).

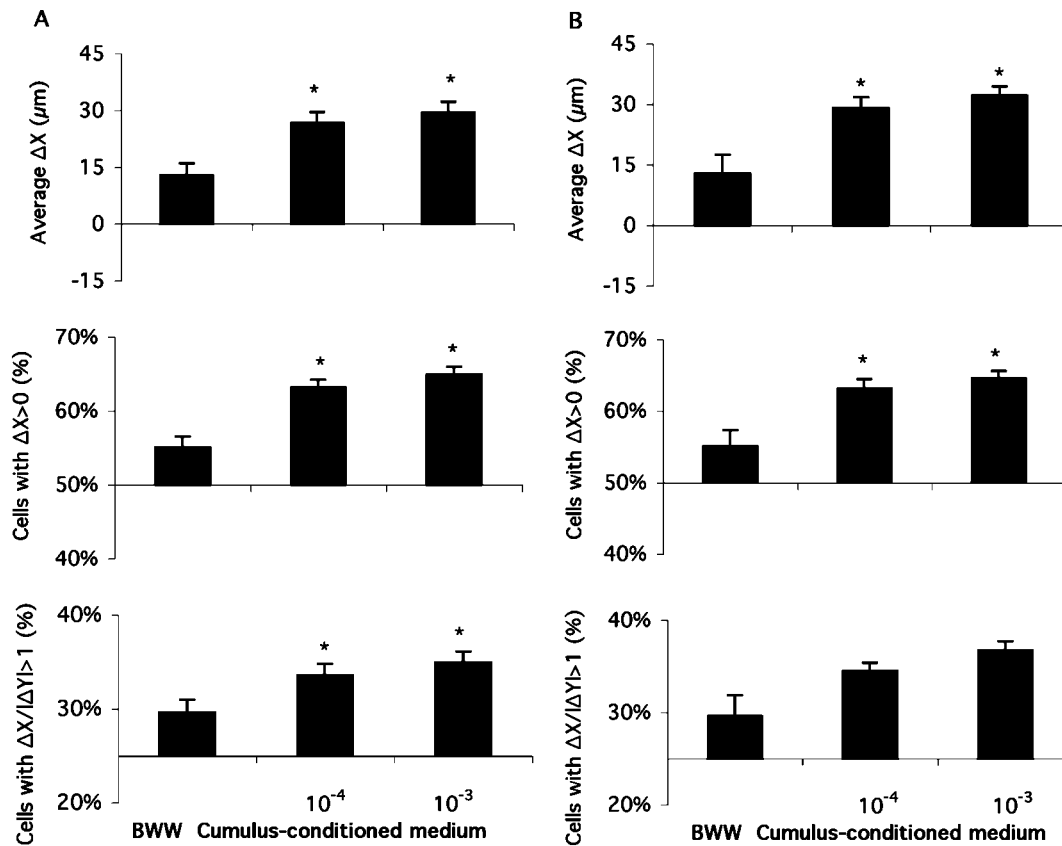


Figure 2. Human sperm chemotaxis to cumulus-conditioned media. (A, B) Cumulus-conditioned media #1, #2 (defined in Materials and methods) respectively. The experiment and analysis were carried out as in Figure 1. The results, shown with respect to the expected values in the case of random movement, are averages \pm SEM of five to eight experiments, with two or three microscope fields in each experiment. An asterisk above the columns indicates a statistically significant difference between the column and the BWW column ($P < 0.05$). (The $P = 0.06$ for the columns without asterisk.) The total numbers of sperm analysed for chemotaxis at each dilution of the cumulus-conditioned medium and at the BWW control were 5991–11 804.

