The effect of ovarian follicular fluid and peritoneal fluid on Fallopian tube ciliary beat frequency

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BACKGROUND: The Fallopian tube undergoes well-recognized changes during the ovarian cycle. Ciliary beat frequency (CBF) increases during the secretory phase of the cycle. The stimulus is unknown, although CBF is known to be hormone responsive. At ovulation, follicular fluid is released into the peritoneal cavity and enters the Fallopian tube. We hypothesized that this fluid may provide the stimulus for the increase in CBF detected after ovulation.

METHODS: Using a technique which records changes in light intensity, we have studied the effect of pre-ovulatory follicular fluid on CBF of Fallopian tube epithelial cells, and compared this with the effect of either peritoneal fluid or culture medium alone. Follicular fluid samples from 13 women undergoing IVF were collected by selective aspiration of individual follicles. Peritoneal fluid was collected from six women undergoing laparoscopic sterilization. Fallopian tubes were collected from 10 women who underwent hysterectomy for benign conditions. RESULTS: After 24 h incubation, there was a highly significant difference in CBF between the Fallopian tube samples bathed in follicular fluid (mean CBF ± SEM: 6.34 ± 0.02 Hz) compared with explants bathed in either medium (4.20 ± 0.06 Hz) or peritoneal fluid (5.24 ± 0.03 Hz) (P < 0.005). There was also a significant difference in CBF between tissues bathed in secretory (5.47 ± 0.03 Hz) compared with proliferative phase peritoneal fluid (4.75 ± 0.02 Hz) (P < 0.005).

CONCLUSIONS: The increase in CBF detected after ovulation may aid ovum pick-up and transport along the Fallopian tube. Factor(s) within human follicular fluid and secretory phase peritoneal fluid may be responsible for this increase in CBF.

Key words: cilia/Fallopian tube/follicular fluid/ovary/peritoneal fluid

Introduction

The Fallopian tube plays an essential role in gamete transport, fertilization and early embryogenesis. For successful intruterine implantation to occur, both gametes and embryo need to be transported within a well-defined time frame (Hafez and Blandau, 1969). This precisely timed process is affected by contractions of the tubal musculature, ciliary activity and the flow of tubal secretions (Jansen, 1984).

There is evidence that ciliary action may play the dominant role in tubal transfer. If muscular activity is inhibited in rodents, there is no difference in total transit times through the ampulla, suggesting that the cilia alone are capable of ovum transport within a normal time frame (Halbert et al., 1976, 1989). Conversely, impairment of CBF as in the ‘immotile cilia syndrome’ or as demonstrated in women with endometriosis is associated with reduced fertility (McComb et al., 1986; Lyons et al., 2002a).

The majority of the literature supports a physiological increase in CBF after ovulation (Critoph and Dennis, 1977; Lyons et al., 2002b). The factors responsible for this post-ovulatory increase in CBF are unknown. CBF is a calcium-dependent process requiring hydrolysis of ATP (Verdugo, 1980; Villalon and Cardina-Danovaro, 1994). In previous studies, we have found that CBF is affected by levels of both angiotensin II and progesterone (Saridogan et al., 1996; Mahmood et al., 1998). It has been hypothesized that, after ovulation, rising progesterone levels in an estrogen-rich environment may increase the release of ATP from apically situated mitochondria within the ciliated cells, and thus increase CBF (Jansen, 1984). Prostaglandins E₂ and F₂α are also known to increase CBF, possibly by intracellular release of calcium ions (Verdugo, 1980; Verdugo et al., 1980). Since the follicular fluid of human pre-ovulatory ovarian follicles contains high concentrations of estradiol, progesterone and prostaglandins (Edwards et al., 1972; McNatty et al., 1979; Seibel et al., 1984), we hypothesized that the influx of follicular fluid may be responsible for the increase in CBF observed after ovulation.

Using contrast analogue enhancement, a well-established and highly reproducible technique based on changes in light intensity, we investigated the effect on CBF in vitro of follicular fluid collected after administration of HCG to women having undergone ovulation induction for IVF treatment. We compared this to the effect on CBF of peritoneal fluid aspirated from women undergoing laparoscopic sterilization.
Materials and methods

Subjects
Follicular fluid was obtained from 13 women undergoing ovulation induction as part of an IVF programme. All patients started a long GnRH agonist protocol with 200 mg s.c. buserelin (Suprecur; Shire Pharmaceuticals Ltd, Andover, UK) from either day 1 or day 21 of the menstrual cycle. When pituitary–ovarian axis down-regulation was achieved, ovarian induction with gonadotrophins was commenced. Eleven women were treated with purified FSH (Metrodin High Purity; Serono Pharmaceuticals Ltd, Feltham, UK), whilst two women were given recombinant human FSH (Gonal F; Serono Pharmaceuticals Ltd). When at least three follicles were present with an average follicular diameter >16 mm in diameter, free from blood contamination and containing a single oocyte. Once the oocyte had been removed, the fluid was immediately centrifuged at 2000 g for 10 min to separate the supernatant from any cellular debris.

