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Artificial insemination: an update

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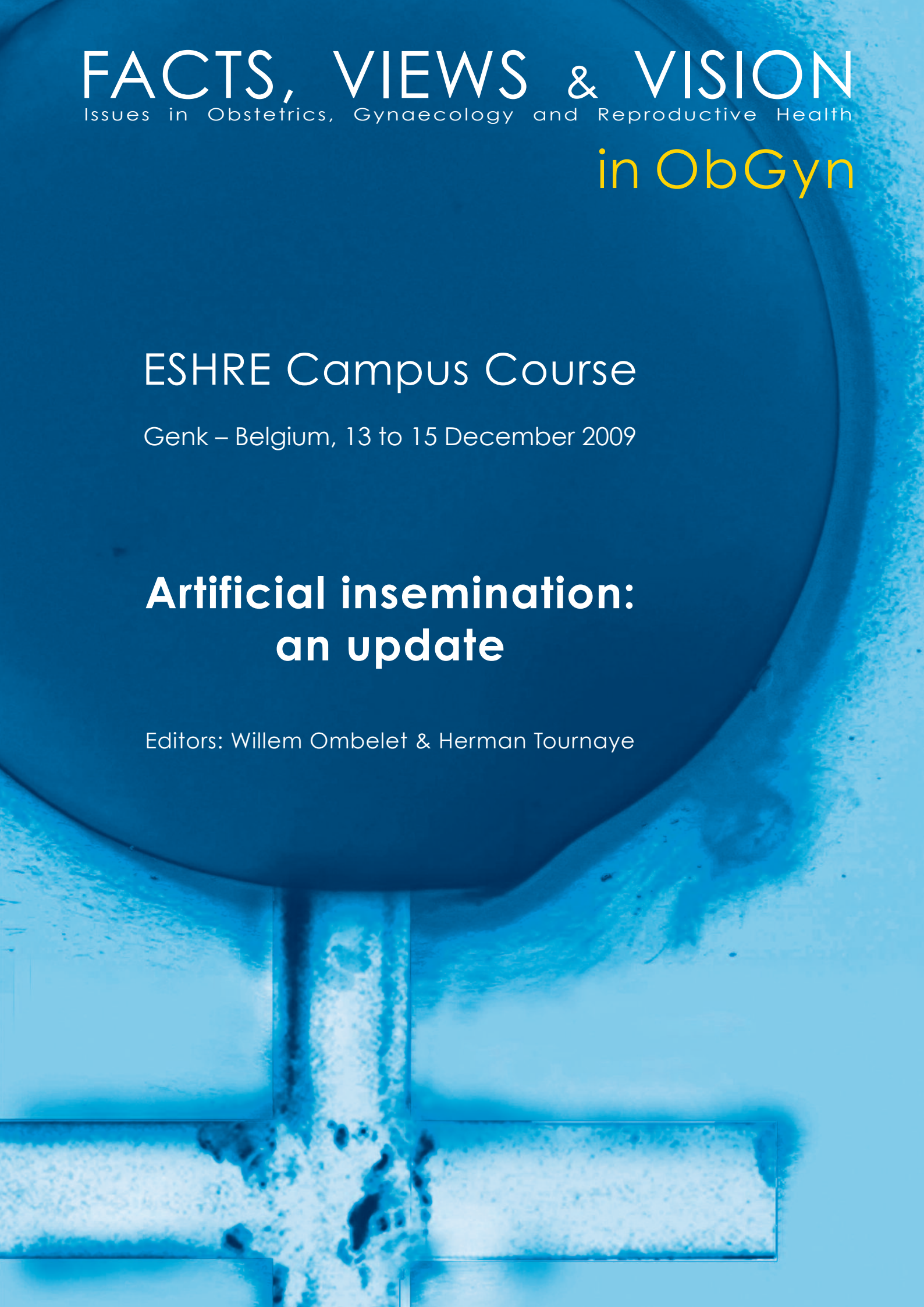

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Introduction to the Monograph

Willem Ombelet, Herman Tournaye

Although being under continuous scrutiny, artificial insemination still remains a widely used treatment option for many couples. Insemination with donor semen is used in case of azoospermia, for genetic reasons and in lesbian couples and for single-parent requests. Homologous intrauterine insemination (IUI) is frequently used in couples with unexplained infertility, cervical factor subfertility and mild to moderate male subfertility. But despite the extensive literature on the subject of artificial insemination, controversy remains about the effectiveness of this very popular treatment procedure, particularly since the introduction of ICSI.

