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Value of microsurgical varicocelectomy for severe oligo-asthenospermia patients failed in fertilization assisted by *in vitro* fertilization

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Abstract. – OBJECTIVE: To investigate the clinical effect of microsurgical varicocelectomy on severe oligo-asthenospermia patients failing in fertilization assisted by intracytoplasmic sperm injection.

PATIENTS AND METHODS: From January 2013 to August 2014, forty-nine patients with severe oligo-asthenospermia and serious varicoceles were treated by microsurgical varicocelectomy after failing in fertilization assisted by intracytoplasmic sperm injection (ICSI), eleven of whom had varicoceles on the left side and thirty-eight had bilateral varicoceles. Patients were followed up for the natural pregnancy condition, changes of routine semen parameters and reproductive hormone level and the embryonic development and outcome of next IVF-ET (ICSI) cycles within 6 months.

RESULTS: After surgery, 61.2% (30/49) of spouses obtained clinical pregnancy. Among whom 22.4% (11/49) were naturally pregnant, 32.65% (16/49) were conceived after second IVF-ET assistance, and 6.1% (3/49) were conceived with the third or further assistance of ICSI-ET. The overall miscarriage rate was 16.7% (5/30). All of the patients had improvement in the sperm concentration and forward motility. The sperm concentration increased from (10.53 ± 8.76) × 10^6/ml to (20.23 ± 11.76) × 10^6/ml. The ratio of forward motile sperm was increased to (30.52 ± 18.78) % from (8.75.52 ± 6.36) % ($p < 0.01$). The serum total testosterone (T) improved from (2.19 ± 1.03) ng/ml to (4.05 ± 0.64) ng/ml ($p < 0.05$). Serum follicle-stimulating hormone (FSH) changed from (5.23 ± 1.26) mIU/ml to (3.76 ± 2.22) mIU/ml after the procedure. Luteinizing hormone (LH) changed from (4.38 ± 1.36) to (3.98 ± 1.38) mIU/ml. Estrogen (E2) changed from 40.28 ± 7.26 pg/ml to 35.24 ± 5.75 pg/ml. Prolactin (PRL) level elevated from (18.24 ± 4.28) to (17.16 ± 2.16) ng/ml ($p > 0.05$). The fertility rate of in vitro fertilization significantly improved to (83.36 ± 19.36) % from (72.36 ± 17.88) % ($p < 0.05$). The rate of 2PN ratio increased from (66.73 ± 17.93) % to (75.96 ± 20.39) %. The cleavage rate increased from (83.26 ± 32.33) % to (90.35 ± 23.66). The abnormal fertility rate were (5.36 ± 12.58) % and (7.26 ± 13.89) % before and after the procedure ($p > 0.05$), while the rate of high-quality embryos increased significantly from (34.36 ± 33.27) % to (55.67 ± 23.36) % ($p < 0.05$). The rate of transferable embryos remained without significant change (70.67 ± 30.6% before and 60.53 ± 30.27% after the procedure). The anabiosis rate of frozen embryo increased from (66.32 ± 30.69) % to (89.72 ± 29.69) %. The further blastocyst rate improved from (10.98 ± 9.7) % to (30.27 ± 15.33) % ($p < 0.01$).

CONCLUSIONS: The microsurgical varicocelectomy effectively improved sperm parameters, the fertility rate of oocyte fertilized *in vitro* and the anabiosis rate and blastocyst rate of the frozen embryo for on patients with severe oligo-asthenospermic, and further increased the odds of natural pregnancy, the rate of high-quality embryos and the success rate of *in vitro* fertilization.

Key Words:
Microsurgical varicocelectomy, Severe oligo-asthenospermia, In vitro fertilization, Intracytoplasmic sperm injection.

