Journal of Assisted Reproduction and Genetics, Vol. 17, No. 2, 2000

GYNECOLOGY

High Progesterone Levels and Ciliary Dysfunction—A Possible Cause of Ectopic Pregnancy

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Submitted: May 20, 1999
Accepted: October 11, 1999

Objectives: *To investigate the effects of different levels of hormones on the ciliary activity of human oviducts and, consequently, to assess their possible role in tubal implantation of the fertilized egg.*
Design: *Fallopian tube epithelial samples were incubated in media with the addition of Estradiol (E_2), progesterone (P), human menopausal gonadotropin (hMG), LH, or pure FSH (Metrodin) in different concentrations. The ciliary beat frequency (CBF) was measured after 24 h of incubation. Then the media were exchanged to media without the addition of hormones and the CBF was measured again 24 h later by using the photoelectric technique.*
Setting: *University teaching hospital, IVF unit.*
Results: *Twenty-four hr after the addition of P to the culture medium in concentrations of 0.5 or 1 ng/ml a significant decline of the CBF down to 63% of the control level was observed ($P < 0.001$) and with P in concentration of 2 ng/ml or greater, 50–70% of the cilia were paralyzed. These effects of P were found to be reversible. Incubation with E_2 induced a slight increase of 4% in the mean CBF ($P = 0.002$). Twenty-four hr incubation with Metrodin, Pergonal, or LH did not affect ciliary motility.*
Conclusions: *Higher levels of progesterone cause ciliary dysfunction and subsequently may be a possible cause of ectopic pregnancy.*

KEY WORDS: fallopian tubes; ectopic pregnancy; ciliary activity; organ culture.

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INTRODUCTION

Implantation of the embryo in the Fallopian tubes is a condition unique to humans. An experimental model for tubal pregnancy in domestic or laboratory animals is lacking. Therefore, much of our knowledge about etiologic mechanisms and pathophisiology of ectopic pregnancy (EP) is based on circumstantial evidence. Tubal pregnancies make up 95–97% of all ectopic gestations. The ampulla is the most common site of implantation, accounting for 78% of ectopic pregnancies; 12% are located in the isthmus, 5% in the fimbria, and 2% are cornual or interstitial. The reminder are abdominal, cervical, or ovarian (1). Approximately 50% of all tubal pregnancies develop from a delay in ovum transport secondary to a diseased Fallopian tube caused by tubal infection (2). Hormones are known to affect many aspects of growth differentiation and function in the human Fallopian tubes. E_2 and P concentrations in monkey oviductal fluid are consistently lower than those in plasma (3). Alteration of the hormonal milieu can result in disturbance of the function of tubal cilia and muscles and can lead to tubal implantation. Therefore, hormonal factors have been implicated in the pathogenesis of the disease. This hypothesis is supported by the association between several conditions such as ovulation induction and the use of certain contraceptive techniques, with an increased incidence of EP (2).

To date, reliable information about the hormonal control of ciliary activity in the human Fallopian tubes is sparse. The aim of the present study was to investigate the effects of different levels of progesterone (P), 17-Estradiol (E_2), LH, pure FSH, and hMG on the ciliary beat in organ cultures of human Fallopian tubes

1058-0468/00/0200-0103$18.00/0

and, as a result, their possible role in tubal implantation.

MATERIALS AND METHODS

Description of the Photoelectric Method for CBF Measurements (Fig. 1)

The slide coverslip preparation of the ciliated epithelium is placed on an electrically controlled warm stage at 37°C and examined by phase-contrast inverted microscopy (I-135, Zeiss, Germany) under objective lens 40×. The cilia are positioned to interrupt the passage of light through an adjustable slit and the output is monitored with a photodiode (UV-444 BQ, EG&G, USA) and amplifier (2061, Tektronix, USA). The electrical signals are fed into a digital storage oscilloscope (2212, Tektronix, USA) for viewing of ciliary activity. CBF values are obtained by counting the number of ciliary beats recorded in 0.5-0 intervals and are expressed in Hz.