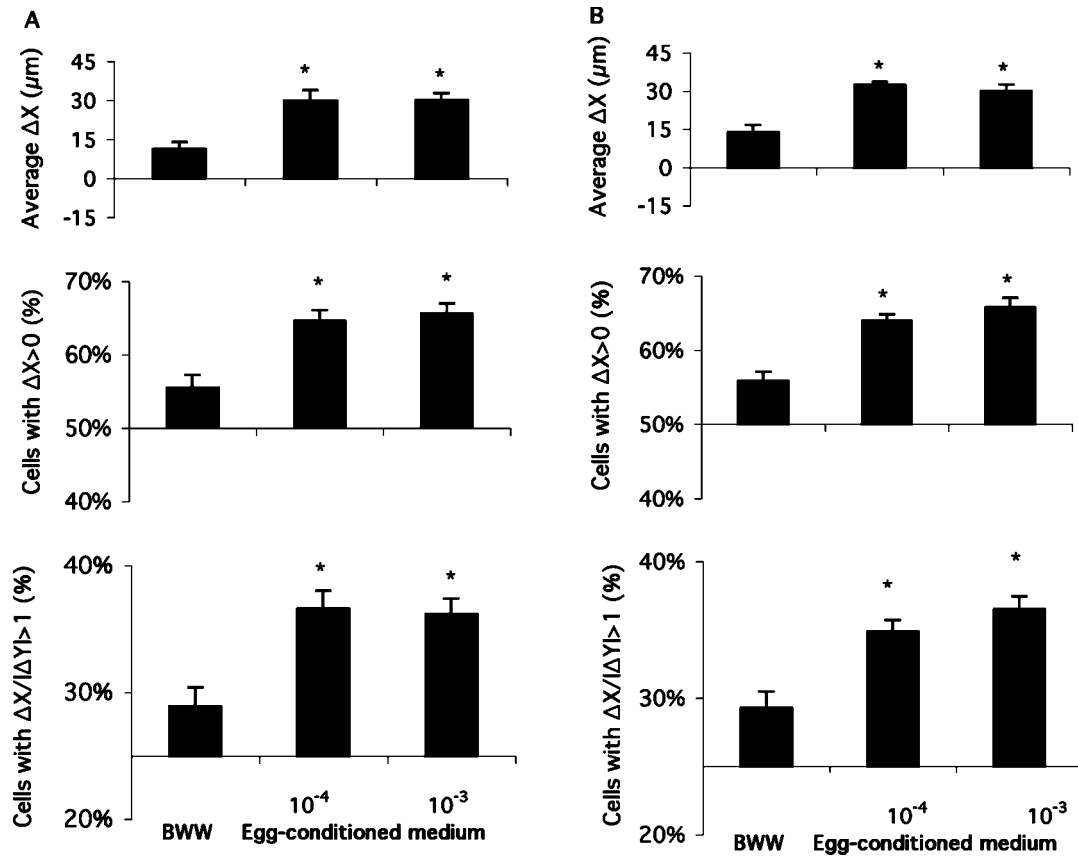


Figure 3. Human sperm chemotaxis to oocyte-conditioned media. (A, B) Oocyte-conditioned media #1, #2 respectively. The experiment and analysis were carried out as in Figure 1. The results, shown with respect to the expected values in the case of random movement, are averages + SEM of five to eight experiments, with two or three microscope fields in each experiment. An asterisk above the columns indicates a statistically significant difference between the column and the BWW column ($P < 0.01$). The total numbers of sperm analysed for chemotaxis at each dilution of the oocyte-conditioned medium and at the BWW control were 6478–10 794.

Discussion

In this study we provided evidence that sperm chemoattractants are secreted not only prior to ovulation within the follicle, as has been known for the last decade (for a review, see (Eisenbach, 1999), but also after oocyte maturation outside the follicle. We further found that the chemoattractant(s) are apparently secreted from two different sources: the mature oocyte and the surrounding cumulus cells. The significance of these findings is discussed below.

Validity of the conclusion that the cumulus cells secrete a chemoattractant

In this study we employed conditioned media from three different origins. Our conclusions based on the results obtained with the oocyte–cumulus-conditioned medium and with the two types of preparations of oocyte-conditioned medium seem straightforward. Conversely, in the case of the cumulus-conditioned media, the conclusion is not straightforward because these media may have contained remnants of oocyte secretions. To reduce this possibility we studied cumulus-conditioned media prepared by two different procedures. Cumulus-conditioned medium #1, from which the oocyte was removed after stripping, could contain some oocyte secretions. However, this possibility appears remote

