Technology assessment of assisted reproduction

Westerlaken, Lucette van der

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Lucia Alida Johanna van der Westerlaken
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Promotor:	Prof. Dr. F.M. Helmerhorst
Co-promotor:	Dr. N. Naaktgeboren
Referent:	Prof. Dr. J. van der Elst, Universiteit van Gent, België
Overige leden:	Prof. Dr. J.B.M.Z. Trimbos
	Prof. Dr. H.H.H. Kanhai
	Prof. Dr. M.H. Breuning

Contents

List of abbreviations

AIH	artificial insemination with husband's semen
ART	assisted reproduction technology
ASRM	American Society for Reproductive Medicine
CBAVD	congenital bilateral absence of the vas deferens
CC	clomiphene citrate
CF	cystic fibrosis
CVS	chorion villus sampling
DOT	direct oocyte transfer
ED	egg donation
FER	frozen embryo replacement
FSH	follicle stimulating hormone
GIFT	gamete intrafallopian transfer
GnRH	gonadotrophin-releasing-hormone
GV	germinal vesicle
hCG	human chorionic gonadotrophin
hMG	human menopausal gonadotrophins
ICSI	intracytoplasmic sperm injection
IUI	intrauterine insemination
IVF	in vitro fertilization
LH	luteinizing hormone
LHRH	luteinising hormone releasing hormone
MESA	microsurgical epididymal sperm aspiration
NICE	National Institute of Clinical Excellence
OAT	oligoasthenoteratospermia
OCC	ooyte cumulus complex
OHSS	ovarian hyperstimulation syndrome
OPU	oocyte pick-up
PCOS	polycystic ovarian syndrome
PGS	preimplantation genetic screening
PN	pronuclei
PND	prenatal diagnosis

PZD	partial zona dissection
PVP	polyvinylpyrrolidone
SART	Society for Assisted Reproductive Technology
SD	standard deviation
SET	single embryo transfer
SPSS	Statistical Package for the Social Sciences
SUZI	subzonal insemination
TESA	testicular sperm aspiration
TESE	testicular sperm extraction
TEST	tubal embryo stage transfer
TFF	total fertilization failure
LF	low fertilization
TMC/TMSC	total motile sperm count
WHO	World Health Organization
ZIFT	zygote intrafallopian transfer

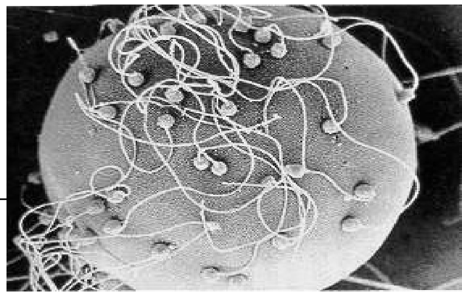
Preface

This thesis entitled “Technology assessment of assisted reproduction” comprises a variety of reproduction technologies that are nowadays common practice in IVF clinics all around the world. The assisted reproduction technology treatments (ART) that were under investigation are intra-uterine insemination (IUI), in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Each study focussed on certain aspects of ART that were thought to influence the result of the treatments mentioned.

The setting of the studies presented in this thesis was the every day practice of the IVF laboratory in the Leiden University Medical Center. This means that the subject of each study resulted directly from the questions that arose from the sometimes difficult clinical situations we were confronted with and to which we wanted an answer. This also means that all 6 studies presented in this thesis are embedded in the whole spectre of assisted reproduction technology.

The first chapter is an introduction to the various technologies and provides background information, showing the relevance of the studies. In the next 6 chapters the studies that have been performed are presented. The last chapter discusses the results that were found and presents the conclusion.

Chapter 1



General introduction

1.1 History of assisted reproduction technology treatments (ART)

1.1.1 Intrauterine insemination

The use of artificial insemination with husband's semen (AIH) goes back as far as the end of the 18^{th} century (1). In those times the main reason for it was the inability of the man to deposit his ejaculate in the vagina due to anatomical, neurological or psychogenic causes (e.g. severe hypospadia, retrograde ejaculation, impotence or vaginism). The sperm was injected with a syringe into the vagina and later through the cervix into the uterus.

In 1866 Sims (2) reported the first successful case after intrauterine insemination (IUI), where sperm was transferred directly into the uterine cavity. The famous gynaecologist who had his office at Central Park in New York considered the condition of the husband's sperm that failed to penetrate the cervical mucus a good indication for the treatment. Unfortunately the pregnancy ended in a miscarriage in the fourth month of the gestation.

The low success rate of IUI together with possible complications, such as mild to severe cramping or risk of pelvic inflammatory disease, led to the conclusion that intrauterine insemination should be abandoned unless techniques were improved (3,4). In parallel with the development of IVF technology many technical modifications have been proposed, such as methodology of sperm selection and preparation, hormonal stimulation of the ovaries, timing of insemination and methods of sperm transfer (4).

In the late 1980s a modification in the procedure for sperm preparation, the density gradient, became a breakthrough. Up till then one of the most frequently used methods of sperm preparation had been washing of the sperm in order to remove the seminal plasma, which contains fertilization inhibiting factors and prostaglandins that can cause uterine cramping. Thereafter the sperm was concentrated by centrifugation. This method however lacks any type of sperm selection. It may even account for poor results in some studies because repeated washing may reduce sperm motility (5,6). Another commonly used sperm preparation technique was called the swim-up method. By this method a seminal plasma-free sperm with increased motility was recovered based on the migration of spermatozoa through a culture medium that had been placed in contact with the semen (7). Both the washing and swim up methods yielded very low recovery of motile spermotozoa (2%- 13%) (8, 9, 10, 11). With the introduction of the density gradient procedure sperm recovery rates increased significantly. This procedure allows a rapid fractioning of spermatozoa according to their density: by centrifugation the spermatozoa with the best morphology and the highest motility will be concentrated in the higher density fractions (12). This technique resulted in the method of choice for in vitro sperm work up (13, 14). The advantages of this technique are particularly important for patients with oligoasthenozoospermia, since it yields high recovery rates of motile spermatozoa. Nowadays IUI is a widely used technique for the treatment of infertility in patients with cervical factor and/or idiopatic infertility, minimal to mild endometriosis, ovulation disorders, one-sided tuba pathology and mild male factor infertility (15). Because of its lower health risks, easier execution, lower cost and relatively high success rates compared to IVF, IUI is often offered before the more rigorous and costly IVF procedure.

1.1.2 In vitro fertilization

While in IUI the fertilization process takes place in vivo in the female, in IVF the fertilization process takes place in vitro in the laboratory by bringing the oocytes that are retrieved from the ovaries and the sperm that has been processed together in a petridish. The in vitro fertilized oocytes are cultured to embryos in the laboratory as well until they are transferred to the uterine cavity.

After many years of experiments with flushing embryos from oviducts of several species which already started in the late nineteenth century (16), it was not until 1959 that Chang successfully carried out in vitro fertilization with rabbit oocytes and sperm (17).

In vitro fertilization in humans was hampered by several problems. First there was the necessity of developing suitable media for in vitro culture of oocytes, sperm and embryos (18). Secondly there was the recovery of suitable, acrosome - reacted sperm and thirdly the recovery of oocytes at a time point where they were mature enough to be fertilized but had not been ovulated into the tubae yet. All those obstacles have been resolved to some extent: culture media have been developed, more knowledge about sperm function has been gained and oocyte recovery can be timed by administration of human chorionic gonadotrophin (hCG) shortly before the expected time of spontaneous ovulation. Another obstacle was the method of oocyte recovery. For some years the ovaries were approached by laparotomy but in the late 1960s laparoscopy was introduced (19). It was at the same time that ovarian stimulation for superovulation was introduced by injecting human menopausal gonadotrophins (hMG) during the follicular phase of the menstrual cycle to obtain more than one oocyte thereby enhancing the chance of achieving a pregnancy (19). Using these methods the first human pregnancy was established in 1976 by Steptoe and Edwards, but unfortunately it turned out to be ectopic (20). However in 1977 they succeeded to achieve a pregnancy without the use of hMG as they believed that it would hinder implantation. This pregnancy resulted in the birth of the first “test tube baby” Louise Brown in 1978 (21).

Since that time several variant methods have been proposed and tested for the treatment of infertility, making use of the knowledge gained from IVF. Among them are the gamete intrafallopian transfer (GIFT) in which the oocytes and washed sperm are transferred directly to the fallopian tubes, the zygote intrafallopian transfer (ZIFT) in which the transfer of pronuclear stage oocytes to the Fallopian tubes is effected 18-24 hours after insemination in vitro, and tubal embryo stage transfer (TEST) in which the transfer to the Fallopian tubes is effected later when early cleavage has been reached (22). These last two techniques represent a modification of the GIFT, which is performed in couples in whom evidence of fertilization is important before transfer (23). It was thought that because the GIFT technique was closer to the natural process and to the natural location of fertilization it would give higher pregnancy rates compared to other techniques. Limitations of GIFT are the need for presence of patent and normally functioning Fallopian tubes, the necessity of laparoscopy and the inability to demonstrate fertilization. Although GIFT has majorily been abandoned as IVF treatment it is still offered in some fertility clinics nowadays (24).

After the birth of Louise Brown in 1978 it took several years before IVF became an established method for the treatment of infertility majorily because of the low success rate. In the first years of IVF the common opinion among the small circle of specialists was that superovulation by the use of hMG hindered implantation by producing an abnormal hormonal environment. Most treatments were therefore performed in a natural cycle. Later with the application of endocrine monitoring and the development of ovarian imaging by ultrasound superovulation cycles became easier and safer to manage. Once it was established that the replacement of more than one embryo increased pregnancy rate the natural cycle was largely abandoned in favour of superovulation cycles (25, 26). The oocyte retrieval from the ovaries underwent also simplification. It had been simplified from laparotomy to laparoscopy by Steptoe (19) but in 1981 it became possible to puncture follicles under transabdominal and since 1985 under transvaginal ultrasound guidance (27, 28).

A further significant improvement in IVF treatment was the introduction of fully controlled ovarian stimulation by inducing pituitary down regulation before ovarian stimulation (29). These new ovarian stimulation protocols were based on the use of luteinising hormone releasing hormone (LHRH) analogue to prevent spontaneous endogenous secretion of LH in combination with exogenous gonadotrophins. Also the formulation of gonadotrophin hormone preparations have changed over the years from urinary derived gonadotrophins over highly purified urinary FSH preparations to recombinant gonadotrophic hormones. Whether this change contributes to higher results is controversial (30).

Next to the progress in clinical IVF treatment modalities, IVF laboratory techniques have been optimised and new ones have been introduced. The most important ones are the development of the density gradient procedure for sperm preparation, the development of intracytoplasmic sperm injection (ICSI) for patients with a severe andrological indication who would have no chance of producing offspring with IVF and the development of cryopreservation for the freezing of surplus embryos that would otherwise be discarded. Survival of cryopreserved mammalian embryos after thawing had been demonstrated already in 1972 in the mouse, before the first successful human IVF (31). With the development of human IVF the potential application for cryopreservation of human embryos was acknowledged. In 1983 the first pregnancy following a frozen thawed embryo transfer was achieved (32) and in 1984 the first live births (33).

In contrast to the development of the techniques with regard to oocyte retrieval is the status quo of the techniques with regard to embryo transfer. It was not until the last couple of years that more attention has been drawn to the embryo transfer technique. There have been studies that show higher pregnancy rates if the embryos are transferred further away from the fundus (34) and others that did not find any effect of the site of embryo transfer (35). Not only is the technique of embryo transfer under attention, but also the number of embryos that is transferred. When it became clear that pregnancy rates increased if more embryos were transferred the number of multiple pregnancies also increased. Although not recognized as a problem at first, multiple pregnancies are considered nowadays as a complication of ART in general. Although there are many differences between countries and between IVF centres in coping with this complication there certainly is an irreversible tendency to reduce the number of embryos for transfer, according to the many publications on this subject (36, 37, 38, 39, 40, 41, 42).

1.1.3 Intracytoplasmic sperm injection

Although IVF became a powerful technique in the treatment of infertility it is primarily useful in the treatment of female and mild male infertility. Severe male infertility due to oligoasthenoteratozoospermia cannot be overcome by applying IVF due to lack of sperm penetration potential. Fertilization was obtained in these cases by a new technology bypassing the oocyte barriers to sperm penetration in an artificial way by mechanical intracytoplasmic sperm injection (ICSI).

The major barrier to fertilize for patients with low numbers and/or low quality of spermatozoa is the zona pellucida that among others protects the oocyte from polyspermy and physical damage. Failure of the spermotozoa to penetrate the zona pellucida and fertilize the oocyte could result from poor motility, abnormal capacitation and/or acrosome reaction. In the late 1980s and early 1990s methods to circumvent the zona by introducing the spermatozoon directly behind the zona have been developed. Various techniques have been tried: complete or localized removal of the zona with an acidic medium or enzyme or cutting a hole in the zona before adding the sperm, thereby allowing free access. Results in the human were not promising: a high rate of polyspermia when the zona was completely removed (43). When the zona was partially removed (partial zona dissection: PZD) the results were better but varied with the different types of male infertility (44). The best results were obtained with oligozoospermic semen. For more severe cases of male infertility subzonal insemination (SUZI) was developed. With this technique spermotozoa were injected under the zona pellucida. However, the success rate in terms of normal fertilization rate never exceeded 20-25% (45).

A major step forward was made in 1991 when the first successful intracytoplasmic sperm injection (ICSI) treatments were reported (46). By injecting one single spermatozoon directly into the cytoplasm fertilization was obtained in cases of severe male factor infertility where fertilization was not obtainable by IVF. The fertilization as well as pregnancy rates in ICSI are similar to IVF. Later it was shown that this technique could also be used in patients with azoospemia: by the use of surgically retrieved sperm at the site of the epididymis (MESA) or testis (TESE) fertilization could be established (47). Nowadays ICSI is offered as a standard ART treatment for severe male infertility.

1.2 Clinical and biological principles of ART procedures

1.2.1 Intrauterine insemination

Basic principle: follicular development is monitored in the female partner and processed semen is inseminated into the uterine cavity after which fertilization takes place in vivo.

Normally ovulating women are monitored by means of ovarian ultrasound with regard to follicular growth. This can be combined with the analysis of the estrogen level in the blood and luteinizing hormone (LH) levels in the blood or in the urine. Follicular growth can optionally be stimulated by the administration of clomiphene citrate (CC) or follicle stimulating hormone (FSH) to increase the number of follicles that will develop to preovulatory follicles slightly.

Once the follicles have reached a diameter large enough for ovulation (the size of preovulatory follicles may vary from 15 to 30 mm (48, 49) and the estrogen levels are accordingly, ovulation is induced by administration of human chorionic gonadotrophin (hCG). When ovulation has been triggered spontaneously as can be measured by the rise of the LH level in the blood and/or urine there is no need to administer hCG.

Freshly ejaculated sperm will be processed in the laboratory usually by density gradient centrifugation. The ejaculate is placed on top of the density media and then centrifuged. Mostly used are the discontinuous gradients where clear boundaries between the layers are shown. Before 1996 a density medium that consisted of polyvinylpyrrolidone (PVP) coated silica particles, was the most widely used substance for density gradient centrifugation. After its withdrawal from the market for clinical use replacements based on silica particles that are coated by silane were introduced. The highly motile spermatozoa can penetrate the boundaries quicker than the poorly motile or immotile spermatozoa. By this method the seminal plasma will be removed, bacterial contamination will be reduced and the spermatozoa with the best morphology and highest motility will be isolated and concentrated. Insemination is performed around the time of ovulation, which is about 38-40 hours after hCG administration or 30-38 hours after the start of the serum LH surge (50, 51). Spermatozoa that are placed in the uterine cavity have a limited period for fertilization and therefore IUI needs to be closely timed with ovulation. The sperm is deposited into the uterine cavity by means of a catheter that is passed through the cervical canal. By inseminating only a small volume of sperm that contains none or only small amounts of seminal plasma the problems caused by the direct transfer of sperm in the uterus as severe cramps and a potential risk of infection as were seen in the past is greatly reduced (4).

1.2.2 In vitro fertilization

Basic principle: follicular development is monitored in the female partner, oocytes are retrieved and become inseminated in vitro with processed semen (figure 1) after which fertilisation and embryo development take place in vitro.

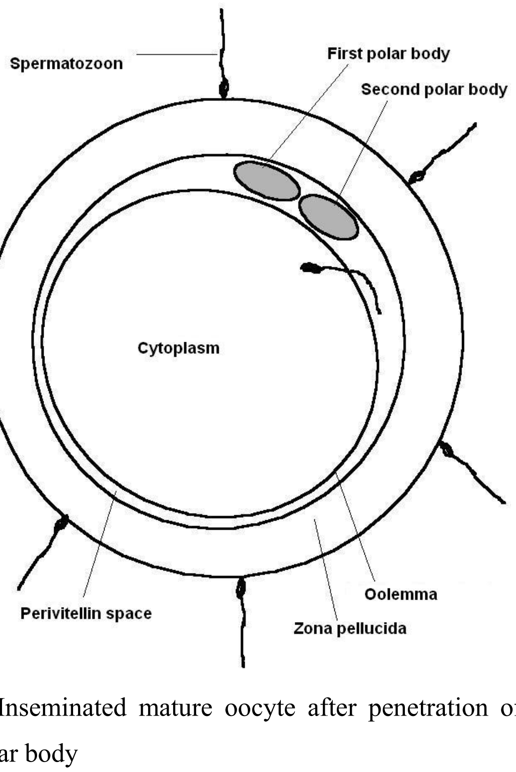


Figure 1: Inseminated mature oocyte after penetration of a spermatozoon and the extrusion of the second polar body

In general to increase the chance on pregnancy the retrieval of several oocytes is wanted. To obtain more than one oocyte the ovary is hyperstimulated by administration of follicle stimulating hormone (FSH) while at the same time a gonadotrophin-releasing-hormone agonist (GnRH-agonist) or antagonist is given to down-regulate the woman's own hormonal cycle. This down-regulation is necessary to prevent a spontaneous rise of the luteinizing hormone (LH), resulting in a spontaneous ovulation in approximately 40% of the cycles (52). By monitoring the stimulated cycle with ultrasonography of the ovaries the growth of the follicles in the ovary can be followed. Also the level of oestradiol in the serum can be measured and in combination with the ultrasounds the hyperstimulation can be monitored. When there are at least 3 follicles of a diameter large enough for ovulation and when the oestradial levels are in accordance with the follicles present, ovulation will be triggered by administration of human chorionic gonadotrophin (hCG), which has the same biological effect as LH.

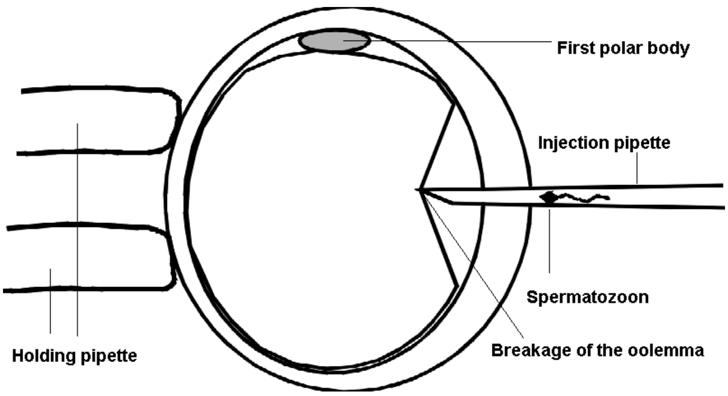
Because ovulation takes place at about 38-40 hours after hCG is administered oocyte retrieval will be planned at 34-36 hours, so 2-4 hours early. The oocyte retrieval is done by ultrasound guided transvaginal aspiration of the follicular fluid. Oocytes are collected from the follicular fluid under the stereomicrosmope in the laboratory and are cultured at 37 ^{0}C and under a 5% CO_2 atmosphere. In vitro insemination of the oocytes takes place the same day by adding semen (standard protocol 75.000 motile spermatozoa per oocyte) that is processed by density gradient centrifugation such that the seminal plasma is washed out and the most motile fraction of the semen sample is left over. Some spermatozoa bind to the outer shell of the oocyte: the zona pellucida. One spermatozoon penetrates the zona and fertilizes the oocyte. This process of fertilization involves the digestion of zona proteins by enzymes that are released from the acrosome, a membrane located in the head of the spermatozoon. After passing the zona pellucida the spermatozoon reaches the oolemma and fuses with it, allowing the spermnucleus to enter the cytoplasm of the oocyte (figure 1). Eighteen to 20 hours after insemination the oocytes are checked for the presence of 2 pronuclei, which are the visible evidence that fertilization has taken place (figure 2a). After another 2 days in culture the fertilized oocytes (zygotes) have become embryos (figure 2b and 2c) that will be transferred to the uterine cavity, by introducing a catheter through the cervical canal into the uterus. The morphologically best embryo(s), evaluated by the number of blastomeres and the amount of fragmentation, will be selected for transfer.

