Sperm pooling and intrauterine tuboperitoneal insemination for mild male factor infertility

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Summary

Purpose of investigation: To evaluate the efficacy of sperm pooling in the treatment of male infertility with the use of intrauterine tuboperitoneal insemination (IUTPI). *Materials and Methods:* A total of 169 cycles of IUTPI were performed in 69 couples with mild male factor infertility. Pooled semen samples were used in 115 cycles (Group A), whereas a single sample was used in 54 (Group B). The same mild ovarian stimulation protocols were used in all cycles. *Results:* The mean inseminate motile count (IMC), following sperm pooling was 6.63 x 10^6 in Group A and 3.74 x 10^6 in Group B ($p = 0.0001$) with a single semen sample. In total, 33 clinical pregnancies were achieved; 28 (24%) in Group A and five (9%) in Group B ($p = 0.036$). *Conclusions:* The results of this study indicate that sperm pooling may prove a useful technique in the treatment of mild male infertility when combined with IUTPI.

Key words: Sperm pooling; Mild male factor infertility; Intrauterine insemination; IUTPI; IMC; Pregnancy rates.

Introduction

Male factor infertility affects approximately 50% of infertile couples [1] either alone, or combined with female infertility. Many hormonal therapies have been proposed, in an attempt to improve the sperm count, such as clomiphene citrate [2, 3], aromatase inhibitors [4, 5], testosterone [6], and follicle stimulating hormone (FSH) [7, 8]. However, the aforementioned therapies have shown to be time-consuming, patient-specific [7, 8], and not entirely effective [9].

Couples suffering with mild male factor infertility are most of the time directly referred for in vitro fertilization (IVF) or intracytoplasmatic sperm injection (ICSI) treatment due to the low success rate of intrauterine insemination (IUI). However, IUI is a less invasive and inexpensive treatment, requiring less hormonal prescriptions, and is regarded as the first line treatment according to WHO guidelines [10]. The success rate of IUI depends on sperm parameters, the woman's age, and the ovarian stimulation protocol used [11].

Regarding sperm parameters, previous studies agree that there is a direct relationship between the number of spermatozoa and IUI success rate; the higher the inseminate motile sperm count (IMC), the higher the success rate of IUI [12], with a threshold level of > 5 x 10^6/ml [11, 13, 14].

The use of more than one sperm sample for IUI, in cases of mild male infertility, was first reported in 1990 [15]. Since then, studies have shown that two sperm samples obtained on the same day or in consecutive days can improve the total motile sperm count and therefore, the outcome of IUI [15-17].

The aim of this study was to evaluate the use of sperm pooling, in couples with mild male factor infertility by combining two semen samples, followed by intrauterine tuboperitoneal Insemination (IUTPI). IUTPI is a novel IUI method that utilizes ten ml of inseminate including the sperm. The method and success rates of IUTPI have been previously described [18].

Materials and Methods

Patient selection

Retrospective analysis was conducted on 169 cycles performed between January 2010 and October 2011. Only couples with sperm concentration between 6 x 10^6/ml and 20 x 10^6/ml were included in the analysis. All female partners had a regular menstrual cycle of 25 – 33 days and had undergone a full diagnostic workup including, hysterosalpingography, prolactin, sex hormone binding globulin, thyroid hormones, and chlamydia screen. On day 2 or 3 of the cycle, transvaginal ultrasound (TVUS) check scan and baseline hormone assays including FSH, luteinising hormone (LH), and estradiol (E2) were performed.

Controlled ovarian stimulation

The female partners underwent the same controlled ovarian stimulation protocol that included, clomiphene citrate from day 2 of the cycle followed by human menopausal gonadotropin (hMG) 150IU from day 6 to day 10 of ovarian stimulation and gonadotropin-releasing hormone (GnRH) antagonists 1.25 mg from day 8 to day 10. This protocol was continued until maturing follicles reached 18 mm in diameter, when 5,000 to 10,000 IU of human chorionic gonadotropin (hCG) were administered. IUTPI was performed 36 – 40 hours after hCG administration, as previously described [18].

Sperm preparation

The male partners of the sperm pooling group (Group A) were asked to provide the first sperm sample (sample 1), by masturbation, on the day of the check scan. Following liquefaction, microscopic examination in the Makler counting chamber was performed. Cryoprotectant was then added slowly at a 1:1 ratio to the ejaculate volume and the sample was kept in room tem-

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perature for ten minutes. It was then aliquoted in vials and kept in liquid nitrogen vapour for 25 minutes before it was plunged and stored in liquid nitrogen dewars (vapour freezing technique).

