Treatment of male infertility

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Summary

Purpose: To present various treatment options for male subfertility. *Methods:* Surgical therapy for varicocele, hormonal therapy, mechanical therapy, e.g., intrauterine insemination, special treatment for antisperm antibodies and low hypoosmotic swelling test scores with protein digestive enzymes, and in vitro fertilization (IVF) with and without intracytoplasmic sperm injection are discussed. *Results:* Questions have been raised as to the efficacy of varicocelectomy. Perhaps only a minority of the best of males respond to this therapy. Clomiphene citrate or gonadotropins or hCG may be effective but usually only when serum FSH, LH and/or testosterone levels are low or are in the low normal range. Intrauterine insemination is helpful for oligoasthenozoospermia but is not so beneficial for antisperm antibodies or low hypoosmotic swelling test scores unless first pretreated with chymotrypsin. *Conclusions:* Obstructive or non-obstructive azoospermia requires sperm aspiration from the testes or testicular biopsy followed by IVF with intracytoplasmic sperm injection (ICSI). In vitro fertilization with ICSI is needed for extremely low counts or motility. Otherwise less costly or invasive therapy can be tried first but IVF with ICSI can eventually be performed if more conservative therapy fails to achieve a pregnancy.

Key words: Intrauterine insemination; Chymotrypsin; Intracytoplasmic sperm injection; Clomiphene citrate; Gonadotropins.

Introduction

Varicocele and varicocelectomy

Varicocele was considered a leading cause of infertility over 50 years ago [1, 2]. Varicocelectomy as a treatment of varicocele-associated infertility was also reported over 50 years ago [3] and its efficacy was allegedly confirmed by subsequent uncontrolled studies [4, 5].

From my own anecdotal experiences I have not been impressed with varicocelectomy producing a clinically important improvement in male semen parameters or an improvement in male fecundity. Since I usually do not recommend the therapy, my anecdotal experience is not very extensive for males having the procedure then having semen evaluation within a few months of the procedure. However, those males that I have been able to follow have not had a good enough response to change my mind about not recommending the procedure.

My greatest experience has been with males having the procedure over a year before and then the couple still failing to conceive seeks help with a reproductive endocrinology group. Often the male partner has been told that the operation corrected the problem (maybe one semen analysis showed an improvement four months later and none was checked since) assuming the problem is with the female partner. However very frequently the semen analysis is no better or worse than the preoperative specimens.

I considered that maybe there is temporary improvement or I am not seeing the males with more extensive improvement because their female partners conceived. There have been some controlled studies evaluating varicocelectomy. A Cochrane Database of systematic review of five trials found only one where there was statistically significant improvement in pregnancy rates which occurred following high ligation of the left spermatic vein [6, 7]. However, the other four studies found no benefit of the procedure [8-11]. The combined relative risk of the four studies was 1.06 (95% CI 0.73-1.83) [7]. The reviewer's conclusion was that "insufficient evidence exists that treatment of varicocele in men from couples with otherwise unexplained subfertility does improve the couple's spontaneous pregnancy chances" [7].

Hormonal therapy of male infertility

Oligoasthenozoospermia

One may simplify the etiology for oligoasthenozoospermia as either from damage to the testes or inadequate hormonal stimulation of spermatogenesis. The two main hormones needed for spermatogenesis are testosterone and FSH [12]. Other hormones that may have an adverse effect are prolactin and estradiol (E2) elevations [12].

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Measurement of serum FSH, LH, testosterone, prolactin and E2 may help to determine therapy for oligoasthenozoospermia [12]. In 51 males with low motile densities 20 (39%) had elevated FSH and normal testosterone. This group of males would not likely respond to any hormonal therapy. Only four (7.8%) had an elevated serum FSH and low testosterone. This group might respond to human chorionic gonadotropin (hCG) injection, e.g., 2000 units three times/week. Thus slightly less than half of males with low motile densities have elevated FSH but possibly about 17% in this group could maybe benefit from medical therapy. However there are no controlled data to determine if the anecdotal findings of apparent improved semen parameters are not merely fortuitous [12].

