Mini-reviews

In the mini-review section this month, there are four reviews on different subjects: intracytoplasmic sperm injection, photodynamic therapy in prostate cancer, and the roles of re-TUR and intravesical chemotherapy in superficial bladder cancer.

There is a fifth paper in the section which is not a mini-review, but rather a short appreciation of Terence Millin, a pioneer in urological surgery, and a great surgical innovator.

Intracytoplasmic sperm injection: a review of risks and complications

GWENDOLYN H. WOLDRINGH, JAN A.M. KREMER, DIDI D.M. BRAAT and ERIC J.H. MEULEMAN*

*Department of Obstetrics and Gynaecology, and *Department of Urology, University Medical Centre St Radboud, Nijmegen, the Netherlands*

Accepted for publication 18 March 2005

KEYWORDS

intracytoplasmic sperm injection, risks, complications

INTRODUCTION

The incidence of couples seeking fertility treatment in the western world is estimated to be 15% [1], and male factors account for about half of the cases [2]. Since the birth of Louise Brown in 1978, *in vitro* fertilization (IVF) has become a widely used treatment for infertile couples. The introduction of intracytoplasmic sperm injection (ICSI) in 1992 revolutionized the management of severe male-factor infertility, with high success rates [3]. ICSI involves injecting a pre-selected spermatozoon into a mature oocyte (Fig. 1) after ovarian superovulation and oocyte retrieval. For men with azoospermia, ICSI with sperm retrieved at the level of the epididymis or testis is the sole possibility to father their own genetic progeny.

In Europe, >250 000 IVF/ICSI cycles are started annually, which accounts for 500–1500 cycles per million inhabitants per year [4]. The numbers of reported cycles continued to increase in the last few years, with an increase of 37% from 1997 to 2000. The mean (range) proportion of ICSI in different countries is 44 (24–68)%. In the European countries in 2000, the clinical pregnancy rate per transfer was 28.4% for IVF and 28.7% for ICSI. It can be estimated that ≈3% of the overall number of live births are the result of IVF or ICSI [5]. These figures indicate that artificial reproductive techniques (ARTs) are practised successfully on a large scale. Along with the success story, less attention is paid to safety and the potential risks for the offspring, and to the consequences of the high rate of multiple pregnancies [6].

THEORETICAL RISKS OF ICSI

Natural conception is associated with failures in all stages, ranging from fertilization through birth defects to developmental abnormalities and infertility in the offspring. In ICSI the risks are theoretically increased for several reasons [7]. Firstly, there are risks to the female gamete: injection of the oocyte might cause damage to the ooplasm or meiotic spindle apparatus; foreign substances or contaminants might be injected in the oocyte; and moreover an anomalous female gamete, that otherwise would be bypassed by natural selection, might be fertilized. Secondly, there are risks to the male gamete: sperm carrying DNA anomalies, i.e. breaks and aneuploidy [8,9], Y-chromosome deletions [10], or structural defects, might be injected. Most infertile men with congenital bilateral absence of the vas deferens (CBAVD) carry mutations in the cystic fibrosis transmembrane regulator (CFTR) gene [11]; their offspring have an increased risk of cystic fibrosis, so genetic testing of the partner and

counselling of the couple is recommended. When sperm is surgically retrieved from the epididymis or from the testis, there are additional risks, e.g. incomplete maturation and ageing after a prolonged stay in the epididymis (in case of obstruction) [12].

Finally, the process of genomic imprinting might be incompletely installed during gametogenesis, or maintained during embryonic development [13]. Mouse developmental genetic research and the genetics of some rare developmental disturbances in humans, such as Angelman syndrome, Prader Willy and Beckwith-Wiedemann syndrome, clearly show that some chromosome regions derived from oocytes rather than sperm are different for a subset of ≈50 genes. Depending on the gene, during prenatal life or into adulthood, there is monoallelic expression of either the male-derived allele or the female-derived allele. When mouse zygotes are reconstructed to contain genetic material from the father only, the balance during early embryogenesis between the embryo proper and the embryonic part of the placenta shifts towards the latter. These observations agree with the developing insight that genomic imprinting is specifically important for placental functioning. Similarly, during mouse *in vitro* development before implantation, the pattern of imprinting can be upset by the type of medium used and most easily for the placenta [14]. Of most human imprinting defects that are associated with ART, involving IVF and ICSI, there was hypomethylation on the genomic areas concerned for the maternal allele.