An aliquot from each follicular fluid sample was assayed to determine estradiol and progesterone levels. The samples were measured using direct chemiluminescence in a competitive immunoassay. The inter- and intra-assay coefficients of variation for estradiol were 8.5 and 4.0% respectively; and for progesterone 8.5 and 3.9%. The cell-free supernatant was then stored at −70°C.

Follicular fluid collection
Follicular fluid was collected from individual follicles 34 h after 10 000 IU HCG injection, by transvaginal ultrasound-guided needle aspiration, using a 16 gauge Cook needle. Each sample was collected into a sterile tube without culture medium. Follicular fluid was selected from follicles >16 mm in diameter, free from blood contamination and containing a single oocyte. Once the oocyte had been removed, the fluid was immediately centrifuged at 2000 g for 10 min to separate the supernatant from any cellular debris.

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Peritoneal fluid collection
Peritoneal fluid was obtained by aspiration from the pouch of Douglas immediately after introduction of the laparoscope. Fluid samples were again collected into a sterile tube and immediately centrifuged at 2000 g for 10 min to separate the supernatant from the cell pellet. Estradiol and progesterone levels were determined from each peritoneal fluid sample, prior to freezing and storage of the sample at −70°C.

Fallopian tube collection
Normal Fallopian tubes were collected from 10 patients undergoing hysterectomy for benign conditions, after obtaining written consent and local ethical committee approval. All women had regular menstrual cycles and no woman had used hormonal medication within 3 months of surgery. Six women were in the proliferative and four in the secretory stage of the menstrual cycle.

Fallopian tubes were collected into ice-cold minimum essential medium (MEM) with Earle’s salts and l-glutamine and supplemented with heparin (CP Pharmaceuticals, Wrexham, UK; 1.8 IU/ml), streptomycin (Evans Medical, Surrey, UK; 50 μg/ml), penicillin (Glаксo Laboratories, Middlesex, UK; 100 IU/ml) and HEPES (Life Technologies, Paisley, UK; 10 mmol/l). Fallopian tubes were rinsed several times to remove all visible evidence of blood. Small 1–2 mm pieces of tissue were dissected free from the fimbrial portion of the Fallopian tube and placed into multwell plates containing 200 μl of either culture medium, or undiluted follicular or peritoneal fluid. These were incubated for 24 h at 37°C, 100% humidity and 5% CO2/95% air, commencing within 30 min of collection.

Measurement of CBF
Baseline CBF of each Fallopian tube was determined in culture medium at room temperature prior to incubation. After 24 h, specimens were removed from the incubator and allowed to equilibrate to room temperature, after which measurement of CBF was taken. In order to control for inherent variations in CBF between individual tubes, each fluid sample (13 follicular fluid; six peritoneal fluid; one medium) was tested on every tube. Thus CBF readings were recorded from a total of 200 Fallopian tube explants.

CBF was assessed under an Olympus inverted microscope. Readings were taken randomly over a 1 min period from several areas of each tissue so that 50–100 readings of CBF were made on each tissue explant and the average taken. The recordings were analysed using a CE-1 contrast enhancer (Brian Reece Scientific Instruments, Newbury, UK) which increased the contrast of the video signal so that it was possible to analyse the cilia on a television monitor. After obtaining a sharply focused image, a cross-hair light-detecting sensor was placed over the real-time image of individual cilia beating on the television screen. As the cilia beat they produce changes in light intensity on the screen; these changes are detected by the sensor at its cross-point and computed into frequencies (in Hertz) by the use of a computer incorporating a PCX 1 video digitizer card (version 7.1) and cell track 8 software (Brian Reece Scientific Instruments).

Statistics
A histogram showing the frequency with which each value of CBF was recorded (Figure 1) demonstrated that the data fit a normal distribution. Univariate analysis was therefore used to investigate the findings. Results are expressed as mean ± SEM.

![Figure 1. The distribution of ciliary beat frequency (CBF). The columns refer to the total number of readings of CBF taken from the ampullary region of every tube. A normal curve is superimposed using SPSS Version 10.](image-url)
Results

The mean (± SEM) follicular fluid estradiol level was 2618 ± 1390 nmol/l and the mean progesterone level was 38 012 ± 16 727 nmol/l. The mean (± SEM) peritoneal fluid estradiol level was 4.7 ± 1.4 nmol/l and the mean progesterone level was 105 ± 12 nmol/l. The ratio of peritoneal fluid to follicular fluid estradiol and progesterone was therefore 1:557 and 1:362 respectively.

The mean (± SEM) baseline CBF of tubal explants was 4.54 ± 0.05 Hz. Figure 2 shows the distribution of CBF at 24 h of tubal epithelial explants incubated in follicular fluid, peritoneal fluid or culture medium. There was a significant difference in CBF between the mucosal explants bathed in peritoneal fluid (5.24 ± 0.03 Hz) compared with those bathed in culture medium alone (4.20 ± 0.06 Hz) (P < 0.03). There was a highly significant difference in CBF of explants incubated in follicular fluid (6.34 ± 0.02 Hz) in comparison to those bathed in either peritoneal fluid or medium (P < 0.005).