The group with normal FSH and low testosterone represented the second highest frequency (13/51, 25.5%). In 1977 in a case series we demonstrated that not only could 25 mg daily of clomiphene citrate increase motile density, but with concomitant evaluation and treatment of the female partners a very high six-month pregnancy rate was achieved [13]. Of course, since low motile densities do not necessarily predict the subnormal male, it is possible that the 90% pregnancy rate was more related to correcting female infertility factors than improving the motile density [13]. The possibility exists that the defect could be at the pituitary level and thus the man may not respond to clomiphene but would respond to hCG with or without gonadotropins. Thus it is important after a month of treatment to repeat the serum testosterone to see if it is then normal. Interestingly the same anecdotal experience was found in males with oligoasthenozoospermia and varicocele treated with clomiphene citrate where similar improvement was seen without surgery [14].

The group with normal FSH and testosterone could also respond to clomiphene citrate therapy. It could be argued that low normal testosterone and FSH and/or LH would be an inappropriate response of the pituitary to low motile densities. My own bias is to use clomiphene for this group when the FSH is below mid-normal; with a serum FSH above top normal and testosterone below mid-normal, I prefer to use hCG injections. Tamoxifen is a similar drug to clomiphene citrate and can probably be substituted for it.

In some instances a low serum testosterone may be associated with an increase in aromatase enzyme converting more testosterone to E2. For these circumstances the male could be treated with an aromatase inhibitor, e.g., anastrozole (arimidex). Sometimes, in fact, clomiphene may raise the serum testosterone but the serum E2 may also rise and may negate to some extent the effect of improved testosterone. Sometimes aromatase inhibitors may be added to clomiphene or gonadotropin therapy. If a high serum prolactin is associated with low serum gonadotropins or testosterone, then either bromocryptine or cabergoline can be tried, but this is very unusual.

Intrauterine insemination (IUI) as a treatment of male infertility

Oligoasthenozoospermia

The peak cervical mucus occurs when the serum E2 is maximal at mid-cycle prior to the LH surge. With the initiation of the rise in LH, which will trigger oocyte release 36 hours later, the serum E2 begins to drop and the serum progesterone begins to rise. Thus the cervical mucus will frequently not be conducive to sperm transport right at the time of ovulation. That is not a problem for males with normal motile densities and females with normal cervical mucus since longevity of sperm in the mucus frequently exceeds one and a half days and may even be three to four days. Therefore, intercourse two to three days before ovulation is sufficient. However, males with low motile densities may not have sufficient numbers to achieve a pregnancy > 36 hours later related to depletion of the sperm. Exposure to sperm right at the time of ovulation or shortly thereafter with IUI, i.e., within the first eight hours after egg release from the follicle, might allow similar numbers of sperm to reach the egg in the fallopian tube from a male with oligoasthenozoospermia vs males with normal motile densities having intercourse two to three days prior to ovulation [15].

Antisperm Antibodies

When there is a great percentage of sperm coated with antisperm antibodies (especially as the percentage approaches 100%) intrauterine insemination has not proven very effective. Couple studies reported improved success with IUI for antisperm antibodies when the male partner ejaculated into medium where 50% serum was added [16, 17]. However it was shown that treating the sperm with the protein digestive enzyme chymotrypsin was much more effective [18]. One study found a 25% pregnancy rate per cycle with chymotrypsin pretreatment prior to IUI vs only 3% with IUI with sperm ejaculated into medium with 50% albumin added [18].

