Effects of Varicocele Repair on Spontaneous First Trimester Miscarriage

A Randomized Clinical Trial

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Purpose: To evaluate the effects of varicocelectomy on semen parameters, pregnancy rates, and live birth in couples with first term recurrent miscarriage.

Materials and Methods: One hundred and thirty-six women with recurrent miscarriage were recruited into this study. All of the husbands had normal semen parameters according to World Health Organization criteria and clinical varicocele. In order to evaluate the causes of recurrent pregnancy loss, we looked for chromosomal abnormalities and endocrine, chronic inflammatory, and infectious diseases. Both groups were well matched according to male/female age, varicocele grade, and smoking history. These couples were assigned randomly into two groups: group one (n = 68), in which male partners underwent varicocele repair, and group two (n = 68), which underwent expectant therapy. All of the couples were followed up monthly up to 12 months. All of the women who conceived were followed up until delivery. In each 3-month follow-up visits, two semen analyses were performed.

Results: Mean sperm concentration, sperm progressive motility, and sperm with normal morphology improved significantly after elapsing 6 months from varicocelectomy by 75.0%, 15.9%, and 14.3%, respectively, versus the expectant group ($P < .01$). The overall pregnancy rate was 44.1% and 19.1% within a 12-month period in groups 1 and 2, respectively ($P = .003$). Of women who conceived in groups 1 and 2, 13.3% and 69.2% developed miscarriage ($P = .001$). Sperm density/mL (r = 0.072; $P = .001$), time elapsed from varicocelectomy (r = 0.068; $P = .001$), and female age (r = -0.062; $P = .002$) were three most significantly related independent factors to pregnancy rate by multiple regression analysis.

Conclusion: Varicocelectomy improves semen quality, increases pregnancy rate, and decreases miscarriage rate significantly. Further controlled studies to confirm our results seem warranted.

Keywords: varicocele, pregnancy trimesters, abortion, randomized controlled trial

INTRODUCTION

The incidence of varicocele in both men and adolescent boys varies between 10% and 20%.[1-3]

The association of a varicocele with infertility has been well documented. It has been demonstrated that testicular development is compromised in teenagers with varicocele; this impairment can adversely affect sperm quality.[4,5] It is also important to note that varicocele can result in sperm DNA damage, and elevated reactive oxygen species (ROS) and apoptosis rates.[6,7] When ROS are in excess, they can cause pathological impairment by inducing oxidative changes in cellular lipids, proteins, and DNA.[8] Men whose semen contains increased levels of ROS may have diminished fertility for both invitro and invivo procedures, and there may be adverse effects on embryo development.[8,9]

In some cases, apoptosis may commence, but terminate prematurely, in a process known as abortive apoptosis, leading to ejaculation of mature sperm with apoptotic traits, such as fragmented DNA.[10,11] Chen and colleagues reported that patients with varicocele have elevated levels of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage.[12] Excessive levels of DNA damage have been associated with a decrease in several fertility indices, including embryo cleavage rate, implantation rate, pregnancy rate, and live birth rate.[13]

Furthermore, examination of biopsies obtained from varicocele-affected testes by atomic force microscopy reveals structural and morphological alterations in the sperm neckpiece and flagella, as well as changes in head dimensions.[14] Surgical varicocelectomy improves seminal parameters and is associated with decreased ROS production and increased levels of seminal plasma antioxidants.[15]

Recurrent miscarriage (RM) is the spontaneous loss of three or more consecutive pregnancies in the first trimester with the same biological father. It affects about 1% of all fertile couples trying to conceive.[16,17] Despite extensive investigations, no clear cause is found in more than half of cases and they are categorized as idiopathic RM.[17,18]

There is an interaction between the male and female genomes during the time of both natural and assisted conception. The paternal genome plays its role during early embryonic development by providing the centrosome in the first mitotic division.[19] Sperm chromatic structure assay (SCSA) measures increased sperm chromatin susceptibility to acid denaturation. There is a significant delay in the time from unprotected intercourse to conception in men with high SCSA values.[20,21] In a study by Evenson and associates, higher values of the SCSA were able to predict 39% of the miscarriages.[20]