On the day of the insemination, the desired ampoules were removed from the liquid nitrogen dewars and thawed at room temperature, to be prepared, along with the fresh semen sample (sample 2) provided on the same day. Each sample was gently layered on top of two separate density gradients: a lower gradient of 90% (v/v) and an upper layer of 45% (v/v), in separate centrifuge tubes. The samples were then centrifuged at 1,500 rpm for 20 minutes, the supernatants were discarded, and the pellets that contained the motile spermatozoa from the fresh and thawed samples were combined and re-suspended in culture medium in the same centrifuge tube. A second centrifugation followed at 1,800 rpm for ten minutes and the final pellets, which contained the motile spermatozoa originating from the fresh and the frozen-thawed samples were re-suspended in ten ml of culture medium. The samples were then re-analyzed and loaded into a syringe and the insemination took place.

Male partners in Group B provided a single semen sample on the day of insemination that was processed in the same way as the fresh sample in Group A.

Statistical analysis

Pregnancy rates between Group A and Group B were calculated using the paired chi-square (χ^2) test and the remaining group characteristics with the paired t test.

Results

A total of 169 cycles were included in the study, 115 in Group A and 54 in Group B. The mean age of female partners was 33.68 years in Group A and 34.57 years in Group B. There was no statistical difference in the baseline characteristics of the female partners in the two Groups, apart from the E2 levels, which were higher in Group B (p = 0.0423).

The sperm samples provided by the male partners in both Groups had comparable semen characteristics (Table 1). However, in Group A, the IMC following sperm pooling was 6.63 x 10^6 compared to 3.74 x 10^6 in Group B with a single semen sample, a large difference, which was statistically significant (p = 0.0001) (Table 2).

A total of 33 pregnancies were achieved, 28 in Group A and five in Group B. The difference between the pregnancy rates was statistically significant (p = 0.036). Two twin and one triplet pregnancies, as well as a missed abortion, were recorded in Group A. No ectopic pregnancies were recorded in either of the Groups.

Discussion

Many studies have reported the benefits of using multiple semen samples in couples suffering from mild male factor infertility, undergoing IUI. This study was designed to evaluate the combination of sperm pooling with the use of IUTPI in cases of mild male factor infertility and its effect on pregnancy rates. This retrospective analysis shows that the use of a cryopreserved semen sample along with the fresh sample provided on the day of IUTPI, significantly increases the inseminate motile count and clinical pregnancy rates per cycle.

Table 1. — *Semen characteristics prior to sample preparation.*

	Group A (pooled samples) (n = 115)		Group B (single samples) (n = 54)
Volume (ml)	2.31 (± 0.63)	2.18 (± 0.61)	2.35 (± 0.72)
Concentration (spermatozoa x 10^6/ml)	12.80 (± 2.47)	13.15 (± 2.14)	12.53 (± 1.57)
Total sperm number (spermatozoa per ejaculate x 10^6/ml)	29.60	28.60	29.40
Motility (%)	34.73 (± 2.05)	37.03 (± 1.34)	35.17 (± 2.44)
Degree of rapid forward progression (%)	28.78 (± 1.16)	27.55 (± 1.70)	26.93 (± 1.56)
Morphology	13.88 (± 1.14)	14.56 (± 1.03)	15.52 (± 1.19)

Semen characteristics of the pooled samples consisting in cryopreserved (sample 1) and fresh (sample 2) samples. Their characteristics are indicated separately, along with the single samples. All values are similar between the different groups.

Table 2. — *Cycle characteristics.*

	Group A (n = 115)	Group B (n = 54)	p
Woman's age	33.68 ± 3.72	34.57 ± 3.23	0.1328
Follicles	3.21 ± 0.75	3.39 ± 0.66	0.1330
E2	683.49 ± 184.14	744.72 ± 175.30	0.0423
Endometrial Thickness	8.97 ± 0.79	9.17 ± 0.61	0.1022
IMC	6.63 (± 1.70)	3.74 (± 1.12)	0.0001
Pregnancies*	28	5	0.036

Characteristics of ovarian stimulation of the female partners in each Group, the Inseminate Motile Count (IMC) of the male partners in Group A following sperm pooling, and in Group B with a single semen sample, and showing the number of pregnancies achieved in each group. *Clinical pregnancies were diagnosed ultrasonographically by the presence of a fetal heart beat.

Previous studies, where multiple semen samples had to be provided on the same or consecutive days, showed that multiple samples were very demanding for the male partners. Kücük *et al.* [12] reported that 35 out of 137 couples did not consent to providing two consecutive semen samples on the same day and 11 that did, were finally unable to provide a second sperm sample. The authors believe that sperm pooling with cryopreservation is more friendly and acceptable by the male partners as they are allowed to provide each sample many days apart.

Furthermore, some may argue that cryopreservation of the first semen sample in Group A, may influence semen characteristics following thawing. For this reason, all thawed samples were reviewed again to assess their microscopic characteristics before proceeding to preparation for insemination. No significant changes were recorded in the thawed samples. Recent studies further support that rapid freezing of the semen samples shows better survival characteristics compared to other methods of cryopreservation [19].

To conclude, the authors believe that sperm pooling, followed by IUTPI, is a procedure that results in higher pregnancy rates that can assist many couples who suffer from mild male factor infertility and for personal reasons do not want to proceed with IVF treatment.

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