Figure 2: A: Fertilized oocyte; B: 4 cell embryo; C: 8 cell embryo

To support the endometrium progesterone is vaginally administered until the result of the pregnancy test (serum hCG determined about 15 days after oocytes retrieval) is known. If the serum hCG is positive a second assessment is performed 2 to 4 days later. If there is an increasing serum hCG the administration of progesterone will be continued until the 7th week of pregnancy.

1.2.3 Intracytoplasmic sperm injection

Basic principle: follicular development is monitored in the female partner, oocytes are retrieved and become mechanically injected in vitro with one single spermatozoon per oocyte using micromanipulation (figure 3) after which fertilization and embryo development take place in vitro.



Figuur 3: Intracytoplasmic sperm injection

The ICSI treatment is similar to IVF treatment with the exception of the way fertilization is established. This means that the patients do not notice any difference whether their oocytes are treated with IVF or ICSI since the only difference between the two treatments takes place in the laboratory where the oocytes are injected with one spermatozoon in case of ICSI or inseminated with tenth of thousands of spermatozoa in case of IVF. The oocytes that are retrieved after aspiration of the follicles need to be assessed for their maturational stage: only oocytes that have matured to metaphase two have the chromosomal constitution that is suitable for fertilization. The morphological proof that an oocyte has reached metaphase two is the presence of a polar body in the perivitelline space of the oocyte. This can be visualized by removal of the cumulus cells that surround the oocytes when they are recovered. Once the maturational status is determined the injection can be performed using micromanipulation techniques. A spermatozoon is aspirated into a microinjection pipet, the oocyte is immobilised with the help of a holding pipet in such a way that the polar body is at the 6 or the 12 o'clock position at the moment of injection. Then the spermotozoon is injected into the cytoplasm of the oocyte, thereby ensuring the breakage of the oolemma by aspirating cytoplasm into the injection pipet and back into the oocyte. This procedure is repeated until all retrieved mature oocytes are injected. Sixteen to 18 hours after injection the oocytes are checked for the presence of 2 pronuclei, the evidence that fertilization was successful. In general the time between injection and the appearance of the pronuclei is 2-4 hours shorter than between insemination and the appearance of the pronuclei. This is because the natural process of fertilization requires the approach of the spermotozoon to the zona, binding with the zona and penetration of the zona and of the oolemma. These processes are bypassed with ICSI, resulting in a faster appearance of the pronuclei. The way embryo selection and transfer are performed are identical to the IVF procedure.

1.3 Effectiveness and success rates of ART

The improved probability to become pregnant with ART treatment compared to no treatment is dependent on many factors such as duration of infertility, female age, pregnancy history, aetiology of the infertility problem, number of embryos transferred, number of previous treatment cycles, life style (alcohol and caffeine consumption, smoking habits), body weight and the laboratory techniques used.

The effectiveness of IUI, IVF and ICSI based on data derived from randomized controlled trials is rigidly investigated by the National Institute of Clinical Excellence (NICE) and described in guideline 11 (15) on which this chapter is based.

In addition success rates are reported based on national and international registries on ART (24, 53, 54, 55, 56).

1.3.1 Intrauterine insemination

For IUI two main influencing factors have been reported, namely the aetiology of the infertility problem and the stimulation protocols for IUI (15, 57). The effect of these factors can be illustrated by the results of a meta-analysis by Cohlen et al. (1999) (57) This analysis reveals significant differences in success rates between different treatment protocols in patients with different indications for IUI (table 1).

Table I: Pregnancy percentages per treatment cycle in patients with male factor infertility and unexplained infertility with or without ovarian stimulation and with or without intrauterine insemination (from Cohlen et al. 1999 (57))

	Intercourse		IUI	
Ovarian stimulation	-	+	-	+
Male factor infertility	0.8	5.1	5.0	9.3
Unexplained infertility	3.2	8.6	3.8	16.4

1.3.2 In vitro fertilization and intracytoplasmic sperm injection

The chance of a live birth per IVF/ICSI treatment cycle decreases with increasing age (greater than 20% for women of 23-35 years to 6% for women aged 40 years and older) and with increasing number of IVF/ICSI cycles (the chance of a live birth is only consistent for the first three IVF/ICSI cycles). The effectiveness of IVF/ICSI treatment in women who have previously been pregnant and/or had live birth is higher. Another important influencing factor is the number of embryos to be transferred. However, the chance of a live birth and the risk of multiple pregnancy and its consequences should be balanced by transferring no more than 2 embryos in any IVF cycle. The effectiveness of ICSI is higher than that of IVF when there is no or poor fertilization after IVF, but once fertilization is achieved the pregnancy rate is no better than with in vitro fertilization.

Table II presents the success rates after Intra-uterine insemination (IUI), In vitro fertilization (IVF), Intracytoplasmic sperm injection (ICSI), Frozen embryo replacement (FER) and Egg donation (ED) in four different ART registries. Different definitions are used in the different registries. The published percentages may reflect the number of clinical pregnancies, the number of ongoing pregnancies, and the number of live births either per oocyte aspiration, per embryo transfer or per started cycle. Min et al (2004) (58) suggested a definition that tried to express the ideal outcome: the number of singleton, term gestation, live birth per cycle. However up till now debates are going on whether this is the best outcome variable (59, 60). As is shown in table 2 the ART registries vary immensely not only in their definition of success rate but also in the way they report their results, making comparisons difficult.

Table II: Success rates of IUI, IVF, ICSI, FER and ED in four different registries

	IUI	IVF	ICSI	FER	ED
The Netherlands (2003) (5)	n.a	20.8%[1]	23.3%[1]	16%[2]	n.a.
Europe (2001) (6a)	12.6%	25.1%[3]	26.2%[3]	16.4%[2]	33.4%[2]
USA (2001) (3)	n.a.	28.1%[4]		23.8%[5] 27.3%[6]	47%[2]
Australia and New Zealand (2000) (4)	n.a.	15.1%[7]	15.1%[7]	14.2%[8] 20.6%[9]	17.1%[10]

[1] clinical pregnancies per cycle
[2] clinical pregnancies per transfer
[3] clinical pregnancies per oocyte aspiration
[4] live births per cycle; no discrimination between IVF and ICSI, 50% of the cycles is ICSI
[5] live births per transfer; only from non-donor eggs
[6] live births per transfer; only from donor eggs
[7] viable pregnancies per cycle
[8] viable pregnancies per transfer; only from non-donor eggs
[9] viable pregnancies per transfer; only from donor eggs
[10] viable pregnancies per transfer; only from donor eggs

The success rates of ART treatments have improved over the years. Relative increases of pregnancy rates can be calculated from the annual reports of different ART registries. In The Netherlands the relative increase in clinical pregnancy rate per oocyte retrieval was 28.9% from 1996 (18%) to 2003 (23.2%) (56). The European registries show a relative increase in clinical pregnancy rate per transfer of 11% for IVF (26.1% in 1997 to 29.0% in 2001) and of 7.0% for ICSI (26.4% in 1997 to 28.3%

in 2001) (53, 54). The ASRM/SART (USA) reports a relative increase in live birth rate per ART cycle of 20% (from 22.5% in 1996 to 27.2% in 2001) (55). The Australian registries show a relative increase of 24.0% in clinical pregnancy rate per oocyte retrieval for IVF (17.1% in 1997 to 21.2% in 2002) and of 14.4% for ICSI (18% in 1997 to 20.6% in 2002) (24). Currently with pregnancy rates ranging from 20-30% per IVF/ICSI attempt the cumulative conception rate for a fertile woman achieving a pregnancy in spontaneous natural cycles is the same as that for an infertile woman of the same age undergoing successive cycles of IVF/ICSI and transfer of several embryos (61).

1.4 Complications of ART

1.4.1 Complications with regard to the female patient

Two major complications resulting from ART treatments are the ovarian hyperstimulation syndrome (OHSS) and multiple gestation.

The ovarian hyperstimulation syndrome is the result of the hormonal stimulation of the ovary. While some degree of ovarian hyperstimulation occurs in all women who respond to ovulation induction severe OHSS is a life-threatening complication. In the most severe cases thromboembolic phenomena related to coagulation disturbances might occur (62). Severe OHSS occurs in 0.5-2% of otherwise healthy patients who undergo ART treatment (63, 64, 65, 66).

While at first not recognized as a complication of ART treatments, multiple pregnancies are nowadays considered as a major complication. The most serious risk for the mother is death: a three-fold increase in maternal mortality was shown in Europe (14.9 per 100,000 live births in multiple pregnancies versus 5.2 per 100,000 live births in singleton pregnancies) (67). Maternal morbidity is also significantly increased and risks include a.o. hypertensive disorders, thromboembolism, premature labor and delivery, urinary tract infection, anemia and vaginal-uterine hemorrhage, (68). Multiple pregnancy rates of 10% after IUI have been reported in Europe (9.6-10.2% twins and 0.6-1.1% triplets) (54, 69). According to a Dutch publication (2003) the majority of high order multiple pregnancies results from mild ovarian stimulation in combination with IUI (70). In a recent study by Goverde et al (2005) multiple pregnancy rates after IUI of 4% in a natural cycle to 27% in a mildly hyperstimulated cycle were presented (71). In addition multiple births accounted for 25.5% of IVF/ICSI associated live births in Europe in 2001 (54) and 36% in the USA in 2001 (55).

The effect of using gonadotrophins to stimulate ovarian follicular growth on the incidence of ovarian cancer has been examined in different studies (72, 73, 74, 75). So far no significant increases have been found.

1.4.2 Complications with regard to the offspring

The main complication for IVF/ICSI offspring is perinatal and infant morbidity. This is partly caused by the ART procedure itself (76) and partly by the high number of multiple pregnancies (77, 78, 79). In addition there are ICSI related risks such as possible chromosomal abnormalities (80, 81, 82).

The influence of the ART procedure itself was investigated by Helmerhorst et al. (2004) (76). Their study showed that the perinatal outcome after IVF was worse for both singletons and twins compared with singletons and twins from natural conception. However, for twins the perinatal outcome was less worse than for the singletons and perinatal mortality was even lower after assisted conception compared with natural conception. The major reason that the outcomes among twins are reaching unity may be found in the assumption that the assisted conception factor among twins is apparently overwhelmed by the multiple conception problem: a comparison between bad and bad remains equal. In order to detect an assisted conception factor, the best comparison we can make is among singletons.

The high number of multiple pregnancies results in more complications because almost all perinatal and infant morbidity occurs more frequently in twins than in singletons. Studies in Europe and in the USA and in Australia show an increase in neonatal mortality and cerebral palsy in twins compared to singletons (77, 78, 79). The percentage of infants born as singletons, twins and triplets in Europe in 2001 was 74.5%, 24% and 1.5% respectively (54), which is an improvement compared to 1999 with percentages of 58%, 37% and 5% for singletons, twins and triplets, respectively (83). Now there is a general agreement that there is a need to prevent multiple pregnancies by transferring fewer embryos. Studies on single embryo transfer (SET) have reported promising results (36, 37, 38, 39, 40, 41, 42).

Two types of risks resulting specifically of the ICSI treatment can be discriminated: ICSI procedure-independent and ICSI procedure-dependent risk factors. In the first case, the risk relates to the cause of infertility, leading to the need to perform ICSI with spermatozoa that otherwise would not have been able to achieve fertilization. ICSI bypasses natural barriers of sperm selection and there is a potential danger of transmission of genetic disorders and inducing genetic anomalies into the offspring. Spermatozoa that are used for ICSI in case of severe male factor infertility may have increased levels of aneuploidy (84) and involve higher risks of transmitting gene defects related to the fertility problem, such as congenital bilateral absence of the vas deferens (CBAVD)/cystic fibrosis (CF) (85) and microdeletions of the Y chromosome (86, 87). A study by Bonduelle (2002) showed an increased risk of 1% of de novo chromosomal abnormalities, mainly related to sex chromosomes (81). This finding has been shown to be related to the source of sperm used for ICSI in extreme oligoasthenoteratospermia (OAT).

The ICSI-dependent risk factors are directly related to the laboratory procedure itself. ICSI is a very invasive procedure: penetration of the zona pellucida and oocyte cytoplasm may result in problems such as possible damage to the internal structures of the oocyte leading to aneuploidy and an increased risk of chromosomal anomalies, which are one of the causes of congenital anomalies. Other risk factors are the injection of biochemical contaminants, e.g. polyvinylpyrrolidone that is used to slow down the sperm motility, which possible damages cell organelles or interferes with normal embryo development. However, despite the risk factors mentioned there is as yet no evidence for ICSI -procedure related risk for the offspring.

1.5 Trends in assisted reproduction technologies (ART)

1.5.1 Demand for ART

In the human, assisted reproduction technology treatments (ART) are common practice these days. The development of ART treatments such as intra-uterine insemination (IUI), in vitro fertilization (IVF), frozen embryo replacement (FER) and later on of intracytoplasmic sperm injection (ICSI) and egg donation (ED) have become widely applied methods since the last two decennia in the treatment of subfertile couples as registered in Europe (53, 54, 83, 47, 78, 48), the United States (55 49) and Australia (24).

In the Netherlands an increase in the number of IVF/ICSI cycles of 41% (11,154 to 15,775 cycles) was registered within the period 1996 to 2003 (56 50), a similar figure as observed in Europe: an 42% increase of ART (203,893 to 289,690) cycles (without IUI) in the period between 1997 to 2001 (47,48). A 66% increase from 1996 to 2001 (64,724 to 107,587 cycles) was reported by the US Centre of Disease Control in 2003 (55 49), and an 80% increase (20,288 to 36,483 cycles) was found in Australia from 1996 to 2002 by the independent Australian Institute of Health and Welfare National Perinatal Statistics Unit (24). Although these numbers might be influenced by an in time more adequate coverage of reporting systems, the expansion of ART treatment cycles cannot be neglected.

The European registry started to publish data on IUI for the first time for the year 2001: a total of 52,949 cycles were included. However only 15 countries were reporting, compared to 23 countries that reported on IVF/ICSI/FER/ED. Only one national authority, the French Health Ministry published an figure of the number of IUI cycles from 1996 to 2000, which corresponded to 20% (36,820 to 44,271 cycles) increase (69).

1.5.2 Development of new treatments

Major improvements in infertility treatment can be attributed to innovations that have resulted from scientific research in human reproduction. Research on ovarian stimulation, oocyte retrieval, fertilization capacity, embryonic development and freezability of embryos resulted in the first successful IVF treatment, successful cryopreservation of embryos and the first successful ICSI. The development of preimplantation genetic diagnosis enabled patients with a genetic disease to become pregnant of a healthy baby by screening the embryos obtained by ICSI before they were being transferred. Other techniques that are under investigation are the preimplantation genetic screening (PGS) to detect aneuploidy in embryos related to maternal age or that is derived from patients with a history of spontaneous abortions or failed implantations after several IVF attempts. Also in vitro maturation and the freezability of human oocytes and ovarian tissue are under investigation.

Further major improvements may be expected from research focussing on the regulation of embryonic implantation. The implantation rate per transferred embryo does not exceed 30-35% using assisted reproduction technologies (88, 89). The responsibility for this high embryonic wastage has to be shared between the embryo and the endometrium. Embryonic implantation will only succeed when there is a perfect communication between a good quality embryo and a receptive endometrium (90, 91, 92). The quality of the endometrium is affected during hormone treatment in the ART cycle that influences the implantation process. In addition to the criteria that are commonly used for embryo quality, like the number of blastomeres and the percentage of fragmentation, other criteria are being developed like chromosomal constitution (93) and biochemical markers (90, 91, 92) to be able to select the embryo with the highest implantation chance for transfer.

1.5.3 Optimizing existing treatments

Success rates of ART can also be improved by fine tuning the use of existing ART treatments. Success rates are influenced not only by optimal technical procedures but also by the effectiveness of choosing the right treatment for the right patient. Subgroups of patients may benefit substantially from patient –tailored treatments. We studied in this thesis the appropriateness of treatment of infertility with IUI, IVF and ICSI in specific patients groups. We studied the effect of indication, age, and sperm parameters on pregnancy rates after intrauterine insemination (chapter 2). The success rates of ICSI versus IVF were compared in patients with specific fertilization disorders (chapter 3, 4, 5). For patients needing ICSI the effect of variations in the technological procedure (chapter 6) was examined. In addition the hypothesis whether pregnant ICSI patients were anxious to have prenatal testing was tested (chapter 7).

1.6 Scope of this thesis

Aim of this study

The aim of this study was to improve success rate of ART treatments in subgroups of patients by choosing the optimal patient – group tailored treatment. Therefore patient characteristics as well as laboratory techniques with a clinical pregnancy as outcome were studied. In addition the hypothesis was tested whether parents who underwent the invasive ICSI procedure where one spermatozoon is inserted inside the oocyte are anxious to have prenatal testing.

Outline of the thesis

Chapter 2: presents an evaluation of the effect of indication, age, and sperm parameters on pregnancy rates after intrauterine insemination to identify prognostic factors for the chance on pregnancy.

Chapter 3: presents a prospective study in which intracytoplasmic sperm injection was performed as a treatment for unexplained total fertilization failure or low fertilization after conventional in vitro fertilization. To determine whether IVF or ICSI should be the treatment of choice in case of a previous IVF attempt with unexplained total fertilization failure or low fertilization (<25%) both IVF and ICSI was performed on sibling oocytes in a second attempt.

Chapter 4: presents a prospective study in which patients with borderline semen are treated with both IVF and ICSI on sibling oocytes. To determine possible differences between patients with and without fertilization in IVF that could be used for future treatments in deciding for IVF or ICSI.

Chapter 5: reports a case of successful intracytoplasmic sperm injection after failed in vitro fertilization due to multipronuclear oocytes. This shows that ICSI can also be indicated in case of the development of multipronuclear oocytes after conventional IVF and not just for male infertility and unexplained fertilization failure.

Chapter 6: reports a prospective study in which the influence of the position of the polar body during ICSI and of the operator performing the injections was examined.

Chapter 7: summarizes the results of the policy of offering invasive prenatal diagnosis (PND) to pregnant ICSI patients: do they choose PND indicated by ICSI?

Chapter 8: discusses the obtained results and gives the conclusion.

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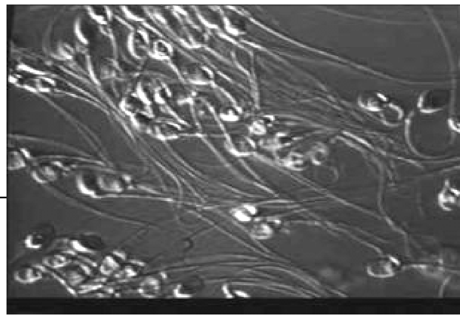
Chapter 2



Evaluation of pregnancy rates after intrauterine insemination according to indication, age and sperm parameters

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LAJ van der Westerlaken[1], N Naaktgeboren[1], FM Helmerhorst[1]

[1]*Department of Gynaecology, Leiden University Medical Center, Leiden, The Netherlands.*

Abstract

Purpose: Our purpose was to evaluate intrauterine insemination results obtained in our clinic and identify prognostic factors for the chance of pregnancy.