Introduction

Varicocele (VC) is a common treatable form of male infertility. The incidence of varicoceles in the general male population is around 15%, and found in 19 to 41% of infertile man[1]. It has been suggested that up to 45 to 81% male infertility may be contributed by VC[2]. The mechanism of varicoceles causing male infertility remained a controversy, and relevant pathogenesis of varicocele include hyperthermia, hormonal dysfunction, increased or decreased testicular blood flow rates, reflux of renal or perirenal metabolites and hypoxia[3]. Clinical randomized controlled trials showed that seminal density increased and forward movement enhanced after treatment of varicoceles[4], as well as overall improvement of pregnancy outcome[5]. Therefore, it

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is inferred that decreased sperm viability and reduced density caused by varicoceles may be directly lead to the problem of conceiving[6].

A variety of surgical and nonsurgical approaches have been advocated for varicocelectomy, including minimally invasive procedures, such as laparoscopic varicocelectomy and transvenous percutaneous embolization, and the traditional open surgical approach[7]. Microsurgical varicocelectomy is the most effective and least morbid method among the three varicocelectomy techniques for treating varicocele in infertile men[8]. With the development of assisted reproductive technology, intracytoplasmic sperm injection (ICSI) has been widely used in patients who had failed to become pregnant and presented with serious abnormal semen parameters[9]. For men with azoospermia or oligoasthenospermia, modest improvements in semen quality after varicocele repair may have a significant impact on couples' fertility options. Yet the impact of microsurgical varicocelectomy on the outcome of future pregnancy outcome among patients failed ICSI and their further need of *in vitro* fertilization remains to be studied[8]. In this article, we recruited couples failed ICSI and performed microsurgical varicocelectomy to male patients with varicoceles, the natural pregnancy condition and pregnancy outcome of those received further *in vitro* fertilization. Microsurgical varicocelectomy was selected considering that it can completely ligate the varicose spermatic vein and meanwhile effectively protects the testicular artery, and the postoperative pregnancy rate is higher after microsurgical varicocelectomy than those received approaches such as high ligation of the spermatic vein and laparoscopic spermatic vein ligation[8]. Reproductive hormone levels after surgery were also compared. Increases in serum testosterone levels in varicocelectomized patients were statistically insignificant in some studies, while some have reported that serum testosterone levels have become normal after varicocelectomy[10]. Besides, to our knowledge, the present analysis represents the first study to date characterizing treatment outcomes after microsurgical varicocelectomy in men with azoospermia and severe oligoasthenospermia, and to assess the effects of microsurgical varicocelectomy on serum FSH, total testosterone, luteinizing hormone, prolactin, and to investigate the interrelationships between the procedure and pregnancy outcome.

Patients and Methods

Patients

A total of 49 male patients with oligozoospermia or asthenospermia accompanied with severe varicoceles evaluated between January 2013 and August 2014 in Reproductive Center of Anhui Provincial Hospital were recruited; their female partners failed to get pregnant with intracytoplasmic sperm injection (ICSI). All patients underwent microsurgical varicocelectomy according to Informed Consent. Preoperative and postoperative semen samples were submitted for sperm analysis, including sperm variables, serum follicle-stimulating hormone (FSH), testosterone level, luteinizing hormone (LH) and prolactin (PRL), the pregnancy condition and further necessity for *in vitro* fertilization were followed up.

Besides the experience of failing at least once ICSI assistance, subject should meet the following inclusion criteria: (1) Couples were in reproductive age and had a normal sexual life without using contraceptive measures over 1 years; (2) The wife's side had normal ovarian reserve function, routine hysteroscopy showed no abnormalities, with no adverse factors affecting reproductive outcome, such as endometriosis and hydrosalpinx; (3) Examination suggested men with grade 3 or more severe varicoceles (mass of vermiform appearance can be touched within erected scrotum or varicose veins found on surface of scrotum); (4) Scrotal ultrasound performed by professional radiologist indicated about left or bilateral varicoceles, with the diameter of at least one side over 3.0 mm, and significant regurgitant flow showed after Valsalva experiment; relevant data was recorded; (5) Computer-aided semen analysis (CASA, WLJY 9000, Weili New Century Science & Tech Dev., Beijing, China) results showed sperm density $< 5.0 \times 10^6$, and sperm motility A+B level < 10%, or post-treatment motile sperm count < 10%; (6) Karyotype analysis and Y chromosome microdeletion analysis are normal; (7) The wife's part received pituitary down-regulation of long protocol and superovulation, and intracytoplasmic sperm injection (ICSI) under the control of micromanipulator[11]; (8) High-quality embryos were selected for transplantation, with two embryos planted once at most; women who failed to conceive after transferring embryos were transferred with thawed embryo two months later. Carnegie stage 3 embryos were discarded.

