Case Reports

Endometrial fluid accumulation during controlled ovarian stimulation for ICSI treatment. A report of three cases

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Summary

The occurrence of endometrial fluid accumulation was examined in patients undergoing ovarian stimulation in a program of intracytoplasmic sperm injection (ICSI), by vaginal ultrasound. Endometrial fluid accumulation was recorded in three cycles out of 124. In one case, the fluid was absorbed before embryo transfer (ET), but in the other cases it was present in ET. In these three cases, the endometrium had been evaluated as normal before ovarian stimulation. Fluid accumulation in the endometrial cavity possibly affects the implantation process negatively. Therefore, alternative options should be considered as cancellation of the embryo transfer and cryopreservation of embryos to be available in a subsequent mild stimulated cycle.

Key words: Endometrial fluid; Hydrometra; In-vitro fertilisation; ICSI; Embryo transfer; Implantation.

Introduction

Accumulation of fluid in the endometrial cavity is a rare phenomenon in in-vitro fertilisation (IVF). A few studies have been published, where in most of the cases, hydrosalpinx coexisted [1-7]. Cycles with endometrial fluid accumulation are considered to have low implantation and pregnancy rates as well as a high incidence of abortions [1-7]. The mechanism of endometrial fluid accumulation during ovarian stimulation is not entirely known. In the present article, we present three cases of endometrial fluid accumulation during ovarian stimulation for intracytoplasmic sperm injection/embryo transfer (ICSI/ET) treatment and we discuss possible alternative options to overcome the implantation and pregnancy failure.

Materials and Methods

Patients.

A total of 124 cycles with 99 women were included. All women followed ICSI/ET treatment in "Otmar Bauer" IVF centre (Alexandroupolis, Greece) from 2000 to 2002. In every cycle, before the ovarian stimulation, tranvaginal ultrasound examination was performed to detect pelvic pathology. Hysterosalpingography, laparoscopy with chromopertubation and ultrasonography were used for the evaluation of the tubal condition.

Ultrasonography examination.

Ultrasonographic examinations were performed with a Siemens Sonoline SI-250 equipped with a 5 MHz frequency transvaginal probe. The endometrium was scanned sagittally along the mid-line axis of the uterus before and during the ovarian stimulation, on the day of hCG administration, on the day of follicle puncture and on the day of embryo transfer. All the ultrasonographic examinations were performed by the same person (N.N.).

Ovarian stimulation and ICSI/ET procedure.

Ovarian stimulation was performed using a long protocol as previously described [8, 9]. Pituitary desensitization was achieved with the gonadotropin-releasing hormone (GnRH-a) triptorelin (Arvekap Depot 3.75, Ipsen Biotech, France). For ovarian stimulation recombinant follicular stimulating hormone (rFSH) was administrated (Gonal-F, Ares-Serono, U.K.). Oocyte maturation was induced with 10,000 I.U. of human chorionic gonadotropin (hCG) (Pregnyl, N.V. Organon, Oss, Holland).

The cumulus and corona radiata were removed mechanically under dissecting microscope, with simultaneous exposure to 0.5% hyaluronidase (Sigma Co., Deisenhofen, Germany) for 30 seconds. ICSI was performed as previously described [10, 11]. After 18 hours of incubation at 37°C in a humidified atmosphere with 5% CO_2, oocytes were examined for the presence of two or more pronuclei as a sign of fertilisation. Uterine embryo transfer was performed 48 hours after oocyte retrieval using an embryo transfer catheter (Sherwood medical vessel catheter, Tullamore, Ireland).

Results

Endometrial fluid accumulation was detected in three cycles of two different women, with an incidence of 2.42% (3/124).

Case 1. A 36-year-old woman followed controlled ovarian stimulation for ICSI/ET due to male factor infertility. In the previous years she had two unsuccessful IVF treatments in other centres, with poor response to ovulation induction. She did not suffer from hydrosalpinx or cervical stenosis.

In our centre, she received a total of 72 rFSH ampoules, the maximum estradiol level was 900 pg/ml and three

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mature oocytes were harvested. Endometrial fluid accumulation was detected on the seventh day of gonadotropin administration, having a diameter of 7.6 mm. The diameter was maintained stable until the day of oocyte retrieval. On the day of embryo transfer, the fluid diameter was 6.2 mm. Three embryos were transferred without success.

Case 2. The same woman participated in another ICSI/ET cycle eight months later. During the controlled ovarian stimulation, she received 66 ampoules of rFSH. The day before hCG administration the estradiol level was 849 pg/ml. On the day of follicle puncture, four oocytes were harvested. The endometrial fluid was detected on the seventh day of gonadotropin administration. The fluid diameter was 8.2 mm. It remained stable, without further increase until the day of follicle puncture. On the day of embryo transfer, the fluid diameter was reduced in 6.7 mm. Four embryos were transferred without conception.