Methods: A retrospective study of data from 1989 to 1996 was undertaken. Only first attempts were included in this study except for the part on the cumulative pregnancy rates. Couples with one-sided tubapathology, hormonal dysfunction, idiopathic infertility, or andrological indication were selected. All women were stimulated with clomiphene citrate. Five hundred sixty-six couples that underwent 1763 cycles were included in the study.

Results: The overall pregnancy rate for first pregnancies was 6.9% per cycle and 21.4% per patient. For first intrauterine insemination attempts this was 8.8% per cycle/patient, varying between 5.0% for andrological indication, 10.6% for tubapathology, 10.0% for idiopathic indication, and 10.3% for hormonal indication. These differences were not significant. Age did not have a significant effect either, although there were no pregnancies observed in women of 40 years or older. The number of inseminated spermatozoa significantly affected the pregnancy rate: <2 million, 4.6%; ≥ 2 to <10 million, 3.9%; and ≥ 10 million, 11.3%.

Conclusions: Unless semen characteristics are insufficient, intrauterine insemination is a useful treatment for infertile couples.

Introduction

Intrauterine insemination (IUI) with husband's semen is a treatment of infertility used for a variety of indications. It is often offered before the more rigorous and costly in vitro fertilization procedure. IUI involves the deposition of washed and concentrated motile spermatozoa directly in the uterine cavity. To achieve fertilization and pregnancy, the insemination has to be synchronized with ovulation, either in a natural cycle or in a cycle with ovarian stimulation. Different protocols are used for that purpose (1). Many studies report on the effects of single variables on the success rate of IUI, such as the influence of age (2, 3, 4). It has also been found that the number of motile spermatozoa influences the chance of pregnancy (2, 5). Only a few studies have examined the role of indication on pregnancy outcome after IUI (6, 7, 8). This probably causes selection, because not all patients undergo the same number of cycles. Therefore we selected only first IUI cycles for this study.

To identify prognostic factors for IUI outcome, pregnancy rates for first IUI attempts were calculated in relation to indication, age of the woman and man, and the total number of inseminated motile spermatozoa. We also looked at the relationships between indication and age of the woman and man and between the age of the man and the number of motile spermatozoa.

In addition, cumulative pregnancy rates were calculated based on data from our IUI program to evaluate the effect of the number of IUI attempts on conception in relation to indication.

Materials and Methods

Patient selection

A total of 566 couples, which underwent a total of 1763 IUI cycles between January 1, 1989, and October 1, 1996, was included in this study. They were selected for the IUI program based on sperm characteristics, one-sided tubapathology, hormonal dysfunction, or idiopathic infertility. All women in this study were stimulated, according to our standard protocol, with clomiphene citrate.

Definition of indications

(1) One-sided tubapathology: Only one patent tube as evaluated by hystersalpingogram or laparoscopy.

(2) Andrological: At least two abnormal semen analyses, according to World Health Organisation standards (9)

(3) Idiopathic: No evident fertility disorders as investigated by the standard fertility workup schedule (10); postcoitus test negatives were also included in this group, because of the low predictive value of the test as determined by a study in our clinic (11)

(4) Hormonal: Couples in whom the woman has an irregular or anovulatory cycle but responds to clomiphene citrate administration. Some of these women belonged to the group with polycystic ovarian syndrome, as demonstrated by an increased luteinizing hormone (LH): follicle stimulating hormone (FSH) ratio.

Ovulation monitoring

The standard protocol for IUI was to combine the inseminations with mild ovarian stimulation with clomiphene citrate (100 mg daily, cycle days 3-7, p.o.), to obtain a slight increase in number of follicles and to facilitate the detection of ovulation. The cycles of all patients were monitored by ultrasound scan of the ovaries and determination of estradiol (E_2) levels and LH levels in the serum between day 11 and day 13 of the cycle. Depending on the cycle length and the ovarian response, more frequent determinations were performed, with extremes varying from cycle day 6 to cycle day 20. When one to three follicles 16-18 mm in diameter were present and the E_2 level was in accordance with the size and the number of follicles, 10,000 IU of human chorionic gonadotrophin (hCG; Profasi; Serono Benelux) were given to initiate ovulation. When the serum LH test was positive, no hCG was administered. The time of insemination was 18 to 42 hr after hCG injection or ±30 hr after the LH surge.

Sperm Preparation

Freshly ejaculated sperm was allowed to liquefy. Volume was determined and concentration and percentage of motile sperm were assessed in a Makler counting chamber and the total number of motile sperm was calculated. Hepes-buffered Earle's medium with 0.5% human serum albumin was added to the semen sample and mixed by pipetting. Depending on the total number of motile sperm the mixed sample was pipetted on top of either a 1-ml 70% or a 1-ml 80% Percoll layer and centrifuged (800g, 10 min). The supernatant was removed and the pellet was resuspended in Hepes-buffered Earle's medium. Depending on the total number of motile sperm, this suspension was either pipetted on top of an 80% Percoll layer and washed two times in the medium or washed two times in the medium after the first Percoll treatment. Volume, concentration, motility, and total number of motile sperm were redetermined after processing. The sperm was kept at 37^0C in an incubator until insemination took place.

Insemination and monitoring

A 1-ml syringe was filled with Earle's medium and connected to a Frydman (MDT; Oisterwijk, The Netherlands) or a TDT catheter (MDT). After flushing the catheter, the sperm suspension was aspirated into the catheter. The catheter was passed through the cervical canal and into the uterine cavity. The suspension (less than 0.5 ml) was slowly injected, after which the catheter was withdrawn gradually. Twelve days after the insemination a quantitative ßhCG determination was performed on the serum. When the ßhCG was positive an ultrasound was made at 6 weeks of gestation to confirm pregnancy.

Statistical analysis

Pearson's χ^2 test was used to compare the proportion of conception and non-conception cycles in women assigned to the different groups.

Cumulative pregnancy rates were calculated for the total number of IUI cycles present in our database and for subgroups of cycles according to the indication for IUI.

Results

Overall results

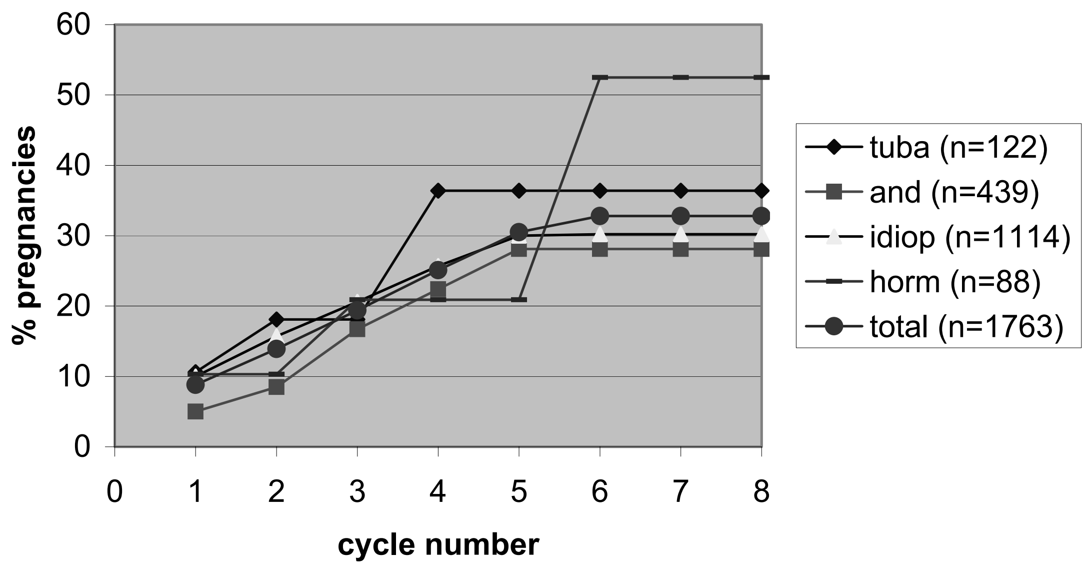
A total of 566 patient underwent a total of 1763 IUI cycles in this study, which resulted in 121 first pregnancies (6.9% per cycle, 21.4% per patient). The mean number of IUI cycles per patient was 3.1, varying from 1 to 8 attempts to achieve a first pregnancy.

The mean age ± SD of the women was 31.8 ± 4.7 years (range 19 to 46 years); the mean age ± SD of the men was 34.3 ± 5.9 years (range 19 to 59 years).

Cumulative pregnancy rates

Figure 1 shows the cumulative pregnancy rate. The total cumulative pregnancy rate reached a maximum of 31.1%, and no additional pregnancies were obtained after six IUI attempts. However, this number differed between indications: in the group with tuba pathology, no additional pregnancies occurred after four IUI cycles; in the idiopathic and hormonal groups, there were still pregnancies observed in the sixth cycles. Also, the cumulative pregnancy rate after six IUI attempts differed between indications (e.g., 30.2% in the idiopathic group versus 52.2% in the hormonal group).

Figure 1: Cumulative pregnancy rates after IUI in relation to indication



Pregnancy rates in relation to indication

Only first IUI attempts were included in this part of the study. No significant differences in pregnancy rates between the different indications were found (Table I). The pregnancy rate in the andrological group was low (although not significantly lower) compared with the other groups. To investigate whether age contributed to this difference by a difference in age between the indications, the age distribution within each indication was calculated. The only significant difference found was the higher number of women ≥40 years old in the tubapathology group compared with the other groups ($P<0.01$). Although not proven to be significant, there was a tendency for the andrological group to consist of a relative young population (40% were younger than 30 years and 80% were younger than 35 years) in comparison with the other indications (nonandrological indication: 30% younger than 30 years and 67% younger than 35 years). Both of these observations, however, cannot explain the observed differences in pregnancy rates.

Table I. Pregnancy rates per patient in the first IUI cycle, according to indication

Indication	# Patients	Age (mean yr ±SD)	# Pregnancies	% Pregnancies[1]
Tubapathology	47	34.3 ± 5.1	5	10.6
Andrological	139	30.8 ± 4.9	7	5.0
Idiopathic	351	31.8 ± 4.4	35	10.0
Hormonal	29	31.6 ± 5.0	3	10.3
Total	566	31.8 ± 4.7	50	8.8

[1] $\chi^2 = 3.33$, df=3, P=0.34

Pregnancy rates in relation to the number of motile sperm inseminated

An increase in the number of motile sperm that was inseminated resulted in a significant increase in pregnancy rate (Table II). This is in agreement with the difference in pregnancy rate found between the group with an andrological indication and the other indication groups: the mean number of motile sperm cells inseminated in the andrological group was 8.3 ± 10.8 million, versus 30.3 ± 34.4 million in the groups without andrological factors.

To investigate whether there was a difference in the chance of becoming pregnant with the same number of inseminated motile sperm cells but belonging to the andrological versus the nonandrological groups, these groups were compared. However, there was no significant difference in pregnancy rate between them (<2 million, $\chi^2 = 0.48$, P=0.49; ≥2 to <10 million, $\chi^2 = 0.29$, P= 0.59; ≥ 10 million, $\chi^2 = 0.42$, P=0.52). This shows that there are probably no factors other than semen characteristics that are responsible for the pregnancy rates found in both the andrological and the nonandrological groups.

Table II: Influence of the total number of inseminated motile sperm on the pregnancy rate with regard to andrological (And.) and nonandrological (Nonand.) indications

Total motile sperm ($x10^6$)	# Patients			# Pregnancies			% Pregnancies		
	And.	Nonand.	Total	And.	Nonand.	Total	And.[1]	Nonand.[2]	Total[3]
<2	35	8	43	2	0	2	5.7	0	4.6
≥2 to <10	67	85	152	2	4	6	3.0	4.7	3.9
≥10	37	334	371	3	39	42	8.1	11.7	11.3

[1] $\chi^2 = 1.35$, df = 2, P = 0.51
[2] $\chi^2 = 4.55$, df = 2, P = 0.10
[3] $\chi^2 = 8.28$, df = 2, P = 0.02

Pregnancy rates in relation to the age of the man and woman

The pregnancy rates according to the age of the men and women were calculated for the first IUI cycles. No differences were observed, either between the different age groups of women or between the different age groups of men (Table III). However, no pregnancies occurred in women 40 years and older.

With regard to the age of the men, two notable differences were observed. First, although the semen quality of men younger than 30 years was comparable to that of men of 30 years and older (25.7 ± 28.0 versus 24.8 ± 32.6 million), the pregnancy rate was half of that in the other age groups. Because the partners of men younger than 30 years did not belong to the group of women of 40 years and older this cannot explain this observation either. Second, comparing the role of age in the andrological indication versus the nonandrological indication groups, no pregnancies occurred in the andrological group when the men were 40 years or older (n=24). This was not observed in the nonandrological groups (n=77). That no pregnancies were established in the andrological group when the man was 40 years or older cannot be explained by either the extremely low number of inseminated spermatozoa (9.3×10^6 versus 8.1×10^6 in men younger than 40 years) or the high proportion of women 40 years or older (only 2 of the 24 in the andrological group, versus 9 of 77 in the nonandrological groups).

Table III: Pregnancy rates per patient in relation to age

Age (yr)	# Patients	# Pregnancies	% Pregnancies
Woman[1]			
<30	180	16	8.9
≥30 to <35	218	22	10.1
≥35 to <40	147	12	8.2
≥40	21	0	0
Man[2]			
<30	110	5	4.5
≥30 to <35	198	19	9.6
≥35 to <40	157	16	10.2
≥40	101	10	9.9

[1] $\chi^2 = 2.55$, df = 3, P = 0.47, for percentage pregnancies

[2] $\chi^2 = 3.16$, df = 3, P = 0.37, for percentage pregnancies

Discussion

From the results of this study it is concluded that the factor with the highest impact on the chance of pregnancy after IUI treatment is the number of motile spermatozoa that is inseminated. Although other factors such as the age of the man and woman and the indication caused some differences in pregnancy rate, they did not reach significance.

The pregnancy rates found in this study are similar to those found in other studies on the outcome of IUI treatment: overall pregnancy rates of 18.7 and 21% per patient and 5.6 and 10% per cycle were reported by Campana et al (2) and Frederick et al (4), respectively, versus the 21.4% per patient and 6.9% per cycle found in this study. For most of the published studies it is difficult to compare IUI results in more detail, due to differences in patient selection, ovarian stimulation, definition of sperm parameters, indication, etc.

With regard to the figures for first IUI attempts, differences between indications are not significant. The somewhat lower pregnancy rate of the relatively young andrological group is not the result of an age bias. On the contrary, it is expected that younger women have a higher chance of pregnancy (12, 13). Slight differences in IUI outcome among male factor infertility (5.45%), idiopathic infertility

(12.12%), endometriosis related infertility (6.09%), and ovulation dysfunction (5.0%) have been reported (14), but these figures are difficult to compare with our results because they do not represent only first IUI attempts.

In this study an overall significant increase in pregnancy rate was found with an increasing number of motile spermatozoa. However, when andrological and nonandrological indications were examined separately, this significance was not maintained. There was a trend toward significance in the nonandrological group. Several studies have reported on the influence of semen characteristics on IUI outcome: a significant increase in pregnancy rate was found when less than 0.5 million versus more than 0.5 million spermatozoa were inseminated (2). A trend toward an increasing pregnancy rate with an increasing total motile sperm count was also reported. This trend reached significance when 20 million or more spermatozoa were inseminated (5). Other findings were similar to our results: a significant lower pregnancy rate was found when 2 million or fewer sperm cells were inseminated (15). However, comparisons are difficult to make because studies vary greatly in the definition of their treatment groups. In our study, the fact that there was no difference in pregnancy rates between andrological and nonandrological indications when the same number of motile spermatozoa was inseminated indicates that probably only semen characteristics are responsible for the number of pregnancies obtained. This is difficult to explain because the low number of inseminated spermatozoa in the nonandrological group should be a coincidence and factors other than semen characteristics should play a major role in obtaining pregnancies in this group. These results however suggest that the number of motile spermatozoa that is inseminated plays a dominant role in IUI outcome.

The influence of age on the pregnancy rate after IUI in this study starts at 40 years: women 40 years or older did not become pregnant. Again, results presented in the literature vary greatly. Agarwell and Buyalos (16) found a decrease in pregnancy rate after IUI already at 35 years of age, while Corsan et al (14) reported a pregnancy rate per cycle in women 40 years or older than was less than half that in women younger than 40 years of age (6.69% versus 17.95%) but still able to become pregnant. This ability rapidly declined at 42 years of age. The influence of the age of the man was observed only in the andrological group and only when the age was 40 years or older. This is not in agreement with the findings of Mathieu et al (3), who reported a significant effect of age of the husband on pregnancy rate: increasing age proved to cause an increasingly poor prognosis for pregnancy after IUI.

From our cumulative figures it is concluded that performing more than six IUI attempt does not result in a higher percentage of pregnancies, as was also shown by Campana et al (2). There are some slight differences between indications. The overall higher cumulative pregnancy rate in the hormonal group (52.2%) might be explained by the fact that the disorder that caused the subfertility/infertility is more or less removed by the clomiphene citrate and hCG administration. Many unknown factors, such as uterine environment, unidentifiable semen characteristics, and dysfunction in the interaction between sperm and oocyte, may be involved in the idiopathic group and therefore cannot be treated adequately. This might result in the overall lower cumulative pregnancy rate (31.9%). However, the low number of six cycles (two of five in total) must also be taken into consideration when interpreting the results. Although there are differences between indications, there is no substantial evidence that we should discriminate between them in performing fewer or more attempts. This is not in agreement with the findings of Lalich et al (8) and of Friedman et al (6), whose results showed a significant difference between cumulative pregnancy rates for different indications. Differences in choices and definitions of indications may be responsible for this discrepancy. In our study the plateau at 31.9% after six IUI attempts might be explained by the fact that some patients are more likely to react positively to IUI treatment than others. Another explanation may be a possible negative effect (desensitization) of several clomiphene citrate treatments every other month, which might result in a decreased chance of pregnancy.

From this study it can be concluded that the number of motile sperm cells that is inseminated has predictive value for the chance of pregnancy. Indication does not play a major role in predicting the chance of pregnancy. The probability of becoming pregnant is greatest during the first six cycles. Overall it can be concluded that IUI is a useful treatment for subfertile couples unless semen characteristics are insufficient.

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Chapter 3

Intracytoplasmic sperm injection as a treatment for unexplained total fertilization failure or low fertilization after conventional in vitro fertilization

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L.van der Westerlaken[1], F. Helmerhorst[1], S, Dieben[1], N. Naaktgeboren[1]

[1]*Department of Gynaecology, Leiden University Medical Center, Leiden, The Netherlands.*

Abstract

Objective: To determine whether IVF or ICSI should be the choice of treatment in case of a previous IVF attempt with unexplained total fertilization failure or low fertilization (<25%).

Design: Prospective study.

Setting: Leiden University Medical Center.

Patients: 38 couples undergoing IVF and ICSI on sibling oocytes after a first IVF attempt with total fertilization failure or with low fertilization (<25%).

Intervention: Performing IVF and ICSI on sibling oocytes.

Main outcome measures: Fertilization- and (ongoing) pregnancy rate.

Results: A total of 271 oocytes were collected in 24 oocyte retrievals in the total fertilization failure group. Hundred-nine oocytes were randomly allocated to IVF and 12 were fertilized (11%) and 162 sibling oocytes were allocated to ICSI and 78 were fertilized (48%). In 8 of the 24 patients fertilization occurred after IVF. The pregnancy rate after transfer of 1 IVF and 1 ICSI embryo (n=3) was 67% and after the transfer of 2 ICSI embryos (n=21) this was 52% (ns). In the low fertilization group 169 oocytes were collected in 14 oocyte retrievals. Seventy-two oocytes were randomly allocated to IVF and 16 were fertilized (22%). Ninety-seven sibling oocytes were allocated to ICSI and 58 were fertilized (60%). In 7 of 14 patients fertilization occurred after IVF. The pregnancy rate after the transfer of 1 IVF and 1 ICSI embryo (n=5) was 80% and after the transfer of 2 ICSI embryos (n=9) this was 33% (ns).