Low HOST scores

A small pilot study found that eight of 12 males could improve low HOST scores > 50% by treatment with chymotrypsin galactose [19]. There were four of eight who achieved a pregnancy in 12 cycles by pretreatment with chymotrypsin prior to IUI [19]. However, a subsequent larger study found only a 3% pregnancy rate per cycle with chymotrypsin sperm instead of the 33% seen in the pilot study [19, 20]. One possibility why the second study had such low pregnancy rates could be that the couples were not cautioned about having protected intercourse since the sperm from the cervical mucus could also reach the oocyte in the fallopian tube, attach to the zona pellucida, and then transfer the toxic protein to the zona pellucida which then becomes incorporated into the embryo membrane, and then subsequently impairs the functional integrity of the embryo membrane, thus inhibiting implantation.

More credence to the hypothesis that chymotrypsin treatment of sperm may improve fertility potential of semen samples with low HOST scores was provided by a study where the chymotrypsin treated sperm used for conventional oocyte insemination with IVF-ET showed a clinical pregnancy rate per transfer of 42.9%, a delivered pregnancy rate of 32% per transfer and an implantation rate of 21.3% [21]. This study thus supported the theory that unprotected intercourse diminished the success rate with IUIs. Thus couples where a low HOST score is present in the male partner should be cautioned against unprotected intercourse at least before ovulation.

Poor motility

The absence of any sperm with rapid linear progression is associated with a poor prognosis of achieving a pregnancy with intercourse. The prognosis remains poor if following washing and separation there still is an absence of sperm with rapid linear progression [22]. One study found that the pregnancy rate per cycle was only 2.5% (2/80) if rapid linear motion remained absent vs 10.2% (130/1368) if it was present [22]. However 95% of most specimens with no rapid linear motion will improve with IUI preparation [22]. Even conventional oocyte insemination for IVF-ET found similar results (6.3% per transfer with no rapid linear sperm vs 21.9% with them present [22].

Intracervical insemination

Sometimes males will present with normal semen parameters but low volume such that following intercourse, the sperm do not reach the cervical mucus. Some men may have retrograde ejaculation so that all the sperm go back into the urinary bladder. Sometimes a small antegrade specimen can be achieved by treating the male with sympathomimatic amines e.g., pseudoephedrine. These problems can be overcome merely by inseminating the small sperm volume directly into the cervical mucus at the appropriate time. Intracervical insemination may be useful for mechanical problems, e.g., hypospadius, erectile dysfunction, obesity or vaginismus.

It could be questioned if there is any benefit of doing intracervical insemination rather than just doing an IUI. The timing for intracervical insemination can be more forgiving and can be two of three days before ovulation and still be effective whereas IUI has about a 12-hour window and timing is more critical. The washing procedure for IUI is also more costly.

Intracervical insemination can also be effective for oligoasthenozoospermia. The sperm for IUI cannot be concentrated as in intracervical insemination because the separation process leads to capacitation of the sperm which inhibits their longevity. However one way to naturally increase the sperm concentration is to split the ejaculate since the majority of the sperm in a given ejaculate is present in the first portion of the ejaculate [23].

Sometimes when a male produces a sperm specimen intended for a split ejaculate intracervical insemination, the motile density is too low to be effective. In some instances, a markedly improved specimen may occur by having the man produce a second specimen after a short interval [24].

In vitro fertilization

The advent of in vitro fertilization (IVF) has enabled many infertile couples with male factor infertility to conceive. In in vivo circumstances it takes an ejaculate of many millions to finally allow ~400 sperm to reach the zona pellucida. With IVF the zona pellucida is usually exposed to 50,000 sperm. Thus this technique has been extremely effective for males with low motile densities especially < 2.5 mil/ml. However, extremely low motile densities could still lead to poor fertilization rates and thus poor pregnancy rates.

Other sperm abnormalities similarly were not universally improved by IVF. Low fertilization rates with antisperm antibodies have been reported [25-27]. However, IVF with ICSI was found to be an effective therapy for antisperm antibodies [28].

The presence of a low HOST score < 50% does not allow reasonable pregnancy rates despite normal fertilization rates with conventional IVF-ET unless the sperm is pretreated with chymotrypsin as previously mentioned [21]. However, treatment with ICSI is highly effective and supports the contention that sperm may be associated with subfertility independent of oocyte fertilization related to some toxic effect of the supernumerary sperm that attach to the zona pellucida [29, 30]. Pretreatment of the sperm by chymotrypsin followed by conventional insemination is another treatment option for this defect [31].