Furthermore, correlations exist between sperm DNA integrity and outcomes of invitro fertilization treatment cycles.[22,23] Another study, investigating sperm chromosome anomalies, demonstrated a significant raise in sex disomy of couples with RM compared with controls (0.84% versus 0.37%).[24]

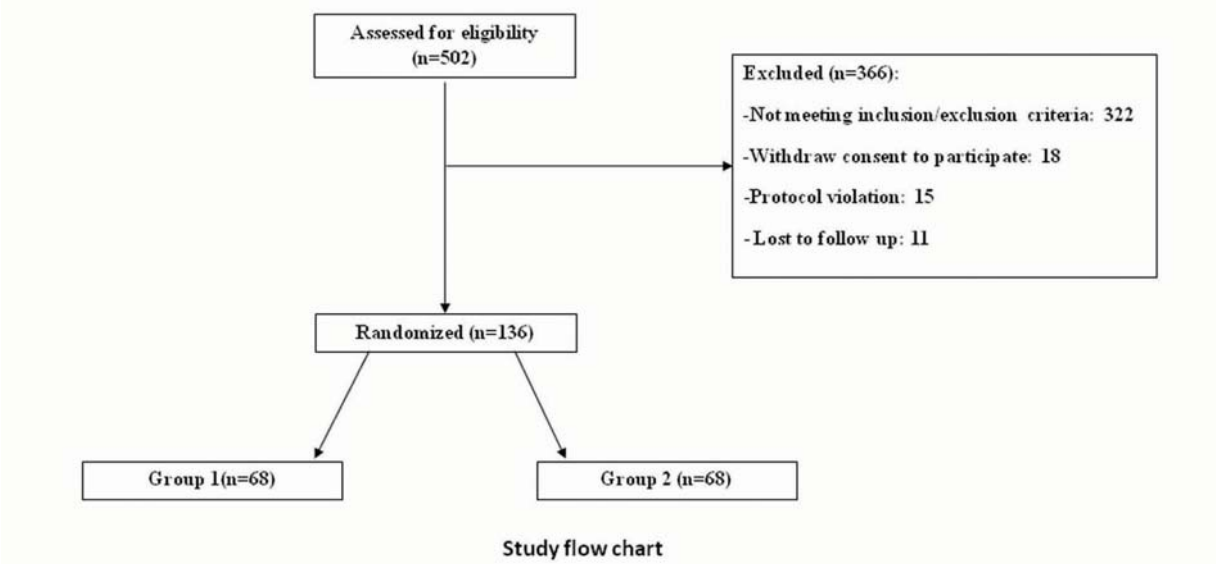
We therefore designed a prospective randomized, double blind study to determine pregnancy outcome after varicocele repair in normozoospermic men.

MATERIALS AND METHODS

Study Population

In a randomized clinical trial, consecutive women were recruited from Alzahra Hospital's gynecology clinic (Rasht, Iran), which is the tertiary referral center for the region. Starting from 1 January 2004, we selected 502 outpatient women referred to our gynecology clinic due to recurrent pregnancy loss (RPL).

All patients with 2 or more first trimester miscarriages were included in this study. In order to evaluate the causes of RPL, we looked for chromosomal abnormalities and endocrine, chronic inflammatory, and infectious diseases. When these women



Study flow chart

were first seen in the gynecology clinic, patient's information leaflets regarding the trial were given to them for consideration of their participation in this study. Couples were excluded if their husband had abnormal semen analysis according to the World Health Organization (WHO) criteria (1992), abnormal reproductive endocrine profiles, or positive antisperm antibody assay.

Evaluations

Women willing to participate were encouraged to ring for an appointment. At this appointment, a full history was taken and the results of previous investigations were noted to make certain that there no cause was found for RPL. In all the cases, the wives had undergone gynecologic workups and were found to be fertile. The trial was then explained further and a written informed consent was obtained.