Conclusion: Performing ICSI on some oocytes of a cohort may avoid total fertilization failures both in patients with a history of total fertilization failure and in patients with a history of low fertilization, since the fertilization % is higher after ICSI compared to IVF and the recurrence of total fertilization failure and low fertilization is high after IVF treatment.

Introduction

Intracytoplasmic sperm injection (ICSI) rather than conventional IVF in case of severe male factor infertility has been accepted as a highly successful method in terms of fertilization and pregnancies resulting in live births. The results in couples with mild male factor infertility were discordant (1, 2) as were those in case of non-male factor infertility. Using sibling oocytes Khamsi et al (3) reported a higher fertilization- and pregnancy rate after ICSI relative to IVF whereas Staessen et al (4) found no difference.

In case of normospermia total fertilization failure and low fertilization (defined as less than 25% fertilization) occurs in 5-15% and 20% respectively of the couples undergoing IVF, with a recurrence rate of about 30-50% (5, 6, 7). This failure of oocytes of the female patient to be fertilized by the spermatozoa of the male partner undergoing infertility treatment may be explained by lack of penetration of the zona pellucida, an oocyte activation failure or a defect in the oocyte. Intracytoplasmic sperm injection circumvents those obstacles and might therefore be effective. Only one study on the efficacy of IVF and ICSI after a previous attempt with total fertilization failure has been published, reporting in favour of ICSI, despite the use of a high insemination concentration in the IVF procedure (8). However, most of the couples included in that study suffered from oligoasthenoteratozoospermia.

The aim of this study is to compare the efficacy of IVF and ICSI in case of a previous IVF attempt with either total fertilization failure or low fertilization in couples with normospermia. It is a prospective study using sibling oocytes that have been randomly assigned to either an IVF or an ICSI procedure with fertilization- and pregnancy rate as primary outcome.

Materials and methods

Patients

The couples that were included in this study showed total fertilization failure or low fertilization (less than 25%) in a first attempt (between September 1995 and September 2002) in which all retrieved oocytes were treated with (conventional) IVF.

In the second attempt (between November 1995 and February 2003) part of the retrieved sibling oocytes of those patients were treated with IVF and the other part with ICSI.

Other inclusion criteria were the retrieval of at least 5 oocytes (sufficient number for an IVF as well as an ICSI procedure) and semen characteristics of >20% motility after sperm preparation (see below) and $>1x10^6$ total motile sperm count after sperm preparation. The criteria were fulfilled both on the day of the first IVF attempt as well as on the day of the second attempt. A total number of 24 different couples fulfilled the inclusion criteria for the total fertilization failure group, as did 14 different couples for the low fertilization group.

The mean age (years) of the women was 32.2 ± 3.9 (range 23-41). The results reported all refer to the second treatment.

Each couple included in this study was informed that due to the possibility of recurrence of total fertilization failure or low fertilization, ICSI and IVF would be performed on sibling oocytes. Because ICSI is a proven method for the treatment of infertility there was no need for Institutional Review Board approval. However, written informed consent of each included couple to perform ICSI on at least part of the retrieved oocytes was obtained.

Ovarian stimulation

Ovarian stimulation was performed by a combination of gonadotrophin-releasing hormone agonist: Decapeptyl (Ferring, Hoofddorp, The Netherlands); Synarel (Pharmacia, Woerden, The Netherlands), FSH: Gonal F; Pergonal; Metrodin HP (Serono Benelux, Den Haag, The Netherlands), and human chorionic gonadotrophin (HCG): Profasi (Serono Benelux); Pregnyl (Organon, Oss, The Netherlands). Luteal phase supplementation was given by intravaginally administered progesterone, Progestan (Organon) and a single HCG injection, Pregnyl (Organon).

Semen preparation

Freshly ejaculated semen from the male partner was allowed to liquefy. After measuring the volume of the sample, concentration and percentage of motile spermatozoa was assessed in a Makler counting chamber (Sefi-Medical Instruments, Israel). Earle's medium buffered with HEPES and supplemented with 0.5% human serum albumin (Cealb, CLB, Amsterdam, The Netherlands) was added to the semen sample and mixed by pipetting. Depending on the total number of motile spermatozoa, the mixed sample was pipetted on top of either a layer of 70% or 80% Percoll (from 1995-2000)(Pharmacia, Woerden, the Netherlands) or PureSperm (from 2000- 2003) (Nidacon, Goteborg, Sweden) layer and centrifuged (800 g, 10 min.).

The supernatant was removed and the sperm pellet was resuspended in HEPES-buffered Earle's medium. Depending on the total number of motile spermatozoa, this suspension was either pipetted again on top of an 80% Percoll or PureSperm layer and then washed twice, first in HEPES-buffered medium and the second time in Universal IVF medium (Medicult; Lucron, Milsbeek, the Netherlands), or washed twice with these media after the first Percoll or PureSperm treatment.

Volume, concentration, motility and the total motile sperm count were redetermined after processing. The spermatozoa were kept at 37^0C and 5%CO_2 in air in an incubator until the insemination or injection procedure. The same semen sample was used for both the insemination and the injection.

Oocyte retrieval and preparation

The retrieved oocyte-cumulus complexes (OCCs) were pooled and washed in HEPES-buffered Earle's medium and then randomly transferred in groups of 2-6 OCCs (depending on the total number of OCCs retrieved) to droplets of 25 µl culture medium (Universal IVF medium (Medicult)) under mineral oil (Sigma, Brunswig Chemie, Amsterdam, the Netherlands) and then stored into an incubator (37 ^{0}C, 5% CO_2).

Before injection or insemination the OCCs were taken out of the incubator and the OCCs in the first droplet(s) were assigned to ICSI and the OCCs in the last droplets were assigned to IVF in a ratio of 3:2, respectively. A higher number of oocytes were assigned to ICSI to secure occurrence of fertilization, since not every oocyte can be injected (about 10-20% due their maturational stage) or will survive after injection (about 10%).

The OCCs that were assigned to ICSI were denuded of their surrounding cumulus cells both enzymatically and mechanically between 0-4 hours after retrieval (9). The maturation stage was checked and the oocytes that had extruded a polar body were selected for injection. The ICSI was performed as described in detail elsewhere

(9). After injection the oocytes were transferred to 25 µl droplets of Universal IVF medium, where they were cultured individually.

The OCCs that were assigned to IVF kept their surrounding cumulus cells and they were cultured individually in 25 µl droplets of Universal IVF medium. In the second treatment each oocyte was inseminated with 100,000-150,000 motile spermatozoa (instead of the standard number of 75,000) 2-6 hours after oocyte retrieval.

Assessment of fertilization

Fertilization was scored 16-18 hours after injection and insemination. For the IVF oocytes the surrounding cumulus cells were removed mechanically by repeatedly pipetting of the OCCs in and out of a hand-drawn Pasteur pipette and the oocytes were transferred to new 25 µl droplets of Universal IVF medium. Both the IVF and the ICSI oocytes were checked for fertilization (normal fertilization was when 2 pronucleï were present, abnormal fertilization when less or more than 2 pronucleï were present). Cleavage and embryo quality were evaluated at day 2 and 3 after oocyte pick-up.

Embryo transfer and pregnancy testing

Embryo transfer took place 3 days after oocyte retrieval. The best-quality embryos were transferred, regardless whether they were derived from ICSI or from IVF. Depending on the woman's age and the embryo quality 1-3 embryos were transferred. Good quality excess embryos were cryopreserved. Details of the embryo transfer technique are described elsewhere (9).

Pregnancy was defined by an increasing ß-HCG >= 50 IU/l at 15 days after oocyte retrieval. Ongoing pregnancy was defined by the presence of gestational sac with fetal heartbeat after 12 weeks of gestation. Implantation rate was based on ongoing pregnancies and calculated by dividing the number of embryos implanted by the number of embryos transferred.

Statistical analysis

The Mantel-Haenszel test was used to compare the proportions of fertilized oocytes between the ICSI and IVF treatment. Pregnancy results were analysed by Pearson's X^2 test. Statistical significance was set at $P<0.05$.

For estimates of population parameters mean and standard deviation were used. For comparison of these parameters the Student's t-test was used. All tests were performed by using the SPSS statistical package.

Results

Fertilization results (Table I)

Total fertilization failure group

A total of 271 oocytes was collected in 24 oocyte retrievals (11.3 oocytes per oocyte retrieval). In 8 of the 24 oocyte retrievals there was fertilization both after IVF and after ICSI (29.3% (12/41) and 55.6% (30/54), respectively). In 16 of the 24 oocyte retrievals there was no fertilization in the oocytes allocated to IVF, while in the sibling oocytes treated with ICSI there was a fertilization percentage of 44.4% (48/108).

Low fertilization group

A total of 169 oocytes was collected in 14 oocyte retrievals (12.1 oocytes per oocyte retrieval). In 7 of the 14 oocyte retrievals there was fertilization both after IVF and after ICSI (42% (16/38) and 62% (32/52), respectively). In the remaining 7 oocyte retrievals there was no fertilization in the oocytes allocated to IVF, while in the sibling oocytes treated with ICSI the fertilization percentage was 58% (26/45).

The overall fertilization percentage of the injected oocytes was significantly higher than that of the inseminated oocytes both in the total fertilization failure group and in the low fertilization group ($P<0.001$). In patients with fertilization after both IVF and ICSI this difference was also found significant in the total fertilization failure group ($P=0.004$), but not in the low fertilization group ($P=0.077$).

Table I: Fertilization results after IVF or ICSI of sibling oocytes.

	Patients with fertilization by IVF + ICSI				**Patients with fertilization only by ICSI**			
	Total Fertilization Failure group (n=8)		Low Fertilization group (n=7)		Total Fertilization Failure group (n=16)		Low Fertilization group (n=7)	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Oocytes retrieved	95		90		176		79	
Oocytes by IVF	41	43.2	38	42.2	68	38.6	34	43.0
IVF Fertilized	12	**29.3**	16	**42.1**	0	**0**	0	**0**
Oocytes by ICSI	54	56.3	52	57.8	108	61.4	45	57.0
Injected	48	88.9	48	92.3	91	84.3	45	100
ICSI fertilized	30	**55.6**[a]	32	**61.5**	48	**44.4**[a]	26	**57.8**[a]

[a] significantly different from IVF result (P<0.001)

For all patients the sperm parameters of both the total fertilization failure group and the low fertilization group are shown in table II. The total motile sperm count after preparation was higher in the group of patients with fertilization in IVF than in the group of patients without fertilization in IVF ((11.5 ± 9.4 x10^6 versus 8.8 ± 12.4 x10^6 (total fertilization failure group); 8.3 ± 6.4 x10^6 versus 4.0 ± 2.5 x10^6 (low fertilization group)). This difference was significant (P=0.05) for the low fertilization group.

Table II: Sperm parameters before and after preparation (mean ± sd) in all patients

	Total Fertilization Failure group (n=24)	**Low Fertilization group (n=14)**
Concentration (x10^6)	41.4 ± 24.8	42.4 ± 34.2
Motility (%)	72.0 ± 19.9	66.5 ± 15.4
TMC (x10^6)	9.7 ± 11.4	6.1 ± 5.2

TMC: Total Motile Sperm Count

Embryo transfer and pregnancy results (Table III)

In both the total fertilization failure group and the low fertilization group embryos were available for transfer in all patients. However, no transfers were performed with only IVF embryos.

In the total fertilization group 3 transfers of a combination of 1 IVF and 1 ICSI embryo were performed resulting in 2 (ongoing) pregnancies and 21 transfers of only ICSI embryos (mean number of transferred embryos was 2.05) resulting in 11 pregnancies of which 10 are ongoing.

In the low fertilization group 5 transfers of a combination of 1 IVF and 1 ICSI embryo were performed resulting in 4 pregnancies of which 3 were ongoing and 9 transfers of only ICSI embryos (mean number of transferred embryo's was 2.00) resulting in 3 (ongoing) pregnancies.

No significant differences were found with regard to pregnancy rates between both the mixed transfers and the ICSI transfers.

Table III: Embryo transfer, pregnancy rate and implantation rate

	Total Fertilization Failure group		**Low Fertilization group**	
	IVF-ICSI[1]	ICSI[2]	IVF-ICSI[1]	ICSI[2]
# ET	3	21	5	9
# embryo's transferred	6	43	10	18
# embryo's /ET	2.00	2.05	2.00	2.00
# pregnancies	2[a] (67%)	11 (52%)	4[a] (80%)	3 (33%)
# ongoing pregnancies	2[a] (67%)	10 (48%)	3[a] (60%)	3 (33%)
implantation rate	50%	30%	30%	22%

[1] Transfers with a combination of 1 IVF and 1 ICSI embryo
[2] Transfers with only ICSI embryo's
[a] not significantly different from the transfers with only ICSI embryo's

Discussion

This study showed that normospermic patients, who had total fertilization failure or less than 25% fertilization in a previous cycle where all oocytes were treated with IVF, show in a second cycle a significantly higher fertilization rate in sibling oocytes treated with ICSI compared to the sibling oocytes treated with IVF. Moreover there was a high recurrence rate of total fertilization failure in the IVF treated oocytes in the second cycle (67% in the total fertilization failure group and 50% in the low fertilization group). There was no significant difference in pregnancy rate between transfers of ICSI embryos and transfers of a combination of 1 ICSI and 1 IVF embryo, suggesting that once being fertilized oocytes are very well capable of developing into embryos that are competent to establish ongoing pregnancies, regardless of whether they were derived from IVF or ICSI. However, 9 of the 11 ICSI pregnancies in the total fertilization failure group and 2 of the 3 ICSI pregnancies in the low fertilization group were obtained from cycles without fertilization after IVF.

In other studies the apparently better performance of ICSI compared to IVF is also observed in couples with male subfertility (2, 8, 10, 11). In a controlled comparison between ICSI and IVF in sibling oocytes Verheyen et al (2) found a fertilization rate of 64% and 22% respectively with a total fertilization failure after IVF of 50% (10 of 20 oocyte retrievals). In that study 4 pregnancies were also obtained from cycles without fertilization in IVF. Calderon et al. (11) reported a randomised study which showed a fertilization rate of 49.5% after ICSI and 19.5% after IVF with a total fertilization failure after IVF of 21% (7 of 34 oocyte retrievals).

Despite the fact that all patients included in this study were diagnosed as normospermic, the number of patients without fertilization after IVF for the second time was relatively high (16 of 24 patients and 7 of 14 patients in the total fertilization failure group and low fertilization group respectively). To find an explanation for the observed difference between patients in fertilizing capacity after IVF, we compared retrospectively sperm characteristics in the groups with and without fertilization in IVF. We found that the total motile sperm count (after preparation) in the cycles with fertilization in IVF was higher than the total motile sperm count in cycles without fertilization in IVF, suggesting that semen samples differ even when they have been classified within the range of being normal according to routine standards in the laboratory. This was also found in a study by Khamsi et al (3) that showed a difference in fertilization rate after IVF between a sperm motility of ≥50% and >30%-<50%, suggesting at least in part an andrological factor. Also the study of Verheyen et al (2) suggested a sperm influence: there was a higher motility percentage in the group with fertilization after IVF than without fertilization after IVF. In addition Audibert et al (12) suggested the presence of undiscovered sperm abnormalities and Atiken et al (13) reported already in 1982 the weakness of conventional criteria in semen analysis.

Another possible influence on the fertilization results might be factors within the oocyte. Since in this study sibling oocytes were randomly assigned to either IVF or ICSI treatment the only possible variation in oocyte quality must be between patients. Given the fertilization percentages after ICSI (between 48 and 60 percent) it appears that, if oocyte quality is involved, ICSI can circumvent a possible defect in the oocyte that may be responsible for the inability to be fertilized in IVF. This is not in agreement with the results of a cohort study by Gabrielsen et al. (14) that compared the fertilization- and pregnancy rates after ICSI performed in case of severe oligoasthenozoospermia and in case of a previous attempt with total fertilization failure with normal sperm parameters. They found a lower fertilization and pregnancy rate in the total fertilization failure group than in the andrological group after ICSI. Olds-Clarke (15) reported the need of both a certain velocity of the sperm and a certain quality of the zona pellucida to enable sperm penetration. Also the presence of many dead sperm cells might affect the quality of the oocyte by causing oxidative stress. Whether the failure to fertilize is caused by sperm factors or by oocyte factors or a combination of both still needs to be elucidated. However, regardless of the possible cause ICSI seems to be a useful tool to avoid this unexplained fertilization failure.

From this study it may be concluded that performing ICSI on at least part of the oocytes will avoid unnecessary total fertilization failure both in patients with a history of total fertilization failure and in patients with a history of low fertilization. Because the contribution of IVF to the total of fertilized oocytes is very low it might be proposed to treat all oocytes with ICSI. Regardless of whether the oocytes have been treated with ICSI or IVF once being fertilized oocytes seem to be very well capable of developing into embryos that are competent to establish ongoing pregnancies.

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Chapter 4

Conventional in vitro fertilization versus intracytoplasmic sperm injection in patients with borderline semen: a randomised study using sibling oocytes

Fertility and Sterility: in press

L. van der Westerlaken[1], N. Naaktgeboren[1], H. Verburg[1], S. Dieben[1],

F.M. Helmerhorst[1]

[1]*Department of Gynaecology, Leiden University Medical Center,*

Leiden, The Netherlands.

Abstract

Objective: To determine whether patients with borderline semen should be treated with conventional IVF or ICSI.

Design: Randomized study.

Setting: Leiden University Medical Center.

Patients: 106 couples with borderline semen undergoing IVF and ICSI on sibling oocytes.

Intervention: Performing IVF and ICSI on sibling oocytes.

Main outcome measures: Fertilization- and pregnancy rate.

Results: 1518 oocytes were collected in 106 oocyte retrievals: 849 oocytes were randomly allocated to ICSI of which 761 were microinjected and 669 oocytes were randomly assigned to IVF. In 26 of the 106 patients there was fertilization only after ICSI and not after IVF (IVF – group). The fertilization rate was 51% (92/182 oocytes). In 78 patients there was fertilization after both IVF and ICSI (IVF + group), the fertilization rate was 51% for both the IVF and ICSI treated oocytes (271/528 oocytes and 334/658 oocytes, respectively). In 2 patients there was no fertilization after either IVF (0/6 oocytes) or ICSI (0/9 oocytes). Patients of the IVF + group had a higher total motile sperm count after preparation than those of the IVF – group. More high quality embryos were obtained after ICSI in patients of the IVF + group. In 101 patients embryo transfer was performed: 26 in the IVF – group and 75 in the IVF + group. No significant differences were found with regard to pregnancy rates between those two groups: 54% in the IVF – group and 48% in the IVF + group.

Conclusion: Performing ICSI on at least part of the oocytes will avoid unnecessary fertilization failure in patients with borderline semen: in this study 26 of 104 cycles (25%) were rescued by ICSI.

Keywords: ICSI, IVF, borderline semen, fertilization rate, embryo quality, pregnancy rate.

Introduction

Threshold values of sperm parameters for assisted procreation are mainly based on the World Health Organization (WHO) standard (1) and are widely used to discriminate between male fertility and subfertility (2, 3, 4). However, the prognostic value of those parameters is questionable (5, 6, 7). In a review by Mahutte et al (2003) different screening tests have been evaluated. Their conclusion was that more sophisticated methods such as sperm-zona binding ratios and zona pellucida induced acrosome reaction tests may improve the ability to predict fertilization capacity, but unfortunately no test can exclude the possibility of fertilization failure. In patients with borderline semen the decision to choose for either IVF or ICSI is very critical because the chance on total fertilization failure after a conventional IVF or on performing an unnecessary ICSI procedure is hard to predict.

