Effect of seminal plasma application to the vaginal vault in in vitro fertilization or intracytoplasmic sperm injection treatment cycles—a double-blind, placebo-controlled, randomized study

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Abstract

Purpose To study whether intravaginal application of seminal plasma after follicle aspiration has the potential to increase implantation and clinical pregnancy rates after IVF-ET.

Methods We conducted a prospective, double-blind, placebo-controlled randomized study of 230 patients undergoing IVF-ET cycles. 500 μ L of Fresh seminal plasma from the patient's partner or culture medium (placebo) were injected in the vaginal vault just after follicle aspiration. The main outcome measured was ongoing clinical-pregnancy rate.

Results After ET cancellation in ten patients due to lack of fertilization or embryo cleavage, 220 embryo transfers (103 and 117 in the study and control groups) resulted in a clinical pregnancy rate of 36.9 % and 29.1 % for the study and control groups, corresponding to a relative increase of 26.8 %. After an early pregnancy loss of 13.1 % (5/38) and 23.5 % (8/34) in the study and control groups respectively an ongoing pregnancy rate of 32.0 % (33/103) and 22.2 % (26/117) was achieved corresponding to a relative increase of 44.1 %. Multivariate logistic regression analysis adjusted for study group, age, infertility, and cycle

characteristics did not demonstrate any parameter that could predict occurrence of clinical pregnancy rates after IVF-ET.

Conclusions Patients who underwent SP intravaginal insemination after oocyte pick-up reached higher implantation and clinical pregnancy rates following ET compared to controls, although the difference did not reach statistical significance. More studies and variable methodologies may clarify the potential clinical effect of SP in improving live birth rates after ART.

Keywords Seminal plasma · IVF · Intercourse · Implantation · Pregnancy rate

Introduction

A variety of studies first in animal models such as mice, [1, 2] and pigs [3], and then in human in vitro cell culture models [4–6] indicate that seminal plasma (SP) reaching the endometrial cavity after intercourse may have an important role in affecting the uterine receptivity. SP contains a variety of factors such as Transforming Growth Factor b, [1, 4], prostaglandin E and interleukin- 8 [7], that may trigger a cascade of events through pro-inflammatory cytokines synthesis leading to maternal endometrial immunomodulation increasing uterine receptivity. A beneficial effect of SP as an adjunctory factor to increase implantation and clinical pregnancy rates after in vitro fertilization and embryo transfer (IVF-ET) was indicated in a few clinical studies that used variable methodologies [8–11]. Others did not corroborate these observations [12]. Based on the hypothesis that local application of SP into the vagina and the cervix at the end of follicle aspiration may prime the endometrium as mentioned in the studies cited above, we decided to conduct a double blind prospective randomized

Capsule SP intravaginal insemination after oocyte pick-up may have a positive contribution to increase implantation and clinical pregnancy rates following IVF-ET.

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study aiming to evaluate the effect of SP insemination after oocyte pick-up (OPU) on implantation and clinical pregnancy rates after IVF-ET.

Material and Methods

After approval of the hospital's IRB (local Helsinki committee approval # 06/10), a prospective randomized and double blinded study was designed.

Study population

Participants were all couples undergoing IVF or ICSI between April, 2010 and March, 2011 at our IVF unit, aged ≤ 40 years, who have failed to conceive at least after one previous IVF-ET cycle. Exclusion criteria included patients whose previous cycle was characterized by lack of aspirated oocytes, endometriosis, or sperm not from the partner's ejaculate (i.e., epididymal, testicular or donor sperm). Also males with presence of hepatitis B, hepatitis C, HIV, or leukocytospermia or other signs of infection, were excluded. Participation was limited to one treatment cycle per couple.

Clinical treatment and patients' randomization

Patients included in the study were treated by routine controlled ovarian hyperstimulation (COH) protocols for IVF using a long GnRH agonist or GnRH antagonist, as previously described [13].

Oocytes were retrieved by ultrasound guided transvaginal follicular aspiration 35 to 38 h after administration of 250 μ g recombinant hCG (Ovitrelle, Serono). Fertilization was assessed 16 to 18 h following routine IVF or intracytoplasmic sperm injection (ICSI) and presence of two pronuclei was recorded. Embryonic cleavage and morphologic appearance were assessed 40 to 44 h and 64 to 68 h after ICSI/IVF. A morphologic score was given for each embryo according to degree of fragmentation: 1, no fragmentation; 2, $<20\%$ fragmentation; 3, 20 % to 50 % fragmentation; and 4, $>50\%$ fragmentation.