Together with observations in farm animals, this led to the suspicion that *in vitro* embryo culture conditions are responsible. Consequently, imprinting disorders might result indirectly from ICSI and are probably unrelated to sperm differentiation.

MULTIPLE PREGNANCIES

The major concern of ICSI is the high rate of multiple pregnancies (26%), because two or more embryos are transferred per cycle. The result is that 40% of ICSI-derived offspring are a part of multiple pregnancy [4]. In natural conception a twin is the result of the fertilization of two separate oocytes (1.2% of pregnancies) or a single fertilized oocyte that subsequently divides into two identical structures (0.4% of pregnancies) [15]. Although, singleton pregnancies after ICSI have a worse perinatal outcome than unassisted singleton pregnancies, twin pregnancies after ICSI have a better outcome than after natural conception [16]. Perinatal and maternal mortality and morbidity are increased, because of the higher rate of prematurity (<37 weeks of gestation), low birth weights (<2.5 kg) and maternal complications (pre-eclampsia, anaemia, postpartum haemorrhage).

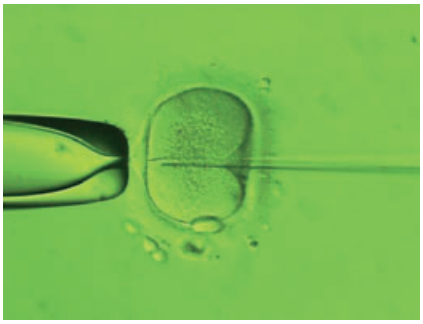
Children born after multiple gestation may suffer long-term consequences of perinatal complications, including cerebral palsy and learning disabilities. Furthermore, parents of multiple births experience more stress, and siblings of multiple births are more likely to have behavioural problems [17].

Until recently, physicians and patients underestimated the negative consequences of multiple pregnancies [15]. Currently however, elective single embryo transfer (eSET), with the aim of reducing the number of multiple pregnancies while maintaining an acceptable pregnancy rate, is practised in increasingly many institutions [18]. In the eSET scenario, a multiple pregnancy is regarded as a complication [19].

EARLY PREGNANCY

The percentage of abortion after ICSI is 17.6%, which is similar to the rates after IVF, irrespective of the cause of male infertility and the origin of the sperm [20]. Moreover, it is similar to the overall risk of spontaneous abortion after naturally conceived pregnancies, which is 14–21%. This proportion depends on the woman's age and previous history of spontaneous abortion [21]. However, the percentage of aneuploidy in ICSI conceptus is significantly higher [22], possibly due to abnormalities in the sperm of patients with ICSI [8,23]. Lower fertilization and implantation rates are reported when testicular sperm of men with unobstructive azoospermia is injected [24]. These data suggest that the theoretical risk in ICSI is partly expressed in reproductive failure in the preclinical stage of pregnancy before implantation, i.e. during fertilization and early embryo development [25]. There was a significantly higher rate of *de novo* chromosomal anomalies (1.6 vs 0.5% in the normal population, on amniocentesis for a mean maternal age of 33.5 years) in ICSI offspring, relating mainly to more sex chromosomal anomalies and partly to more autosomal structural anomalies [26]. This finding was related to sperm concentration and motility, and not morphology. The significantly higher rate of observed inherited anomalies (1.4 vs 0.3–0.4% in prenatal tests in the general population) was related to a higher rate of constitutional chromosomal anomalies, mainly in the fathers [27].

