

THE EFFECT OF VARICOCELE LIGATION ON OOCYTE FERTILIZATION AND PREGNANCY AFTER FAILURE OF FERTILIZATION IN *IN VITRO* FERTILIZATION-EMBRYO TRANSFER

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Although varicocelectomy is generally believed to improve semen quality and the hamster egg penetration assay, these parameters do not correlate well with *in vitro* fertilization (IVF) success. The effect of varicocelectomy on IVF of human oocytes was examined on thirteen couples with normal female fertility, but severe oligozoospermia and the presence varicocele in the males with failure of fertilization preoperatively in *in vitro* fertilization-embryo of transfer (IVF-ET) attempts. The couples were readmitted for the IVF-ET procedure following varicocelectomy. A 31% pregnancy rate was achieved after the operation, while no pregnancies occurred before surgery. Sperm density and motility improved significantly after operation, resulting in a coinciding improved fertilization and pregnancy rates.

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Key words: Varicocelectomy, Fertilization, Pregnancy, IVF

INTRODUCTION

Varicocele, abnormal dilatation of the spermatic vein is a common pathological finding in human males with the clinical complaint of infertility¹⁾. Abnormal testicular histology and seminal aberrations associated with varicocele have been well described²⁾, but the cause-effect relationship between varicocele and human infertility remains a matter of controversy. Nevertheless, many infertile patients with varicocele undergo a surgical repair procedure in the hope of removing the putative ill-effects of the usually unilateral, left-sided varicocele. There are conflicting reports about whether or not varicocele repair actually improves semen quality³⁻⁵⁾. Often the criteria for the evaluation of semen quality are not well defined. Furthermore, these criteria do not depict the most important qualification of the sperm; namely, its fertilizing ability.

Rodgers et al.⁶⁾ reported the relationship between the improved result of hamster egg penetration test and an increased pregnancy rate after varicocelectomy. How-

ever, semen quality and hamster egg penetration assay do not correlate well with *in vitro* fertilization (IVF) success. Thus, the effect of varicocelectomy on IVF of human oocytes is unknown. After failure of fertilization preoperatively in *in vitro* fertilization-embryo transfer (IVF-ET), the infertile men with varicoceles received surgical repair of the varicocele. Then they were readmitted for the IVF-ET procedure. This is the first report providing evidence of the positive effect of varicocelectomy on IVF success.

MATERIALS AND METHODS

The present study was performed on 13 couples with over 2 years duration of infertility, in whom the male partner had a clinical varicocele. Most of them were severe oligozoospermic ($<5 \times 10^6/\text{ml}$), asthenospermic (motility of $<40\%$) or teratospermic ($<30\%$ normal morphology).

In all cases, the presence of varicocele was confirmed by scrotal thermography. The female partners were found to be fertile in an evaluation including hormonal, mechanical, immunological and cervical

factors. The couples were subjected to IVF and embryo transfer therapeutic cycles, without success, before varicocelectomy. In view of the failure of the treatment or poor fertilization rates, the male partners were referred for an infertility evaluation and were found to have varicocele with several degree. They underwent an operation for repair of a varicocele. This procedure was done according to the high retroperitoneal ligation of internal spermatic vein with preservation of the testicular artery. One month after surgery, the women underwent additional IVF-ET treatment cycles. IVF-ET before and after varicocelectomy was performed by the same IVF team.

IVF-ET induction of ovulation protocol consists of pure follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG) starting on the third day of the cycle, with administration of 3 ampules daily of either. Ovulation was induced with 5,000 IU HCG when the leading follicle had reached a mean diameter of 21 mm. Transvaginal follicle puncture, which was carried out 35 h later under local anesthesia. Insemination was performed 3 to 6 hours after oocytes were obtained at pickup. Fertilization was identified by the presence of 2 pronuclei and/or a second polar body, as well as 2 equal blastomeres. Pregnancy was considered to have been established only after the identification of a fetal heart at ultrasound scan.