because, prior to the conditioning of the medium with the cumulus cells, the incubation with the oocyte was only a few minutes (the time needed for oocyte stripping) and the cumulus cells were washed from potential oocyte secretions. Cumulus-conditioned medium #2 essentially contained lysates of cumulus cells made subsequent to the removal of the cells from the oocyte and centrifugation. Although this medium may also have contained remnants of oocyte secretions not fully removed by the centrifugation, it is unlikely that these remnants were responsible for the measured sperm chemotaxis. Had this been the case, we should have observed the chemotactic activity at lower dilutions. Taken together, the results suggest that the cumulus cells also secrete sperm chemoattractants. This suggestion is consistent with the observation that cumulus cells secrete a substance that alters the pattern of sperm movement (Bronson and Hamada, 1977) and with the finding that cumulus secretions improve the fertilizing ability of sperm penetrating into the oocyte (Tanghe *et al.*, 2003).

Validity of the chemotaxis assays

The best criterion for distinguishing between chemotaxis and other processes that may cause sperm accumulation is the criterion of directionality, i.e. the directional change of

movement of sperm towards the source of the chemoattractant—a unique feature of chemotaxis (for reviews, see Eisenbach, 1999, 2004; Eisenbach and Tur-Kaspa, 1999). As thoroughly analysed by Fabro *et al.* (2002), the assay used in the current study measures solely movement in the direction of the gradient and, as such, it is a measure of chemotaxis only. However, several issues related to the results obtained in this study with this assay require clarification. First, the measured values of the directionality parameters in the negative controls were somewhat higher than the values expected for random movement. This phenomenon, observed earlier with human sperm (e.g. Sun *et al.*, 2003), is due to the fact that sperm are added to only one of the wells of the Zigmond chamber. Although the Zigmond chamber is equilibrated before the initiation of the assay, the distribution of sperm remains asymmetric: high sperm density in the well into which the sperm were introduced, and low sperm density in the other well. However, because the measured values are always compared to the negative control rather than to the theoretical random values, the asymmetry and the resulting higher-than-random values do not interfere with the analysis of chemotaxis and its reliability. Therefore, whenever a significant difference from the control is observed, it is only due to chemotaxis and not to asymmetric sperm distribution or any other reason. Second, the fraction of responsive cells was relatively small. This is an intrinsic property of mammalian sperm chemotaxis and is due to the facts that only capacitated sperm are chemotactically responsive and that the fraction of capacitated sperm in humans is low (Cohen-Dayag *et al.*, 1994, 1995; Oliveira *et al.*, 1999; Fabro *et al.*, 2002). Third, the chemotactic response was observed over a relatively narrow dilution range of the conditioned media (at $\times 1000$ and $\times 10000$ dilutions only). This limited concentration range strengthens the confidence that the results reflect true chemotaxis, because one of the characteristics of any chemotactic response in any chemotaxis system is a small concentration range: no response below a threshold concentration, and no response at saturating and above-saturating concentrations. The reason for this is that fully occupied receptors cannot sense a further increasing concentration gradient. In earlier studies that investigated the dependence of the chemotactic response on the concentration of follicular fluid, a similarly narrow concentration range was observed (e.g. Ralt *et al.*, 1994). The same holds for the well-investigated system of bacterial chemotaxis: the response of *Escherichia coli* to the potent chemoattractant galactose in the capillary assay has a sharp peak; the responses at concentrations 10-fold lower or 10-fold higher are much reduced (Adler, 1969).

The significance of lack of effect on the sperm motility

A true chemotactic response of human sperm is expected to be expressed by swimming of the capacitated sperm, which constitute a small fraction of the sperm population (Cohen-Dayag *et al.*, 1995), in the direction of the chemoattractant gradient. Such a response is not expected to affect the average values of the kinetic parameters. The lack of effect of the conditioned media on these parameters (Table I) endorses

the conclusion that the measured effect (Figures 1–3) reflects true chemotaxis and not some other effect on the sperm motility. Earlier studies, which employed non-diluted cumulus cells, had conflicting observations on the effect of these cells on the sperm motility: Tesarik *et al.* (1990) found that cumulus cells increase the linearity and decrease the speed of human sperm, whereas Fetterolf *et al.* (1994) reported that cumulus cells slightly reduce the linearity and slightly increase the sperm velocity.