All the patients underwent karyotype analysis in order to determine chromosomal abnormalities, such as balanced translocations. The anatomy of the uterus was evaluated by transvaginal ultrasound scan and hysterosalpingography and/or hysteroscopy in order to diagnose mullerian malformations or the presence of uterine fibroids or polyps.

All men underwent a basic infertility evaluation, including history taking, complete physical examination, and hormonal profile (follicle-stimulating hormone and testosterone). A documented history of varicocele was diagnosed by physical examination and confirmed by scrotal ultrasonography. In order to confirm the diagnosis of normal semen parameters, at least two semen analyses were performed at 1 month interval to remove inadvertent and possible adverse effects of various issues on spermatogenesis. The normal WHO values included $\geq 20 \times 10^6$/mL concentration with grade A motility in 25% or grade A + B motility in 50% of spermatozoa and normal morphology in at least 30% of the spermatozoa. Exclusion criteria included wives aged older than 40 years, abnormal testes on physical examination, total testicular volume of less than 12 mL on ultrasonography, alcohol, drug, or substance abuse, severe general diseases, and endocrinopathies.

Clinical pregnancy was defined as serum human chorionic gonadotropin (hCG) level > 25 IU/L and was confirmed with ultrasonography.

Of 502 screened women, 136 were eligible and entered the study (Figure). All couples gave their informed consent and Local Medical Ethics Committee approved the study protocol.

Randomization

Women meeting the inclusion criteria were visited

Table 1. Baseline demographic and clinical characteristics of study groups.*

Characteristics	Group 1 (n = 68)	Group 2 (n = 68)	*P*
Age, y			
Male	36.1 ± 4.2	36.8 ± 4.6	.67
Female	29.1 ± 3.7	28.8 ± 3.4	.46
Infertility duration, y	5.6 ± 2.8	5.4 ± 2.6	.52
Prior miscarriage, No.	3.8 ± 1.4	3.7 ± 1.3	.72
Body mass index, kg/m^2			
Male	27.3 ± 2.4	27.6 ± 2.2	.72
Female	24.1 ± 2.7	24.3 ± 2.6	.64
Varicocele grade, No. (%)			
Grade I	17 (25.0)	15 (22.1)	.08
Grade II	41 (60.3)	42 (61.7)	.24
Grade III	10 (14.7)	11 (16.2)	.09
Testis volume, mL	23.7 ± 2.6	23.6 ± 2.7	.12
Serum hormones			
Male			
Testosterone, nmol/L	16.4 ± 4.7	16.2 ± 4.6	.27
FSH, IU/L	12.2 ± 3.6	12.6 ± 4.1	.34
Female			
SHBG, nmol/L	62.8 ± 14.2	63.2 ± 14.4	.47
LH, IU/L	8.6 ± 2.4	8.9 ± 2.2	.62
FSH, IU/L	9.7 ± 2.4	9.4 ± 2.3	.46
Estradiol, pmol/L	88.6 ± 45.2	91.2 ± 47.1	.26
Estrone, pmol/L	98 ± 24	102 ± 25	.52
PRL, pmol/L	378 ± 117	368 ± 114	.38

*FSH indicates follicle-stimulating hormone; SHBG, sex hormone-binding globulin; LH, luteinizing hormone; and PRL, prolactin.

after a negative pregnancy test. If the consent was obtained, couples were given consecutive study numbers and sent to Department of Urology. A dedicated urologist (SAA) then allocated the couples to the varicocelectomy (group 1, n = 68) or expectant groups (group 2, n = 68) using their study number.

We used minimization to ensure comparability between women with respect to parity, type of miscarriage, and gestation. Both groups were well matched according to male/female age, varicocele grade, and smoking history. Inguinal standard varicocelectomy was performed using a loupe magnification.

