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ANDROLOGY

Effect of heat-induced hypermotility on pregnancy rate in intrauterine insemination for male factor infertility associated with asthenospermia: a prospective, randomized, controlled study

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Abstract

Objective To assess the effect of mild heat for the enhancement of sperm fertilizing capacity in intrauterine insemination for male factor infertility associated with asthenospermia.

Material and method Prospective, controlled, clinical study. Male factor infertility associated with asthenozoospermia was the criteria for inclusion. Ninety-seven couples were randomized to the study group while 100 couples were randomized to serve as the control group. Semen samples from the study group were processed with Percoll gradient and were left for incubation at 40°C for 2 h. Semen samples from the control group were processed with Percoll gradient method and were incubated at 37°C. Main outcome measure was the pregnancy rate.

Results The mean concentration of total motile sperm (TMS) in the study group was $11.20\pm4.22\times10^6$ (range 7–18) after wash with Percoll while was increased to $62.41\pm12.49\times10^6$ (range 44–71) after heat treatment. The mean concentration of TMS in the control group was $13.90\pm5.66\times10^6$ (range 8–19) after wash with Percoll while was increased to $17.73\pm3.67\times10^6$ (range 14–22) after incubation at 37°C. The difference in TMS concentrations between the study and the control group after incubation at different temperatures was statistically significant ($p<0.005$). There were 24 pregnancies in the study group, providing a pregnancy rate of 24.7%. In the control group eight pregnancies were achieved (8%) ($p=0.001$).

Conclusion Mild heat was found remarkably effective in asthenozoospermic males for increasing the concentration of inseminated total motile sperm and the pregnancy rate correspondingly.

Keywords Heat · Sperm motility · Asthenozoospermia · Intrauterine insemination

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Introduction

As sperm motility is strongly linked to successful fertilization [1], asthenozoospermia is among the causes of male infertility. It is well documented that the human fertilization rate in vitro is directly affected by the severity of oligospermia, which, if associated with asthenozoospermia, in partial or total failure of fertilization can occur [2].

There are numerous publications on the adverse effect of heat associated with varicocele on spermatogenesis. Interestingly, semen out of the testes is not adversely affected by mild heat; on the contrary, heat induced activation of spermatozoa is an established phenomenon [3]. Hyperactive motility in sperm was shown to occur in capacitated state of hamster sperm [4]. Heat enhanced fertility was shown in a previous study [5]. Heat-induced hyperactive motility was significantly higher in sperm of the male partner of pregnant patients compared with nonpregnant patients after test yolk buffer processing followed by 4 h of incubation at 40°C. This was also observed after colloid (Percoll) processing.

Data on heat induced sperm hyperactivation and pregnancy rate is scarce, only in two clinical studies were published by the date [4, 5]. We conducted a study to assess the effect of mild heat on the pregnancy rate in intrauterine insemination for male factor infertility associated with asthenospermia.

Material and method

The study was conducted between 2002 and 2007 in a private setting. Local ethic committee approval was obtained prior to the study and all couples consented for the study. All couples had undergone a diagnostic work-up including hysterosalpingography, baseline levels of FSH, LH, estradiol, TSH, T3, T4, prolactin, midluteal progesterone, transvaginal ultrasonography. Male infertility was diagnosed when sperm abnormalities according to WHO criteria were seen in at least two semen samples. Motility assessments were done after wash with Percoll. The definition of asthenospermia in this study was the observation of less than 50% progressive motility and a total motile sperm count below 20×10^6 after wash with Percoll. Sperm viability was assessed with hypo-osmotic swelling (HOS) test. Types of male factor infertility other than asthenospermia, and any female factor for infertility were criteria for exclusion. One hundred ninety-seven couples with male factor infertility associated with asthenospermia were included in the study. Randomization was done by the first author using a computer generated random number table prior to the recruitment of the couples. Ninety-seven couples were randomized to the study group while 100 couples were randomized to serve as the control group.

The female partners underwent controlled ovarian stimulation protocol with recombinant FSH (Gonal-F, Serono, Switzerland). 75 IU of recFSH was started on day 2 of menstruation. Same dose was continued for 4 days; for day 5 and onwards FSH dose was individualized according to follicular measurement on transvaginal ultrasonography and serum estradiol level. RecFSH administration was continued until the dominant follicle exceeds 17 mm in diameter. Then, 10,000 IU hCG (Profasi, Serono, Switzerland) was injected. Inseminations were scheduled to between 36 and 38 h after hCG administration.

Semen samples were obtained with masturbation after 3–7 days of sexual abstinence. For standard preparation of semen samples, a washing procedure was performed following the liquifaction of semen. Then, followed by centrifugation of the pellet on 40% Percoll gradient at 600 *g* for 20 min. Samples were left for incubation at 37°C. For the study group, semen sample was layered on Percoll gradient and centrifuged. Then the sample left for incubation at 40°C for 2 h. The pellet was resuspended in 0.3 mL of washing medium (Irvine Scientific, CA, USA), re-evaluated and inseminated.

Insemination was performed with a catheter (IUI, CCD Laboratories, Paris, France). No mucus cleaning was done. Intrauterine inseminations were performed at dorsal litotomy position. Patients were kept in rest for 30 min. No luteal support was given. Couples were advised to refrain from sexual intercourse until the next visit.

The main outcome measure was the pregnancy rate indicated by elevated β-hCG level (>10 mIU/mL) on the first or second day of missed menstruation. Comparison between the pregnancy rates was made by using paired χ^2 test in SPSS (SPSS Inc, Chicago, USA). Comparison of total motile spermatozoa counts was made by using student's *t*-test.