At least three semen samples were obtained within 4 months preoperatively and from 3 months to 1 year postoperatively. Sperm penetration assay for assessment of sperm function was not performed because of lack of research equipment. The significance of postoperative seminal data changes was analyzed by Wilcoxon signed-rank test. Chi square test was used to compare the fertilization rate or pregnancy rate before and after varicocelectomy.

RESULTS

The results of 13 IVF-ET cycles were compared before and after the operation.

Table 1. Comparison of results of 13 cycles of *in vitro* fertilization (IVF) before and after varicocelectomy

	Before operation	After operation
No. of IVF cycles	13	13
No. of oocytes retrieved	82	76
No. of embryos	8	31
No. of embryos transferred	5	27
Fertilization rate (%) ^a	9.8	41
Pregnancy rate (%) ^b	0	31

^aP<0.001, ^bP<0.001.

No pregnancies occurred before surgery, whereas a 31% success rate was obtained after the operation (Table 1). In 13 treatment cycles prior to operation, 82 oocytes were retrieved and fertilizations resulted in only 8 (9.8%). This fertilization rate was low when compared with that observed in parallel IVF cycles done for couples with a normal male factor, during the same period (60% to 80%). After operation, 31 of 76 oocytes were fertilized (41%). Of these, 27 embryos were transferred into the uterus. Subsequently, four pregnancies occurred (31%).

Table 2 shows the results of semen quality before and after varicocelectomy. Sperm density increased from $2.1 \pm 0.5 \times 10^6$ /mL to $4.6 \pm 0.4 \times 10^6$ /mL. The percent of forward progressive motility also increased from $22 \pm 1.9\%$ to $31 \pm 1.3\%$. There was significant improvement in sperm density ($p=0.001$) and motility ($p=0.001$) after varicocelectomy, but the percent of normal morphology did not reach the level of statistical significance. Consequently, it was obvious that the four men who impregnated their wives had improved sperm density and motility after varicocelectomy.

DISCUSSION

The relationship between the presence of varicocele and infertility is still controversial, since this condition may be recognized in at least 10% to 15% of fertile males⁷. The available medical literature suggests that treatment of a varicocele does improve testicular function. Johnsen found objective improvement in morphologic studies of testes biopsies after varicocele

Table 2. Semen parameters before and after operation in 13 men with varicocele and fertilization rate on *in vitro* fertilization before and after varicocelectomy

No.	Before operation				After operation				P
	Sperm Density ($\times 10^6/\text{mL}$)	Motility (%)	Morphology* (%)	FR	Sperm Density ($\times 10^6/\text{mL}$)	Motility (%)	Morphology* (%)	FR	
1	2	20	40	0	4	32	41	25	
2	1	28	32	0	4	40	38	35	+
3	4	31	30	12	5	35	41	29	
4	2	28	25	0	3	30	28	25	
5	7	22	26	30	6	38	25	29	
6	3	20	31	11	5	29	29	28	
7	0.2	15	28	0	4	39	29	38	+
8	0.5	10	25	0	3	30	26	31	
9	1	10	38	0	6	32	31	35	+
10	2	25	32	10	8	31	33	33	+
11	3	22	28	12	5	25	27	29	
12	2	25	25	0	4	28	24	30	
13	0.1	30	32	0	3	29	30	31	
	2.1 $\pm$ 0.5*	22 $\pm$ 1.9*	30 $\pm$ 1.3*		4.6 $\pm$ 0.4*	32 $\pm$ 1.3*	31 $\pm$ 1.6*		

* The values are expressed as mean values $\pm$ SE.

Percentage of normal morphology.