Preparation of the Organ Cultures

Normal-looking Fallopian tubes from women undergoing hysterectomy were collected into DMEM/F-12 medium (Beit Haemek, Israel) containing Streptomycin (100 g/ml), Penicillin (100 IU/ml), 10% Fetal Calf Serum, and L-Glutamine (4mM) buffered with 15 m*M* HEPES buffer. Tubes were placed on ice and taken to the laboratory within 30 min of removal. The ampullary region was dissected out in a laminar flow cabinet, opened longitudinally, and pieces of 3–4 mm^2 were cut with curved scissors and transferred to tissue culture dishes. Each dish contained two pieces in 2 ml medium. The pieces, originating from the same tube, were placed in the following media: DMEM:F12; DMEM:F12 with E_2 in concentrations of 200 or 600 pg/ml; steroid-free medium (IVF Medium without phenol red, Medi-Cult, Denmark); DMEM:F12 with progesterone in concentrations of 0.5 or 1, 2, 4, and 6 ng/ml; human menopausal gonadotropin (hMG) (Pergonal, Teva, Israel) or LH (Sigma) or pure FSH (Metrodin, Teva, Israel) all in concentrations of 15, 150, 300 mIU/ml. The CBF was measured after 24 hr of incubation. Then the media were exchanged with DMEM:F12 medium without the addition of hormones and the CBF was measured again 24 hr later. In every experiment, for each type of medium, the CBF were measured in 4–16 pieces (originated from the same tube), 10 times per piece, using the photoelectric technique. The effects of the tested hormones on the ciliary activity were determined as the percentage of change in CBF compared to the controls.

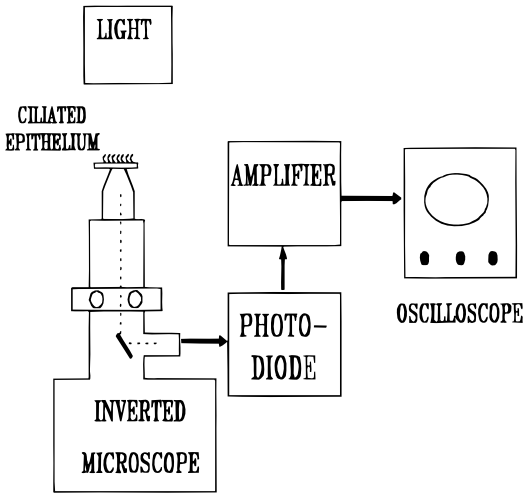

Fig. 1. A schematic diagram of the photoelectric system.

Statistical Analysis

Results are expressed as means (SD). Mean values of CBF in DMEM/F12 and IVF media were compared by unpaired *t* test. Comparisons between the treated groups were carried out by one-way analysis of variance (ANOVA). If significance was obtained with ANOVA, the Newman-Keuls test was used for multiple comparisons between groups. $P < 0.05$ was considered significant.

RESULTS

Incubation with P for 24 hr at concentrations of 0.5, 1, 2, 4, and 6 ng/ml, reduced the CBF to 74.6% (8.2%, 71.0%) (9.0%, 66.0%) (1.4%, 64.0%) (1.8% and 62.6%) (1.54%), respectively, of the CBF measured in controls (Fig. 2A). All the treated groups were found to be significantly different from the control group ($P < 0.001$). It was also observed that in culture media with P of 2 ng/ml or greater, about 50–70% of the cilia were paralyzed. When the medium with P was replaced with DMEM:F12 alone, after 24 hr incubation, the mean CBF in all the treated groups were between 94% and 96% of the control level ($P < 0.001$) (Fig. 2A). Cilia which did not move after 24 hr incubation with high levels of P regain their motility. Incubation with E_2 in concentrations of 200 pg/ml or 600 pg/