Case 3. Another 37-year-old woman followed ICSI/ET treatment due to male factor infertility. Seven months before she had followed another ICSI/ET cycle unsuccessfully. She did not have a medical history of hydrosalpinx or cervical stenosis. In our centre, during controlled ovarian stimulation she received 36 ampoules of rFSH, the maximum estradiol level was 1,640 pg/ml and five oocytes were harvested at the day of follicle puncture. Endometrial fluid was detected at the eighth day of gonadotropin administration. The fluid diameter was 7.9 mm. It remained stable, without further increase until the day of hCG administration and was absorbed in the following two days. Four embryos were transferred without conception.

Discussion

Careful monitoring for detection of endometrial fluid accumulation during controlled ovarian stimulation is a measure of great importance due to the detrimental effects of endometrial fluid on embryo implantation. Ultrasound sonography is recognised as an easy and effective method for that detection [2-4]. Endometrial fluid accumulation is transient, present during gonadotropin and hCG administration but undetectable on the day of ET, or persistent, present from the gonadotropin administration to the day of ET. The incidence of fluid accumulation during IVF treatment is rare but the impact on embryo implantation is of great importance. Chien *et al.* [7] reported an incidence rate of 4.7% and a pregnancy rate of 5%. Our data confirm both the low incidence rate (2.42%) and the detrimental effect on the implantation procedure. Persistent endometrial fluid accumulation seems to have more detrimental effects on embryo implantation than transient does [7].

Hydrosalpinx is one of the main predisposing factors for endometrial fluid accumulation during ovarian stimulation for IVF treatment [2, 4, 5, 7]. Cervical stenosis, endometriosis and polycystic ovarian syndrome (PCOS) are also considered as predisposing factors [6, 7]. However, fluid accumulation during IVF treatment has also been detected in women without any obvious pathological condition. In the cases of the two women presented in this study, there was neither tubal disease nor endometriosis. They did not suffer from PCOS as well. According to Chien *et al.* [7], in a considerable part of cases with fluid accumulation during ovarian stimulation there are no obvious predisposing factors.

The mechanism of fluid accumulation and the way it affects embryo implantation is not sufficiently known. In the presence of hydrosalpinx, it has been proposed that there is a reflux of fluid into the uterine cavity [3, 5]. Stenosis or obstruction of the cervical canal can easily explain the fluid accumulation. In cases of endometriosis, one may hypothesize that pelvic adhesions cause tubal obstruction and consequently fluid accumulation. However, this hypothesis does not explain all the cases. Moreover, the mechanism of endometrial fluid accumulation in the cases without any predisposing factor remains inexplicable. Factors such as age, medication and the number of IVF cycles before the appearance of endometrial fluid have not been investigated.

Repeated accumulation in successive cycles is a very rare phenomenon. It has been reported in women who underwent surgical operations before IVF treatment [7]. In our study, one of the two women presented fluid accumulation in two successive cycles although she had not undergone any surgical operations.

As for the mechanism of how endometrial fluid affects embryo implantation, various explanations have been proposed. A number of in-vitro studies have demonstrated a reduction in embryo development when embryos are exposed to concentrations of hydrosalpingeal fluid, especially in high concentrations [12-15]. This detrimental effect may be partially explained by the high PH values and the impaired cytokines profile of hydrosalpingeal fluid [12, 16]. However, opposite opinions also exist [17]. Unfortunately, there are no in-vitro studies evaluating the effect of endometrial fluid on embryo developmental potential.

Wittek *et al.* [18] reported that there is atrophy of the uterine glands in goats with endometrial fluid accumulation. This finding suggests a mechanical effect of fluid accumulation that may explain the implantation hindrance.

Chien *et al.* [7] reported that endometrial thickness on the embryo transfer day was statistically significantly lower in cycles with fluid than cycles without fluid.

The detrimental effects of this condition on embryo implantation require solutions. In the cases of hydrosalpinx, Strandell *et al.* [19] and Statdmauer *et al.* [20] proved that surgical correction before IVF treatment improves the pregnancy rate. In cases of cervical stenosis, dilatation before IVF treatment is also a reasonable solution. Aspiration of fluid before embryo transfer is not always successful, as re-collection may happens [2, 4]. Therefore, we believe that the most reasonable solution in cases of endometrial fluid accumulation is the cancel-

lation of embryo transfer and the cryopreservation of embryos. In this way, the woman can have a second chance in a mild stimulated transfer cycle, with the hope that fluid accumulation will not happen again. However, the benefits of such practice remain to be examined in future studies.

References

[1] Welker B. G., Gembruch U., Diedrich K., Al-Hasani S., Krebs D.: "Transvaginal sonography of the endometrium during ovum pick up in stimulated cycles for in vitro fertilization". *J. Ultrasound Med.*, 1989, *8*, 549.

[2] Mansour R. T., Aboulghar M. A., Serour G. I., Riad R.: "Fluid accumulation of the uterine cavity before embryo transfer: a possible hindrance for implantation". *J. In Vitro Fert. Embryo Transf.*, 1991, *8*, 157.