The majority of failed fertilized oocytes do not contain sperm nuclei after conventional IVF (9, 10), indicating that most cases of fertilization failure relate to an inability of the spermcell to penetrate the oocyte. Oocyte related factors that might account for fertilization failure in some cases could be defects in the pronuclear formation or an oocyte activation failure (8).

The aim of this study was to determine the optimal treatment (IVF or ICSI) for patients with borderline semen, while minimizing the risk of total fertilization failure with fertilization rate as primary outcome and pregnancy as secondary outcome. We therefore performed IVF and ICSI on sibling oocytes in a first cycle in a cohort of patients with borderline semen.

Materials and methods

Patients

Between September 1995 and September 2003 hundred and six couples suffering from mild male factor infertility were treated in a first cycle with IVF and ICSI on sibling oocytes. Mild male factor subfertility was defined by the presence of at least one abnormal semen parameter, i.e. concentration <20 mln/ml and/or <40% motility according to the World Health Organization (WHO) (1) criteria.

Patients were included in this study based on previous diagnostic semen analyses and when on the day of oocyte retrieval their semen fulfilled the above criteria again. The other inclusion criterion was the retrieval of at least 5 oocytes. The mean age of the women was 31.3 ± 4.4 (years ± sd).

Institutional Review Board approval was obtained to perform ICSI and all patients were informed that due to the low semen quality total fertilization failure after conventional IVF was possible and that therefore ICSI and IVF would be performed on sibling oocytes. Written informed consent was obtained of each included couple to perform ICSI on at least part of the retrieved oocytes.

Ovarian stimulation

The patients were stimulated according to a standard short stimulation protocol, starting by down regulation with an GnRH agonist on the first day of the menstruation as a nasal spray (Synarel, Pharmacia, Woerden, The Netherlands) or subcutaneous (Decapeptyl, Ferring, Hoofddorp, The Netherlands). This was followed by ovarian stimulation with purified FSH (Metrodin, Serono Benelux, Den Haag, The Netherlands) or recombinant FSH (Gonal F, Serono Benelux) starting either on day 4 with 150 IU when the women were 37 years of age or younger, or on day 2 with 225 or 300 IU if the women were 38-39 or 40-41 years of age respectively. The patients were monitored by vaginal ultrasound scans and serum estradiol measurement. If necessary the dose was adjusted during stimulation. When the desired follicle growth was achieved hCG was given s.c. (Profasi 10.000 IU, Serono; Pregnyl, Organon, Oss, The Netherlands), followed by oocyte pickup 36 hours later. Luteal phase supplementation was given by intravaginally administered progesterone, Progestan (Organon) and a single HCG injection, Pregnyl (Organon).

Semen preparation

Freshly ejaculated semen was allowed to liquefy. Volume was determined and concentration and percentage of motile spermatozoa was assessed in a Makler counting chamber and the total number of motile spermatozoa was calculated. The semen samples were diluted 1:1 with HEPES-buffered Earle's medium supplemented with 0.5% human serum albumin. The diluted sample was pipetted on top of a 1 ml layer of 70 % Percoll (from 1995-2000) (Pharmacia, Woerden, The Netherlands) or a

1 ml layer of 70 % PureSperm (from 2000- 2003) (Nidacon, Goteborg, Sweden) in a 12 ml tube and centrifuged (800 g, 10 min.). The supernatant was removed and the sperm pellet (0.1-0.5 ml) was resuspended in 5 ml of HEPES-buffered Earle's medium. This suspension was then washed twice, first in HEPES-buffered medium and the second time in Universal IVF medium (Medicult; Lucron, Milsbeek, The Netherlands). Volume, concentration, motility and the total motile sperm count were redetermined after processing. The spermatozoa were kept at 37^0C in a CO_2 incubator until IVF or ICSI took place.

Oocyte retrieval and preparation

The retrieved oocyte-cumulus complexes (OCC's) were pooled and washed in HEPES-buffered Earle's medium and then randomly transferred in groups of 2-6 OCC's (depending on the total number of OCC's retrieved) to droplets of 25 µl culture medium (Universal IVF medium (Medicult)) under mineral oil (Sigma, Brunswig Chemie, Amsterdam, The Netherlands) and then put into an incubator (37 ^{0}C, 5% CO_2).

Before injection or insemination the OCC's were taken out of the incubator and the OCC's in the first droplet(s) were assigned to ICSI and the OCC's in the last droplet(s) were assigned to IVF in a ratio of 3:2, respectively. A higher number of oocytes were assigned to ICSI to secure the occurrence of fertilization: not all oocytes can be injected because of their maturational stage (about 10-20%) and not all oocytes will survive the injection (about 10%).

The OCC's that were assigned to ICSI were denuded of their surrounding cumulus cells both enzymatically and mechanically between 0-4 hours after retrieval (11). The maturation stage was checked and the oocytes that had extruded a polar body were selected for injection. The ICSI was performed as described in detail elsewhere (11). After injection the oocytes were transferred to 25 µl droplets of Universal IVF medium, where they were cultured individually.

The OCC's that were assigned to IVF kept their surrounding cumulus cells and they were cultured individually in 25 µl droplets of Universal IVF medium. Each oocyte was inseminated with 75,000-150,000 motile spermatozoa (standard number is 75,000) 2-6 hours after oocyte retrieval in a total volume of 25-30 µl.

The same semen sample was used for both the insemination and the injection.

Assessment of fertilization

Fertilization was scored 16-18 hours after injection and insemination. For the IVF oocytes the surrounding cumulus cells were removed mechanically by repeated pipetting of the OCC's in and out of a hand-drawn Pasteur pipette and the oocytes were transferred to new 25 µl droplets of Universal IVF medium.

Both the IVF and the ICSI oocytes were checked for normal fertilization (the presence of 2 pronucleï) or no and abnormal fertilization (0 and 1, >2 pronucleï, respectively) or whether the oocyte had degenerated.

Assessment of cleavage

Cleavage and embryo quality were evaluated at days 2 and 3 after oocyte retrieval. According to the number and size of blastomeres and the amount of fragmentation the embryos were assigned to 4 different quality types: type 1, equal sized blastomeres and no fragmentation; type 2, <20% fragmentation; type 3, 20-50% fragmentation; type 4, >50% fragmentation.

Embryo transfer and pregnancy testing

Embryo transfer took place 3 days after oocyte retrieval. The best-quality embryos were transferred, regardless whether they were derived from ICSI or from IVF. Depending on the woman's age and the embryo quality 1-3 embryos were transferred. Good quality excess embryos were cryopreserved. Details of the embryo transfer technique are described elsewhere (11).

Pregnancy was defined by an increasing ß-HCG >= 50 IU/l at 15 days after oocyte retrieval. Ongoing pregnancy was defined by the presence of a gestational sac with fetal heartbeat after 12 weeks of gestation.

Statistical analysis

The Mantel-Haenszel test was used to compare the proportions of fertilized oocytes and the proportions of type 1 and type 2 embryos between the ICSI and IVF treatment. Pregnancy results were analysed by Pearson's X^2 test. Statistical significance was set at $P<0.05$.

For estimates of population parameters mean and standard deviation were used. For comparison of these parameters the Student's t-test was used. All tests were performed by using the SPSS statistical package.

Results

In 106 oocyte retrievals 1518 oocytes were collected, of which 669 oocytes were randomly assigned to the conventional IVF procedure and 849 oocytes were randomly allocated to the ICSI procedure; 761 of them were microinjected.

Fertilization results (Table I)

Among 78 of the 106 treated couples fertilization occurred both after IVF (271/528 oocytes; 51%) as after ICSI (334/658 oocytes; 51%) (IVF+ group), while among 26 of the 106 couples fertilization was observed only after ICSI (92/182 oocytes; 51%) and not after IVF (IVF – group). In 2 couples there was no fertilization after IVF (0/6 oocytes) and ICSI (0/9 oocytes. Both these patients had mature oocytes: 8 of the 9 were injected. No morphological abnormalities of the oocytes were observed.

Table I: Fertilization- and cleavage results after conventional IVF and ICSI performed on sibling oocytes in 106 patients with borderline semen

	Patients without fertilization after ICSI and IVF (n=2)		**Patients with fertilization only by ICSI (n=26)**		**Patients with fertilization after IVF and ICSI (n=78)**	
	Number	Percent	Number	Percent	Number	Percent
Oocytes retrieved	15		317		1186	
Oocytes by IVF	6	40	135	43	528	45
IVF fertilized	0	0	0	0	271	51
Type 1-2 embryos	n.a	n.a.	n.a.	n.a.	194	72[a]
Oocytes by ICSI	9	60	182	57	658	56
Injected	8		164	90	590	90
ICSI fertilized	0	0	92	51	334	51
Type 1-2 embryos	n.a.	n.a.	71	77	278	83[b]

n.a.: not applicable

[a b] significantly different from each other (P<0.05)

Sperm parameters before and after preparation in the IVF – and the IVF + group are shown in Table II. Significant differences were found with regard to motility before preparation (32.6 ± 14.3 % versus 39.7 ± 20.1 %: P=0.033) and the mean total motile sperm count (TMC) (± SD) after preparation, (2.3 ± 1.5 x10^6 versus 3.4 ± 2.8 x 10^6: P=0.009). For the 2 patients with no fertilization in both the IVF and ICSI treated oocytes the sperm parameters were not significantly different from the other 2 groups.

Table II: Sperm parameters of the 106 patients included in this study

	Patients with no fertilization after IVF and ICSI (n=2)	**Patients with fertilization only after ICSI (n=26)**	**Patients with fertilization after IVF and ICSI (n=78)**
Before processing			
Concentration (mean ± sd)	17.0 ± 19.8 x10^6/ml	19.3 ± 14.0 x10^6/ml	26.2 ± 33.5 x10^6/ml
Motility (% ±sd)	28.0 ± 7.1	32.6 ± 14.3[a]	39.7 ± 20.1[b]
Total motile count (mean ± sd)	5.5 ± 2.2 x10^6	19.4 ± 18.9 x10^6	25.7 ± 28.0 x10^6
After processing			
Concentration (mean ± sd)	23.0 ± 8.5 x10^6/ml	30.9 ± 17.2 x10^6/ml	33.4 ± 27.1 x10^6/ml
Motility (% ±sd)	62.5 ± 44.5	46.4 ± 23.0	57.2 ± 18.9
Total motile count (mean ± sd)	2.8 ± 4.9 x10^6	2.3 ± 1.5 x10^6 [c]	3.4 ± 2.8 x10^6 [d]

[a,b] significantly different from each other (P<0.04)

[c,d] significantly different from each other (P<0.01

Cleavage results (Table I)

Significantly more type 1 and 2 embryos developed after ICSI compared to IVF (83% versus 72%) in the group of patients with fertilization after both IVF and ICSI. There was no difference in embryo quality between ICSI embryos developed in cycles with and without fertilization in IVF (83% versus 77%).

Embryo transfer and pregnancy results (Table III)

In 5 of the 106 patients there was no transfer: in 2 patients because there was no fertilization and in 3 patients because of OHSS. Overall more ICSI embryos were transferred compared to IVF embryos (140 versus 50). In the IVF + group (n=78) 91 ICSI embryos and 50 IVF embryos were transferred: 14 transfers of only IVF embryos (18%), 27 transfers of a mixture of IVF and ICSI embryos (36%) and 34 transfers of only ICSI embryos (44%).

In total 50 patients became pregnant: 14 in the group with fertilization only after ICSI (54% per transfer) and 36 in the group with fertilization after both IVF and ICSI (48% per transfer). No significant differences were found with regard to pregnancy rates and ongoing pregnancies between the three groups of embryo transfers (IVF/ IVF-ICSI / ICSI).

Table III: Embryo transfer, pregnancy rate and implantation rate (101 patients)

	Patients with fertilization only by ICSI (n=26)		**Patients with fertilization after IVF and ICSI (n=75)***		
	Number (%)		Number (%)		
	ICSI	IVF	IVF	IVF + ICSI	ICSI
Transfers	26	0	14	27	34
Pregnancies	14 (54)	0	6 (43)	12 (44)	18 (53)
Ongoing pregnancies	11 (42)	0	5 (36)	11 (41)	17 (50)

* In 3 patients there was no transfer because of OHSS

Discussion

In case of male subfertility the ICSI treatment results in higher fertilization rates per oocyte compared to conventional IVF treatment in this study (50% versus 41%). However when the fertilization percentage is calculated per patient three groups of patients can be discriminated: one without fertilization after either conventional IVF or ICSI, one with fertilization only after ICSI and one with fertilization after both conventional IVF and ICSI. In the latter group the fertilization percentages after IVF and ICSI are similar. These results suggest an all-or-nothing effect with regard to the fertilization capacity in conventional IVF. Pregnancy rates were similar after transfer of embryos derived from ICSI or IVF or both, suggesting that once fertilization has been established there is no difference in developmental competence between IVF and ICSI treatment.

Patients with fertilization after conventional IVF apparently do not need to be treated with ICSI. The question is how to discriminate between patients that do and do not need ICSI to fertilize. In the literature there is no answer to this question. Overall, studies in which sibling oocytes are treated with conventional IVF and ICSI in case of borderline semen show absence of fertilization after conventional IVF in 25-50% of the cycles; the cycles with fertilization after both IVF and ICSI show similar fertilization rates of the IVF and ICSI treated oocytes (2, 4, 12, 13, 14). However, the definition of borderline semen differs immensely between these studies, which may influence differences in the results found. Alboughar et al. (13) included patients that showed normal semen (based on WHO criteria) in at least one test and subnormal semen in one or more tests. No information is given on semen quality on the day of oocyte retrieval. The study by Plachot et al. (12) also use the WHO criteria, however it is unclear whether patients were included based on previous semen analyses or on the semen parameters found on the day of oocyte retrieval. Verheyen et al. (4) included patients with ≤5% rapid progressive type A motility in fresh semen and ≥500,000 progressive motile spermatozoa (type A and B) after preparation of the entire sample. The semen had to fulfil these criteria both in the diagnostic semen examinations and on the day of oocyte retrieval. A study by Pisarska et al. (2) compared the fertilization results after IVF and ICSI in patients with borderline semen defined as concentration $\leq 20 \times 10^6$ or motility <40%. It was not clear whether this was on the day of oocyte retrieval and/or in diagnostic semen analyses.

A dominant effect of a single suboptimal semen parameter on the fertilization results after IVF and ICSI was reported for sperm morphology and for motility. Two studies on the effect of severe teratozoospermia in the presence of normal count and motility on fertilizing ability after IVF and ICSI showed lower total fertilization failure and a higher fertilization percentage after ICSI compared to IVF in case of severe teratozoospermia (2,15). A study on the effect of motility (4) showed that in case of asthenozoospermia there is a high risk of absence of fertilization with conventional IVF (50%) and a tendency of the fertilization rate to be lower in sibling oocytes treated with IVF (46%) compared to ICSI (59%). However, none of the studies mentioned could identify cut-off values for one or more sperm parameters that can predict the occurrence of fertilization and thus be used to determine the optimal treatment for individual patients.

In order to find an indication for the differences in fertilization we retrospectively analysed the sperm characteristics for the 3 groups mentioned and found a significant lower motility before processing and a significant lower total motile sperm count after processing in the group with fertilization only after ICSI. Also the results of the semen analyses that were performed prior to IVF/ICSI treatment and on which the choice for IVF and/or ICSI was primarily based (data not shown) show that sperm parameters in patients that fertilize only after ICSI are lower than those in patients that fertilize in ICSI as well as in IVF. Even though we found differences in sperm parameters between the groups that do and do not fertilize in IVF it is impossible to predict the fertilizing capacity of the sperm of individual patients based on the semen analyses.

With regard to embryo quality we found significantly higher quality embryos after ICSI compared to IVF in patients that fertilized both after IVF and ICSI. This difference did not reach significance when ICSI embryos from patients with fertilization only after ICSI were compared with the IVF fertilized embryos. This is not in agreement with other studies that did not find differences in embryo quality between IVF and ICSI (2, 4, 12, 16). Whether it is the technique (IVF or ICSI) that is responsible for this observation is not clear. Patient variation as well as differences in sperm characteristics can be excluded as a possible explanation because sibling oocytes and the same semen sample have been used to establish fertilization. It might be that the ICSI embryos develop faster than the IVF embryos. This is a known phenomenon inherent to the ICSI technique (17). It might also be that exposure of the IVF embryos to large numbers of spermatozoa, creating suboptimal culture conditions, affects embryo quality negatively. This negative effect might be avoided by using the short insemination method in conventional IVF (18,19). The fact that more ICSI embryos are transferred than IVF embryos can be explained by the fact that there are more ICSI embryos available and that the ICSI embryos are of a higher quality. The choice of embryos for transfer was based on embryo quality regardless whether they were derived from IVF or ICSI. So the IVF and ICSI embryos that were transferred were of similar quality. This explains the similar (ongoing) pregnancy rates between IVF and ICSI treatment.

From the present study it may be concluded that in patients with subfertile semen the treatment of sibling oocytes with both IVF and ICSI remains the optimal tool to prevent total fertilization failure after conventional IVF. Whether the results of this treatment can be used as a guideline for future cycles in the same patients is now under investigation. Preliminary results show that the choice for IVF or ICSI is only marginally based on the results obtained in previous cycles. The dominating factors in this decision seem to be sperm parameters, which can vary a lot between treatments within patients. Until more results are available it is recommendable to apply the IVF/ICSI treatment in case of borderline semen when enough oocytes are available and to apply ICSI when the number of oocytes is too small for a fair chance on fertilization in either IVF or ICSI.

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Chapter 5

Successful intracytoplasmic sperminjection after failed IVF due to multipronuclear oocytes

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L. van der Westerlaken[1], F. Helmerhorst[1], H.Verburg[1], N. Naaktgeboren[1]

[1]*Department of Gynaecology, Leiden University Medical Center, Leiden, The Netherlands.*

Abstract

Objective: To report a case of IVF with apparently normal female and male gametes which resulted in the development of only multipronuclear oocytes instead of oocytes showing 2 pronuclei, which was successfully treated by ICSI.

Design: Case report.

Setting: An university hospital.

Patient(s): A 35-year-old woman with an unexplained infertility, and her partner, a 38-year-old man with normozoospermia.

Intervention: Intracytoplasmic sperm injection.

Main Outcome Measure: Oocyte fertilization and pregnancy.

Result(s): Normal fertilization and an ongoing pregnancy after transfer of 2 embryos.

Conclusion: Although the indication for ICSI is generally male subfertility, it can also be successful in the treatment of defects in the oocyte.

Introduction

The presence of more than 2 pronuclei (multipronuclei) after IVF treatment can be caused by defects of maternal as well as paternal origin (1). The development of multipronuclear oocytes in a IVF procedure might be the result of failure by the oocyte to extrude the second polar body after the penetration of the spermatozoa, resulting in three pronuclei. Cytoplasmic vacuoles enclosed by a plasma membrane, lacking DNA or nucleoli is a second option and are designated as pseudo-pronuclei (2). Fragmentation of initially normal pronuclei may offer an alternative explanation for the phenomenon, although never mentioned in the literature. The smaller size of these pronuclei compared with normal pronuclei might be an indication for fragmentation. Finally, multipronuclear oocytes can be the result of penetration of more than one spermatozoon in an oocyte, probably caused by an impairment of the zona reaction resulting in a failure to protect against multiple sperm entry.

In order to try to prevent the development of multipronuclear oocytes in a patient with a history of multipronuclear oocytes in a IVF procedure, ICSI was applied with a part of the retrieved oocytes, the remaining oocytes were treated with the conventional IVF procedure.

CASE REPORT

A 35 year old woman and a 38 year old man with unexplained primary infertility of more than 4 years, were admitted for IVF treatment after 6 attempts of (unsuccessful) intrauterine insemination treatments. The first IVF treatment started in April 2001. Ovarian stimulation was performed by a combination of GnRH-agonist, Synarel (Searle, Maarssen, The Netherlands), recombinant FSH, (Puregon, Organon, Oss, The Netherlands) and hCG (Pregnyl, Organon). Luteal support was given by intravaginally administered progesterone (Progestan, Organon). After oocyte pick up (OPU), 8 oocytes were collected. Insemination took place four hours after OPU with normal sperm (46 mln/ml, 61% motile after preparation with PureSperm (NidaCon, Göteborg, Sweden). Per oocyte 75.000 motile spermcells were inseminated.