At first high DNA fragmentation scores on the sperm chromatin structure assay seemed to be associated with not only very poor outcome with IVF-ET with conventional oocyte insemination, but even with ICSI [32-34]. However some studies found that achieving a pregnancy with ICSI and high DNA framentation index (DFI) scores was not much different than normal scores but suggested that an abnormal SCSA test may be associated with a higher miscarriage rate [35, 36]. Other studies found that high DFI scores were not associated with any decreased pregnancy outcome even with conventional insemination [37, 38]. Nevertheless the study by Bungum *et al.* did find lower pregnancy rates with IUI [38]. At the recent 2005 American Society of Reproductive Medicine meeting there was a poster claiming that high DFI scores did not even predict lower pregnancy outcome following IUI. Based on these data I would not order a SCSA test initially but if it was abnormal on later testing I would still recommend proceeding to IVF with ICSI.

The advent of ICSI has enabled pregnancies with some male factor problems that certainly would not have occurred with conventional insemination. Sperm from men with Kartagener's syndrome that have defective ciliary bodies and thus they are not motile (necrospermia) can successfully achieve fertilization and pregnancies with ICSI. Sperm normally achieves final maturation after remaining in the epididymus for about three weeks. However immature sperm from both the epididymus and testes in males with obstructive azoospermia have been found to be equally effective [39-41]. In fact live pregnancies were achieved from aspiration of sperm from the testes in males with obstructive azoospermia and found to be equally effective [39-41]. Moreover a live pregnancy was achieved from aspiration of sperm from the testes many hours after the man died with no attempts to put him on life support [42]. For completely non-motile immature sperm sometimes a viable sperm can be distinguished solely after exposure to hypoosmolar median and the sperm with tail swelling is then used for oocyte injection.

Though IUI with extraction of alkalinized sperm from urine from males with retrograde ejaculation has achieved pregnancies [43], there is no question that IVF with ICSI is more effective.

For non-obstructive azoospermia, sometimes using techniques that evaluate the entire semen specimen, enough sperm can be isolated over many hours to perform single sperm injection of ten eggs or more. Generally speaking if there are less than five spermatozoa per seminiferous tubule, sperm may not be found in the ejaculate. However in at least 50% of the time when males with azoospermia associated with increased serum FSH levels and decreased inhibin B levels have no sperm despite a careful search of the entire ejaculate, sperm can be detected on testicular biopsy or even aspiration [41]. There are techniques to get better odds of retrieving sperm on testicular aspiration, e.g., testes mapping [44]. The methods of sperm retrieval available are TESE – testicular sperm extraction where an incision is made, TESA – testicular sperm aspiration with a needle, PESA – percutaneous epididymal aspiration, and MESA – microepididymal sperm aspiration. Some couples have donor sperm backup in case of failure to find any sperm. Otherwise the oocytes may be frozen but oocyte freezing is still in its infancy. If none or a low percentage of oocytes are fertilized with conventional insemination, it is usually related to failure of the sperm to bind to the zona pellucida. Subsequent cycles using ICSI are generally successful. Thus many physicians recommend ICSI for sperm with subnormal semen parameters for fear of failed fertilization or in patients undergoing IVF-ET for unexplained infertility. Often times the ICSI is used for sperm that never was much of a problem in the past for use with conventional insemination, or for that matter even with IUI. Other than increased expense for the couple and more work and time consumption for the embryologist, the question has been posed as to whether the process could lead to a lower pregnancy rate. In fact my group recently presented data suggesting that conventional insemination may result in a 50% higher pregnancy rate without much reduction in fertilization rate or increase in percentage of failed fertilization [45-47].