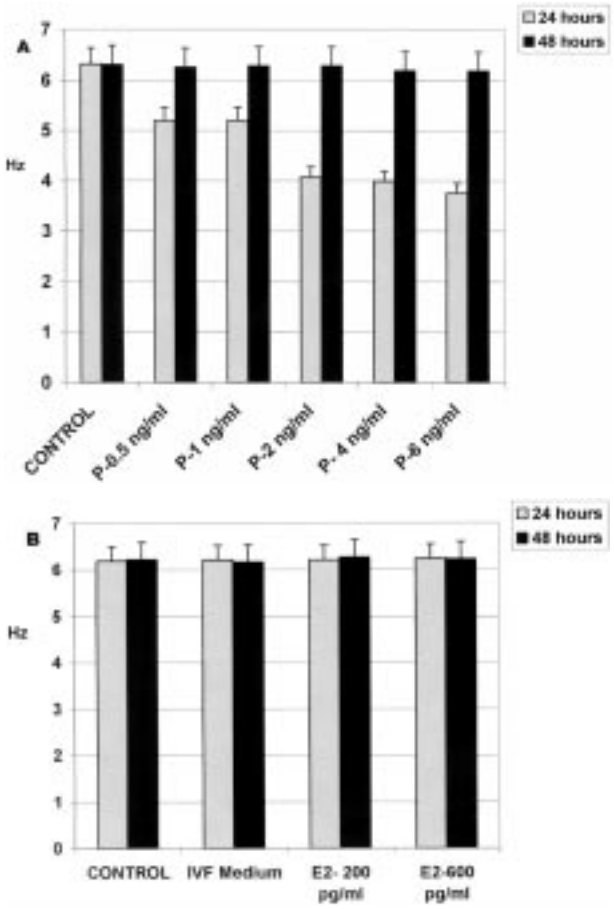

Fig. 2. (A) Mean ± SD of CBF after 24 hr of incubation (gray bars) with progesterone (P) and with 17-Estradiol (E_2); (B) and after replacement of the culture media to DMEM/F12 alone for 24 hr (black bars).

ml for 24 hr induce a slight increase in the mean CBF (103.7%) (2.7% and 104.3%) (1.7%) ($P = 0.002$) (Fig. 2B). When the mediums with E_2 were replaced with DMEM:F12 alone, after 24 hr of incubation, the mean CBF in the treated groups was not significantly different from the control group ($P = 0.08$) (Fig. 2B). Incubation for 24 hr with Metrodin, Pergonal, or LH at concentrations of 15, 150, and 300 mIU/ml did not affect ciliary motility ($P = 0.23$, $P = 0.55$, $P = 0.41$, respectively).

DISCUSSION

Ovum transport in the human oviducts involves two main effectors: ciliary motility and muscle contractility. Decrease in oocyte cumulus complex pick up and transport as a result of a decrease in CBF may increase the incidence of tubal infertility and ectopic pregnancy (4). Data about the effects of hormones on the tubal muscles and ciliary function are conflicting. Pauerstein (5) suggested that estrogens increase and progestins decrease smooth muscle contractile activity particularly in the isthmic portion. On the other hand, Pulkkinen and Jaakkola (6) observed that when the serum progesterone level is low, the oviduct, mainly the isthmus segment, shows characteristics of dysfunction. Jansen (7) suggested that changes in the ciliary activity and ciliary morphology are estrogen-dependent in the sense that E_2 causes differentiation of ciliated cells, including ciliogenesis. Progesterone has an antagonistic effect to estrogen, and prolonged exposure to progesterone causes cilial regression. A high correlation was found between the number of ciliary cells and the ciliary function (8).

There is evidence that high levels of progesterone are related to the occurrence of ectopic pregnancies. The risk of ectopic pregnancy among women using progestin oral pills or progestin implants for contraception is two to five-fold compared to other women of childbearing age (9,10). The incidence of ectopic pregnancy among the progesterone-bearing IUDs is also considerably greater (16.3% of the pregnancies) than observed with placebo device (5.1% of the pregnancies) (11). It seems to be well established that women who undergo treatment with human gonadotropins run a greater risk of tubal pregnancy. The risk is more than three times as high as in the population of women with similar risk factors. If hyperstimulation occurs, the risk is further increased (12). A higher rate of ectopic pregnancy (4.9%) has also been reported among IVF pregnancies in the United States and Canada (13). McBain *et al.* (14) observed in women without evidence of tubal pathology, an association between ectopic pregnancy and elevated urinary estrogen excretion in the peri-ovulatory phases of induced ovulatory cycles. They implied that high estrogen levels may induce abnormal tubal embryo transport.