FIG. 1. Injection of a preselected spermatozoon into a mature oocyte.



BIRTH DEFECTS

After the introduction of ICSI in 1992, several researchers expressed concerns about the possible adverse effects on birth defects, and on the health and development of children, especially when unejaculated sperm is used. Some critics even suggested that ICSI might have a negative impact on the genetic composition of the human race [28]. In the Netherlands this has led to a moratorium on the application of ICSI in azoospermic men using unejaculated sperm. That reasoning appears to be valid from the viewpoint that ICSI bypasses the effective biological mechanisms of sperm selection, and has not been preceded by research. Consequently, the human experience with ICSI is only the experimental record [29].

In particular, the risk of boys born to couples with male factor subfertility has attracted attention, because for many patients with male factor subfertility, a genetic cause can be suspected. These include Y-chromosomal microdeletions, X-chromosomal and autosomal aberrations (i.e. Robertsonian translocations), syndromal disorders featuring infertility (i.e. Kallmann's syndrome) and ultrastructural sperm defects with a

genetic origin [30]. Theoretically, with ICSI these defects can be transmitted to the next male generation.

Furthermore, there is some evidence that the risk of congenital malformations is increased [31–33], especially after ICSI with surgically-retrieved sperm in unobstructive azoospermia [25,34]. The results of these studies are of interest because the chance of chromosomal aberrations and genetic abnormalities is higher in men with unobstructive azoospermia. Unfortunately, too few infants have been investigated to draw valid conclusions.

The major malformation rate (those causing functional impairment or requiring surgical correction) varies in different studies, at 0.7–9.1% for ICSI patients and 0.5–7.2% for naturally-conceived children [35,36]. However, the case for a causal relationship between ICSI and adverse effects on the offspring is difficult to make, because in most studies maternal characteristics (e.g. age and parity), comorbidity, life-style (smoking, drinking, drugs), and that ICSI children are more often born prematurely with a low birth weight, are confounding factors. Moreover, the low incidence of malformations demands large-scale studies. In recent large Swedish studies, hypospadias was found more often in a cohort of ICSI male infants [37]. The investigators associated this malformation with paternal subfertility. However, there was also a greater incidence of hypospadias in a cohort of IVF boys, and it was related to maternal progesterone administration [38].

Imprinting diseases like Beckwith-Wiedemann syndrome and Angelman syndrome are very rare, but for the first syndrome, ART (irrespective of the fertilization technique) increases the relative risk by a factor of ≈6 [39]. Large-scale and long-term follow-up studies are necessary to confirm an association between imprinting disorders and ART. Further laboratory research is required to understand the pathogenesis of this association.

INFANT DEVELOPMENT

Since the late 1990s only a few studies have been published on the development of children conceived by ICSI. Most of these studies compared the development of such children and control children during the first 2 years of life, using either the Bayley scales of Infant Development or comparable scales. In 1998, an Australian study [40] found lower scores in the Bayley mental scale for the ICSI offspring at 1 year than for the IVF and natural-conception controls. These differences were statistically significant for boys but not for girls. However, other studies could not corroborate these data. Bonduelle *et al.* [41] reported a prospective study with a follow-up of 2 years; the overall Bayley mental development scores were similar in the ICSI and IVF offspring and naturally conceived children. Sutcliffe *et al.* [42] found no difference in neurodevelopment using the Griffiths scales of mental development at up to 15 months old in ICSI-conceived children compared with their naturally conceived peers. A pilot study in Belgium showed that the ICSI-conceived children had a similar psychomotor and intellectual development as the IVF- and naturally conceived children at the age of 5 years [43].

RISKS OF ICSI FOR WOMEN

Short-term medical complications of inducing ovulation or retrieving oocytes for IVF are rare. Ovarian hyperstimulation syndrome (1.8%), intraperitoneal bleeding (0.2%), pelvic infections (0.4%) and adnexal torsion (0.13%) have been reported [44]. Another short-term effect of IVF or ICSI treatment is the emotional impact. After an unsuccessful treatment cycle, couples score higher on depression scales and many women have a clinically relevant form of depression [45].

