Does the absence or presence of seminal fluid matter in patients undergoing ovulation induction with intrauterine insemination?

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Sperm preparations for intrauterine insemination (IUI) generally do not include seminal fluid, and it is not known whether the absence of this component affects pregnancy rates. Therefore we evaluated the effect of high intravaginal seminal fluid deposition on clinical pregnancy rates in patients undergoing ovulation induction and IUI therapy. A prospective, randomized, double-blind study was designed for an infertile population in a university-based infertility practice. Patients were randomized to receive high vaginal deposition of either seminal fluid separated from the husband's ejaculate (study group) or normal saline solution (control group). Intercourse was restricted. A comparison of clinical pregnancy rates per cycle between study and control groups showed no significant difference between them (22/164 (13.4%) and 19/155 (12.3%) respectively). Further, in non-participants with unregulated intercourse, the pregnancy rate per cycle was not significantly different (40/307; 13.0%). Miscarriage rates between the study and control groups were similar. As high intravaginal deposition of seminal fluid at the time of IUI does not improve the clinical pregnancy rate in patients undergoing ovulation induction and IUI therapy, our study suggests that, after ejaculation, clinically significant biological contributions of seminal fluid to the achievement of pregnancy are bypassed by well-timed IUI.

Key words: gonadotrophins/insemination/ovulation/semen/ seminal fluid

Introduction

Current methods effectively separate seminal fluid from the inseminate in the preparation of semen for in-vitro fertilization (IVF; Levay et al., 1995). Such sperm preparations are also used for intrauterine insemination (IUI). Separated spermatozoa are resuspended in appropriate medium for the IUI procedure, while seminal fluid is discarded. It is not known to what degree the absence of the seminal fluid component in IUI therapy affects the chances of conception and the establishment of pregnancy. For this purpose, we designed a prospective, randomized, double-blind study to investigate whether the presence or absence of seminal fluid at the time of IUI significantly influences the pregnancy rate.

Materials and methods

Between December 1992 and October 1994, a prospective, randomized, double-blind study was undertaken on infertile patients undergoing ovulation induction followed by IUI. This study was approved by the Institutional Review Board (University of Medicine and Dentistry of New Jersey — Robert Wood Johnson Medical School, New Brunswick, NJ, USA). All patients had a basic work-up, with testing of the ovulatory status, the documentation of tubal patency by a hysterosalpingogram, a semen analysis of the partner and the correction of tubal or peritoneal problems when applicable. All patients were advised to refrain from intercourse once human chorionic gonadotrophin (HCG) was scheduled. To participate in the study, patients also had to agree to refrain from intercourse for at least 48 h following their insemination. A total of 218 patients undergoing 319 treatment cycles were enrolled. During this time, another 307 treatment cycles were performed in 206 patients who did not enrol, largely because patients indicated a preference for intercourse following IUI. Briefly, ovulation induction treatments consisted of clomiphene citrate (50 mg/day orally) on cycle days 3–7, followed by 150 IU i.m. human menopausal gonadotrophin (HMG; Pergonal/Metrodin; Serono Laboratories, Norwell, MA, USA), or 150 IU HMG i.m. alone beginning from cycle day 3 onwards. The response to treatment was monitored using the determination of serum oestradiol concentrations and transvaginal follicle sonography. Dosages were individualized based on the responses of the patients. When one or more follicles measuring ≥18 mm in diameter with an appropriate serum oestradiol concentration were present, patients received 10 000 IU i.m. HCG (Profasi; Serono Laboratories) followed by a single IUI 36 h later. The IUI inseminate of 0.5 ml was placed in a high fundal position by using a Shepard cannula (Cook Corporation, Bloomington, IN, USA). The cycles were prospectively randomized by a randomization table to utilize the intravaginal deposition of either seminal fluid separated from the husband's ejaculate or normal saline solution. The randomization table was maintained by the laboratory, and the physician performing the IUI was not informed of the contents of the specimen. The fluid was injected into the posterior fornix of the vagina immediately after the IUI, and maintained by a vaginal sponge (Milex Products, Chicago, IL, USA) for 4–6 h. This sponge was covered by a plastic sheath to preclude sperm absorption and was used to prevent transvaginal semen loss.