December 13-15, 2009, an **ESHRE Campus Course** entitled **"*Artificial insemination: an update*"** was organized by the ESHRE Special Task Force 'Developing countries and infertility', the ESHRE 'Special Interest Group Andrology' and the Genk Institute for Fertility Technologies. It was our aim to cover the most important issues considering artificial insemination. On the occasion of this meeting a Monograph is published covering most lectures.

Beside a brief overview of the history of artificial insemination, both human and from the veterinaries perspective, the monograph deals with many important issues in artificial insemination such as (1) evidence-based data on the effectiveness of artificial insemination, (2) the use or abuse of ovarian stimulation, (3) the value of different washing procedures and media supplements and (4) the risk factors associated to artificial insemination. In order to asses the value of IUI, results after IUI have to be weighed against expectant management, medical and surgical treatment, timed intercourse, IVF and ICSI. But this comparison should not only involve success rates but also cost-benefit, a complication rate of the different treatment options, the invasiveness of the techniques and couple compliancy.

The monograph also covers the use of semen cryopreservation in cancer patients as well as important ethical issues e.g. reproductive tourism, insemination in lesbian couples and single-parents and preconceptional gender selection.

Finally, a lot of attention has been given to the specific situation in developing countries and the value of artificial insemination in these countries.

Although IUI is a simple, non-invasive and inexpensive first-line treatment, the future of IUI will depend on our ability to control the multiple pregnancy rates which will undoubtedly be the most important challenge in the near future. We also have to convince infertility specialists to consider IUI as a first-line treatment option. If not, governments will make their own rules and recommendations, purely based on economic arguments.

Artificial insemination in livestock production: the Vet's perspective

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Abstract

Artificial insemination (AI) can undoubtedly be considered as the oldest and most widely used technique in the assisted reproduction spectrum for livestock production. Following a brief introduction on the history of artificial insemination, an overview is given on available bovine assisted reproduction techniques. Subsequently, indications for use of AI in livestock production are discussed and the outlines of the cattle AI business are given. In addition, current and new applications of AI are described such as sperm sexing and the accompanying new interest in low dose and deep intra-uterine insemination

Key words: AI, cattle, artificial insemination, assisted reproduction, sperm sexing, deep intra-uterine insemination.

Introduction

Artificial insemination (AI), defined as the introduction of sperm in the female reproductive tract by means of an instrument, is undoubtedly the oldest technique within the assisted reproductive spectrum. Although many Arabian horse breeders banned assisted reproduction techniques until recently, it were the Arabs to first report on AI around 1322 (Verberckmoes et al., 2004b). Several centuries later, Anthony van Leeuwenhoek took the first step to what would be andrology later on by inventing a small microscope by which he was able to study gross movement of spermatozoa. While Spallanzani (1784) discovered that a bitch could be impregnated by 'cells' present in semen, we had to wait until 1900 when professor Ivanoff, hired by the Russian throne, started to develop AI procedures in horses. By 1922, he had AI procedures in place to be used in horses, cattle, sheep and swine (Ivanoff, 1922). The first commercial AI cooperative was founded in 1936 in Denmark, by Sørensen (Foote, 2002). Originally, AI was mainly performed with fresh semen, whereas from 1965 on, mostly frozen-thawed semen is used. Nowadays, AI is performed in all livestock species: cattle, horses, sheep, goats, pigs, poultry, rabbits etc... The evolution AI has gone through over the past decades is best illustrated by its use in cattle breeding business, as summarized by Vishwanath in 2003. Also in pig industry, AI became more widely used over the past decades with an increase in the number of AI matings of 5% in 1990 up to 60-70% in 2000 (Vyt et al., 2004).