Although ciliary beat was increased in peritoneal fluid from the secretory (5.47 ± 0.03 Hz) as compared with the proliferative phase of the ovarian cycle (4.75 ± 0.02 Hz) (P < 0.005) (Figure 3), follicular fluid remained a significantly greater stimulus for CBF. The cilia bathed in follicular fluid beat on average 21% faster than control samples in peritoneal fluid and 51% faster than samples in culture medium.

Discussion

Follicular fluid is known to affect reproductive parameters. It can stimulate the acrosome reaction in capacitated sperm (De Jonge et al., 1993; Saaranen et al., 1993) and enhance the cleavage of human embryos during IVF (Hemnings et al., 1994). Follicular fluid also increases contractility of the tubal fimbria, possibly through the effect of its high prostaglandin content (Sterin-Speziale et al., 1978).

Our study is the first to assess the effect of follicular fluid on ciliary action. Using our technique of analogue contrast enhancement, we have shown that the incubation of tubal mucosal explants in pre-ovulatory follicular fluid significantly increases CBF in comparison with incubation in either culture medium or peritoneal fluid. We have also detected a significant increase in CBF of explants incubated in natural cycle secretory as compared with proliferative phase peritoneal fluid.
Follicular fluid has been shown to enter the porcine Fallopian tube at ovulation (Hansen et al., 1991), and in women ovulation instantaneously increases by >100-fold the peritoneal fluid sex steroid concentrations, which remain elevated until the mid-luteal phase (Loumaye et al., 1985; Bouckaert et al., 1986). This marked rise in steroid levels appears to be initially solely dependent on the mechanical rupture of the ovarian follicle (Loumaye et al., 1985). We postulate that the influx of follicular fluid into the Fallopian tube may initiate the increase in CBF detected post-ovulation and that the raised hormone concentrations within secretory phase peritoneal fluid maintain the heightened activity of the tubal cilia throughout ovum transit.

One limitation of our approach is that follicular fluid from women undergoing hormonal manipulation during IVF may differ in composition from that of women in natural cycles, and consequently our observations may only be of relevance for women undergoing intrauterine insemination after ovulation induction. However, although one group did find differences in follicular fluid sex steroid levels during controlled ovarian stimulation (Frederick et al., 1991), most other studies have detected similar concentrations of estrogen, progesterone and prostaglandins in the follicular fluid of stimulated and unstimulated ovaries, irrespective of the type of gonadotrophin regimen used (Yilikorkala et al., 1984; Mantzavinos et al., 1997; Teissier et al., 1999). Ovarian steroid levels in the follicular fluid samples used in this study are comparable to those previously reported in mature follicles (Seibel et al., 1989; Mantzavinos et al., 1997).

The same limitations do not apply to the assessment of the effect of proliferative and secretory phase peritoneal fluid on CBF, as the peritoneal fluid samples were all obtained from women in natural cycles. Although secretions from the tubal epithelium will exert a dilutional effect on intraluminal follicular and peritoneal fluid, this effect may not be significant, as tubal secretions are small in volume. However, in order to replicate the in vivo environment, it would be necessary to collect intraluminal fluid pre- and post-ovulation and assess its effect on CBF.

As in our previous studies, we noted an effect after 24 h, within the time frame of action of steroid hormones (Mahmood et al., 1998; Lyons et al., 2002a). In earlier in vitro work, we have shown that, whilst estrogen has no effect, addition of high levels of progesterone has an inhibitory effect on the cilia, reducing CBF by 40% (Mahmood et al., 1998). Although total progesterone levels within pre-ovulatory follicles are high, most of the sex steroid hormones are protein-bound, with <5% free and biologically active (Andersen, 1991). This raises the possibility that other factors in antral fluid may be responsible for its stimulatory effect on CBF.

Human follicular fluid has been found to contain high levels of prostaglandins, which increase in the pre-ovulatory period (Darling et al., 1982; Seibel et al., 1984). Prostaglandins play a pivotal role in oocyte maturation and in the mechanism of follicle rupture (Kobayashi et al., 1981; Seibel et al., 1984). In animal studies prostaglandins have been shown to increase both oviductal contractility and fimbrial CBF (Barbosa et al., 1980; Verdugo et al., 1980). Prostaglandins could therefore be possible mediators of the increased CBF demonstrated in the human Fallopian tube after ovulation.

In summary, this study has shown a significant stimulatory effect of follicular fluid and secretory phase peritoneal fluid on CBF of human Fallopian tube explants in vitro. It is possible that this may be the mechanism by which CBF is increased after ovulation. Further work is required to identify the active component(s) which modulate this increase, but possible mediators include the ovarian steroid hormones or prostaglandins. In order to assess the biological relevance of these findings, further research is required on the relationship between the absolute frequencies of ciliary beat and tubal transit times, and to determine which alterations in tubal transport have an impact on fertility. An increase in CBF may aid ovum pick-up and the transport of gametes and embryos along the Fallopian tube, and thus may improve the chances of a successful pregnancy.

References


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