

# Pregnancy Outcome with Intrauterine Insemination in Patients with Unexplained Recurrent Abortion Whose Partners Have Abnormal Hypo-Osmotic Swelling Test

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## Abstract

**Background:** Recurrent abortion is defined as three or more consecutive pregnancy losses. We aimed to determine the relationship between male sperm parameters and hypo-osmotic swelling score and recurrent abortion. We also studied whether washing of spermatozoa and intrauterine insemination in patients with recurrent miscarriage and low score hypo-osmotic swelling test in male partners could improve the outcome of pregnancy.

**Methods:** Between February 2003 and September 2006 in gynecology clinics affiliated to Shiraz University of Medical Sciences, 176 women with a history of recurrent abortion and their male partners were selected as study group. And 159 healthy and fertile couples without a history of recurrent abortion were selected as control group. The relationship between male sperm parameters and hypo-osmotic swelling test and recurrent spontaneous abortion in their female partners was evaluated after intrauterine insemination for those with abnormal hypo-osmotic swelling test. Outcome of pregnancy in patients with recurrent abortion whose male partners had low score hypo-osmotic swelling test was evaluated after intrauterine insemination.

**Results:** Low scores of hypo-osmotic swelling test were more frequently seen in the study group than the controls: 116 (65%) versus 24 (15%). The mean hypo-osmotic swelling score was significantly lower in the study group ( $P < 0.001$ ). The outcome of pregnancy improved after intrauterine insemination in those with low score hypo-osmotic swelling test. The pregnancy success rate in the treated group (pregnant with intrauterine insemination) was 77.77% while in the untreated group (pregnant without intrauterine insemination) was 30.76% with a success rate ratio of 2.04.

**Conclusion:** There was a positive relationship between low hypo-osmotic swelling test score in male partners and recurrent abortion in their wives. Intrauterine insemination improved the outcome of pregnancy in these couples.

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**Keywords** • Recurrent abortion • intrauterine • insemination

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## Introduction

**S**pontaneous miscarriage is the most important complication of pregnancy, affecting 15% to 20% of pregnancies. The prevalence of recurrent miscarriage, defined as three or more consecutive pregnancy losses, may be as high as 3% with unexplained etiology in half of the patients.<sup>1-3</sup> The contribution of abnormalities in male partners of women with recurrent miscarriages is unexplained, although abnormal sperm morphology has been associated with increased miscarriage rates in patients undergoing in vitro fertilization and embryo transfer.<sup>4,5</sup> The hypo-osmotic swelling test (HOST) assesses the functional integrity of the sperm membrane and the hypo-osmotic swelling score is the proportion of sperms with functionally intact membranes. It has been reported that a score < 60% is suboptimal.<sup>1,5</sup> Recent studies have indicated that a low hypo-osmotic swelling score correlates with recurrent miscarriage.<sup>1</sup>

On the other hand, some investigators showed that sperm washing by centrifugation alters the HOST result, whereas the swim-up method selects a population of spermatozoa with intact head and tail membranes.<sup>6</sup> The aims of the present study were to find the relationship between male sperm parameters and hypo-osmotic swelling score and recurrent abortion, and to find whether washing of spermatozoa and intrauterine insemination in women with recurrent miscarriage whose male partners have low score hypo-osmotic swelling tests could improve the outcome of pregnancy.

## Patients and Methods

Between February 2003 and September 2006, all of the patients referred to gynecology clinics affiliated to Shiraz University of Medical Sciences were evaluated. A diagnosis of unexplained recurrent abortion was made when no abnormalities were detected in:

1. Peripheral blood karyotype of the couples
2. Hysterosalpingogram
3. Serum levels of TSH, T4 and Prolactin in women
4. Anticardiolipin Antibody titer and lupus anticoagulant (measured by partial thromboplastin time)
5. Thrombophilia test (protein C & S panel, homocysteine and factor V Leiden)

According to previous studies, the incidence of abnormal HOST in the male partners of women with recurrent abortion is 3% - 9%. Therefore the measured sample size would be

178 patients who met the above criteria ( $\alpha=0.05$  and Power 80%). Their ages ranged from 22-35 years with 3-9 previous abortions. Semen samples from their male partners were taken for analysis and HOST. One hundred fifty nine consecutive healthy and fertile couples without any history of abortion were selected and asked for taking part voluntarily by giving sperm samples as control group. Informed consents were taken from all the participants. All demographic, clinical, and paraclinical data including husbands' semen analyses were gathered and recorded. Semen analysis was performed using the standard protocol,<sup>7</sup> and in controlled conditions including sexual abstinence for at least 4 days. The analysis performed during 4 hours after sampling. Ejaculate volume, sperm count, motility, and morphological alternations were the four investigated parameters.