Nineteen hours after insemination the oocytes were mechanically denuded from the surrounding cumulus and spermcells and checked for the presence of 2 pronuclei. All 8 oocytes showed more than 3 pronuclei, which were smaller than average. The number of spermcells in the zona pellucida was normal. All oocytes had extruded either one or 2 polar bodies.

The next day one oocyte had cleaved to the 3-cell stage with less than 20% fragmentation. Three days after OPU a total of 3 oocytes had cleaved: one to the 2 cell stage with no fragmentation, one to the 3 cell stage with less than 20% fragmentation and one to the 5 cell stage with less than 20% fragmentation. No embryo transfer was performed.

In August 2001 the couple consented to perform ICSI in at least part of the oocytes. The ovarian stimulation was protocol was similar to the last time, with the exception that Suprefact (Hoechst, Frankfurt, Germany) was used instead of Synarel as GnRH-agonist. A total of 9 oocytes were retrieved after OPU. Of those 9 oocytes 5 were denuded of their surrounding cumulus cells both mechanically and enzymatically. All 5 oocytes had extruded their first polar body. They were injected with semen with normal characteristics (107 mln/ml, 93% motility after preparation with PureSperm) (3). The remaining 4 oocytes were inseminated with spermatozoa from the same semen sample 4 hours after OPU (75.000 motile sperm cells per oocyte).

Eighteen hours after injection 2 of the 5 oocytes showed 2 pronuclei, one oocyte showed no pronuclei and 2 oocytes were degenerated. Nineteen hours after insemination the inseminated oocytes were mechanically denuded from the surrounding cumulus- and sperm cells. Three of the 4 oocytes showed more than 3 pronuclei, which were smaller than average. In 1 oocyte no pronuclei were observed. All oocytes had extruded one or two polar bodies. On day 2 after OPU the normally fertilized oocytes (2 pronuclei) had cleaved to the 2-cell stage. One showed no fragmentation, the other less than 20%. The other oocytes remained in the one cell stage. Three days after OPU the two normally fertilized oocytes had developed to a 4-cell embryo without fragmentation and a 6-cell embryo with less than 20% fragmentation, respectively. The other oocytes were still in the one cell stage.

Both the 4- and 6-cell embryo were transferred on day 3 after OPU with a Wallace catheter (3). Three weeks after OPU the β-hCG testing was positive (9500 IU/l) and a single intrauterine pregnancy was confirmed by ultrasound 8 weeks after OPU. The patient delivered a healthy female infant at 40 weeks and 4 days of gestation, with an Apgar of 10 after 5 minutes. She weighted 3035 g. Congenital abnormalities were not found.

Discussion

Although ICSI is well known as a treatment for male factor infertility and unexplained total fertilization failure, this case report shows that ICSI may also offer a solution for multipronuclear oocytes.

The fact that injection of just 1 spermcell resulted in normal fertilization leads to the assumption that polyspermia could have been the cause of the multipronuclear oocytes found in this case. Polyspermia seems to be a multifactorial phenomenon involving the competence of both male and female gametes (1).

Because the size of the pronuclei found in the IVF oocytes was smaller than one would expect of normal pronuclei it cannot be excluded that those were fragmented pronuclei. The fact that with ICSI no fragmentation of pronuclei was observed while of the sibling oocytes that were inseminated 3 out of 4 showed fragmentation of the pronuclei is hard to explain. Cytoplasmic factors responsible for the development of normal nuclear envelopes might be influenced in different ways by ICSI and by IVF. Another explanation for our observations might be a possible relationship between impairment of the zona reaction and a defect to develop normal pronuclei. This also would explain the different results after IVF and ICSI treatment with apparently normal gametes.

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Chapter 6

Intracytoplasmic sperm injection: position of the polar body affects pregnancy rate

Human Reproduction 1999; 14: 2565-2569

LAJ van der Westerlaken[1], FM Helmerhorst[1], J Hermans[2], N Naaktgeboren[1],

[1]*Department of Gynaecology, Leiden University Medical Center, Leiden, The Netherlands.*

[2] *Department of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands*

Abstract

A prospective study on intracytoplasmic sperm injection (ICSI) was performed to evaluate the effect of the position of the polar body relative to the opening of the injection needle during sperm injection, and of the person who performs the injections on fertilization, cleavage and pregnancy rates. This study included 173 couples undergoing 313 ICSI cycles from September 1995 to December 1997. All injections were performed by two persons. For each injected oocyte the person who performed the injection was recorded as well as the position of the polar body during injection (6 o'clock: animal pole towards the opening of the needle; 12 o'clock: animal pole away from the opening of the needle). Of 2630 oocytes retrieved, 2232 were injected. Significantly more oocytes developed two pronuclei after injection with the polar body at 6 o'clock versus 12 o'clock ($P = 0.01$; 51 versus 45% respectively) and after injection by person 1 versus person 2 ($P = 0.02$; 50 and 45% respectively). Higher pregnancy rate ($P = 0.046$) was found after transfer of embryos from oocytes injected with the polar body at 6 o'clock (36%) versus 12 o'clock (18%). This was the result of a significant interaction ($P = 0.03$) between the position of the polar body and the person performing the injections. Given the higher fertilization rate in the 6 o'clock group, it is recommended that oocytes be injected with the polar body at 6 o'clock. The higher pregnancy rates as a result of polar body position and the operator suggest variations in injection technique.

Introduction

Intracytoplasmic sperm injection (ICSI) is the injection of a single spermatozoon into the cytoplasm of a mature oocyte. This treatment is offered in cases of severe male factor infertility (1, 2, 3). The overall fertilization cleavage and pregnancy results of ICSI using semen with low sperm concentration, low motility, and/or abnormal morphology are similar compared to the results in the conventional in-vitro fertilization (IVF) procedure with normal semen parameters (1, 3, 4, 5) as evaluated by World Health Organization recommendations (6).

During the ICSI procedure the oocyte is fixed by a holding pipette in such a way that the polar body of the oocytes is at the 6 or the 12 o'clock position at the moment of injection. When the needle is entering the oocyte at the 3 o'clock position, the opening of the bevel of the needle is facing the 6 o'clock position. During injection it is checked if the tip of the needle is inside the cytoplasm by aspirating cytoplasm into the needle to make sure that the oocyte membrane is broken. During this aspiration of cytoplasm different structures might be damaged or lost. There might be a difference between the risk of loosing or damaging structures in oocytes injected with the polar body at the 6 o'clock position or the 12 o'clock position. In the human it can be assumed that the chromosomes of the oocyte are found in the periphery of the oocyte near the location of the first polar body (7), and therefore the chance of damaging the meiotic spindle and/or disturbing the microtubule organization in the oocyte that has an important role in fertilization and embryonic development (8) might differ with the position of the polar body relative to the opening of the injection needle. Those structures may be responsible for embryo development. Differences in damage in those structures might be expressed in fertilization rates, cleavage rates, embryo quality, and pregnancy and abortion rates.

Differences between operators that perform the injections may also influence ICSI outcome. Although the ICSI procedure itself is performed by a standard protocol, inter-individual differences in the injection technique itself may occur.

The effect of the ICSI procedure on oocyte characteristics and sperm characteristics after injection has been studied at both the cytological and developmental levels of oocytes and embryos (9). The effect of the position of the polar body relative to the opening of the injection needle at the moment of injection has been studied at the developmental level of oocytes and embryos (10, 11). However, those studies did not include pregnancy rates and abortion rates that resulted from the transfer of embryos originating from oocytes injected with the polar body at different positions relative to the opening of the injection needle. So far no studies have been published on variations between operators performing injections.

In this study the influence of the position of the polar body during injection and of the person performing the injection on fertilization, cleavage, pregnancy, and delivery rates is examined.

Materials and Methods

Patient selection

From September 1995 to January 1998 a total of 173 patients underwent 313 oocyte retrievals followed by ICSI. In 31 oocyte retrievals there was no embryo transfer because of total fertilization failure (29 ICSI) or because of the risk of ovarian hyperstimulation syndrome (2 ICSI). These last two ICSI were not included in this study.

The mean age of the female patients was 32.2 ± 4.7 (range 20-45) and of the male patients 36.3 ± 6.5 (range 24-62).

The indication for undergoing ICSI treatment was poor semen parameters (i.e. $<1x10^6$ total number of morphologically normal motile spermatozoa) or fertilization failure in previous conventional IVF despite normal sperm parameters according to World Health Organization standards (6).

Ovarian stimulation

Ovarian stimulation was performed by a combination of a gonadotropin-releasing hormone agonist, Decapeptyl (Ferring, Hoofddorp, The Netherlands); Synarel (Searle, Maarssen, The Netherlands), human menopausal gonadotropin, Metrodin HP (Serono Benelux, Den Haag, The Netherlands); Pergonal (Serono Benelux), and human chorionic gonadotropin (HCG), Profasi (Serono Benelux); Pregnyl (Organon, Oss, The Netherlands). Luteal phase supplementation was given by intravaginally administered progesterone, Progestan (Organon) and an HCG injection, Pregnyl (Organon) 6 days after oocyte retrieval.

Semen preparation

Fresh ejaculated sperm was allowed to liquefy. For seven patients, frozen sperm was thawed (12 cycles). Volume was determined, concentration and percentage of motile spermatozoa were assessed in a Makler counting chamber and the total number of motile sperm was calculated. HEPES-buffered Earle's medium with 0,5% human serum albumin was added to the semen sample and mixed by pipetting. Depending on the total number of motile sperm the mixed sample was pipetted on top of either a 1 ml 70% or 80% Percoll layer and centrifuged (800 x g, 10 minutes). The supernatant was removed and the pellet was resuspended in Hepes buffered Earle's medium. Depending on the total number of motile sperm, this suspension was either pipetted on top of a 80% Percoll layer and washed two times, first in the HEPES-buffered medium and the second time in IVF-50 medium (Medicult), or washed two times in the medium after the first Percoll treatment (first in the Hepes-buffered medium and the second time in IVF-50 medium (Medicult). Volume, concentration, motility and the total number of motile sperm were redetermined after processing. The sperm was kept at 37^{o}C in a CO_2 incubator until ICSI took place.

Oocyte preparation

Between 0 and 4 hours after oocyte-cumulus complex (OCC) collection the OCC were denuded of their surrounding cumulus cells by incubation in 80 IU/ml hyaluronidase (Hyase; IVF science, Götenborg, Sweden) in HEPES-buffered Earle's medium for 20 s and by repeated pipetting of the OCC in and out of a hand-drawn Pasteur pipette. After denudation the oocytes were washed in HEPES-buffered Earle's medium with 0.5% human serum albumin and the maturational stage of the oocytes was checked: the oocytes which had extruded a polar body were selected for ICSI and transferred to Universal IVF medium (Medicult) droplets under mineral oil (Sigma, Brunswig Chemie, Amsterdam, The Netherlands) until ICSI took place. Just before starting the ICSI procedure all oocytes were checked again for the presence of a polar body.

ICSI procedure

Microinjection was carried out on the heated stage of an inverted microscope (Olympus, IX70, Paes, Zoetremeer, The Netherlands), using Hoffman modulation optics at 300x magnification. The injection and holding pipette were obtained from Humagen (Gynotec, Malden, The Netherlands). They were connected to two microinjectors (IM-6; Narishige, Paes), which were fitted to two micromanipulators (Narishige) by Teflon tubing (CT-1; Narishige). Petri dishes (Falcon type 1006, Micronic, Lelystad. The Netherlands) were prepared with two central droplets of 3 µl polyvinylpyrrolidone (PVP) solution (Medicult) with one containing prepared sperm and one to flush the injection pipette when necessary. Five droplets of 5 µl HEPES-buffered Earle's medium were arranged around these droplets, each containing one oocyte. All droplets were covered with mineral oil (3.5 ml/Petri dish).

A single motile spermatozoon from the central spermatozoa droplet was immobilized by pressing the tail of the spermatozoon against the bottom of the Petri dish until it stopped moving. The spermatozoon was then aspirated in the injection pipette, tail first. The Petri dish was then moved in order to visualize an oocyte in one of the surrounding droplets. The oocyte was firmly attached to the holding pipette. The position of the polar body was chosen without any preference for the 6 or 12 o'clock position and without any knowledge of the patient. The position was then recorded for each oocyte. The injection pipette always entered the oocyte at the 3 o'clock position with the opening of the bevel directed to the 6 o'clock position. The breakage of the oolemma was checked by gentle aspiration of cytoplasm into the pipette and the spermatozoon was injected into the cytoplasm. The person who performed the injection was recorded for each oocyte. This depended on a weekly work schedule. All injections were performed by two operators with equal ICSI experience.

After injection the oocyte was washed and incubated in well-equilibrated Universal IVF medium at 37 ^{0}C in5% CO_2 in air.

Embryo transfer

Embryo transfer took place 3 days after oocyte retrieval. In our standard protocol two embryos were transferred. In some circumstances, depending on age and/or number of available embryos, one or three embryos were transferred. These transfers were excluded from this study.

For transfer, a 1-ml syringe was filled with IVF medium (Medicult) and connected to a Wallace catheter (SIMS Portex Ltd, Hythe, UK). After flushing the catheter the selected embryos were aspirated into the catheter. The catheter was passed through the cervical canal and into the uterine cavity. The embryos were slowly injected, after which the catheter was withdrawn gradually.

Assessment of fertilization parameters, embryo quality, and pregnancy evaluation

Fertilization was scored 16-18 hours after injection. Fertilization was considered normal when two pronuclei (PN) were present. The presence of no, one, and more than two PN were also recorded as well as the number of degenerative oocytes.

For all oocytes, the cleavage and the quality were evaluated at day 2 and day 3 after injection. According to the number and size of blastomeres and the amount of fragmentation the embryo's were assigned to four different quality types: type 1, equal-sized blastomeres and no fragmentation; type 2, <20% fragmentation; type 3, 20-50% fragmentation; type 4, > 50% fragmentation.

Pregnancy was defined by an increasing serum ß-HCG ≥ 50 IU/l at 15 days after oocyte retrieval. Spontaneous abortion was defined as pregnancy ending in a miscarriage up until 16 weeks after the last menstrual period. No ectopic pregnancy occurred in this study.

Statistical analysis

Pearson's χ^2 test and logistic regression were used to compare the proportions of fertilization, type of cleavage, and pregnancy and abortion rates.

Results

Overall results

A total of 2630 oocytes was collected in 313 oocyte retrievals (8.4 oocytes per oocyte retrieval) in 173 patients (1.8 oocyte retrievals/patient). At the moment of retrieval 290 of the oocytes (11%) were in the germinal vesicle stage (GV), 121 were in metaphase I (4.6%), 14 were degenerative (0.5%) and 2205 were in the metaphase

II stage (83.8%), which was shown by the presence of an extruded first polar body. A few (n=27) oocytes were injected later after they had developed in vitro to the metaphase II stage. Only oocytes that showed a polar body were injected.

Fertilisation results

Logistic regression on the number of normally fertilized oocytes showed that both the person who performed the injections (P= 0.02) and the position of the polar body (P= 0.01) significantly affected the number of 2PN oocytes (Table I): the percentage of 2 PN oocytes that developed in the 6 o'clock group was higher than in the 12 o'clock group and this effect was very similar for both operators. Operator 1 obtained higher fertilization rates than operator 2 for the 12 o'clock group as well as for the 6 o'clock group. To exclude the possibility that these effects were the result of a general gain in experience with time, the study period was divided into two. The second period showed higher fertilization rates for both operators and for both positions of the polar body. However, in both periods the same effects of the operator performing the injection and of the position of the polar body were present. In addition there was a significant effect of the position of the polar body and of the person performing the injection on the number of 1PN oocytes. Significantly more 1PN oocytes developed after injection with the polar body at the 12 o'clock position versus the 6 o'clock position [118/1167 (10%) versus 78/1065 (7%) respectively] (χ^2 = 5.40, df = 1, P= 0.02) and after injection by person 2 versus person 1 [90/832 (11%) versus 106/1400 (8%) respectively] (χ^2 = 6.86, df = 1, P= 0.01). No significant differences between the 12 and 6 o'clock positions and between operators performing the injection were found with regard to the number of oocytes with >2PN, no PN and the number of degenerated oocytes (data not shown).

Table 1. Number of two pronuclear (2PN) oocytes developing after injection by operator 1 or 2 and with the polar body held at the 12 or 6 o'clock position during injection

Polar body position	12 o'clock	6 o'clock	Total
Operator			
1	343/734 (47)	355/666 (53)	698/1400 (50)^{a}
2	187/433 (43)	186/399 (47)	373/832 (45)^{a}
Total	530/1167 (45)^{b}	541/1065 (51)^{b}	1071/2232 (48)

Values in parentheses are percentages.
^{a,b}Values with the same superscript were significantly different, ^{a} P=0.02; ^{b} P=0.01 respectively

Cleavage results

A significant difference was found between the 12 o'clock and the 6 o'clock position of the 2 PN embryos with regard to the quality of cleavage (χ^2 = 10.52, df = 4, P= 0.032) (Table II). Detailed analysis showed that this was caused by a lower number of type 4 embryos in the 12 o'clock group (χ^2 = 5.14, df = 1, P= 0.02). No differences were found between persons with regard to quality of cleavage in the group of 2PN embryos (χ^2 = 2.68, df = 4, P= 0.61).

Table II. Number of cleavage type 1, 2, 3 and 4 embryos developed from oocytes with two pronuclei after sperm cell injection of the oocyte by operator 1 or 2 with the polar body (PB) at the 6 or 12 o'clock position

	Polar body position			
	12 o'clock		6 o'clock	
Operator	1	2	1	2
Cleavage type[a]				
1	83 (24)	56 (30)	94 (26)	41 (22)
2	163 (48)	78 (42)	152 (43)	79 (42)
3	48 (14)	25 (13)	58 (16)	39 (21)
4	10 (3)	4 (2)	21 (6)	8 (4)
No cleavage	39 (11)	24 (13)	30 (8)	19 (10)
Total	343 (100)	187 (100)	355 (100)	186 (100)

Values in parentheses are percentages
[a]Cleavage type 1 = equal-sized blastomers and no fragmentation; type 2 = <20% fragmentation; type 3 = 20-50% fragmentation; type 4 = >50% fragmentation.

Pregnancy results

When all embryo transfers were included, no significant differences in pregnancy rate between the two operators were found [34/121 (28%) and 23/73 (32%) respectively, χ^2 = 0.25, df = 1, P= 0.61]. For the comparison of the pregnancy rate with regard to the position of the polar body the transfers of a combination of 6 o'clock and 12 o'clock embryos were excluded and all transfers of embryos both originating from either the 6 o'clock or from 12 o'clock injections were included. The transfers of a combination of 6 o clock and 12 o'clock embryos resulted in a pregnancy rate of 32% (32/100). The transfers of embryos both originating from the 6 o'clock injected oocytes resulted in a significantly higher pregnancy rate compared with the 12 o'clock injected oocytes (χ^2 = 3.98, df = 1, P= 0.046) (Table III). Logistic regression showed that this difference was the result of a significant interaction between polar body position and the person performing the injections (P = 0.03) For operator 1 the success rates were similar for both polar body positions. For operator 2 a clear difference in success rates occurred between the two positions: the 6 o'clock position scored 48% and the 12 o'clock position scored 6% (P = 0.004 (Table III). Adding the age of the patient into this logistic regression as an explanatory variable, age turned out to be non-significant (P = 0.21). Again, to exclude the possibility that these effects were the result of a general gain in experience with time, the study period was divided into two. Overall, the second period showed higher pregnancy rates. In both periods the pregnancy rate of the 6 o'clock group was twice as high compared with the 12 o'clock group (Table III).