Hormonal and reproductive factors are involved in the causes of breast cancer and cancers of the female genital tract. Therefore, the long-term effect of fertility drugs on the risk of these cancers has been investigated. Many studies have not been able to reach firm conclusions because of low statistical power, lack of control for important confounders (e.g. causes of subfertility and parity) and short duration of follow-up. In a large-scale cohort study in the Netherlands, after a follow-up of 5–8 years, there was no increased risk of breast and ovarian cancer in women who had undergone IVF compared with subfertile women who had received no IVF. For endometrial cancer there was a greater risk in those exposed to IVF and in the unexposed group of women with subfertility caused by hormonal disorders [46]. The ESHRE consensus group concluded that there is currently no evidence that ART has any effect on the incidence of genital or breast cancer [4].

RISKS OF ICSI FOR MEN

Surgical sperm retrieval by percutaneous epididymal sperm aspiration or testicular sperm extraction in case of obstructive or unobstructive azoospermia is a procedure with only minor complications; pain, bleeding, bruising and scarring are the most common [47]. There might be a risk of a decrease in serum testosterone levels after testicular sperm extraction, especially in cases of small testicular volume and hypogonadism, such as patients with nonmosaic Klinefelter syndrome [48]. After an unsuccessful treatment cycle, couples score higher on depression scales [45]. A greater subjective responsibility for the infertility, impact of childlessness on daily life and treatment-related stresses (particularly for sperm aspiration/extraction methods) is described for men after an ICSI treatment. The men only reported marginally higher depression scores than their controls [49].

CONCLUSIONS

The theoretical risks of ICSI may result in fertilization failure, abortion, birth defects, genetic abnormalities, developmental abnormalities and infertility in the offspring. According to the current state of knowledge, it appears that the incidence of chromosomal abnormalities, including *de novo* abnormalities, is higher after ICSI than in the general population. This might be a result of the infertility *per se* rather than the ICSI technique. The incidence of congenital malformations might be slightly higher after ICSI, but more large prospective studies, with naturally conceived children as controls, are needed to address this question definitively.

Special attention is needed for children born after ICSI using epididymal or testicular sperm obtained from men with obstructive or unobstructive azoospermia. Concerns about the use of immature testicular spermatozoa from men with testicular failure require further study. One of the ESHRE consensus recommendations is to offer chromosomal analysis in cases of unobstructive azoospermia and oligozoospermia with $<5 \times 10^6$ sperm/mL, and offer Y-

microdeletion testing in men with unobstructive azoospermia and oligozoospermia of $<1 \times 10^6$ sperm/mL. CFTR gene analysis must be offered in cases of CBAVD. If the genetic abnormality is confirmed, counselling of the couple by a trained genetics specialist must be involved [4]. Another recommendation is to offer mid-trimester ultrasonographic screening for congenital malformations, and amniocentesis may be considered.

The conclusion that children conceived by ICSI have similar psychomotor and intellectual development at the age of 5 years as have those conceived by IVF or spontaneously need to be confirmed by multicentre studies.

The increased risk of congenital malformations seems also to be related to preterm and multiple births, so a twin pregnancy is regarded as a complication. The ESHRE consensus meeting agreed that the essential aim of IVF and ICSI is the birth of one healthy child, so elective single-embryo transfer is proposed in a first IVF/ICSI cycle in women aged <36 years if at least one good quality embryo is available.

The question of possible risks of infertility in the offspring, especially boys, is still unanswered, because the oldest children are only 12 years old. It is very difficult to follow these children to their fertile age, because of privacy and the possible stigmatization of these children.

Before starting ICSI treatment it is important to counsel all patients, verbally and by standard patient information material. They must be informed that not everything is known about the health and development of children in their puberty and beyond, and about their fertility.