Results

In the study group, the mean duration of infertility was 34.1 months (range 16–60); the mean age of male partner was 29.3 years (range 23–42) and was 25.4 years (range 20–30) for female partner. In the control group, the mean duration of infertility was 29.9 months (range 13–62); the mean age of male partner was 28.6 years (range 23–40) and was 21.8 years (range 20–33) for female partner. Vitality by HOS test was 78±8% (range 69–89) in the study group, while was 75±6% (range 67–85) in the control group. There was no statistically significant difference in any of the baseline characteristics (Table 1).

The mean concentration of total motile sperm (TMS) after wash with Percoll in the study group was $11.20\pm4.22\times10^6$ (range 7–18) while was increased to $62.41\pm12.49\times10$ (range 44–71) after heat treatment. The mean concentration of total motile sperm in the control group was $13.90\pm5.66\times10^6$ (range 8–19) after wash with Percoll while was increased to $17.73\pm3.67\times10^6$ (range 14–22) after incubation at 37°C. The difference in TMS concentrations between the study and the control group after incubation at different temperatures was statistically significant ($p<0.005$).

There were 24 pregnancies in the study group, providing a pregnancy rate of 24.7%. In the control group eight pregnancies were achieved (8%). The difference between the pregnancy rates was statistically significant ($p=0.001$). There was two abortions in the study group and one abortion in the control group. There were three set of twins in the study group while no multiple pregnancy was seen in the control group. Overall, 25 babies were born in the study group and seven babies were born in the control group. There was a congenital bilateral inguinal hernia in one of the male twins in the study group (Table 2).

Table 1 Baseline characteristics of the couples

Characteristic	Study group *n*:97	Control group *n*:100	*p*
Mean age of male partner (years)	29.3±4.2 (23–42)	28.6±6.1 (23–40)	NS
Mean age of female partner (years)	25.4±5.1 (20–30)	21.8 (20–33)	NS
Duration of infertility (months)	34.1±8.2 (16–60)	29.9±6.6 (13–62)	NS
HOS test (%)	78±8 (69–89)	75±6 (67–85)	NS
Mean TMS count after wash[a] ($\times 10^6$)	11.20±4.22 (7–18)	13.90±5.66 (8–19)	NS

Comparisons were made by using χ^2 test
TMS Total motile sperm, *HOS* hypo-osmotic swelling
[a] Percoll

Discussion

In the present study, we tested the efficiency of mild heat for sperm motility enhancement in IUI for the treatment in male factor infertility associated with asthenozoospermia. IUI associated with controlled ovarian stimulation is a simple and inexpensive treatment for infertility. The pregnancy rates per cycle vary between 4% and 40% [6, 7]. Majority of the studies assessing the effect of sperm parameters on pregnancy rate of IUI have reported that male factor infertility have low pregnancy rates with IUI [8, 9]. Previous studies showed increasing conception rates with increasing inseminating motile count [10, 11]. Before proceeding to more expensive and more invasive methods like in vitro fertilization, IUI with heat induced sperm motility enhancement method provided 24.7% pregnancy rate in our hands which was far better than that of conventional Percoll sperm preparation for IUI. The expected rate of pregnancy for IUI was around 12% in our center. Lower (8%) rate of pregnancy in the control group indicates the insufficiency of sole sperm preparation for IUI in infertility associated with asthenospermia.

Hyperactivation is a movement pattern observed in spermatozoa at the site and time of fertilization. Hyperactivated movement of spermatozoa was first reported by Yanagimachi [12]. Yanagimachi's proposal that hyperactivated motility enables spermatozoa to penetrate zona pellucida was later confirmed by other studies. Strauss et al used various methods that prevented hyperactivation but did not inhibit the acrosome reaction, and showed that hyperactivated hamster spermatozoa were more successful at penetrating the zona pellucida of oocytes in vitro than spermatozoa that were not hyperactivated [13].

Heat-induced hyperactivation was previously tested in sperm after colloid or pentoxiphylline wash methods [14]. The percentage of sperm hyperactive motility was reported as five times greater after 40°C heat treatment than with the 37°C, for both the colloid and the pentoxiphylline-washed sperm. Miller et al have found that total motility and progression were also improved by heat treatment; it has been suggested that heat might have triggered the release of sperm motility factors like heat shock proteins. Heat treatment was shown to be effective if applied for 4 h. We applied the heat for 2 h, it was as effective as 4 h application.

Heat was shown to be detrimental to germ cells during their genesis in testicles [15]. Miller et al have analysed of a sentinel gene to determine whether mild heat had an effect on DNA [14]. They have found no change on sperm DNA. In the current study, we have found no congenital abnormality other than single case of bilateral inguinal hernia among the babies born after the treatment in whom karyogram was found normal.

Current study showed a significant improvement in the pregnancy rate of the study group whose semen was treated with mild heat. The sperm was returned to 36.5–37°C after being inseminated. The sperm velocity is 1 cm per minute, hyperactivated spermatozoa must be swimming much faster than that. The inseminated aliquot of sperm must have reached the oocyte in minutes, while still having the capacitated state induced by heat treatment.

Table 2 Results

Characteristic	Study group *n*:97	Control group *n*:100	*p*
Mean post-treatment TMS count ($\times 10^6$)	62.41±12.49 (44–71)	17.73±3.67 (14–22)	<0.005
Pregnancy	24 (24.7%)	8 (8%)	0.001
Abortion	2	1	NS
Multiple pregnancy	3	0	NA

Comparisons were made by using χ^2 test
TMS Total motile sperm

In conclusion, we have shown that mild heat was remarkably effective in asthenozoospermic males for increasing the concentration of inseminated total motile sperm and the pregnancy rate correspondingly. Future modifications of the technique can produce much better results in asthenozoospermy and possibly in other subgroups of patients. Safety issue must be powered by broader studies.

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