Patients satisfying any of the following conditions were excluded from further examination: (1) The poorer ovarian response was observed in *in vitro* fertilization-embryo transplant (IVF-ET) cycles[12], and less than 5 oocytes were retrieved. Retrieved oocytes with uneven size, rough cytoplasmic granules, pale luster, abnormal zona pellucida, or without forming first polar bodies cannot be used for transplant. (2) Male subject with low sperm motility due to reproductive system infection, medication, overworking, smoking, drinking or other common factors lead to a temporary decline in sperm motility.

Surgical Approach

All male patients received microsurgical varicocelectomy by dissection of the spermatic cord at the external inguinal ring. Unilateral and bilateral varicocele concurrent with regurgitation detected by color Doppler ultrasound received unilateral and bilateral varicocelectomy, respectively. No medication or contraception was used after surgery and pregnancy outcome was followed at an interval of one month. Those who failed to get pregnant six months later would participate into *in vitro* fertilization or medical treatment based on patients' willingness and seminal parameters. Couples preferred *in vitro* fertilization received *in vitro* fertilization and embryo transfer (IVF-ET) or ICSI. Microsurgical subinguinal varicocelectomy was performed as previously described. Briefly, the location of around 2 to 3 cm below external inguinal ring was identified by invaginating the scrotal skin and 2 cm long lesion was made along spermatic cord. Isolate subcutaneous tissue with sharp dissection to expose the spermatic cord. Fascia around the spermatic cord was separated with blunt dissection to isolate the cord out of the skin lesion. Across the rubber band, blow the cord and fix the band. Facilitate varicocelectomy under operating microscope with 10-fold magnification. Open longitudinally the external spermatic fascia, cremaster muscle, and internal fascia to expose the internal spermatic vein and testicular artery. Isolate the distinct vas deferens, deferential artery and deferential vein. Separate the vas deferens and vessel with another rubber belt to avoid potential injury to the vessels. The spermatic vein was separated, ligated and cut under a surgical microscope while preserving testicular artery and lymph vessel. The wound was closed in layers after the procedure.

Assisted Reproduction Protocol

The wife's part was administrated with short-acting triptorelin 0.1 mg by intramuscular injection, once daily. Those achieved satisfactory control of ovarian hyperstimulation were injected with recombinant follicle stimulating hormones (r-FSH). The dosage was adjusted according to the rate of follicular development. Intravenous injection of human chorionic gonadotropin (Ovidrel, EMD Serono, Inc. Merck KGaA, Darmstadt, Germany.) 250 IU was given when the average diameter of dominant folliele exceeded 18 mm, and egg retrieval was performed 36 hours later. The way of fertilization was selected depending on the quality of sperm taken on the date of egg retrieval. IVF-ET was selected as long as the total sperm number with forward movement after treatment was over 5 million. Otherwise, ICSI was selected. Oviductal fluid (COOK, Brisbane, Australia) was used for culturing and eggs were kept in a CO_2 incubator at 37°C. Fertilization condition was observed 16 to 18 hours after ICSI procedure, including fertilization, tow pronuclear fertilization, abnormal fertilization (single pronucleate or multiple pronucleate embryos), and unfertilized embryos. The embryos were cultured for 48 hours to monitor the development of blastomeres and cleavage, and count the number of transferrable embryos, high-quality embryos and untransferrable embryos 72 hours later. The quality of embryos on day 3 of development was evaluated based on WIH embryo grading system[13]: < 6 points: untransferrable; 6-7: transferrable; > 7, high-quality. 1 to 2 best quality oocytes were selected for implantation first. Serum free β-hCG level was detected 14 days after embryo transfer to evaluate conception and pregrent subjects were examined at one and two months later with transvaginal sonography for monitoring embryo development. The follow-up lasted through pregnancy to one month after delivery. Those failed to conceive after 2 months were transferred with thawed embryos proceed with blastocyst culture.