For the purpose of this study, all semen specimens were processed by density gradient centrifugation. A Percoll preparation (Pharmacia Biotech, Inc., Piscataway, NJ, USA) consisting of three 1 ml layers of 92, 70 and 50% Percoll solution was used to separate seminal fluid from the spermatozoa to be used for IUI. Semen specimens were overlaid on top of the 50% layer, and the column was centrifuged at 250 g for 30 min at room temperature. The resultant sperm pellet was aspirated and washed with 2 ml human tubal fluid medium (HTF; Irvine Scientific, Santa Ana, CA, USA) containing 0.5% human...
serum albumin (HSA; Baxter Healthcare Corp., Glendale, CA, USA). This pellet was then centrifuged at 190 g for 8 min at room temperature. After removal of the supernatant, the sperm pellet was then resuspended in 0.5 ml HTF medium with 0.5% HSA to be used for IUI. In the study group, the top layer of seminal fluid was aspirated off and transferred into microfuge tubes, and then centrifuged for 8 min in a Beckman microfuge E to remove the remaining spermatozoa. A maximum of 2 ml of this seminal fluid was then collected in a sterile syringe to be used for vaginal insemination. Control patients received 2 ml of 0.9% sodium chloride solution (McGraw, Inc., Irvine, CA, USA). In addition, those patients who did not participate in the study were evaluated in terms of pregnancy achievement.

A clinical pregnancy was defined as sonographic evidence of a gestational sac. Clinical pregnancy rates per cycle were compared in patients receiving seminal fluid versus saline. Pregnancy outcome was followed to ≥24 weeks of gestation in all cases. In addition, factors including maternal age, serum oestradiol concentration and number of follicles ≥18 mm in mean diameter prior to HCG administration, as well as total number of motile spermatozoa inseminated, were compared between the two groups.

A statistical analysis was performed using a χ² and Fisher’s exact test for the comparison of pregnancy rates. The effects of seminal fluid versus saline were analysed using contingency methods to combine 2×2 tables. The Mantel–Haenszel method was employed to assess the heterogeneity of risk. Patient factors were calculated as means ± SD, and compared using analysis of variance statistics. A P value <0.05 was considered significant, and a power statistic was calculated for the results obtained.

**Results**

A total of 319 cycles in 218 patients were randomized in the study. In 164 treatment cycles (130 patients) seminal fluid was applied intravaginally, and in 155 control cycles (127 patients) normal saline solution was applied; 39 patients received both forms of therapy. Of the 319 cycles, 257 were clomiphene/HMG treatment cycles (in 176 patients) and 62 were HMG-alone treatment cycles (in 44 patients); two patients received both treatment regimens.

In using contingency methods to combine 2×2 tables, the application of intravaginal seminal fluid was not found to yield a superior pregnancy rate to saline [odds ratio 1.11; 95% confidence interval (CI) 0.57–2.14; Table I]. In addition, using the Mantel–Haenszel method of calculating a χ² statistic for the different forms of ovulation induction therapy, no statistically significant heterogeneity of risk was found across the strata (clomiphene–HMG versus HMG alone; χ² = 0.07). In non-participants, the cycle pregnancy rate was 13.0% (40/307). This was not significantly different from the cycle pregnancy rates for study patients with respect to the use of seminal fluid, saline or total participants. Assuming an α (type I) error value of 0.05 (with a 95% CI), this study had a power of 80% to detect a 12.7% increase in the pregnancy rate for exposed (seminal fluid) versus non-exposed (saline) cycles.

Table II gives a breakdown of patient factors with respect to treatment cycles utilizing seminal fluid versus saline. There was no significant difference between the two groups with respect to patient age, serum oestradiol concentration prior to HCG administration, the number of follicles ≥18 mm in

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<td>Triplets</td>
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Odds ratio for first trimester loss: 1.52 (95% confidence interval 0.34–6.74). *Triplet pregnancy lost after amniocentesis. *Spontaneous absorption of one twin.

mean diameter or the total number of motile spermatozoa inseminated.