Outcome Measures

The primary endpoint was clinical pregnancy and live birth. The secondary outcome was to determine whether the varicocelectomy influenced the pregnancy rate. The study consisted of a 2-month screening phase and a 12-month follow-up period. Patients and their husbands were visited every month during the whole study period. Since be-

Table 2. Semen parameters and pregnancy data at various assessment points.[£]

	Assessment points after varicocelectomy					Assessment points during expectant therapy				
Variables	**Baseline**	**3 months**	**6 months**	**9 months**	**12 months**	**Baseline**	**3 months**	**6 months**	**9 months**	**12 months**
Semen parameters, (mean ± SD)										
Total sperm count, $\times 10^6$	110.6 ± 15.3	114.2 ± 14.4[d]	159.4 ± 18.5[b]	176.2 ± 15.1[c]	184.2 ± 18.3[c]	113.4 ± 12.2	115.2 ± 14.2[d]	118.4 ± 14.2[d]	114.2 ± 14.7[d]	115.5 ± 16.7[d]
Sperm density, $\times 10^6$/ml	32.2 ± 6.4	33.2 ± 4.4[d]	55.2 ± 5.2[c]	56.2 ± 6.8[c]	62.6 ± 5.7[c]	32.4 ± 6.4	36.6 ± 5.3[d]	38.7 ± 6.1[d]	36.6 ± 6.2[d]	36.7 ± 6.1[b]
Sperm motility, %	38.4 ± 2.2	41.4 ± 2.5[d]	44.8 ± 4.4[a]	48.8 ± 6.8[b]	54.2 ± 5.7[c]	37.2 ± 2.1	38.4 ± 2.6[d]	40.4 ± 2.2[d]	39.6 ± 2.6[d]	40.6 ± 2.5 [d]
Normal morphology, %	56.7 ± 2.6	59.7 ± 2.6[d]	64.4 ± 3.4[a]	67.6 ± 4.6[c]	66.4 ± 4.2[c]	56.8 ± 2.4	67.7 ± 3.4[d]	58.4 ± 3.5[d]	58.4± 3.5 [d]	58.7 ± 3.8 [d]
Pregnancy data, No. (%)*										
Clinical pregnancy rate	NA	0 (0)	20 (29.4)	7 (10.3)	3 (4.4)	NA	1 (1.8)	5 (7.3)	4 (2.6)	3 (2.6)
Live birth rate	NA	0 (0)	17 (85.0)	6 (85.7)	3 (100.0)	NA	0 (0.0)	1 (20.0)	1 (25.0)	2 (66.7)
Miscarriage rate	NA	0 (0)	3 (15.0)	1 (14.3)	0 (0.0)	NA	1 (100.0)	4 (80.0)	3 (75.0)	1 (33.3)

[a]*P* = .02 to .05, [b]*P* = .01, [c]*P* = .001 to .005, and [d]*P* = not significant.
All *P* values are versus baseline.

*New cases between previous assessment point and current assessment point.

£NA indicates not applicable; and SD, standard deviation.

ginning of the study, to assess fertility outcome, women were visited every month to complete a questionnaire.

We collected data regarding pregnancy, including date of last normal menstrual period, serum level of hCG (> 25 IU/L), and ultrasonography confirmation of clinical pregnancy. Pregnancy testing was performed by the quantitative measurement of serum level of hCG in the absence of menstruation. For every 3-month visit, two semen samples were collected within a 1- to 2-week period from each other. The outcome of pregnancy was defined by time to pregnancy since the start of the trial. All of the clinical pregnancies were followed up until delivery.

Statistical Analysis

Univariate analyses were carried out using Student's *t* test for continuous variables and the Chi-Square or Fischer's exact test for dichotomous variables. A two-sided independent-sample *t* test was used for comparison. The Pearson correlation r was used to determine any potential associations. Cox proportional hazards regression analysis was performed to determine which groups of

Table 3. Summary of multiple regression analysis of factors affecting live birth rates in couples.