ET was performed using the Wallace catheter (Marlow Technologies, Willoughby, OH). The number of embryos to be transferred was decided according to the Israeli Ministry of Health guidelines, i.e., only two embryos for the first three embryo transfers. A third embryo is allowed for women aged >35 years and a fourth for women aged ≥ 40 years or in cases after repeated implantation failure. All patients (in both the intervention and control groups) received the same information regarding the quality and number ET.

Luteal support using micronized progesterone was given to all patients, starting on the day of ET, until serum β hCG measurement 14 days later, and continued until the 8th week of gestation in pregnant patients.

The patients were instructed not to engage in intercourse between 2 days before and 7 days after follicle aspiration.

The flow chart of this study is depicted in Fig. 1.

After obtaining the patients' approval by written consent before OPU, randomization, using computerized random number sequence, was done, selecting the patients for the study or control group. Out of the 230 patients who were asked to give their consent to participate in the study none refused. The randomization was performed in the IVF laboratory by the embryologist, blinded to the treating physician.

SP preparation

Semen samples were obtained from the patient's partner by masturbation, about 30–60 min before follicle aspiration, collected in sterile cups and transferred to the IVF laboratory. Seminal plasma was extracted by centrifugation of ejaculates at $700 \times g$ for 10 min. A second round of centrifugation was performed at $10,000 \times g$ for 10 min to completely extract all spermatozoa. Five hundred μ L of supernatant SP were aspirated in 2-mL syringe.

As placebo, 500 μ L of culture medium was aspirated also in 2-mL syringe. Blinding of the samples was achieved by using a culture medium that was visually indistinguishable from SP.

After oocyte pick-up direct inspection of the vagina using a Cosco speculum was done and bleeding was stopped by local pressure. An insemination catheter was introduced and the 500 μ L of fluid were injected on the cervix and into the vaginal vault containing either partner's SP (study group) or culture medium (control group). The patients as well as the physician performing the insemination were blinded to the content of the syringe. Follicle aspiration and ET were performed by different gynecologists. After injection of the fluid, the patients were transferred to the after-surgery-care

Fig. 1 Study flow chart

unit, for intensive care, being after general anesthesia. They stayed characteristically supine in their own bed for another 30–60 min.

Data concerning patient's background, cycle characteristics, embryo implantation, clinical pregnancy, and pregnancy outcome were analyzed according to patients' age group and the number of embryos transferred.

Clinical pregnancy was defined as a visible intrauterine pregnancy sac by ultrasound at 3 weeks gestational age. Live-birth was defined as a birth of a neonate at 24 weeks gestation and onwards.

Statistical evaluation

Given a two-sided significance level of 0.05 and a difference of 15 % in the clinical pregnancy rate between the two groups, the group size needed for 80 % statistical power was 94 patients in each arm. Statistical analysis was performed using the chi-square test for comparison of the groups' pregnancy rates and other categorical variables, and Student's two-sided *t*-test regarding continuous variables. Implantation rate was calculated by dividing the number of gestational sacs with the number of embryos transferred. The multivariable logistic regression model was used to adjust the pregnancy rates for prognostic factor imbalances. The multivariate logistic regression analysis used occurrence of clinical pregnancy as the dependent variable and investigated whether parameters such as age, type of infertility, number of oocytes retrieved, fertilized, number of embryos cleaved, transferred, study group or control were statistically linked to this variable. A $p < 0.05$ was considered statistically significant.

Results

Out of 230 patients included in this study, 124 were in the study group and 106 were the controls. Comparing the study group to controls, no significant differences were found regarding age, infertility factor (male factor 59 %, tubal 17 %, endometriosis 2 %, unexplained 22 %), length of infertility, grade (primary or secondary), number of previous treatments as well as COH protocol used, mean Estradiol level on the day of hCG administration, mean number of ova retrieved, and mean number of cleaving embryos. In the study and control groups, 3 and 7 patients (respectively) did not reach embryo transfer due to lack of fertilization or embryo cleavage. Comparable mean number of embryos was transferred in both groups, (Table 1).

As presented in Table 2, overall out of 220 embryo transfers (103 and 117 in the study and control groups respectively), 72 clinical pregnancies were achieved (32.7 %).