FR : Fertilization rate on *in vitro* fertilization

P : Pregnancy

repair⁸). Kass and Bellman found catch-up growth in the testis of boys who had varicoceles repaired⁹). The most difficult question, of course, is whether varicocele repair improves fertility. There are many studies which indicate good responses in semen quality and relatively high fertility rates after varicocele repair. Unfortunately, most of these studies were uncontrolled. Nilsson et al. did not show any favorable effect of repair of varicocele on sperm count or fertility¹⁰). However, Gerris et al. found that men treated for varicocele had a significantly higher fertility rate (54%) than men with similar sperm counts treated medically (31%)¹¹). Aafjes et al. obtained similar findings with a pregnancy rate of 40% in the varicocele patients vs. 17% in medically treated patients¹²). Rodgers et al.⁶) demonstrated the relationship between the improved result of the sperm penetration test and an increased pregnancy rate after varicocelectomy. Therefore, there is strong circumstantial evidence that varicocele repair does improve fertility, although this is yet to be documented in a well controlled study.

Such previous studies specifically demon-

strated improved semen quality, sperm penetration assay test and fertility following varicocelectomy. However, semen quality and sperm penetration assay (SPA) do not correlate well with eventual IVF success in male factor infertility. For example, the correlation of a positive SPA with eventual IVF success was only 46% for couples with male factor infertility, while it was 94% for couples with female factor infertility¹³). Because of this poor association between SPA and IVF results in oligoasthenospermic men, Margalioth et al. concluded that this bioassay should not be relied upon for predicting IVF outcome in male factor subfertility¹⁴). Others have reported similar views. Ausmanas et al. reported a good correlation between SPA and human oocyte fertilization, except in cases of male factor infertility. Paradoxically, fertilization of human oocytes could be achieved even when there was very low or no penetration of zona-free hamster ova¹³). Obviously, an SPA result can not predict IVF success in cases of male factor infertility. Therefore, neither improved semen quality nor SPA result can predict the ultimate success of IVF after varicocelecto-

my.

Thus, the effect of varicocelectomy on IVF of human oocytes remained to be elucidated. We examined 13 men with severe oligoasthenospermia resulting in failure of fertilization in IVF, and who underwent varicocelectomy with preservation of the testicular artery. IVF was subsequently resumed with the same partner. Prior to varicocelectomy, 8 of 82 eggs (9.8%) were fertilized by the sperm of these men, but, no pregnancies occurred. Following varicocele repair, 31 of 76 eggs (41%) were fertilized by the sperm of these men, and furthermore, 4 pregnancies were obtained. Although oocyte quality and laboratory factors are known to be related to human IVF failure, defective sperm function is generally recognized as its main cause¹⁵⁾. Defective sperm function is determined by a range of anomalies, i.e., inadequate flagellar movement, increased peroxidative damage, changes in sperm activation, or abnormal oocyte-sperm interaction¹⁶⁾. Kazama et al. demonstrated evidence for the improvement of sperm function after varicocelectomy by using the hypoosmotic swelling test¹⁷⁾. They showed that the percentage of swollen sperm in the hypoosmotic swelling test elevated after operation in varicocele patients¹⁷⁾. On the basis of our clinical experience and their report, varicocelectomy is considered to affect some sperm functions which were not identified in this study. Varicocelectomy can enhance the fertilization in cases with reduced or failed IVF. These four successful pregnancies with IVF-ET after varicocelectomy following failure of fertilization in IVF, in which each male partner served as his own control, provide the first direct evidence of the positive effect of varicocelectomy on IVF success.

In conclusion, the infertile males with varicoceles were successfully treated with IVF-ET procedure after repair of varicocele. These findings suggest that varicocele repair may result in the enhanced human sperm fertilizing capability and in the improved ability of the fertilized embryo to transfer. Therefore, the patients who

have multiple failure of IVF-ET should be searched for the presence of varicocele to amplify the possibility of IVF success.