The physiological significance of chemoattractant secretion by the OCC

Recently, Spehr *et al.* (2003) identified, cloned, and functionally expressed a previously undescribed human testicular olfactory receptor, hOR17-4, and provided evidence that it may function in human sperm chemotaxis. Although the odorant bougeonal was shown to be a ligand for this receptor, the identity of the physiological ligand is not known. The question of whether or not this receptor is the one that mediates the chemotactic response to chemoattractants secreted in the female's gametes is also unresolved. Earlier studies have demonstrated that human follicular fluids (Ralt *et al.*, 1994), as well as follicular fluids of some other mammals (Oliveira *et al.*, 1999; Fabro *et al.*, 2002), contain yet-unidentified sperm chemoattractants [for reviews, see (Eisenbach, 1999, 2004)]. However, in view of the fact that, at least in pigs, only small quantities of follicular fluid are transported into the oviduct (Hansen *et al.*, 1991; Brussow *et al.*, 1998; Hunter *et al.*, 1999), the physiological significance of these findings was not clear. It was therefore assumed that the OCC continues to secrete chemoattractants subsequent to ovulation, but no experimental data have been available to support or negate this assumption. The present study provides the first evidence that the OCC does indeed secrete sperm chemoattractants. Since our findings were with mature oocytes, the results suggest that the OCC, which apparently starts to secrete the sperm chemoattractants within the follicle prior to ovulation, continues doing so after ovulation. This is important in view of the fact that physiologically relevant sperm chemotaxis to the OCC can occur after ovulation only.

The physiological significance of chemoattractant secretion by both the oocyte and cumulus cells

The present study suggests that the oocyte and, very likely, the cumulus cells secrete chemoattractants. The observation that the chemotactic activities of a medium conditioned with a single oocyte and a medium conditioned with as many as ~ 100 cumulus cells are similar (in both cases the activity is observed at medium dilutions of 10^3 – 10^4), suggest that the chemoattractant secreted from the oocyte is more potent or more concentrated than that secreted from the cumulus cells. Although we cannot exclude the possibility that both the oocyte and the cumulus secrete the same chemoattractant, a more appealing possibility is that each of them secretes a different chemoattractant(s). This suggests that, *in vivo*, sperm chemotaxis is a two-step process: first sperm chemoattraction to the cumulus, then chemoattraction to the oocyte. Chemotaxis within the cumulus is not superfluous. Without

chemotaxis, arrival to the oocyte through the dense matrix of the cumulus would be quite difficult (Bedford, 1982). Our finding that the mature oocyte secretes chemoattractants supports the notion of chemotaxis within the cumulus and is consistent with the remarkable efficiency with which sperm reach the oocyte embedded within the cumulus (Bedford and Kim, 1993). The observations that only capacitated sperm can penetrate the cumulus layers (for reviews: Eisenbach, 1995; Jaiswal and Eisenbach, 2002) and that only capacitated sperm are chemotactically responsive (Cohen-Dayag *et al.*, 1995; Fabro *et al.*, 2002) are also consistent with the notion of chemotaxis within the cumulus.

A reasonable sequence of events is that capacitated sperm, released from the sperm reservoir at the isthmus, are first guided by thermotaxis from the cooler sperm storage site towards the warmer fertilization site in the ampulla (Bahat *et al.*, 2003). Passive contractions of the oviduct may assist the sperm to reach the fertilization site. There, the sperm sense the chemoattractant secreted by the cumulus cells and reach the OCC by chemotaxis. Once within the cumulus matrix, the sperm sense the more potent chemoattractant, secreted from the oocyte, and navigate to the oocyte by chemotaxis.

Acknowledgements

We thank Prof. Edna Schechtman, a professional statistician, for assistance with the statistical analysis, and Dr Rony Oren-Benaroya for carrying out the chemotaxis assays with bougeonal. M.E. is an incumbent of the Jack and Simon Djanogly Professorial Chair in Biochemistry. L.C.G is a member of the Conicet (Argentina).

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Received on June 6, 2004; resubmitted on August 10, 2004; accepted on October 26, 2004