The clinical pregnancy rate was 36.9 % and 29.1 % for the study and control groups, respectively ($p = \text{ns}$). The difference

Table 1 Patients characteristics

	Control (mean $\pm$ SD)	SP (mean $\pm$ SD)	<i>p</i>
Age	32.0 $\pm$ 5.7	31.22 $\pm$ 5.3	0.40
Length of infertility	2.7 $\pm$ 1.7	2.5 $\pm$ 1.8	0.71
# of IVF Treatment	3.0 $\pm$ 2.3	3.1 $\pm$ 2.8	0.52
E2 (pg/ml) on the day of hCG administration	2372 $\pm$ 1407	2484 $\pm$ 1351	0.74
# of ova retrieved	11.7 $\pm$ 7.2	12.23 $\pm$ 6.9	0.34
# of cleaving embryos	6.2 $\pm$ 4.5	6.6 $\pm$ 4.4	0.63
#of embryos transferred	2.0 $\pm$ 0.5	2.1 $\pm$ 0.6	0.09

was 7 % corresponding to a relative increase of 26.8 % (odds ratio, 1.72; 95 % confidence interval, 0.8–3.6). Transfer of 224 and 248 embryos in the study and control groups, resulted in an implantation rate of 21.4 % and 16.9 % respectively ($p = \text{ns}$). After an early pregnancy loss of 13.1 % (5/38) and 23.5 % (8/34) in the study and control groups respectively an ongoing pregnancy rate of 32.0 % (33/103) and 22.2 % (26/117) was achieved. The difference was 9.8 % corresponding to a relative increase of 44.1 %.

Multivariate logistic regression analysis adjusted for study group, age, infertility, and cycle characteristics did not demonstrate any parameter that could predict occurrence of clinical pregnancy rates after IVF-ET.

Discussion

Currently many observations indicate that seminal fluid may be considered not just a survival medium for sperm, but as a mean for communication between the male and female reproductive tissues and a key factor in conditioning the female tract for pregnancy.

Indeed a growing body of clinical evidence as well as basic research on human and non-human endometrium suggests that SP may play a role in human implantation. Specifically studies in mice [1, 2] and pigs [3] found SP to be essential for implantation, interacts with uterine epithelial cells and induces a postmating inflammatory cascade. Several inflammation-inducing proteins present in SP are transforming

Table 2 Clinical outcome

	Control	SP	<i>p</i>
Clinical pregnancy rate	34/117=29.1 %	38/103=36.9 %	0.21
Early spontaneous abortion	8/34=23.5 %	5/38=13.1 %	0.25
Ongoing pregnancy rate	26/117=22.2 %	33/103=32.0 %	0.10
Implantation rate	42/248=16.9 %	48/224=21.4 %	0.71

growth factor b, [1, 4], prostaglandin E and interleukin- 8 [7]. Transforming growth factor b triggers a surge in synthesis of cytokines such as granulocyte macrophage-colony stimulating factor and interleukin-6. These pro-inflammatory cytokines trigger the recruitment and activation of inflammatory cells in endometrial stroma, including macrophages and granulocytes, activating maternal immune activity to accept the invading embryo. To prevent the conceptus from maternal immune attack a state of active tolerance mediated by T regulatory (Treg) cells must be functional from the time of embryo implantation. According to Robertson et al., [14] seminal fluid and ovarian steroid hormones play an important role in regulating the size and suppressive function of the T regulatory (Treg) cell pool during the peri-implantation phase. At coitus, seminal fluid delivers male alloantigen, TGF β and PGE₂, causing recruitment and maturation of tolerogenic dendritic cells (DCs) into the uterine tissue, and driving further expansion of subsets of antigen-reactive Treg cells in the lymph nodes draining the uterus. In a recent study by Sharkey et al. [15] it was demonstrated that seminal fluid introduced at intercourse elicits expression of proinflammatory cytokines and chemokines, and a robust recruitment of macrophages, dendritic cells, and memory T cells. They concluded that the leukocyte and cytokine environment induced in the cervix by seminal fluid appears competent to initiate adaptations in the female immune response that promote fertility.

In human in-vitro studies SP was shown stimulating those factors such as leukemia inhibitory factor and interleukin-6 (IL-6) [4, 5] that have been described to play an important role in human implantation. Furthermore, SP has been shown to stimulate or inhibit in Ishikawa cells, in which many of those factors that are down-regulated or up-regulated, respectively, in patients undergoing stimulation with gonadotropins for IVF or ICSI [6]. In a recent study Doyle et al. [16] demonstrated that SP enhances and accelerates P₄-mediated decidualisation of human endometrial stromal cells (ESC) and may enhance endometrial receptivity. Combined SP and P₄ treatment over the course of 27 days induced endometrial stromal cells morphological differentiation, and significantly upregulated mRNA levels of the decidua-specific markers prolactin (PRL) and insulin-like growth factor binding protein 1 (IGFBP1), significantly increased PRL protein secretion and IGFBP1 secretion.