Finally it is important to have good registries in all countries, including all data on maternal and fetal morbidity and mortality, congenital malformations (with a uniform nomenclature), ART and non-ART procedures.

ACKNOWLEDGEMENTS

The authors thank Dr Ir. P. de Boer, Senior Scientist, Department of Obstetrics and Gynaecology, University Medical Centre St Radboud, Nijmegen, for carefully reading the manuscript and giving useful comments on the section of genomic imprinting.

CONFLICT OF INTEREST

None declared.

REFERENCES

1 **World Health Organization**. *WHO Laboratory Manual for the examination of human semen and sperm-cervical mucus interaction*. Cambridge: Cambridge University Press, 1999
2 **Irvine DS.** Epidemiology and aetiology of male infertility. *Hum Reprod* 1998; **13** (Suppl. 1): 33–44
3 **Palermo G, Joris H, Devroey P.** Pregnancies after intracytoplasmatic injection of single spermatozoon into an oocyte. *Lancet* 1992; **340**: 17–8
4 **Land JA, Evers JLH.** Risks and complications in assisted reproduction techniques: report of an ESHRE consensus meeting. *Hum Reprod* 2003; **18**: 455–7
5 **Andersen AN, Gianaroli L, Nygren KG.** Assisted reproductive technology in Europe, 2000. Results generated from European registers by ESHRE. *Hum Reprod* 2004; **19**: 490–503
6 **Land JA, Evers JL.** What is the most relevant standard of success in assisted reproduction? Defining outcome in ART. a Gordian knot of safety, efficacy and quality. *Hum Reprod* 2004; **19**: 1046–8
7 **Patrizio P.** Intracytoplasmic sperm injection (ICSI): potential genetic concerns. *Hum Reprod* 1995; **10**: 2520–3
8 **Bernardini L, Gianaroli L, Fortini D *et al.*** Frequency of hyper-, hypohaploidy and diploidy in ejaculate, epididymal and testicular germ cells of infertile patients. *Hum Reprod* 2000; **15**: 2165–72
9 **Morris ID, Ilott S, Dixon L, Brison DR.** The spectrum of DNA damage in human sperm assessed by single cell gel electrophoresis (Comet Assay) and its relationship to fertilisation and embryo development. *Hum Reprod* 2002; **17**: 990–8
10 **Kremer JA, Tuerlings JH, Meuleman EJ *et al.*** Microdeletions of the Y chromosome and intracytoplasmic sperm injection: from gene to clinic. *Hum Reprod* 1997; **12**: 687–91
11 **Wong LJ, Alper OM, Hsu E, Woo MS, Margetis MF.** The necessity of complete CFTR mutational analysis of an infertile couple before *in vitro* fertilization. *Fertil Steril* 2004; **82**: 947–9
12 **Cremades N, Sousa M, Bernabeu R, Barros A.** Developmental potential of elongating and elongated spermatids obtained after *in-vitro* maturation of isolated round spermatids. *Hum Reprod* 2001; **16**: 1938–44
13 **Niemitz L, Feinberg A.** Epigenetics and reproductive technology: a call for investigation. *Am J Hum Genet* 2004; **74**: 599–609
14 **Mann MR, Lee SS, Doherty AS *et al.*** Selective loss of imprinting in the placenta following preimplantation development in culture. *Development* 2004; **131**: 3727–35
15 **Multiple gestation pregnancy.** The ESHRE Capri Workshop Group. *Hum Reprod* 2000; **15**: 1856–64
16 **Helmerhorst F, Perquin D, Donker D, Keirse M.** Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ* 2004; **328**: 261–6
17 **Cook R, Bradley S, Golombok S.** A preliminary study of parental stress and child behaviour in families with twins conceived by in vitro fertilization. *Hum Reprod* 1998; **13**: 3244–6
18 **Vilska S, Tiitinen A, Hyden-Granskog C, Hovalta O.** Elective transfer of one embryo results in acceptable pregnancy rates and eliminates the risk of multiple birth. *Hum Reprod* 1999; **14**: 2392–5
19 **De Sutter P, Van der Elst J, Coetsier T, Dhont M.** Single embryo transfer and multiple pregnancy rate reduction in IVF/ICSI. a 5-year appraisal. *Reprod Biomed Online* 2003; **6**: 464–9
20 **ASRM/SART.** Assisted reproductive technology in the United States: 1999 results generated from the American Society for Reproductive Medicine/ Society for Assisted Reproductive Technology Registry. *Fertil Steril* 2002; **78**: 918–31
21 **Gray RH, Wu LY.** Subfertility and risk of spontaneous abortion. *Am J Public Health* 2000; **90**: 1452–4
22 **Lathi RB, Milki AA.** Rate of aneuploidy in miscarriages following *in vitro* fertilization and intracytoplasmic sperm injection. *Fertil Steril* 2004; **81**: 1270–2
23 **Levron J, Aviram-Goldring A, Madgar I, Raviv G, Barkai G, Dor J.** Sperm chromosome abnormalities in men with severe male factor infertility who are undergoing *in-vitro* fertilization with