The use of AI in animal reproduction was originally introduced for sanitary reasons. When frozen sperm became readily available later on, the economic advantages through improved fertility rates and accelerated genetically progress became fully clear. By lowering the insemination dose, the number of inseminations and offspring of highly valuable sires could be increased enormously. Since then, several interesting developments guaranteed and increased the importance of AI as an assisted reproduction technique (ART) such as sorting X- and Y-bearing spermatozoa by means of flow cytometry (Seidel, 2003), the development of new sperm diluters and the use of new AI techniques such as deep intra-uterine or utero-tubal junction insemination (Verberckmoes et al., 2002). This brief overview wants to comment on a few of these relatively new

prospects in the use of AI besides of giving a short description on the current state of the art of AI in livestock production, using the bovine model as an example.

(Bovine) assisted reproduction techniques

AI has become the tool of choice for rapidly spreading desired animal genetics in a given population, while emphasizing the male role in genetic selection. A substantial number of bulls have produced hundreds of thousands of insemination doses and numbers of offspring accordingly. However, in a next stage, breeders also wanted to use the valuable genetics on the female side in a more intensive way. This is why commercial embryotransfer (ET) business was established in the early 1970s (Hasler, 2003). Genetically valuable donor cows are superovulated and subsequently inseminated. Following fertilization, they carry on average between 4 and 10 embryos in the uterus until 6 to 7 days later. Through surgical (early 1970s) and later on non-surgical embryo collection (mid 1970s), on average 5 transferable embryos can be collected during uterine flushing and transferred into recipient cows (Hasler, 2003). This procedure can be repeated 4-5 times a year for the same donor cow, depending on its reaction to hormonal superovulation. The overall yield, expressed by the number of calves per donor cow per year could be increased to 15-20 calves born instead of only one through natural mating or AI. From the early 1980s on, effective methods to freeze embryos, utilizing dimethyl sulfoxide or glycerol as cryoprotectants, became available (Hasler, 2003). This way, a successful ET program was no longer dependent on the immediate availability of recipient cattle. Around 754.000 in vivo embryos are produced worldwide each year (Thibier, 2009). The next step to even increase the maternal contribution to the selection process was the introduction of in vitro embryo production (IVP) with the first IVP calf produced from an in vivo matured oocyte in 1982 (Brackett et al., 1982). A few years later, in vitro maturation, fertilization and culture were sufficiently optimized to result in the birth of a calf from the first totally in vitro produced embryo (Fukuda et al., 1990). Oocytes for IVP can be harvested from slaughterhouse ovaries (Hasler, 2003) or transvaginally aspirated from living donor cows through Ovum Pick-Up procedures (OPU) (for review: Bols, 2005). This way, nearly 331.000 IVP embryos were produced worldwide in 2008 (Thibier, 2009). Relatively new technologies include embryo sexing by PCR (Shea, 1999), marker assisted selection (MAS) (Georges, 1999) based on the identification of genetic markers for unknown alleles of valuable production traits, flow cytometric technology used to separate X- and Y-bearing sperm (see below) (Johnson, 2000), and cloning (Wilmut et al., 1997). Although reports are available on relatively large numbers of cattle clones produced (Faber et al., 2003), still several economical and practical hurdles need to be overcome before these high-tech techniques can be applied routinely in livestock production (Hasler, 2003).

Indications for the use of AI in animal (re)production

As stated above, the main reason for the introduction of AI in animal reproduction was out of a sanitary concern that bulls, which were used on different farms, would spread venereal diseases such as vibriosis and trichomoniasis. In addition, the transport of bulls would also facilitate the spread of non-sexually transmitted diseases such as (para)tuberculosis and brucellosis (Thibier and Guerin, 2000). Soon enough farmers understood that AI is the method of choice for the rapid dispersal of valuable genes in a given animal population in order to improve production traits in livestock (Vishwanath, 2003). By introducing AI in the pig, poultry and rabbit industry, a significant improvement in carcass quality could be obtained and breeding units could be expanded (Singleton, 2001). While the success of AI was largely based on the fact that the technique is simple, economical and has a high success rate, the introduction of freezing protocols – with glycerol as a cryoprotectant – for semen has enormously boosted the possibilities of AI. The use of frozen-thawed semen increased the speed with which valuable genes could be dispersed and made it possible to install testing and breeding programmes for promising bulls (Verberckmoes et al., 2004). Dairy bulls for example, will finally be selected based on the production and conformation characteristics of their female offspring, which is currently complemented by the genomics approach and marker assisted selection. However, freezing semen is not as successful in all farm animals as it is in cattle. Semen of horses, pigs, sheep, goats, poultry and rabbits is much more prone to freezing damage.