Parameter Examination

Computer-aided semen analysis (CASA, WLJY 9000, Weili New Century Science & Tech Dev., Beijing, China) was conducted and sperm density and vitality were assessed according to World Health Organization reference values for human semen characteristics[14]. Samples of fasting venous peripheral blood were collected for detecting reproductive hormone levels (Uni-Cel Dxl 800, Beckman Coulter, Kraemer Boulevard Brea, CA 92821, USA).

Statistical Analysis

Data were expressed as mean±SD and analyzed by with SPSS 3.0 (SPSS Inc., Chicago, IL, USA). The two groups were compared using Student's *t*-test, and comparison between more than two groups was performed by one-way ANOVA with Student-Newman-Keuls post-test. Differences with $p < 0.05$ were considered statistically significant.

Results

Pregnancy Characteristics and Clinical Outcome

A total of 49 male subjects were recruited with a average age of 34.8 years and average infertile years of 3.2 years, including 11 cases of unilateral varicoceles and 38 cases of bilateral varicoceles. The number of subjects failed first, second and third or more ICSI were 35, 9 and 5, respectively. The average age of spouses was 30.6 years, among whom 25 had tubal factors and 3 had ovulation obstacles. The rest 21 females had no abnormal findings in physical examination.

All male cases showed improve sperm density and vitality 6 months after the procedure. The pregnancy rate and miscarriage rate was 61.2% (30/49) and 16.7% (5/30), including 11 cases of natural conception and 2 cases of natural miscarriage. 9 deliveries were reported, all singleton pregnancy. 38 cases received second *in vitro* fertilization, 30 received IVF-ET instead of ICSI due to improved sperm condition. 16 received implantation of fresh embryos or thawed embryos, resulting 2 cases of miscarriage, 14 cases of delivery, 9 were singleton pregnancy and 5 were twin pregnancy. 8 cases were continued with a second or further *in vitro* fertilization assistance, resulting 3 cases of conception with singleton or twin pregnancy, 1 were miscarried and 2 were delivered, including 2 twin babies.

Sperm Analysis Results

In men with varicoceles, sperm density and vitality improved significantly after the procedure ($p < 0.01$, Table I). The total testosterone increased significantly as well ($p < 0.05$), while all other tested variables (FSH, LH, PRL) showed no significant change compared with preoperative values (Table I).

Fertilization Results

As indicated in the Table I, the fertilization rate elevated significantly after the procedure ($p < 0.05$); while 2PN ratio, abnormal fertilization rate, and cleavage rate were comparable to preoperative level ($p > 0.05$). The ratio of high-quality embryos, anabiosis rate of frozen embryos, and further blastocyst rate increased significantly compared with preoperative tests ($p < 0.01$, Table I).

Table I. Sperm quality, reproductive hormone level, and treatment outcome for all patients.

Variable	Before surgery	After surgery
Seminal fluid analysis		
Sperm density	10.53 ± 8.76	20.23 ± 11.76†
Forward motility sperm (%)	8.75.52 ± 6.36	30.52 ± 18.78†
Reproductive hormone level		
FSH (mIU/ml)	5.23 ± 1.26	3.76 ± 2.22
LH (mIU/ml)	4.38 ± 1.36	3.98 ± 1.38
T (ng/ml)	2.19 ± 1.03	4.05 ± 0.64*
E2 (pg/ml)	40.28 ± 7.26	35.24 ± 5.75
PRL (ng/ml)	18.24 ± 4.28	17.16 ± 2.16
Fertilization outcome (%)		
Fertility rate	72.36 ± 17.88	83.36 ± 19.36*
2PN ratio	66.73 ± 17.93	75.96 ± 20.39
Cleavage rate	83.26 ± 32.33	90.35 ± 23.66
Abnormal fertilization rate	7.26 ± 13.89	5.36 ± 12.58
Embryonic development outcome (%)		
Transferrable embryos	60.53 ± 30.27	70.67 ± 30.69
High quality embryo	34.36 ± 33.27	55.67 ± 23.36†
Anabiosis rate of frozen embryo	66.32 ± 30.69	89.72 ± 29.69†
Blastocyst rate	10.98 ± 9.72	30.27 ± 15.33†

*$p < 0.05$; †$p < 0.01$.