Variables	Univariate			Multivariate		
	Coefficient	*P*	Odds ratio (95% CI)*	Coefficient	*P*	Odds ratio (95% CI)
Male age, y	-0.024	.03	0.94 (0.87 to 1.00)	-0.026	.03	0.96 (0.87 to 1.00)
Female age, y	-0.062	.002	0.84 (0.61 to 0.90)	-0.064	.002	0.84 (0.63 to 0.92)
Varicoceletomy	0.068	.001	3.4 (2.4 to 5.8)	0.068	.001	3.1 (2.8 to 5.1)
Total sperm count, $\times 10^6$	0.061	.001	3.6 (2.5 to 5.6)	0.067	.001	3.4 (2.4 to 4.8)
Sperm density, $\times 10^6$/mL	0.072	.001	3.7 (2.7 to 6.4)	0.073	.001	3.2 (2.5 to 5.2)
Normal morphology, %	0.064	.001	2.7 (1.7 to 4.7)	0.061	.01	2.7 (1.7 to 4.7)

*CI indicates confidence interval.

factors were independently significantly related to pregnancy rates. The SPSS software (the Statistical Package for the Social Sciences, Version 17.0, SPSS Inc, Chicago, Illinois, USA) was used for statistical analyses and a *P* value < .05 was considered significant.

RESULTS

Baseline demographics and clinical characteristics of study groups are shown in Table 1. Mean total sperm count, sperm concentration, sperm motility, and sperm morphology did not significantly differ between study groups at baseline (Table 2). In the varicocelectomy group, significant improvements in sperm parameters were observed during the 12-month follow-up period.

Baseline mean sperm concentration in groups 1 and 2 was 32.2 ± 6.4 and 32.4 ± 6.4 million per mL, respectively (*P* = .1). At 6-month follow-up period, the mean sperm concentration increased by 75.0% and 12.5% in groups 1 and 2, respectively (*P* = .001). After elapsing 12 months from varicocelectomy, the increases in total sperm counts from baseline were even greater (Table 2). Only patients who underwent varicocelectomy had significant improved sperm motility. Mean sperm motility increased from 38.4 ± 2.2% to 44.8 ± 4.4% at 6 months postoperatively (*P* = .01). In patients who underwent varicocelectomy, the normal morphological sperm level increased from a mean of 56.7 ± 2.6% to 64.4 ± 3.4% at 6 months postoperatively (*P* = .02).

When studying the correlations between the time elapsed after the varicocelectomy and the semen analysis parameters, strong positive correlations were found between elapsed time and sperm concentration up to 12 months (r = 0.61; *P* = .01), sperm motility (r = 0.48; *P* = .01), and sperm morphology (r = 0.37; *P* = .02).

Forty-three of 138 couples conceived clinically during the study period; 30 (44.1%) of these pregnancies were after the varicocelectomy and 13 (19.1%) were in expectant group (*P* = .003; Table 2). Of the above-mentioned clinical pregnancies, 26 (86.7%) and 4 (30.8%) resulted in live birth (*P* = .002). Pregnancy rates of 29.4%, 10.3%, and 4.4% were achieved after 6, 9, and 12 months in the varicocelectomy group, respectively. These rates were 7.3%, 2.6%, and 2.6% in expectant group respectively (*P* < .01). There were 4 (13.3%) and 9 (69.2%) miscarriages in groups 1 and 2, respectively (*P* = .003). About two-thirds of pregnancies

ended with miscarriage in the expectant group.

Correlations

We also addressed the correlations between some variables and live birth. We put them in multivariate analysis. The two factors most significantly related to outcome were the sperm density followed by history of varicocelectomy (Table 3). The chance of pregnancy increased with an increase in the number of sperms/million/mL [r = 0.072; odds ratio (OR) = 3.7; 95% confidence interval (CI): 2.7 to 6.4; *P* = .001] and decreased with rising age of the woman (r = -0.062; OR = 0.84; 95% CI: 0.61 to 0.90; *P* = .002).