REFERENCES

- 1) Dubin L and Amelar RD: Etiologic factors in 1,294 consecutive cases of male infertility. *Fertil Steril* 22: 469-474, 1971
- 2) McFadden MR and Mehan DJ: Testicular biopsies in 101 cases of varicocele. *J Urol* 119: 372-374, 1978
- 3) Getzoff PL: Surgical management of male infertility: Results of a survey. *Fertil Steril* 24: 553-560, 1973
- 4) Cockett ATK, Urry RL and Dougherty KA: The varicocele and semen characteristics. *J Urol* 121: 435-436, 1979
- 5) Rodriguez-Rigau LJ, Smeth KD and Steinberger E: Relationship of varicocele to sperm output and fertility of male partners in infertile couples. *J Urol* 120: 691-694, 1978
- 6) Rogers BJ, Mygatt GG, Soderdahl DW, et al.: Monitoring of suspected infertile men with varicocele by the sperm penetration assay. *Fertil Steril* 44: 800-805, 1985
- 7) Swerdloff RS and Walsh PC: Pituitary and gonadal hormones in patients with varicocele. *Fertil Steril* 26: 1006-1012, 1975
- 8) Johnsen SG and Agger P: Quantitative evaluation of testicular biopsies before and after operation for varicocele. *Fertil Steril* 29: 58-63, 1978
- 9) Kass EJ and Bellman AB: Reversal of testicular growth failure by varicocele ligation. *J Urol* 137: 475-476, 1987
- 10) Nilsson S, Edvinsson A and Nilsson B: Improvement of semen and pregnancy rate after ligation and division of the internal spermatic vein: fact or fiction? *Br J Urol* 51: 591-596, 1979
- 11) Gerris J, Van-Nueten J, Van-Camp C, et al.: Clinical aspects in the surgical treatment of varicocele in subfertile men. I. Comparison of observed and expected pregnancy rates. *Eur J Obstet Gynecol Reprod Biol* 27: 33-41, 1988
- 12) Aafjes JH and Van Der Fijver JC: Fertility of men with and without a varicocele. *Fertil Steril* 43: 901-904, 1985
- 13) Ausmanas M, Tureck RW, Blasco L, et al.: The zona-free hamster egg penetration assay as a prognostic indicator in a human in vitro fertilization program. *Fertil Steril* 43: 433-437, 1985
- 14) Margalioth EJ, Navot D, Laufer N, et al.:

- Correlation between the zona-free hamster egg sperm penetration assay and human in vitro fertilization. *Fertil Steril* 45: 665-670, 1986
- 15) Oehninger S, Ecosta A, Kruger T, et al.: Failure of fertilization in in vitro fertilization: the occult male factor. *J In Vitro Fert Embryo Transfer* 5: 181-187, 1988
- 16) Aitken RJ: Assessment of sperm function for IVF. *Hum Reprod* 3: 89-95, 1988
- 17) Kazama T, Terada T, Satomi S, et al.: Treatment of male infertility: current situation and problems. *Acta Urol Jap* 37: 1355-1360, 1991
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和文抄録

精索静脈瘤根治術の体外授精，胚移植における受精および妊娠におよぼす影響

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精索静脈瘤根治術が精液所見あるいはハムスター卵貫通試験の成績を改善させることはひろく知られている。しかしこれらは，体外授精の成功率とは相関しない。そこで，精索静脈瘤根治術の体外授精におよぼす影響を調べる目的で，高度の乏精子症を呈する夫が精索静脈瘤を有し，かつ体外授精に失敗した13組の夫婦

を追跡した。この13組は，夫の精索静脈瘤根治術終了後再び体外授精を試みた。その結果，術前は妊娠例が認められなかったが，術後4例（31%）に妊娠が確認された。この4例においては，精子濃度および運動率は上昇した。

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Editorial comment

精索静脈瘤の高位結紮術により精液所見が改善することはよく知られている事実であり，本論文は，さらに高位結紮術が体外授精におよぼす影響を検討したものである。試みとしては比較的新しく，高位結紮術後，精索静脈瘤患者の精液所見は改善し，体外授精による妊娠率も向上しており，精索静脈瘤に対する積極的な手術療法を示唆するものである。しかしながら，その

向上した理由として精液所見の改善以外の体外受精が故の特別なデータ等の呈示がなく，考察も含め少し重みの欠けたものとなっている。今後，さらに症例数ならびに検討項目を追加し，その原因の検討を含め，研究を発展させていただきたい。

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