Discussion

Severe oligoasthenospermia is a common cause of male infertility. Varicocele repair resulted in the induction or enhancement of spermatogenesis for most men with azoospermia or severe oligoasthenospermia, and varicocele repair should be considered for all men with azoospermia and severe oligoasthenospermia[15]. The contributing factors for varicoceles included genetic defects, congenital gonadal dysplasia, and hypospermatogenesis. The treatment for varicoceles can be tricky and conducive infertility can be compensated with *in vitro* fertilization such as ICSI techniques. Clinically, however, attention has been overly paid to assisted fertilization, while somehow the treatment for varicoceles has been neglected.

Sperm motility, total number of motile sperm, and percentage of sperm with normal strict morphology can be significantly increased after varicocele repair[16]. Therefore, varicocelectomy may obviate the need for assisted reproductive technology, and to down stage or shift the level of assisted reproductive technology needed to bypass male factor infertility. Besides, the cost-effectiveness of assisted reproduction using *in vitro* fertilization (IVF) with ICSI as a primary treatment for varicocele-associated infertility has been studied, concluding that specific treatment of varicocele-associated male infertility with surgical varicocelectomy is more cost-effective than primary treatment with assisted reproduction; despite the apparent success of ICSI for the most severe cases of male factor infertility, application of assisted reproduction for all cases of male factor infertility also does not necessarily provide as high a delivery rate as specific treatment of the cause of male factor infertility[17].

Usually, treatment with medication or surgical ligation is made dependent on the severity of illness. The clinical outcome of medical and surgical treatments are comparable for mild to moderate varicoceles, while the severe condition is preferred to be handled with varicocelectomy. Specifically, the surgical approaches include conventional high ligation or internal spermatic vein, laparoscopic varicocele high ligation, and laparoscopic varicocele low ligation. Among the three main approaches, microsurgical varicocelectomy provides more thorough ligation of varicose veins, while preserving testicular artery blood supply, which optimizes the surgical outcome.

A significant improvement in testicular blood supply and sperm parameters, and a more evident hemodynamics promotion can be achieved with microsurgical subinguinal varicocelectomy than laparoscopic varicocelectomy. Thus, better improve the testicular microcirculation and function[18]. All subjects in the present study experienced once or repeated unsuccessful ICSI, representing a group of patients with significant reproductive difficulties. However, results showed that nearly half of the studied couples reported natural pregnancy (with no abnormal findings for the wife's part) within six months after varicocelectomy, and some cases received IVF-ET considering the fact that sperm quality has been significantly improved after the procedure. Patients without significant improvement in sperm analysis also reported successful pregnancy, which indicated that the procedure may alleviate the sperm defects and enhance the fertilization capability. Even for those failed to conceive naturally, the fertilization outcome and embryos quality are significantly improved compared with preoperative values, including a higher ratio of high-quality embryos and a higher anabiosis rate of the frozen embryo, all suggesting that patients with varicoceles would benefit from microsurgical varicocelectomy.

Limited by the short follow-up, continued evaluation is warranted to investigate whether the sperm quality would continuously improve with time. Besides, the subjects were featured with relatively advanced varicoceles. Thus, the improvement of testicle blood supply and clinical pregnancy outcome was more remarkable compared with those with a less severe condition. Whether the positive effect remains among patients with mild oligo-asthenospermia would be further studied. Moreover, the effect observed in some patients was less significant than others, suggesting that varicocele-associated infertility may be attributed to concurrent factors.

Conclusions

We observed that sperm quality and pregnancy outcome of infertile couples caused by varicoceles can be largely improved after microsurgical varicocelectomy, suggesting that varicocelectomy should be first tried before *in vitro* fertilization treatment. The increase of natural conception rate on one hand reduces medical cost, and on the other hand achieves better clinical pregnancy outcome.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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