Sometimes, however, special techniques can be used with ICSI when conventional insemination seemingly results in the production of seemingly normal embryos but ones that result in very poor pregnancy rates. As mentioned in the diagnostic section there have been claims that poor nuclear morphology may be one of these factors [48]. The use of high magnification can be used to not only diagnose the problem but to use this technique to isolate the sperm with normal nuclei for purposes of ICSI [49]. Claims of a 66% pregnancy rate have been made following IVF-ET with high magnification ICSI [49]. Recently, these data were corroborated by Hazout *et al.* with a 38% pregnancy rate per transfer [50]. We have recently presented a poster at the 2006 American Society of Andrology meeting where we found a 40% pregnancy rate with high magnification ICSI in couples having failed after at least three previous IVF cycles [51].

One of the questions that arise is whether there are any options available if there is failed fertilization even with ICSI? When all of the sperm displaying 100% round heads (globozoospermia), fertilization rates with ICSI have varied from 0-37% [52-54]. Successful pregnancies have also been reported [52, 55-57]. Treatment of the oocytes after ICSI with calcium ionophore after ICSI has resulted in both fertilization and pregnancies in women with previous failed fertilization with round headed sperm and with normal appearing sperm [58, 59].

References

[1] Russell J.K.: "Varicocele in groups of fertile and subfertile males". *Br. Med. J.*, 1954, *1*, 1231.
[2] Dubin L., Amelar R.D.: "Etiologic factors in 1294 consecutive cases of male infertility". *Fertil. Steril.*, 1971, *22*, 469.
[3] Tulloch W.S.: "Varicocele in subfertility: results of treatment". *Br. Med. J.*, 1955, *2*, 356.
[4] Charny C.W.: "Effect of varicocele on fertility: results of varicocelectomy". *Fertil. Steril.*, 1962, *13*, 47.
[5] Dubin L., Amelar R.D.: "Varicocelectomy as therapy in male infertility: a study of 504 cases". *J. Urol.*, 1976, *113*, 640.
[6] Madgar I. *et al.*: "Controlled trial of high spermatic vein ligation for varicocele in infertile men". *Fertil. Steril.*, 1995, *63*, 120.
[7] Cochrane Database Syst Rev., 2001, CD000479.
[8] Nilsson S., Edvinsson A., Nilsson B.: "Improvement of sperm and pregnancy rate after ligation and division of the internal spermatic vein: fact or fiction?". *Br. J. Urol.*, 1979, *51*, 591.
[9] Breznik R., Vlasisavlijevic V., Borko E.: "Treatment of varicocele and male infertility". *Arch. Androl.*, 1993, *30*, 157.
[10] Yamamoto M., Hibi H., Hirata Y., Miyake K., Ishigaki T.: "Effect of varicocelectomy on sperm parameters and pregnancy rate in patients with subclinical varicocele: a randomized prospective controlled study". *J. Urol.*, 1996, *155*, 16360.
[11] Nieschlag E., Hertle L., Fischedick A., Behre H.M.: "Treatment of varicocele: counseling as effective as occlusion of the vena spermatical". *Hum. Reprod.*, 1995, *10*, 347.
[12] Check J.H., Lurie D., Vetter B.H.: "Sera gonadotropins, testosterone, and prolactin levels in men with oligozoospermia or asthenozoospermia". *Arch. Androl.*, 1995, *35*, 57.