intracytoplasmic sperm injection. *Fertil Steril* 2001; **76**: 479–84
24 **Vernaeve V, Tournaye H, Osmanagaoglu K, Verheyen G, Van Steirteghem A, Devroey P.** Intracytoplasmic sperm injection with testicular spermatozoa is less successful in men with nonobstructive azoospermia than in men with obstructive azoospermia. *Fertil Steril* 2003; **79**: 529–33
25 **Vernaeve V, Bonduelle M, Tournaye H, Camus M, Van Steirteghem A, Devroey P.** Pregnancy outcome and neonatal data of children born after ICSI using testicular sperm in obstructive and non-obstructive azoospermia;. *Hum Reprod* 2003; **18**: 2093–7
26 **Bonduelle M, Aytoz A, Van Assche E, Devroey P, Liebaers I, van Steirteghem A.** Incidence of chromosomal aberrations in children born after assisted reproduction through intracytoplasmic sperm injection. *Hum Reprod* 1998; **13**: 781–2
27 **Bonduelle M, Van Assche E, Joris H *et al.*** Prenatal testing in ICSI pregnancies. incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Hum Reprod* 2002; **17**: 2600–14
28 **Te Velde ER, van Baar AL, van Kooij RJ.** Concerns about assisted reproduction. *Lancet* 1998; **23**: 1524–5
29 **Johnson MD.** Genetic risks of intracytoplasmic sperm injection in the treatment of male infertility: recommendations for genetic counselling and screening. *Fertil Steril* 1998; **70**: 397–411
30 **Meschede D, Lemcke B, Behre HM, De Geyer C, Nieschlag E, Horst J.** Clustering of male infertility in the families of couples treated with intracytoplasmatic sperm injection. *Hum Reprod* 2000; **15**: 1604–8
31 **Wennerholm UB, Bergh C, Hamberger L *et al.*** Incidence of congenital malformations in children born after ICSI. *Hum Reprod* 2000; **15**: 944–8
32 **Ludwig M, Katalinic A.** Malformation rate in fetuses and children conceived after ICSI. Results of a prospective cohort study. *Reprod Biomed Online* 2002; **5**: 171–8
33 **Katalinic A, Rosch C, Ludwig M.** Pregnancy course and outcome after intracytoplasmic sperm injection: a controlled, prospective cohort study. *Fertil Steril* 2004; **81**: 1604–16
34 **Aytoz A, Camus M, Tournaye H, Bonduelle M, Van Steirteghem A, Devroey P.** Outcome of pregnancies after intracytoplasmic sperm injection and the effect of sperm origin and quality on this outcome. *Fertil Steril* 1998; **70**: 500–5
35 **Retzloff MG, Hornstein MD.** Is intracytoplasmic sperm injection safe? *Fertil Steril* 2003; **80**: 851–9
36 **Kurinczuk JJ, Hansen M, Bower C.** The risk of birth defects in children born after assisted reproductive technologies. *Curr Opin Obstet Gynecol* 2004; **16**: 201–9
37 **Ericson A, Kallen B.** Congenital malformations in infants born after *in vitro* fertilization. A population based study. *Hum Reprod* 2001; **16**: 504–9
38 **Silver RI, Rodriguez R, Chang TS, Gearhart JP.** *In vitro* fertilization is associated with an increased risk of hypospadias. *J Urol* 1999; **161**: 1954–7
