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.
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).

C. Prior to the experiment, the media were thawed and centrifuged to remove 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 P-1 medium (containing 10% SSS) for 2 h incubation at 37°C. Following this treatment the oocyte was removed for ICSI. 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 chemotactic 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 chemotactic gradient (i.e. the distance made along the X-axis, ΔX). Another parameter was the percentage of cells whose net distance of swimming is towards the chemotactic well (cells making trajectories with positive projection on the X-axis, i.e. with projection in the direction of the gradient; tracks with ΔX > 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; \( \frac{\Delta X}{\Delta Y} > 1 \), \( |\Delta Y| \) being the absolute value of the distance in the Y direction). In the case of random movement, the expected values are \( \sim 0 \mu m \) for \( \Delta X \), \( \sim 50\% \) 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 VSL/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 (5144–10 794 tracks). The mean values of \( \Delta X \) 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 chemotacticants, 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 \( 1 \times 10^3 \) and \( 1 \times 10^4 \) (Figure 1). In accordance with the expected bell-shaped dependence of a chemotactic response on the chemotactrant concentration (Adler, 1973; Ralt et al., 1994), no response was observed at higher and lower dilutions of the conditioned media, i.e. the values 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 (~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 \( 1–10 nmo/l \) (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 chemotacticants. 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.

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.
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).

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

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

The values are the mean ± 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 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 ± 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.
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

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**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 $6478–10,794$. 

| Dilution | Cells with $\Delta X > 0$ (%) | Cells with $\Delta X = 0$ (%) |
|----------|-----------------------------|-----------------------------|
| BWW      | 20%                         | 70%                         |
| $10^{-4}$| 20%                         | 70%                         |
| $10^{-3}$| 20%                         | 70%                         |

| Dilution | Cells with $\Delta X > 1$ (%) | Cells with $\Delta X = 1$ (%) |
|----------|-----------------------------|-----------------------------|
| BWW      | 20%                         | 70%                         |
| $10^{-4}$| 20%                         | 70%                         |
| $10^{-3}$| 20%                         | 70%                         |
movement of sperm towards the source of the chemoattrac-
tant—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 nega-
tive 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 sig-
nificant 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 capaci-
tated sperm are chemotactically responsive and that the frac-
tion 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 rela-
tively narrow dilution range of the conditioned media
(at ×1000 and ×10,000 dilutions only). This limited concen-
tration 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 occu-
peled 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-investi-
gated system of bacterial chemotaxis: the response of Escher-
ichia coli to the potent chemoattractant galactose in the
capillary assay has a sharp peak; the responses at concen-
trations 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 aver-
age 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 moti-
licity. 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 cumu-
lus 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 function-
ally expressed a previously undescribed human testicular
olfactory receptor, hOR17-4, and provided evidence that it
may function in human sperm chemotaxis. Although the
odorant bourgeonal was shown to be a ligand for this recep-
tor, the identity of the physiological ligand is not known.
The question of whether or not this receptor is the one that med-
iates 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-uniden-
tified 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
ovocytes, 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
ovocyte 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 chemoi-
attraction 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.

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