[13] Check J.H., Rakoff A.E.: "Improved fertility in oligospermic males treated with clomiphene citrate". *Fertil. Steril.,* 1977, *28,* 746.
[14] Check J.H.: "Improved semen quality in subfertile males with varicocele-associated oligospermia following treatment with clomiphene citrate". *Fertil. Steril.,* 1980, *33,* 423.
[15] Check J.H., Bollendorf A., Zaccardo M., Lurie D., Vetter B.: "Intrauterine insemination for cervical and male factor without superovulation". *Arch. Androl.,* 1995, *35,* 135.
[16] Cohen J., Edwards R.G., Fehilly C.B., Fishel S.B., Hewitt J., Rowland G., Steptoe P.C., Webster J.: "Treatment of male infertility: factors affecting fertilization and pregnancy". *Acta Eur. Fertil.,* 1984, *15,* 455.
[17] Elder K.T., Wick K.L., Edwards R.G.: "Seminal plasma antisperm antibodies and IVF: the effect of semen sample collection into 50% serum". *Hum. Reprod.,* 1990, *5,* 179.
[18] Bollendorf A., Check J.H., Katsoff D., Fedele A.: "The use of chymotrypsin/galactose to treat spermatozoa bound with anti-sperm antibodies prior to intra-uterine insemination". *Hum. Reprod.,* 1994, *9,* 484.
[19] Katsoff D., Check J.H.: "Two methods of achieving pregnancies despite subnormal hypo-osmotic swelling test scores". *Fertil. Steril.,* 1997, *68,* 549.
[20] Check M.L., Kiefer D., Check J.H., Hourani W., Long R.: "Treatment of sperm with subnormal HOST scores with chymotrypsin/viable pregnancy after IUI". *Arch. Androl.,* 2002, *48,* 155.
[21] Check M.L., Katsoff D., Check J.H., Summers-Chase D.: "Effect of treating sperm with low hypo-osmotic swelling test scores with chymotrypsin on pregnancy rates after conventional in vitro fertilization-embryo transfer". *Fertil. Steril.,* 2004, *82,* 741.
[22] Bollendorf A., Check J.H., Lurie D.: "Evaluation of the effect of the absence of sperm with rapid and linear progressive motility on subsequent pregnancy rates following intrauterine insemination or in vitro fertilization". *J. Androl.,* 1996, *17,* 550.
[23] Check J.H.: "Improved fertility results with split ejaculate insemination and improved cervical mucus". In: E.S.E. Hafez, K. Semm (eds.), Instrumental Insemination, The Hague, Martinus Nijhoff, 1982, *22,* 157.
[24] Check J.H., Chase J.S.: "Improved semen quality after a short-interval second ejaculation". *Fertil. Steril.,* 1985, *44,* 416.
[25] Ackerman S.B., Graff D., van Uem J.F.H.M., Swanson R.J., Veeck L.L., Acost M., Garcia J.E.: "Immunologic infertility and in vitro fertilization". *Fertil. Steril.,* 1984, *42,* 474.
[26] Brannen-Broch L.R., Hall J.: "Effect of male anti-sperm antibodies on sperm fertilizability in vitro". *Arch. Androl.,* 1985, *15,* 15.
[27] Clarke G.N., Lopata A., McBain J.C., Baker H.W.G., Johnston W.I.H.: "Effect of sperm antibodies in males on human in vitro fertilization". *Am. J. Reprod. Immunol. Microbiol.,* 1985, *8,* 62.
[28] Check M.L., Check J.H., Katsoff D., Summers-Chase D.: "ICSI as an effective therapy for male factor with antisperm antibodies". *Arch. Androl.,* 2000, *45,* 125.
[29] Check J.H., Katsoff D., Check M.L., Choe J.K., Swenson K.: "In vitro fertilization with intracytoplasmic sperm injection is an effective therapy for male factor infertility related to subnormal hypo-osmotic swelling test scores". *J. Androl.,* 2001, *22,* 261.
[30] Check J.H.: "Sperm may be associated with subfertility independent of oocyte fertilization". *Clin. Exp. Obst. Gyn.,* 2005, *32,* 5.
[31] Check M.L., Katsoff D., Check J.H., Summers-Chase D.: "Effect of treating sperm with low hypo-osmotic swelling test scores with chymotrypsin on pregnancy rates after conventional in vitro fertilization-embryo transfer". *Fertil. Steril.,* 2004, *82,* 741.
[32] Evenson D.P., Larson K.L., Jost L.K.: "Sperm chromatin structure assay: Its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques". *J. Androl.,* 2002, *23,* 25.
[33] Saleh R.A., Agarwal A., Nelson D.R., Nada E.A., El-Tonsy M.H., Alvarez J.G.: "Increased sperm nuclear DNA damage in normozoospermic infertile men: a prospective study". *Fertil. Steril.,* 2002, *78,* 313.