Endometrial priming by SP would not be dependent on sperm quality because no differences in cytokine and growth-factor concentrations, nor in the immunomodulatory potential of the SP of men with normal and abnormal sperm counts, were demonstrated [5]. However this issue is still with no closure due to differing findings by others [15, 17].

It is important to mention that physiologically not only spermatozoa but also SP is transported passively through the cervix and reached the uterine cavity as a result of subendometrial and myometrial peristaltic waves (Leyendecker et al. [18] and Kunz

et al. [19]), where they may interact with endometrial components.

However, in patients undergoing IVF or ICSI, SP and spermatozoa do not come into contact with the uterine cavity. The embryos are transferred without any SP and spermatozoa, and patients usually abstain from sexual intercourse several days before and after oocyte retrieval. Moreover, COH for IVF may cause suboptimal endometrial function, caused by dysregulated expression of >300 genes [20].

In theory, stimulation of endometrium by transcervically applied protein mixtures such as seminal fluid could serve as a means to improve uterine receptivity. Indeed a few human clinical studies that used variable methodologies showed a beneficial effect of SP as an adjunctory factor to increase implantation and clinical pregnancy rates after IVF. Bellingue et al. [8], deposited semen in the vagina of patients undergoing IVF treatment at the time of oocyte fertilization and found an implantation rate of 52.6 % (30/57), compared with 23.2 % (13/56) in the control group ($p < 0.05$). Coulam et al. [9] performed a placebo-controlled clinical trial, depositing vaginal capsules containing SP or placebo. They described an implantation rate of 80 % (35/44) in patients in the SP group, compared with one of 67 % (29/43) in the placebo group. Tremellen et al. [10] randomized patients during 478 cycles of IVF (including transfer of 1343 embryos) either to abstain from or engage in vaginal intercourse and found similar pregnancy rates (21.2 % vs 23.6 %, respectively) but a significantly higher proportion of viable embryos at 6–8 weeks of pregnancy in the group engaging in vaginal intercourse (11 % vs 7.7 %). Finally, von Wolff et al., [11] performed a pilot study, injecting cryopreserved seminal plasma from the patient's partner or sodium chloride (placebo) into the cervix and the posterior fornix of the vagina just after follicle aspiration. The clinical-pregnancy rate was 25.7 % (18/70) in the placebo group. The clinical-pregnancy rate in the seminal plasma group reached 37.3 % (25/67), corresponding to a relative increase of 45 %. Even though significance was not reached in this pilot study, possibly due to the small size of the groups, the data suggest that seminal plasma has the potential to improve the pregnancy rate.

Based on the above cited cell culture experiments and the few clinical studies, we hypothesized that the local application of SP into the vagina and the cervix may be able to prime the endometrium at the time of follicle aspiration and thereby could increase pregnancy rates following embryo transfer. Indeed in our prospective double blinded study patients who underwent SP intravaginal insemination after oocyte pick-up reached higher implantation and clinical pregnancy rates following ET compared to controls, with a relative difference of 44 %, however the difference did not reach statistical significance. Thus our findings corroborate the observations by von Wolff et al., [11] cited above.

We admit several limitations in our study. First, the size of the groups in each arm of the study was sufficient to detect a

difference of 15 % in the clinical pregnancy or ongoing/live birth rates but the study is underpowered to examine a smaller difference. Second, no “dose finding studies” concerning SP injection are available, therefore a single injection of the seminal fluid from one ejaculate may be insufficient in the human to inflict a significant change in the endometrial receptivity. Moreover the potency of variable men’s SP in stimulating endometrial receptivity may be substantial. Third, the methodology of injection of the seminal fluid into the patient’s vaginal vault may not be optimal to achieve the maximal effect and different methodology of the SP administration such as intracervical or intrauterine may demonstrate a more significant improvement of implantation and conception rates.

It seems that the advice to refrain from intercourse after oocyte pick up, as advocated in some units, should be questioned.

In summary, patients who underwent SP intravaginal insemination after oocyte pick-up reached higher implantation and clinical pregnancy rates following ET compared to controls, although the difference did not reach statistical significance. However more studies are needed to clarify the potential clinical effect of SP in improving live birth rates after ART and to add relevant information to help policy making about whether patients should or should not refrain from intercourse during IVF-ET.

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