The number of abortions did not differ significantly between the 6 and 12 o'clock polar body positions [4/16 (25%) versus 2/9 (22%) respectively]. The ongoing pregnancies all ended in deliveries after at least 25 weeks of gestation.

Two of the transfers with two embryos involved a mix of embryos originating from the injection by operator 1 and operator 2 and were therefore excluded from this analysis.

Table III. Number of pregnancies per embryo transfer after transfer of two embryos developing after injection by operator 1 or 2 and with the polar body of both transferred embryos held at either the 12 or the 6 o'clock position during injection

Polar body position	12 o'clock	6 o'clock	Total
Operator			
1	8/31 (26)	6/24 (25)	14/55 (26)
2	1/18 (6) [b]	10/21 (48) [b]	11/39 (28)
Total	9/49 (18) [a]	16/45 (36) [a]	25/94 (27)

Values in parentheses are percentages
[a,b]Values with the same superscript were significantly different, [a] P = 0.046 and [b] P = 0.004 respectively

Discussion

This study shows that there is a significantly higher fertilization percentage (2PN) amongst oocytes that have been injected with the polar body at the 6 o'clock position relative to the opening of the injection needle in comparison with oocytes that have been injected with the polar body at the 12 o clock position relative to the opening of the injection needle. Also the person who performed the injections had a significant influence on the fertilization rate. Embryo quality (cleavage) was slightly affected by polar body position in the 2PN group. The pregnancy rate after transfer of embryos derived from oocytes injected relative to the 6 o clock position was significantly higher than that from oocytes injected relative to the 12 o clock position. This was the result of a significant interaction between the person performing the injections and the position of the polar body. Although there was a gain in experience during the whole study period this did not influence the effects of position of the polar body and of the operator.

Overall fertilization percentages, cleavage results and pregnancy rates found in this study were similar to those found in our conventional IVF programme and to those reported by others (2, 3, 10, 12, 13).

It was expected that aspiration towards the site of the polar body (6 o'clock position) would result in more damage of structures important for fertilization, assuming that the nuclear material is located near the polar body (7). This was not conformed by the results of this study. On the contrary, the fertilization rate was higher when the aspiration was closer to the polar body. Perhaps deposition of the sperm cell closer to the meiotic spindle is responsible for this result. One study (11) supports this in which the highest fertilization rate was found when the sperm cell was injected adjacent to the meiotic spindle. The success of the injections could depend on the rotation of ooplasm by a correctly positioned sperm chromosome (14). However, another study (10) did not find a difference between the 6 and the 12 o'clock positions in the number of 2PN oocytes but in the quality of embryos.

The percentage of multi pronuclear ICSI oocytes in this study was 2.5%. This percentage is lower than that in our conventional IVF programme (7.9%). The difference in origin of those multipronuclear eggs might explain this observation. The multipronuclear oocytes inn the ICSI procedure probably result from the incorporation of the second polar body (15), while the majority of the multipronuclear oocytes in the conventional IVF programme arise from dispermic fertilization. However, the position of the polar body did not affect the number of multipronuclear oocytes.

The number of oocytes developing only one pronucleus after ICSI (8.5%) was significantly (P = 0.02) higher than after conventional IVF (3.4%). It is probable that such oocytes are activated by ICSI resulting in the formation of one female pronucleus but fail to form a male pronucleus, although the spermatozoon may have contributed to activation. The formation of a male pronucleus might be impaired due to defects in the sperm cell itself, such as impaired microtubule nucleation and elongation and/or compromised sperm aster function (8). The number of 1PN oocytes might be influenced by PVP, which has a stabilizing effect on the disruption of the sperm plasma membrane. This disruption is needed to give the spermatozoa-assiciated oocyte-activating factor access to the sperm head as it is swelling (13). The observation that significantly more 1PN oocytes develop from oocytes injected with the polar body at the 12 o'clock position in our study suggests that deposition of the sperm cell further from the meiotic spindle decreases the chance of normal fertilization, although activation may be achieved. Rotation of the ooplasm and the way the sperm centrosome is positioned may influence this result.

In agreement with the results of one study (10), degeneration of injected oocytes seems to be independent of the position of the polar body during injection.

The difference in pregnancy rate between the 6 and the 12 o'clock polar body positions was almost solely the result of the significant interaction between the operator and the polar body position. This suggests that there are technical differences between people performing the injection, which are related to the position of the polar body. This might result in a difference in pregnancy rate depending on the position of the polar body, It was previously reported that the rate of development to the blastocyst stage is related to the person performing the injection (16). This supports our observations on interindividual differences. The latter may be related to subtle differences in injection technique that improve fertilization and pregnancy rates: The amount of cytoplasm that is sucked into the injection pipette, the force with which the injection pipette is pushed through the oocyte membrane, or the relative positioning of the sperm head inside the oocyte (e.g. in the more central or the peripheral ooplasm, far away or in the vicinity of the second meiotic spindle). This study shows the importance of recording and evaluation of the individual performances with regard to the ICSI technique.

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Chapter 7

Invasive prenatal diagnosis after intracytoplasmic sperm injection

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L.van der Westerlaken[1], H. Verburg[1], N. Naaktgeboren[1], M. Breuning[2], F. Helmerhorst[1]

[1]Department of Gynaecology, Leiden University Medical Center, Leiden, The Netherlands.

[2]Department of Human and Clinical Genetics, Leiden University Medical Centre, Leiden, The Netherlands

Introduction

Since the introduction of intracytoplasmic Sperm Injection (ICSI) as a treatment for severe male factor infertility, the safety of the procedure has been questioned. Because of the invasive character of the ICSI technique, the risk of damaging the chromosomes during the injection procedure might be substantial. In addition, there may be an increased risk of the use of abnormal sperm cells, because the sperm cells that are injected are chosen randomly, without any natural selection. Increased prevalence of sex chromosomal anomalies (1) and a high prevalence of structural and numerical chromosomal aberrations (2) have been reported.

For these reasons invasive prenatal diagnosis (PND) is routinely offered to all our ICSI patients, in contrast to our IVF patients, who are only eligible for PND if they are 36 years of age or older or unless a special reason emerges. We hypothesized that a large proportion of ICSI patients is willing to undergo reliable prenatal testing for the detection of chromosomal abnormalities.

Materials and methods

All ICSI and IVF pregnancies that occurred in our clinic between September 1995 (start date of our ICSI program) and December 1999 are included in this study. Our ICSI patients were informed both orally and in writing about the risks of ICSI and PND by a gynaecologist before to the procedure. In addition, counseling by a geneticist is noncompulsory but strongly advised.

Results

During the study period a total of 252 ICSI pregnancies (of 787 oocyte retrievals) were established in our clinic. Forty-four pregnancies (17%) ended in a spontaneous abortion before 10 weeks of gestation, and 3 pregnancies (1%) were ectopic. Of the 205 ongoing pregnancies (153 singletons, 52 twins), only 36 patients (17%) (24 singletons, 12 twins) chose invasive PND (5 chorion villus biopsy (CVS) and 31 amniocentesis) (Table I).

In the same period 699 IVF pregnancies (of 2,178 oocyte retrievals) were observed, 159 (23%) of which ended in a spontaneous abortion before 10 weeks of gestation and 13 (3%) of which were ectopic. Of the ongoing 521 pregnancies (373 singletons, 140 twins and 8 triplets) 166 were eligible for PND based on age (131 singletons, 27 twins, 8 triplets) (Table I), and of those 67 (40%) chose invasive PND (52 singletons, 13 twins and 2 triplets). Only 9 pregnant IVF patients younger than 36 years were eligible for PND based on genetic disorders in the family history, and they all underwent invasive PND (6 singletons and 3 twins).

Table I: Number of women pregnant after ICSI or IVF who underwent invasive prenatal testing

Type of pregnancy, age of patient	No. of pregnancies	No. of ongoing pregnancies (%)	No. of prenatal diagnosis (%)	CVS	Amniocentesis
ICSI, <36	206	169 (82)	18 (11)	3	15
ICSI, ≥36	46	36 (78)	18 (50)	2	16
IVF, <36	460	355 (78)	9 (2)	3	6
IVF, ≥36	239	166 (67)	67 (40)	2	65

Conclusion

Despite counseling (3) about the possible genetic risks, prenatal testing of ICSI pregnancies for detection of genetic abnormalities allegedly caused by the ICSI procedure itself is, at least among our patients, not popular in comparison with PND for ICSI or IVF pregnancies based on the indication of maternal age. Maternal age seems to be the predominant factor to consider when deciding whether to undergo invasive PND. This result might lead to reconsider the policy of offering invasive prenatal testing in favour of non-invasive procedures (4).

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Chapter 8

General discussion

General discussion

In medically assisted reproduction several procedures can be offered to infertile patients ranging from intrauterine insemination (IUI) via in vitro fertilisation (IVF) to intracytoplasmic sperm injection (ICSI). In IUI processed semen is inseminated into the uterus, in IVF oocytes are inseminated in vitro with processed semen and in ICSI a single spermatozoon is injected mechanically into the oocyte. The main mission of this thesis was to find the right treatment for specific patients groups without overtreatment. Patient specific characteristics and laboratory related factors that can influence the outcome of the ART treatments were studied. These factors can be used to choose the most effective ART treatment for the patient involved and to optimize the treatment itself.

8.1 Intrauterine insemination (IUI)

8.1.1 In search of a gold standard

If more than 10 million processed sperm was inseminated after mild ovarian stimulation significantly more ongoing pregnancies were observed than if less than 10 million processed sperm was used. This difference found in our study (chapter 2) was more predominant in the non-andrological group than in the group with a male factor. Remarkably, in the andrological group 2 out of 7 pregnancies occurred when less than 2 million processed sperm was inseminated, whereas 3 pregnancies out of 7 were observed when more than 10 million processed sperm was introduced. The lower threshold of 2 million and the upper one of 10 million processed sperm were arbitrarily set as scientific data are lacking. If regularly below 2 million processed sperm is retrieved, IVF is advised. If regularly more than 10 million processed sperm is retrieved, IUI is useful if the chance of a pregnancy is drastically diminished as appears from the in/subfertility period (1, 2). But where are the boundaries lying between the 2 and 10 million where we better can perform IUI rather than IVF or waiting? And should we stick to the number of processed sperm to guide our daily practice?

Iberico and co-workers (3) found a direct relationship between pregnancy rate and the number of processed motile sperm that was inseminated, however below 5 million processed motile sperm inseminated the pregnancy rate was low (3.6%). Presumably, an andrological aetiology might then be involved, although we are not explicitly informed about it. In the study of Khalil et al (4) a significant relationship was found between processed motile sperm inseminated (>5 million) and pregnancy. Van Voorhis et al (5) found in their study that the average total motile sperm count (TMSC) in the ejaculate was a better predictor of pregnancy than the TMSC in the inseminate. In addition they concluded that the average total motile sperm count in the ejaculate of 10 million might be a useful threshold value to decide between IUI and IVF.

For a threshold value for IUI the European Society of Human Reproduction refers to the WHO (6) that suggests IUI when more than 0.3-2 million progressive motile spermatozoa are recovered after processing in case of oligospermia (defined by 5-10 to $20x10^6$ spermatozoa /ml) and no severe teratozoospermia (less than 1-4% normal forms). Below this threshold IVF is recommended. In addition to these reference values the WHO also states that each laboratory should develop its own cut-offs in terms of the numbers of progressively motile spermatozoa available from processing a man's ejaculate in order to guide patient management in terms of the type of ART that can be used (6). The American Society of Reproductive Medicine has no guideline on this subject. The Dutch Society of Obstetrics and Gynaecology bases her recommendations on the so-called VCM (volume × concentration × % grade I en II motility/100) (7) of an *un*processed semen sample: 6 attempts of IUI followed by IVF is suggested in case of a VCM > 10 million, after 3 years of infertility (or after 2 years when the woman is ≥36 yr or earlier when 40 yrs has been reached) and in case of a VCM between 1 and 10 million, after 2 years of infertility (or after 1 year when the woman is ≥36 years) (7). In another guideline of the same organisation published in 2000 it is recommended to perform IUI in a spontaneous cycle in case of a VCM < 10 million and in a stimulated cycle in case of a VCM of >10 million (8).

With so many different recommendations, suggestions and guidelines currently in use and/or published it is impossible to define a gold standard for sperm characteristics suitable for IUI.

8.1.2 Prognostic factors

Factors as woman's age, duration of in/subfertility are firmly established prognostic factors for in/subfertility treatments (9,10). Increasing female age is associated with a decline in fecundity as a result of decreased oocyte quality. Ovarian stimulation as a catch up system for it appeared not to be the explanation since ovarian stimulation was applied in all three studies with age as a significant predictor of pregnancy (3,5,11). In the IUI study described in chapter 2, age was not significantly predictive of IUI success, at least not in women of 40 years and younger. Other studies (with more than 750 cycles) form a miscellaneous picture, not only with regard to age, but also with regard to a.o. aetiology, number of sperm inseminated (Table 1).

Table 1: Prognostic indicators for IUI

		cycles tested	age yr	duration yr	aetiology male	aetiology unexpl.	cycle number	sperm mln	follicle number
Steures [11]	2004	14.968	S	S	S	NS	first 6	nt	nt
Iberico	2004	1.010	30-34	<3	nt	nt	NS	<30	2 & 3
Khalil	2001	2.473	NS	nt	S	S	first 4	>5	3 & 5
Voorhis	2001	3.479	<37	nt	NS	NS	nt	<10	nt
Nuojua-H.	1999	811	<40	≤6	NS	S	first 3	NS	S
Westerlaken	1998	1.763	NS	nt	NS	NS	nt	<10	nt

S significant
NS not significant
nt not tested

Most studies show a decrease in pregnancy rate with increasing number of cycles (3, 12). This was also shown in our IUI study (chapter 2) where we found a cumulative pregnancy rate of 19% after 3 cycles and of 31% after 6 cycles (121 pregnancies). The overall diversity in results might be accounted for by the variation between studies in definition of indication, in inclusion criteria for patients, in the number of cycles per patient, in stimulation protocol as well as the retrospective mode of all the studies. More scientifically derived data are needed to be able to decide when the scale is turned from treatment towards no treatment, whether treatment should be adapted or whether an alternative treatment should be offered. From our study it is concluded that the effectiveness of IUI is lower if less than 10 million motile spermatozoa are inseminated.

8.2 In vitro fertilization (IVF) and/or intracytoplasmic sperm injection (ICSI)

The studies presented in chapter 3, 4 and 5 aimed to determine whether IVF or ICSI should be the treatment of choice in three different subpopulations of patients. It was shown that patients with a history of total fertilization failure or low fertilization after conventional IVF should be indicated for ICSI. Similar results were found in patients with mild male factor infertility and in patients that had a history of abnormal fertilization, i.e. the development of multipronuclear oocytes after conventional IVF.

8.2.1 Total fertilization failure or low fertilization

It is beyond dispute that in case of severe male factor infertility, ICSI and not IVF is the treatment of choice (13, 14). The high chance on fertilization failure in subgroups of patients when treated with IVF counterbalances the possible increased risk on chromosomal anomalies related to ICSI that might occur (see introduction). The number of ICSI treatments in case of non-andrological or mild male factor indications is increasing as is shown by the number of publications on this subject (15, 16, 17, 18, 19).

By randomly assigning half of the normospermic patients to ICSI and the other half to IVF, Bhattacharya et al (2001) (17) found no clinical benefit of the use of ICSI compared to IVF. Although the fertilization rate per inseminated or injected oocyte was lower after IVF than after ICSI (70% versus 61% respectively) the pregnancy rate did not differ significantly (IVF: 33% and ICSI: 26%). In addition in only one couple undergoing IVF, total fertilization failure occurred. However, in other studies (18, 19) ICSI has been shown to be effective in normospermic patients by preventing unexplained total fertilization failure (TFF) that occurs in about 5-15% of the normospermic population that is treated with the conventional IVF procedure. The other side of the picture is that overtreatment may be introduced when all patients with normospermia are currently treated with ICSI, a policy suggested by Khamsi et al. (18) and Ruiz et al. (19): in more than 80% of the couples with normospermia fertilization was established by the conventional IVF procedure while all patients were treated with ICSI besides IVF.

We therefore propose, based on our data described in chapter 3, that first the diagnosis of TFF in a conventional IVF-procedure must be established before treatment with ICSI should be performed. The effectiveness of ICSI in a second attempt after a first conventional IVF attempt with TFF is high: the recurrence rate of fertilization failure after IVF was 67%, whereas all couples showed fertilization after ICSI. Therefore it is recommended in patients with a history of total fertilization failure or low fertilization (<25%) rate to perform ICSI even in case of normospermia.

8.2.2 Mild male factor infertility

The effectiveness of ICSI in a first cycle in case of mild male factor infertility is more evident compared to non-male factor infertility: the absence of fertilization after IVF occurs more frequently in patients with oligoasthenospermia than in normospermic patients: 25-50% versus 5-15% (20, 21, 22, 23).

However, to avoid ICSI in patients that do not need ICSI to fertilize predictive parameters are needed to be able to make the right treatment choice. Studies have been published aiming to identify such predictors for fertilization. The results of those studies have contributed to more knowledge about the factors that are associated with the occurrence of total fertilization failure after IVF such as a low rate of rapid progressive sperm motility and severe teratozoospermia (21, 24). However, no threshold for sperm characteristics defining sperm not capable of fertilization in conventional IVF has been identified to date.

From the data of our study presented in chapter 4 we were able to retrospectively identify differences in sperm motility before processing and in total motile sperm count after processing between the group of patients with and without fertilization after IVF. However, we do not have a tool to predict the chance on fertilization after IVF. Because the chance on total fertilization failure in case of mild male infertility in conventional IVF is relatively high (25-50%) we recommend performing IVF and ICSI on sibling oocytes. We know that by this policy some patients are overtreated and that ICSI is not more effective than IVF. On the other hand patients, who would otherwise have suffered total fertilization failure benefit and for them ICSI is very effective.

8.2.3 Abnormal fertilization

In addition to fertilization failure after conventional IVF as an indication for ICSI we examined whether abnormal fertilization could be prevented by ICSI. Chapter 5 shows that ICSI prevented the development of multipronuclear oocytes in a normospermic couple with a history of multipronuclear oocytes after conventional IVF in a first attempt. The development of multipronuclear oocytes has been reported in a publication of a later date by Matt et al (2004) (25). They performed IVF in a first cycle that resulted in the appearance of polyploidy in the majority of the oocytes (20/23). In a second cycle all oocytes were treated with ICSI and even there 12 out of 17 injected oocytes showed polyploidy. They concluded that because ICSI did not prevent the development of multipronuclear oocytes the mechanism that was responsible probably was not polyspermia. In our case the cause of the pronuclear oocytes probably was polyspermia because the injection of one spermcell resulted in normal fertilization. However, the pronuclei in the IVF oocytes were smaller than those in the ICSI oocytes and therefore nuclear fragmentation might not be ruled out as a possible cause. If the latter is the case the mechanism through which ICSI can prevent fragmentation is unknown. The effectiveness of ICSI for this couple was very high: in the second attempt only the sibling oocytes that were treated with ICSI were fertilized normally, the sibling oocytes that were treated with IVF developed multipronuclear oocytes again. When no ICSI would have been performed no normal fertilization would have occurred and no pregnancy and live birth would have been achieved.

Based on the results found in our study we recommend to perform ICSI in patients with a history of multipronuclear oocytes after conventional IVF.