[34] Larson K.L., De Jonge C.J., Barnes A.M., Jost L.K., Evenson D.P.: "Relationship of assisted reproductive technique (ART) outcomes with sperm chromatin integrity and maturity as measures by the sperm chromatin structure assay (SCSA)". *Hum. Reprod.,* 2000, *15,* 1717.
[35] Virro M.R., Kjersten L., Larson-Cook, Evenson D.P.: "Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles". *Fertil. Steril.,* 2004, *81,* 1289.
[36] Check J.H., Graziano V., Cohen R., Krotec J., Check M.L.: "The effect of an abnormal sperm chromatin structural assay (SCSA) on pregnancy outcome following in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) in previous IVF failures". *Arch. Androl.,* 2005, *51,* 121.
[37] Gandini L., Lombardo F., Paoli D., Caruso F., Eleuteri P., Leter G. *et al.*: "Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage". *Hum. Reprod.,* 2004, *19,* 1409.
[38] Bungum, Humaidan P., Spano M., Jepson K., Bungum Giewercman A.: "The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI". *Hum. Reprod.,* 2004, *19,* 1401.
[39] Ubaldi F., Camus M., Tournaye H., Clasen K., Nagy Z., Smitz J., Van Steirteghem A., Devroey P.: "Results of microsurgical epididymal sperm aspiration (MESA) and testicular sperm extraction (TESE) in azoospermic men using intracytoplasmic sperm injection (ICSI)". *Andrologia,* 1996, 28 (suppl. 1), 71.
[40] Watkins W., Nieto F., Bourne H., Wutthiphan B., Speirs A., Baker H.W.: "Testicular and epididymal sperm in a microinjection program: methods of retrieval and results". *Fertil. Steril.,* 1997, *67,* 527.
[41] Nicopoullos J.D., Gilling-Smith C., Almeida P.A., Norman-Taylor J., Grace I., Ramsay J.W.: "Use of surgical sperm retrieval in azoospermic men: a meta-analysis". *Fertil. Steril.,* 2004, *82,* 691.
[42] Check M.L., Check J.H., Summers-Chase D., Choe J.K., Check D.J., Nazari A.: "Live birth after posthumous testicular sperm aspiration and intracytoplasmic sperm injection with cryopreserved sperm: Case report". *Clin. Exp. Obstet. Gynecol.,* 2002, *29,* 95.
[43] Check J.H., Bollendorf A.M., Press M.A., Breen E.M.: "Noninvasive techniques for improving fertility potential of retrograde ejaculates". *Arch. Androl.,* 1990, *25,* 271.
[44] Turek P.J., Cha I., Ljung B.M.: "Systematic fine-needle aspiration of the testis: correlation to biopsy and results of organ "mapping" for mature sperm in azoospermic men". *Urology,* 1997, *49,* 743.
[45] Katsoff B., Check J.H., Summers-Chase D., Yuan W., Horwath D.: "Pregnancy rates (PRs) per embryo transfer (ET) may be improved by conventional oocyte insemination for male factor rather than intracytoplasmic sperm injection (ICSI)". 2005 Annual Meeting of the American Society for Reproductive Medicine, Montreal, Quebec, Canada, October 15-19, 2005. *Fertil. Steril.,* 2005, *84,* S217 (abstract #P186).
[46] Katsoff B., Check J.H., Choe J.K., Krotec J.W., Amui J.: "Conventional oocyte insemination may result in a better pregnancy outcome than intracytoplasmic sperm injection (ICSI) for unexplained infertility". 2005 Annual Meeting of the American Society for Reproductive Medicine, Montreal, Quebec, Canada, October 15-19, 2005. *Fertil. Steril.,* 2005, 84, S363 (abstract #P585).
[47] Katsoff B., Check J.H., Wilson C., Yuan W., Horwath D.: "Conventional insemination of oocytes may improve odds of successful pregnancy compared to intracytoplasmic sperm injection (ICSI) as determined by a matched controlled study". 2006 Annual Meeting of the American Society for Reproductive Medicine, New Orleans, Louisiana, October 21-25, 2006. *Fertil. Steril.,* 2006, *86,* S351 (abstract #P587).
[48] Bartoov B., Berkovitz A., Eltes F., Kogosowski A., Menezo Y., Barak Y.: "Relationship between human sperm subtle morphological characteristics and IVF-ICSI outcome". *J. Androl.,* 2002, *23,* 1.
[49] Bartoov B., Berkovitz A., Eltes F., Kogosovsky A., Yagoda A., Lederman H. *et al.*: "Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection". *Fertil. Steril.,* 2003, *80,* 1413.