Table III examines pregnancy outcome up to 24 weeks. When analysing the potential impact of the presence of intravaginal fluid at the time of IUI on pregnancy outcome, no improvement in the rate of ongoing viable pregnancies was noted in comparison with saline: odds ratio 1.52 (95% CI 0.34–6.74). There was no statistically significant heterogeneity of risk across strata (miscarriages, ectopies, singletons, twins, triplets; χ² = 2.12). The viable pregnancy rate at 24 weeks for the study group was 17/21 (81%; one pregnancy was lost after amniocentesis), compared with 14/19 (73.7%) for the control group.

**Discussion**

Human seminal plasma is recognized in its role as a vehicle for spermatozoa in the reproductive process (Kanwar et al., 1979). Further, it has been found to contain various substances
or factors which may ultimately affect sperm function (Kanwar et al., 1979). Levay et al. (1995) recently summarized the numerous effects of seminal fluid which could affect fertilization positively or negatively. It has been further suggested that seminal fluid contains factors that protect spermatozoa from the immune system (Kelly, 1995; Wolff, 1995).

The IUI preparation encompasses the separation of a subpopulation of spermatozoa from seminal fluid and residual spermatozoa. Several methods have been shown to effectively remove seminal plasma from the inseminate, including the Percoll gradient method utilized in this study (Levay et al., 1995). After the motile spermatozoa are removed, the remaining seminal fluid is ordinarily discarded, because its introduction into the uterine environment is undesirable. Yet its elimination from the reproductive environment may also deprive the patient of possible benefits in terms of pregnancy establishment. However, our findings demonstrate no significant difference in the pregnancy rates with or without the presence of intravaginal seminal fluid in patients undergoing ovulation induction with IUI. This suggests that seminal fluid functions in terms of transport, sperm enhancement and capacitation are bypassed or supplemented by IUI.

The IVF experience has already established that the presence of seminal fluid is not essential for the establishment of pregnancy when fertilization takes place outside the body. This study extends this observation to natural fertilization. Furthermore, it does not support the notion that the presence of intravaginal seminal fluid at the time of ovulation enhances pregnancy establishment when IUI is utilized.

Our study relies on patient compliance with regards to intercourse instructions. Therefore, patients expressing any possible interest in having intercourse at the time of ovulation were excluded from participation. Interestingly, the cycle pregnancy rate of non-participants (13.0%) did not differ significantly from that of study patients who received saline (12.3%) or those who received seminal fluid (13.4%). As we can assume that the majority of these external control patients had intercourse along with their IUI, this observation seems to refute further the theoretical benefits of seminal plasma, and perhaps even intercourse, with respect to conception in ovulation induction— IUI cycles.

While no studies seem to have investigated the effect of seminal fluid in patients with IUI, investigations have been initiated to assess the potential effect of intravaginal semen (not seminal fluid) in the IVF scenario, specifically with respect to fertilization and implantation. In a prospective trial, Bellinge et al. (1986) demonstrated an improved implantation rate with high vaginal deposition of a portion of their partners’ semen samples compared with controls. However, in a subsequent prospective trial, Fishel et al. (1989) found no significant effect of the use of high vaginal insemination at the time of oocyte recovery in patients undergoing IVF. Neither of these two reports utilized true randomization methods to assign treatment—control protocols.

Recently Coulam and Stern (1995) suggested that higher implantation rates were obtained in a group of women experiencing infertility and/or recurrent spontaneous abortion who received vaginal capsules of seminal plasma versus placebo (80 versus 67%); however this difference was not significant. The same group reported that in women experiencing a history of recurrent spontaneous abortion, seminal plasma enhanced the probability of live birth by 21% (Stern et al., 1992). In our study the viability was higher in the study group (81% vs 74%), but this difference was not statistically significant. Further investigations are needed to examine the hypothesis that seminal fluid affects successful pregnancy maintenance.

In summary, the high intravaginal deposition of seminal fluid does not improve the pregnancy rate in patients undergoing ovulation induction— IUI therapy. While seminal fluid serves as a medium for assisting sperm transport during intercourse, and may enhance sperm function and survival, our data did not demonstrate that its presence at the time of IUI enhances the fertility potential in these women. IUI appears to effectively bypass any physiological effects of seminal plasma.

References

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