39 **DeBaun MR, Niemitz EL, Feinberg AP.** Association of *in vitro* fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am J Hum Genet* 2003; **72**: 156–60
40 **Bowen JR, Gibson FL, Leslie GI, Saunders DM.** Medical and developmental outcome at 1 year for children conceived by intracytoplasmic injection. *Lancet* 1998; **351**: 1529–34
41 **Bonduelle M, Joris H, Hofmans K, Liebaers I, Van Steirteghem A.** Mental development of 201 children at 2 years of age. *Lancet* 1998; **351**: 1553
42 **Sutcliffe AG, Saunders K, McLachlan R *et al.*** A retrospective case-control study of developmental and other outcomes in a cohort of Australian children conceived by intracytoplasmic sperm injection compared with a similar group in the United Kingdom. *Fertil Steril* 2003; **79**: 512–6
43 **Place I, Englert P.** A prospective longitudinal study of the physical, psychomotor, and intellectual development of singleton children up to 5 years who were conceived by intracytoplasmic sperm injection compared with children conceived spontaneously and by in vitro fertilisation. *Fertil Steril* 2003; **80**: 1388–97
44 **Govaerts I, Devreker F, Delbaere A, Revelard Ph, Englert Y.** Short term complications of 1500 oocyte retrievals for in vitro fertilization and embryo transfer. *Eur J Obstet Gynecol Reprod Biol* 1998; **77**: 239–43
45 **Verhaak C, Smeenk J, Kremer J, Braat D, Kraaimaat F.** Emotional impact of the IVF or ICSI treatment: increased anxiety and depression following an unsuccessful first treatment cycle. *NTVG* 2002; **146**: 2363–6
46 **Klip H, Burger CW, Kenemans P, Van Leeuwen FE.** Cancer risk associated with subfertility and ovulation induction: a review. *Cancer Causes Control* 2000; **11**: 319–44
47 **Wood S, Thomas K, Sephton V, Troup S, Kingsland C, Lewis-Jones I.** Postoperative pain, complications, and satisfaction rates in patients who undergo surgical sperm retrieval. *Fertil Steril* 2003; **79**: 56–62
48 **Okada H, Shirakawa T, Ishikawa T, Goda K, Fujisawa M, Kamidono S.** Serum testosterone levels in patients with nonmosaic Klinefelter syndrome after testicular sperm extraction for intracytoplasmic sperm injection. *Fertil Steril* 2004; **82**: 237–8
49 **Beutel M, Kupfer J, Kirchmeyer P *et al.*** Treatment-related stresses and depression in couples undergoing assisted reproductive treatment by IVF or ICSI. *Andrologia* 1999; **31**: 27–35

Correspondence: Eric J.H. Meuleman, Consultant Urologist Sexologist, Department of Urology, Free University Medical Centre, PO Box 7057, 1007 MB, Amsterdam, the Netherlands.
e-mail: e.meuleman@vumc.nl

Abbreviations: **IVF**, *in vitro* fertilization; **ICSI**, intracytoplasmic sperm injection; **ART**, artificial (assisted) reproductive technique; **CBAVD**, congenital bilateral absence of the vas deferens; **CFTR**, cystic fibrosis transmembrane regulator; **eSET**, elective single embryo transfer.