[50] Hazout A., Cohen Bacrie P., Junca A., Tesarik J.: "Outcomes of high-magnification ICSI in cases with low, moderate and high degrees of sperm DNA fragmentation". *Fertil. Steril.,* 2005, *84,* S78.
[51] Check J.H., SanSoucie L., Swenson K., Summers-Chase D., Horwath D.: "A comparison of the efficacy of selecting sperm with normal nuclei by high magnification for intracytoplasmic sperm injection (ICSI) according to age in refractory in vitro fertilization (IVF) cases. 31st Annual Meeting of the American Society of Andrology, Chicago, IL, April 8-11, 2006". *J. Androl.*, 2006 (suppl.), 81 (abstract #104).
[52] Liu J., Nagy Z., Joris H., Tournay H., Devroey P., Van Steirteghem A.: "Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia". *Hum. Reprod.,* 1995, *10,* 626.
[53] Battaglia D.E., Koehler J.K., Klein N.A., Tucker M.J.: "Failure of oocyte activation after intracytoplasmic sperm injection using round-headed sperm". *Fertil. Steril.,* 1997, *68,* 118.
[54] Rybouchkin A.V., Van der Straeten F., Quatacker J., De Sutter P., Dhont M.: "Fertilization and pregnancy after assisted oocyte activation and intracytoplasmic sperm injection in a case of round-headed sperm associated with deficient oocyte activation capacity". *Fertil. Steril.,* 1997, *68,* 1144.
[55] Stone S., O'Mahony F., Khalaf Y., Taylor A., Braude P.: "A normal live birth after intracytoplasmic sperm injection for globozoospermia without assisted oocyte activation: case report". *Hum. Reprod.,* 2000, *15,* 139.
[56] Zeyneloglu H.B., Baltaci V., Duran H.E., Erdemli E., Batioglu S.: "Achievement of pregnancy in globozoospermia with Y chromosome microdeletion after ICSI: case report". *Hum. Reprod.,* 2002, *17,* 1833.
[57] Kim S.T., Cha Y.B., Park J.M., Gye M.C.: "Successful pregnancy and delivery from frozen-thawed embryos after intracytoplasmic sperm injection using round-headed spermatozoa and assisted oocyte activation in a globozoospermic patient with mosaic Down syndrome". *Fertil. Steril.,* 2001, *75,* 445.
[58] Eldar-Geva T., Brooks B., Margalioth E.J., Zylber-Haran E., Gal M., Silber S.J.: "Successful pregnancy and delivery after calcium ionophore oocyte activation in a normozoospermic patient with previous repeated failed fertilization after intracytoplasmic sperm injection". *Fertil. Steril.,* 2003, *79,* 1656.
[59] Chi H.J., Koo J.J., Song S.J., Lee J.Y., Chang S.S.: "Successful fertilization and pregnancy after intracytoplasmic sperm injection and oocyte activation with calcium ionophore in a normozoospermic patient with extremely low fertilization rates in intracytoplasmic sperm injection cycles". *Fertil. Steril.,* 2004, *82,* 475.

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