The time elapsed from varicocelectomy was a continuous variable that was categorized into two different groups, namely ≤ 6 months and > 6 months. The pregnancy rate in the ≤ 6-month varicocelectomy was 0% while it was 44.1% (30/68) in the > 6-month varicocelectomy (r = 0.068; OR = 3.4; 95% CI: 2.4 to 5.8; *P* = .001).

Sperm motility was a significant predictor of pregnancy within the statistical model. With sperm motility of > 40%, the chance of achieving pregnancy was almost 3.5 times more than the chance with motility < 40% (r = 0.062; OR = 3.4; 95% CI: 2.1 to 5.8; *P* = .001). When the normal sperm morphology was > 60%, the chance of achieving pregnancy was 2.7 times greater than when the percentage of normal sperm morphology was < 60% (r = 0.064; OR = 2.7; 95% CI: 1.7 to 4.7; *P* = .001).

DISCUSSION

We found significant association between varicocelectomy and the rate of clinical pregnancy and miscarriage. The risk of child loss decreased significantly with varicocelectomy. This effect remained after correction for male/female age and previous miscarriage. We could not find similar study for comparison. Our data indicate that even in healthy men with varicocele, without overt oligoasthenoteratozoospermia, there is an increased risk of miscarriage in their wives. In the present study, varicocelectomy in normozoospermic men resulted in improved live birth rate in their wives. We could not explain this completely.

Men with varicocele have increased oxidative DNA damage.[12] Seminal antioxidant capacity significantly decreases in men with varicocele.[25] Increased oxidative stress production in seminal plasma attacks the fluidity of the sperm plasma membrane and the sperm DNA integrity.[12] Excessive sperm DNA damage is associated with a reduction in some fertility indices, such as fertilization, embryo cleavage, implantation, and clinical pregnancy rates.[25]

O'Brien and coworkers studied the outcomes in infertile men with varicocele who had female partners older than 35 years.[26] They reported that surgical and nonsurgical approaches resulted in similar pregnancy rates. In this study, we included men with normal semen parameters.

The definite etiology is still unknown. There is a significant association between sperm dysfunction and excessive ROS production.[27] It has been shown that oxidative stress plays a key role in sperm dysfunction in patients with varicocele.[28] Spermatozoa are vulnerable to oxidative damage due to insufficient protection by antioxidants.[27] In men with varicocele, sperm ROS levels are greater than those in normal healthy men.[27,29] It has been demonstrated that varicocelectomy decreases ROS levels and increases the antioxidant capacity of seminal plasma from infertile men with varicocele.[15,30]

Increased level of ROS in the reproductive tract disrupts the integrity of DNA in the sperm nucleus. Spermatozoa containing damaged DNA may result in paternal transmission of defective genetic material with adverse outcomes for embryonic development.[13] It has been reported that infertile men demonstrate improved sperm DNA integrity six months after varicocele repair.[31]

Varicocele is characterized by increased temperature of the scrotum. According to one theory, in-

creased temperature can result in thermal damage of the DNA and proteins in the nucleus of spermatic tubules' cells and/or Leydig cells.[32,33] Furthermore, it has been shown that in men with varicocele, germ cell apoptosis is a very common phenomenon.[30] Indeed, germ cell apoptosis can lead to subsequent oligozoospermia. Varicocele grades in the treated and untreated groups were well matched.

Our study is not without limitations. The main limitation is the small sample size. The sample was limited in size and was taken from a specific geographical area, limiting the generalizability of the findings. In addition, we did not measure functional sperm parameters, such as seminal antioxidant capacity, sperm acrosomal reaction, and sperm DNA integrity.

CONCLUSION

Our results demonstrate that varicocele repair increases the chance for spontaneous pregnancy and live birth. Varicocelectomy may be offered to couples who suffer from recurrent miscarriage. Nonetheless, further studies with a large number of subjects are required to replicate our findings.

CONFLICT OF INTEREST

None declared.

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