8.3 Intracytoplasmic sperm injection (ICSI)

8.3.1 ICSI technique

Not only the choice for a particular treatment but also the techniques within the treatment itself can be performed differently and thereby influence the fertilization, embryonic development and pregnancy rate. After the birth of the first ICSI babies in 1992 (26) many studies have been performed with regard to the effects of different injection protocols, denudation protocols, types of membrane breakage etc. (27, 28, 29, 30). One of the most studied technical parameters is the location of the metaphase II spindle during the injection procedure. It has been postulated that fertilization and embryonic development is affected by damage or disruption of the spindle caused by the injection technique. The cytoskeletal architecture (and in particular the second metaphase spindle) could be disturbed during the ICSI procedure, either by the injection pipette itself or by substances such as culture medium and PVP injected into the oocyte. Aspirating cytoplasm during ICSI would further increase the chance of disrupting the meiotic spindle: a higher incidence of diffuse cytoskeletal assembly and other features suggestive of a damaged or disorganized cytoskeleton in oocytes and zygotes after ICSI compared to IVF have been reported (31). A disturbed spindle could lead to non-disjunction during second meiosis, resulting in an aneuploid zygote developing in an aneuploid embryo. The location of the spindle was believed to be located in the periphery of the ooplasm adjacent to the first polar body (32, 33). Therefore the first polar body was used as a mark for the location of the spindle. The orientation of the injection channel and the sperm deposition site during ICSI were usually chosen with the position of the first polar body at 6 o'clock or at 12 o'clock to minimize potential spindle damage. Later studies showed that the spindle location in denudated oocytes that were fixated and stained was not directly adjacent to the polar body but in the same hemisphere (34). Later technical developments made it possible to visualize the spindle in living oocytes by the use of an orientation-independent polarized microscopy system. It was found that the meiotic spindle of human oocytes that have been exposed to manipulation (such as removal of the cumulus oophorus and the corona radiata) can be displaced with regard to the position of the first polar body (35). A study by Rienzi et al (2003) (36) showed that the spindle of approximately half of the oocytes were almost adjacent to the polar body (0-5^0), 40% showed a moderate (6-45^0) to medium (46-90^0) deviation and 5% deviated more than 90^0.This is different from the location of the spindle in human in-vivo matured oocytes that have not been exposed to any manipulation which always corresponds to the position of the first polar body (36). Only oocytes with an angle deviation of the meiotic spindle with regard to polar body position of more than 90^0 had lower fertilization rates in that study. Therefore the choice for the 6 and 12 o'clock position of the polar body during injection as was examined in chapter 6 is still valid. Several other studies on this subject have been published since (37, 38, 39). In contrast to our study those publications did not report differences in fertilization rate between the 6 and 12 o'clock position. Our study found higher fertilization rates as well as higher pregnancy rates if oocytes were injected with the polar body at the 6 o'clock position. However this last result appeared to be due to a significant interaction between the operator performing the injection and the position of the polar body. The reason for discrepancies between studies is unknown. It might be that the outcome measures may depend much more on the individual differences in the exact technique employed by ICSI operators, as we already saw in the small setting of our laboratory, then upon a general feature of the ICSI technique. This was also shown by Dumoulin et al (2001) (39) who reported differences in blastocyst formation between different ICSI operators. It was shown that there were subtle differences between them in their injection technique: membrane breakage occurred at a significantly shorter distance from the tip of the pipette, reflecting a more sudden aspiration of ooplasm, by the operator with the highest blastocyst yield.

These results show that the effectiveness of performing ICSI can vary with technical details, which can even be influenced by the person performing the ICSI. Therefore it is important to record and evaluate the results in relation to possible influencing factors.

8.3.2 ICSI outcome

> And this," said the Director opening the door, "is the Fertilizing Room."
>
> Bent over their instruments, three hundred Fertilizers were plunged, as the Director of Hatcheries and Conditioning entered the room, in the scarcely breathing silence, the absent-minded, soliloquizing hum or whistle, of absorbed concentration.

These shocking sentences can be read in the first chapter of Aldous Leonard Huxley's (1894-1963) 'Brave New World'. Advances in biotechnology, especially in reproductive medicine seem to worry the public. Recently the media reported on cloning and stem cells as they did in the seventies and nineties of the last century when IVF and ICSI respectively were introduced: fear for the new developments. At the same time that the general reluctance of the great biotechnical developments are broadcasted, the media bring also some individuals to the footlight with their requests to be considered for the new treatments in reproductive medicine. They try to persuade their physicians or via the media the health authorities to accept the new technologies that might benefit them. Known but also unexpected adverse effects are brushed aside in order to enlarge the potential achievement. But, at the time of the treatment, is the patient still discarding the potential adverse effects?

Since the introduction of ICSI studies have been conducted to evaluate the safety of this technique (40,41,42,43). Increased prevalence of sex chromosomal anomalies and a high prevalence of structural and numerical chromosomal aberrations have been reported (44,45). A significantly higher risk of de-novo chromosomal anomalies, mainly due to sex-chromosomal anomalies, and partly related to a higher level of de-novo structural anomalies was reported by Bonduelle et al. (2002) (46). In order to inform the patients who underwent ICSI we offered all those patients prenatal diagnosis (PND) that is chorion villus biopsy or amniocentesis. At that time less invasive methods as nuchal translucency thickness were not routinely available.

In the Netherlands at the end of the nineties of the last century only women of 36 years of age and older and those with special reasons are eligible for PND. Despite the counselling of the genetic risks only 11% of the ICSI patients younger than 36 years of age choose PND. The risk of loosing the pregnancy due to PND (0.3-1%) seemed to dominate the risks associated with ICSI in those patients. The desire to have a child apparently overrules concerns that have been raised regarding the potential for ICSI to facilitate transmission of infertility associated chromosomal or genetic disease and regarding the risk of possible congenital malformations.

8.4 Conclusion

The studies presented in this thesis have been performed to improve success rates of ART treatments in subgroups of patients by fine tuning and optimising existing treatments. The knowledge we gained about the role of these factors can help to make the right decisions with regard to the treatment of choice for individual patients. The results show that we must be aware that we need to evaluate carefully for each patient the most suitable solution to the problem that we have to deal with.

Besides the effectiveness of the treatment there is also the cost-effectiveness that may influence the choice of treatment. There are some publications dealing with this aspect of infertility treatment (47, 48). Although this aspect is getting more and more important in the Netherlands due to the change in policy with regard to reimbursement of treatments by insurance companies it goes beyond the scope of this thesis.

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Summary

This thesis presents six studies each focussing on a different aspect of assisted reproduction.

Intrauterine insemination (IUI) (chapter 2)

This study evaluated intrauterine insemination results retrospectively according to indication, age and sperm parameters. It was found that IUI is a useful treatment for subfertile couples unless semencharacteristics are insufficient: insemination of less than 10 million total motile spermcells significantly reduces pregnancy rate (4% versus 11% per cycle). Other indications examined (idiopathic, age, tubapathology, hormonal, andrological as defined by the WHO) did not significantly affect pregnancy rate. Also age did not have a significant effect on pregnancy rates, however, no pregnancies were established in women of 40 years or older. The total cumulative pregnancy rate reached a maximum of 31.1%. Most of the pregnancies were established in the first 4 cycles. After 6 cycles no additional pregnancies were obtained.

In vitro fertilization (IVF) versus intracytoplasmic sperminjection (ICSI) (chapter 3, 4 and 5)

Three studies were performed focussing on the question whether conventional IVF or ICSI should be the choice of treatment. The first study involved patients with a history of total fertilization failure or low fertilization in a first IVF attempt, the second study involves patients with oligoasthenospermia who came for a first attempt and the third study invloves a couple with a history of multipronucleated oocytes after a first IVF attempt.

The aim of the first study (chapter 3) was to determine whether ICSI was more effective than IVF in patients with a previous IVF attempt that resulted in total fertilization failure or low fertilization. Overall, in 5-15% of the couples undergoing IVF with normal semenparameters according to WHO criteria no fertilization occurs (1). The recurrence rate of this fertilization failure is rather high (30-50%) (2). By performing intracytoplasmic sperm injection this fertilization failure might be avoided. This study showed that ICSI offers a solution in patients with a history of

unexplained total fertilization failure (TFF) as well as low fertilization (less than 25%) (LF) after IVF. By performing IVF and ICSI on sibling oocytes fertilization was established in all patients. However, in 68% of the couples with a history of TFF and in 50% of the couples with a history of LF no fertilization occurred in the IVF treated oocytes. Whether this is caused by sperm factors or oocyte factors is still unknown. The effectiveness of ICSI compared to IVF in those patients is high: no fertilization results in no embryotransfer, and no pregnancies will be established, while the ongoing pregnancy rate when fertilization was established varied between 33% and 67% per transfer. The effectiveness of ICSI compared to IVF in the patients that had fertilization in ICSI as well as in IVF is less pronounced: although fertilization was established with IVF the fertilization rates were lower compared to ICSI.

Whether couples suffering from oligoasthenospermia should be treated with IVF or ICSI was the subject of the second study. Although the WHO has defined threshold values for spermparameters that discriminate between male fertility and subfertility, the prognostic value is questionable. Overall about 25%-50% of the cycles with oligoastenospermia result in total absence of fertilization. Since we do not want to “overtreat” on the one hand and at the same time want a successful outcome on the other hand it is important to carefully balance both methods. By performing IVF and ICSI on sibling oocytes in patients with oligoasthenospermia we were able to examine the fertilizing capacity of the semen in IVF as well as in ICSI (chapter 4). The results showed an all-or-nothing effect with regard to the fertilization rate after IVF. And, although retrospectively lower spermparameters were found in patients that fertilized only after ICSI, no distinct difference in individual semenparameters could be identified that would enable us to decide for each patient with male subfertility whether IVF or ICSI treatment would result in high fertilization rates. The problem regarding effectiveness in this study is the fact that although we know that for some patients ICSI is not more effective than IVF we will offer ICSI because we are not able to distinguish between patients who will and who will not fertilize with IVF.

The fact that ICSI can also be succesfull in the treatment of patients with a history of multipronucleated oocytes after IVF was demonstrated in a case report (chapter 5). A couple that underwent a first IVF cycle that resulted in multipronucleated oocytes, were treated with IVF and ICSI on sibling oocytes in the second cycle. This resulted in normal fertilization in the ICSI oocytes and again in multipronucleated oocytes in the sibling IVF oocytes. Two ICSI oocytes were transferred and resulted in a pregnancy and the birth of a healthy girl. The effectiveness of ICSI versus IVF in this case is high as no normal fertilization would have occurred with IVF.

Intracytoplasmic sperm injection (ICSI) (chapter 6 and 7)

Not only patient characteristics but also differences in the way ART techniques are performed may influence the outcome of the total treatment. This was subject of a study (chapter 6) examining the effect of some characteristics of the ICSI technique: the position of the polar body during injection and the person performing the injection (operator) was examined in relation to the outcome of ICSI in terms of fertilization and pregnancy rates. A significant effect of both the position of the polar body and of the operator was found with regard to fertilization rate. With regard to the pregnancy rate a significant interaction was found between the position of the polar body and the operator, suggesting technical differences between the operators, which are related to the position of the polar body. The effectiveness of injection of the oocytes at 6 o'clock is higher compared to 12 o'clock injections with regard to fertilization results as well as pregnancy results.

In chapter 7 we evaluatued the hypothesis that a high proportion of ICSI patients would choose for invasive prenatal diagnosis (PND) when offered because of the possible genetic risks (see introduction). However, the predominant factor to consider PND in our population was maternal age and not ICSI. This result lead to reconsideration of the policy of offering PND to ICSI patients.

Samenvatting

In dit proefschrift worden 6 studies beschreven die elk een ander aspect van de geassisteerde voorplanting bestudeerd hebben.

Intra-uteriene inseminatie (IUI) (hoofdstuk 2)

Zwangerschapspercentages na intra-uteriene inseminatie zijn geëvalueerd afhankelijk van indicatie, leeftijd en spermakwaliteit. Hieruit bleek dat IUI slechts een zinvolle behandeling is als er meer dan $10x10^6$ spermacellen geïnsemineerd kunnen worden. Bij minder te insemineren spermacellen werd de zwangerschapskans significant verlaagd (4% tegenover 11% per cyclus). Er bleek geen verschil in zwangerschapskans te zijn tussen de verschillende indicaties (idiopatisch, tubapathologie, hormonaal, andrologisch (zoals gedefinieerd door de WHO)). Ook leeftijd had geen significant effect op de kans op zwangerschap na IUI, ondanks het feit dat in vrouwen van 40 jaar en ouder geen zwangerschappen meer zijn waargenomen. Het totale cumulatieve zwangerschapspercentage bereikte een maximum van 31.1%. De meeste zwangerschappen ontstonden in de eerste 4 cycli. Na 6 cycli kwamen er geen zwangerschappen meer bij.

In vitro fertilisatie (IVF) versus intracytoplasmic sperm injection (ICSI) (hoofdstuk 3, 4 en 5)

In drie verschillende studies is onderzocht of conventionele IVF dan wel ICSI de optimale behandelingsvorm is bij paren die in een voorgaande IVF poging onverklaard geen bevruchting hadden (hoofdstuk 3), bij paren met een milde vorm van oligoasthenospermie (hoofdstuk 4) en in geval van een paar waarbij conventionele IVF resulteerde in de ontwikkeling van eicellen met multipronucleï in plaats van 2 pronucleï (hoofdstuk 5).

Het doel van de eerste studie (hoofdstuk 3) was om te achterhalen of ICSI dan wel IVF het meest effectief is in paren die in een eerdere IVF poging geen of weinig bevruchte eicellen hadden. In zijn algemeenheid komt het uitblijven van bevruchting in geval van normospermie (gebaseerd op de WHO normen) in 5-15% van de paren voor. Herhaling van het uitblijven van bevruchting bij die paren komt zelfs in 30-50% van de gevallen voor. Door deze paren te behandelen met ICSI zou het uitblijven van bevruchting voorkomen kunnen worden. De resultaten van deze studie laten zien dat in 68% van de paren die voorheen geen bevruchting hadden in IVF en in 50% van de paren van wie voorheen minder dan 25% van de eicellen bevruchtten in IVF in een tweede poging waarin een deel van de eicellen met IVF en een deel met ICSI behandeld werd wel bevruchtten middels ICSI, maar weer niet middels IVF. De effectiviteit van ICSI vergeleken met IVF voor deze paren is hoog: geen fertilisatie betekent geen terugplaatsing en dus geen zwangerschappen. Het doorgaand zwangerschapspercentage bij wel bevruchten van de eicellen middels ICSI lag tussen de 33% en 67% per terugplaatsing. Bij de paren waarbij er niet alleen bevruchting optrad na ICSI maar ook na IVF is de effectiviteit minder duidelijk, echter het bevruchtingspercentage na IVF was lager dan na ICSI.

De tweede studie richtte zich op paren met oligoasthenospermie. Ondanks dat de WHO grenswaarden vastgesteld heeft die aangeven wanneer er sprake is van mannelijk subfertiliteit is de voorspellende waarde daarvan twijfelachtig. Ongeveer 25-50% van de cycli waarin sprake is van oligoasthenospermie resulteren in het uitblijven van bevruchting. Om te voorkomen dat er enerzijds ICSI gedaan wordt terwijl met IVF volstaan had kunnen worden en dat er anderzijds IVF gedaan wordt waarbij er geen bevruchting optreedt is het noodzakelijk om beide methoden zorgvuldig tegen elkaar af te wegen. Door zowel IVF als ICSI uit te voeren op de eicellen van eenzelfde patiënt, waarbij een deel van de eicellen behandeld wordt met IVF en het andere deel van de eicellen met ICSI is het mogelijk om het bevruchtend vermogen van het semen in IVF en in ICSI te bestuderen (hoofdstuk 4). Dit resulteerde in een alles-of-niets effect met betrekking tot de eicellen die met IVF behandeld waren: ofwel er was geen bevruchting opgetreden ofwel bevruchting had plaatsgevonden in percentages gelijkwaardig aan die in geval van normospermie. Ondanks dat er retrospectief verschillen zijn gevonden in spermaparameters tussen de groep patiënten die alleen bevruchting hadden na ICSI en de groep patiënten die ook in de IVF bevruchtten, is het onmogelijk om op basis van individuele spermaparameters onderscheid te maken tussen sperma dat wel en dat niet bevrucht in de IVF. Als gevolg daarvan zullen we genoodzaakt zijn om in de patiënten populatie met oligoasthenospermie naast IVF ook ICSI uit te voeren om te voorkomen dat in 25-30% van deze patiënten geen bevruchting optreedt.

Aan de hand van een case report is aangetoond dat ICSI een succesvolle behandeling kan zijn voor patiënten met een historie van abnormale bevruchting na

IVF in de vorm van de ontwikkeling van multipronucleï in de geïnsemineerde eicel in plaats van 2 pronucleï, zoals in een normale bevruchting het geval zou zijn (hoofdstuk 5). Een paar dat een eerste IVF cyclus onderging waarbij er slechts eicellen met multipronucleï zich ontwikkelden werd in een tweede cyclus behandeld met zowel IVF als ICSI. Dit resulteerde in normale bevruchting van de eicellen die met ICSI waren behandeld en tot abnormale bevruchting (multipronucleï) van de eicellen waar IVF mee was gedaan. Twee van de met ICSI bevruchte eicellen die zich respectievelijk ontwikkelden tot een 4 – en een 6-cellig embryo zijn teruggeplaatst en dit resulteerde in een zwangerschap en uiteindelijk in de geboorte van een gezond meisje. De effectiviteit van ICSI ten opzichte van IVF in dit geval is hoog omdat er met IVF geen normale bevruchting tot stand gebracht kon worden.

Intracytoplasmic sperm injection (ICSI) (hoofdstuk 6 en 7)

Niet alleen patiënten karakteristieken maar ook verschillen in de uitvoering van ART technieken kunnen de resultaten van de totale behandeling beïnvloeden. Dit is onderzocht in een studie (hoofdstuk 6) naar het effect van enkele karakteristieken van de ICSI techniek: de positie van het poollichaampje tijdens de injectie (6 en 12 uur) en de persoon die de injectie uitvoert (operator) is bestudeerd met betrekking tot bevruchtingsresultaten en zwangerschapspercentages. Een significant effect is gevonden van zowel de positie van het poollichaampje als van de operator op het bevruchtingspercentage. Verder is er een significante interactie gevonden tussen de positie van het poollichaampje en de operator met betrekking tot het zwangerschapspercentage. Dit laatste zou erop kunnen wijzen dat er technische verschillen bestaan tussen operators in hoe de injectie uitgevoerd wordt en die gerelateerd zijn aan de positie van het poollichaampje. De positie van het poollichaampje op 6 uur resulteerde in zowel hogere bevruchtingspercentages als in hogere zwangerschapspercentages.

In hoofdstuk 7 is de hypothese getest dat een groot deel van de ICSI zwangeren zich zouden willen laten testen op mogelijke genetische afwijkingen middels de op dat moment meest betrouwbare prenatale genetische diagnostiek: vruchtwaterpunctie of vlokkentest. Deze hypothese bleek niet juist: de reden voor het testen was met name de leeftijd van de moeder en niet ICSI. Dit resultaat heeft uiteindelijk geleid tot een wijziging in het beleid om invasieve prenatale diagnostiek aan te bieden.

Curriculum Vitae

Lucette van der Westerlaken werd geboren op 2 juli 1963 te Breda. Het Gymnasium diploma heeft zij behaald aan het Newman College in Breda in 1982 . Na anderhalf jaar informatica te hebben gestudeerd aan de Technische Universiteit te Delft is zij overgestapt naar de Landbouw Universiteit te Wageningen. Daar begon zij aan de studie Zoötechniek alwaar zij in 1990 het doctoraal examen met als specialisaties Gezondheid-en Ziekteleer en Veevoeding met goed gevolg heeft afgelegd.

Van maart 1990 tot april 1994 was zij werkzaam als wetenschappelijk onderzoeker in vitro fertilisatie en embryotechnologie, in dienst van Pharming en gedetacheerd bij ID-DLO. Daar heeft zij meegewerkt aan verschillende projecten, waaronder het opzetten van transvaginale follikelpunctie bij het rund onder echografische begeleiding, het opzetten en optimaliseren van een IVF-laboratorium voor rundereicellen en het in vitro matureren van rundereicellen.

Van april 1994 tot heden is zij werkzaam als klinisch embryoloog op het IVF laboratorium van het Leids Universitair Medisch centrum. Gedurende deze periode zijn de studies die in dit proefschrift beschreven zijn uitgevoerd.

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