[3] Andersen A. N., Yue Z., Meng F. J., Petersen K.: "Low implantation rate after in-vitro fertilization in patients with hydrosalpinges diagnosed by ultrasonography". *Hum. Reprod.*, 1994, *9*, 1935.

[4] Bloechle M., Schreiner T., Lisse K.: "Recurrence of hydrosalpinges after transvaginal aspiration of tubal fluid in an IVF cycle with development of a serometra". *Hum. Reprod.*, 1997, *12*, 703.

[5] Sharara F. I., McClamrock H. D.: "Endometrial fluid collection in women with hydrosalpinx after human chorionic gonadotrophin administration: a report of two cases and implications for management". *Hum. Reprod.*, 1997, *12*, 2816.

[6] Sharara F. I., Prough S. G.: "Endometrial fluid collection in women with PCOS undergoing ovarian stimulation for IVF. A report of four cases". *J. Reprod. Med.*, 1999, *44*, 299.

[7] Chien L.-W., Au H.-K., Xiao J., Tzeng C.-R.: "Fluid accumulation within the uterine cavity reduces pregnancy rates in women undergoing IVF". *Hum. Reprod.*, 2002, *17*, 351.

[8] Nikolettos N., Al-Hasani S., Felberbaum R., Demirel L. C., Riethmuller-Winzen H., Reissmann T., *et al.*: "Comparison of cryopreservation outcome with human pronuclear stage oocytes obtained by the GnRH antagonist, Cetrorelix, and GnRH agonists". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2000, *93*, 91.

[9] Nikolettos N., Al-Hasani S., Felberbaum R., Demirel L. C., Kupker W., Montzka P., *et al.*: "Gonadotropin-releasing hormone antagonist protocol: a novel method of ovarian stimulation in poor responders". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2001, *97*, 202.

[10] Al-Hasani S, Küpker W, Baschat A. A., *et al.*: "Mini swim-up: a new technique of sperm preparation for ICSI". *J. Assist. Reprod. Genet.*, 1995, *12*, 428.

[11] Al-Hasani S., Ludwig M., Karabulut O., Al-Dimassi F., Bauer O., Sturm R., *et al.*: "Results of intracytoplasmic sperm injection (ICSI) using microprocessor controlled TransferMan Eppendorf Manipulator system". *J. Middle East Fertil. Soc.*, 1999, *4*, 41.

[12] Mukherjee T., Copperman A. B., McCaffrey C., Cook C. A., Bustillo M., Obasaju M. F.: "Hydrosalpinx fluid has embryotoxic effects on murine embryogenesis: a case for prophylactic salpingectomy". *Fertil. Steril.*, 1996, *66*, 851.

[13] Rawe V .J., Liu J., Shaffer S., Compton M. G., Garcia J. E., Katz E.: "Effect of human hydrosalpinx fluid on murine embryo development and implantation". *Fertil. Steril.*, 1997, *68*, 668.

[14] Freeman M. R., Whitworth C. M., Hill G. A.: "Permanent impairment of embryo development by hydrosalpinges". *Hum. Reprod.*, 1998, *13*, 983.

[15] Spandorfer S. D., Liu H.-C., Neuer A., Barmat L. I., Davis O., Rosenwaks Z.: "The embryo toxicity of hydrosalpinx fluid is only apparent at high concentrations: an in vitro model that stimulates in vivo events". *Fertil. Steril.*, 1999, *71*, 619.

[16] Barmat L. I., Nasti K., Yang X., Spandorfer S., Kowalik A., El-Roely A.: "Are cytokines and growth factors responsible for the detrimental effects of hydrosalpingeal fluid on pregnancy rates after in vitro fertilization-embryo transfer?". *Fertil. Steril.*, 1999, *72*, 1110.

[17] Granot I., Dekel N., Segal I., Fieldust S., Shoham Z., Barash A.: "Is hydrosalpinx fluid cytotoxic?". *Hum. Reprod.*, 1998, *13*, 1620.

[18] Wittek T., Erices J., Elze K.: "Histology of the endometrium, clinical-chemical parameters of the uterine fluid and blood plasma concentrations of progesterone, estradiol-17β and prolactin during hydrometra in goats". *Small Rumin. Res.*, 1998, *30*, 105.

[19] Strandell A., Lindhard A., Waldenstrom U., Thorburn J.: "Hydrosalpinx and IVF outcome: cumulative results after salpingectomy in a randomized controlled trial". *Hum. Reprod.*, 2001, *16*, 2403.

[20] Statdmauer L. A., Riehl R. M., Toma S. K., Talbert L. M.: "Cauterization of hydrosalpinges before in vitro fertilization is an effective surgical treatment associated with improved pregnancy rates". *Am. J. Obstet. Gynecol.*, 2000, *183*, 367.

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