The sperms underwent hypo-osmotic swelling test by mixing 0.1 ml of semen and 1 ml of a hypo-osmotic solution (150 mOs) prepared by dissolving 7.35 g of sodium citrate and 13.5 g of fructose in 1000 ml of distilled water. The mixture was incubated for 60 minutes at 37 °C in 95% air, 5% carbon dioxide (CO<sub>2</sub>). Then 0.2 ml of the mixture was placed on a slide and mounted with a cover slip and examined immediately at a magnification of 40 under a phase contrast microscope.

The percentages of hypo-osmotic swelling reacted sperms (with curled and swollen tails) and non-reacted sperms (with straight tails) were assessed by counting multiples of 100 sperms. All of the tests were performed by a single person.

In the second step of the study, 116 women whose male partners had low scores hypo-osmotic swelling test were divided into two groups. Fifty one women underwent superovulation with clomiphene plus human menopausal gonadotropin (hMG) and after maturation of the follicles which were monitored by ultrasound, human chorionic gonadotropin (hCG) was administered. After 34 hours, sperm washing was performed by Ham's F10 (Sigma-Aldrich, USA) and the selected swim-up spermatozoa were used for intrauterine insemination (IUI, treated group). IUI was repeated up to six cycles until they conceived.

The remaining 65 patients (untreated group) were permitted to conceive spontaneously without IUI.

None of the patients in the treated and untreated groups had infertility problems and they were primary aborters (no live birth in the past). Continuation of pregnancy after 20 weeks of gestation was considered as a successful outcome, because the risk of abortions ends at this time.

Data were analyzed using SPSS software version 11. Chi square test was used for analysis of the correlations.

**Results**

The result of the first step of the study showed that the men's mean age in the study group was  $29.7 \pm 3.5$  years (with a range of 25-55 years). The mean age of the control group was  $31.3 \pm 5.3$  year (with a range of 26 to 50 years). The mean ages of husbands and wives were not statistically different in both groups ( $P=0.2$ ). And also the number of previous abortions was matched in both treated and untreated groups.

The results of semen analysis in both groups showed that there were no differences in terms of mean ejaculate volume ( $P=0.1$ ), sperm count ( $P=0.2$ ), sperm motility ( $P=0.07$ ) and sperm normal morphology ( $P=0.06$ ) between the groups (table 1). However the low scores of HOST occurred more frequently in the study group than the controls: 116 (65%) versus 24 (15%). The mean of hypo-osmotic

swelling score was significantly lower in the study group ( $P<0.001$ ; table 2).

In the second step of the study, wives of the 116 patients with low hypo-osmotic swelling score were divided into two groups. Treated group (51 women) underwent IUI who 18 of them became pregnant. Untreated group (65 women) who were permitted to conceive without IUI and 13 of them became pregnant.

Outcome of pregnancy in the treated and untreated groups is shown in table 3. Fourteen patients in treated group (77.77%) and four patients in untreated group (30.76%) continued their pregnancy over 20 weeks. Four patients (22.22%) in treated group and nine patients in untreated group (69.23%) aborted their fetuses.

The success rate in the treated group was 77.77%, while in the untreated group it was 30.76% with a ratio of 2.04. The success rate in the treated group was significantly higher than untreated group ( $C^2= 4.49, P< 0.05$ ). Table 4 shows the outcome of fetuses in patients who continued their pregnancy after 20 weeks.