In the present study, we showed that the addition of P to the culture medium markedly decreased or even blocked the ciliary activity in a dose-dependent fashion. It has been suggested that in the ampulla, the cilia is the main factor in the propagation of the egg (6,15). Our data may explain earlier findings of increased rates of EPs, especially in the ampulla, among women using progestin-only pills, injectable progestin preparation and progestin coated intrauterine devices. In contrast to previous studies (7), we have found that incubation in media without sex steroids or with high E_2 levels did not affect CBF. We conclude

that E_2 is not essential for ciliary activity and has minor effect on the ciliary activity in vitro. Also, the higher rates of EPs in ovulation induction cycles are not the result of the direct effects of hMG, pure FSH, and LH on the cilia motility. We propose that the higher levels of P in ovulation induction cycles caused by multiple corpora lutea and by P supplement early in the luteal phase may affect ciliary activity and ovum transport. High levels of E_2 in ovulation induction cycles may slow cilia motility via increased P receptors in the oviducts (16). We suggest that treatment with high levels of progesterone early after ovulation in ovulation induction cycles may increase the incidence of ectopic pregnancy. This requires further clinical study.

ACKNOWLEGMENTS

This work was supported by grants from the Lady Davis Grant, and the Israel Ministry of Health

REFERENCES

1. Ectopic pregnancy. ACOG Tech Bull 1990;150:1–7
2. Lavy G, DeCherney AH: The hormonal basis of ectopic pregnancy. Clin Obstet Gynecol 1987;30:217–224
3. Wu CH, Mastroianni L, Mikhail G: Steroid hormones in monkey oviductal fluid. Fertil Steril 1977;28:1250–1256
4. Knoll M, Talbot P: Cigarette smoke inhibits oocyte cumulus complex pick-up by the oviduct in vitro independent of ciliary beat frequency. Reprod Toxicol 1998;12:57–68
5. Pauerstein CJ: Clinical implication of oviductal physiology and biochemistry. Gynecol Invest 1975;6:253–264
6. Pulkkinen MO, Jaakkola UM: Low serum progesterone levels and tubal dysfunction—A possible cause of ectopic pregnancy. Am J Obstet Gynecol 1989;161:934–937
7. Jansen RPS: Endocrine response in the Fallopian tube. Endocrine Reviews 1984;5:525–551
8. Paltieli Y, Weichselbaum A, Hoffman N, Eibschitz I, Kam Z: Laser scattering instrument for real time in-vivo measurement of ciliary activity in human Fallopian tube. Hum Reprod 1995;10:1638–1641
9. Beral V: An epidemiological study on the recent trends in ectopic pregnancy. Br J Obstet Gynecol 1975;82:775–782
10. Liuko P, Erkkola R, Laakso L: Ectopic pregnancies during the use of low-dose progestogens for oral contraception. Contraception 1977;16:575–580
11. Tatum HJ, Schmidt FH: Contraceptive and sterilization practices and extrauterine pregnancy: a realistic perspective. Fertile Steril 1977;28:407–421
12. Gemzel C, Guillome J, Wang CF: Ectopic pregnancy following treatment with human gonadotropin. Am J Obstet Gynecol 1982;143:761–765
13. Assisted reproductive technology in the United States and Canada: 1992 results generated from the American fertility society/ Society for assisted reproductive technology register. Fertil Steril 1994;62:1121–1128
14. McBain JC, Pepperell RJ, Robinson HP, Brown JB: An unexpectedly high rate of ectopic pregnancy following the induction of ovulation with human pituitary and chorionic gonadotropin. Br J Obstet Gynecol 1980;87:5–9
15. Halbert SA, Tam PY, Adams RJ, Blandau RJ: An analysis of the mechanism of egg transport in the ampulla of the rabbit oviduct. Gynecol Invest 1976;7:306–320
16. Brenner RM, Slayden OD: Oestrogen action in the endometrium and oviduct of rhesus monkeys during RU486 treatment. Hum Reprod 1994;9(suppl):82–97