**Table 1:** Results of semen analysis in the study groups

Parameter	Case group	Control group	P value
Semen volume			
Mean±SD (ml)	3.65±1.5	3.87±1.3	
Number of abnormal values	0	0	0.1
Sperm count			
Mean ±SD (millions/ml)	76.3±34.3	73.3±25.5	
Number of abnormal values	1	0	0.2
Sperm motility			
Mean ±SD (percent normal)	67.8±33.2	58.9±29.4	
Number of abnormal values	5	8	0.07
Sperm morphology			
Mean ±SD (percent normal)	65.2±39.2	58.5±41.2	
Number of abnormal values	10	12	0.06
Hypo-osmotic test score			
Mean ±SD	50.2±24.5	68.1±33.2	
Number of abnormal values	116	24	0.001

SD: Standard deviation

**Table 2:** Results of abnormal hypo-osmotic swelling score in the treated and untreated groups

	Total	Abnormal score	Percent	P value
Patients	178	116	65%	
Controls	159	24	15%	0.001

**Table 3:** Pregnancy and outcome of pregnancy in the treated and untreated groups

	Total	Pregnancy	Abortion	Continued pregnancy	P value
Treated group	51	18 (36.46%)	4 (22.22%)	14 (77.77%)	
Untreated group	65	13(20)	9 (69.23)	4 (30.76)	0.002

**Table 4:** Outcome of the fetuses in treated and untreated groups who continued their pregnancy after 20 weeks

	Total	20-27 weeks	28-32 weeks	33-36 weeks	≥37 weeks	Live birth
Treated	14	1(7.14%)	3 (21.42%)	4 (28.57%)	6(42.85%)	13(92.8%)
Untreated	4	-	1 (25%)	1(25%)	2 (50%)	4 (100%)

## Discussion

A routine semen analysis has long been the standard laboratory test for male fertility potential. Although a routine semen analysis quantitates sperm count, motility and morphologic features, it can not ascertain the functional capacity of a given semen sample. HOST can evaluate the functional integrity of the sperm membrane and a score of < 60% is suboptimal.<sup>1,5,8</sup> Esteves, Chan, and co-workers studied semen parameters and their relationship with hypo-osmotic swelling test and found that the men with abnormal semen parameters had a lower degree of sperm swelling compared with those with normal parameters.<sup>6,9</sup> The role of male factor in the recurrent spontaneous abortion remains controversial and the data in the literature are limited.<sup>10</sup>

Larger studies comparing abnormal sperm morphology in partners of women with recurrent spontaneous abortion showed no significant difference with the controls.<sup>1,11</sup> Other studies have shown that low HOST scores in couples undergoing in vitro fertilization (IVF) do not affect the rates of fertilization or pregnancy, but is associated with higher rates of spontaneous miscarriage.<sup>5</sup> Evidence from animal studies suggests that men can contribute to spontaneous miscarriage despite apparently normal semen parameters. Raised scrotal and body temperatures in rams, rats and rabbits are associated with increased early pregnancy loss. This might be a function of spermatozoal abnormality with consequent non-viable embryos.<sup>12</sup>

Direct cytogenetic analysis of human sperm has shown that chromosomal abnormalities in the presence of normal peripheral blood karyotype are not infrequent,<sup>13,14</sup> whereas no association between chromosomal abnormalities and abnormal sperm morphology has been described.<sup>14</sup> Spermatozoal chromosomal abnormalities may cause subtle membrane defects that are detectable by HOST. Our study showed a significantly reduced hypo-osmotic swelling test score in spite of a normal semen analysis in partners of women with recurrent spontaneous abortion. This finding is consistent with a study by Bouckett and colleagues that showed a low hypo-osmotic swelling test score in patients with recurrent miscarriage.<sup>1</sup> On the other hand, we found that the patients whose partners have low score HOST had higher continued pregnancy and lower abortion rate after IUI. Success rate in the treated group was 78.77% while in the untreated group it was 30.76% with a ratio of 2.04% ( $P=0.05$ ).

Some investigators showed that sperm washing by centrifugation alters the HOST result,

whereas the swim-up method selects a population of spermatozoa with intact head and tail membranes.<sup>8</sup>

The present study was based on the hypothesis that if we can separate the best qualified sperms for fertilization, then miscarriage should occur less frequently. Our study is the first investigation that presents an ideal way for selection of recurrent aborters that benefit from IUI. Although the patients who underwent this IUI technique had a better outcome, further studies with larger populations should be conducted to confirm the effectiveness of this approach.

**Conflict of Interest:** None declared

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