State of the art of AI in animal (re)production: the bovine model

As stated by Vishwanath (2003), the cattle dairy industry is the 'reference where technical advancement in AI and semen technology has been captured most successfully'. This is why most of the data referred to in this overview deal with cattle (re)production programmes, which does not mean it's easy

to retrieve reliable data when it comes to the worldwide use of AI. A 1980 survey reported a worldwide use of cattle AI doses of about 130 million (Bonadonna and Succi, 1980). Whereas an updated survey in 1995 reported a total number of doses of semen produced exceeding 200 million (Chupin and Thibier, 1995), the most recent available information mentions more than 260 million doses produced worldwide in 1998 (Thibier and Wagner, 2002), of which 95% was deep frozen. More than half of the frozen doses was produced in Europe. Fresh semen is produced only in limited amounts, more specifically in the Far East (Oceania) and Europe, about 12 million doses altogether. Some of the data of the most recent survey are summarized in Table I. The number of bulls enrolled in semen collection programmes is around 40.000 worldwide, of which 50% were located in Europe. The volume of international trade in frozen semen is given in Table II (Thibier and Wagner, 2002). Around 20 million doses of semen have been shipped around the globe (1998), which was approximately 10% of the total production. North America and Europe were the most important exporters while South America was the biggest importer. This worldwide trade in frozen semen reflects globalisation of animal breeding and a vast interest in the exchange of genetics between different countries and breeds. Obviously, as mentioned by Thibier and Wagner (2002) this raises concerns about animal health aspects because transfer of animal diseases through the use of AI cannot totally be ruled out. The same survey illustrated that about 20% of all breedable females in the world (about 550 million heads) were bred by AI. Large variations in this number exist between the different continents, being 61% in Europe, 25% in North America and the Far East, down to a marginal proportions of 1 to 4,5% in the Near East, South America and Africa. These low percentages can be attributed to the extensive production systems in these countries as well as to a lack of updated information. About 75% of AI's are performed in dairy cattle, the remaining 25% with semen from beef bulls.

It is assumed that, on average, 2 inseminations are needed to establish 1 pregnancy (Thibier and Wagner, 2002), which brings us to the efficiency of the AI procedure. As summarized by Vishwanath (2003), the main factors that determine the extent of the use of AI are the cost of the semen, the cost of the insemination procedure and the success of the technique. The contribution of these 3 factors to the overall cost depends largely on the country/ continent were the AI is used. This is mainly because other variables also play a steering role: commodity milk price, purchase prices of animals and the salvage value of an animal as meat. The success rate of AI can be expressed as the percentage of animals not returning to service within a defined period after first insemination referred to % Non Return Rates (%NRR). In most countries, these NRR's are within the range of 65-70% for 24, 60 or 90 days NRR's (Vishwanath, 2003). As mentioned above, AI demonstrated to be the tool of choice to accelerate genetic gain by using sperm of genetically superior bulls on a large scale within a certain population. Foote (1998) described the potential genetic contribution to genetic improvement of a single sire as the number of progeny of that sire multiplied by the genetic superiority. The number of progeny will be determined by the total sperm output of the bull, the number of sperm used per insemination and the percentage of cows calving to a single insemination. Several highly wanted bulls produced over a million doses of sperm during their lifespan.

Table I. — Partial data of the 'World statistics for artificial insemination in cattle' survey from 2002 (Thibier and Wagner).

Region	No. of bulls	No. of doses produced fresh (× 1000)	No. of doses produced frozen (× 1000)	Total (X 1000) (%)
Africa	646	55	1.484	**1.540 (0.6%)**
North America	9.627	0	43.270	**43.270 (16.4%)**
South America	530	0	5.917	**5.917 (2.2%)**
Far East	9.228	8.875	69.938	**72.812 (27.5%)**
Near East	268	31	2.559	**2.590 (1.0%)**
Europe	20.785	2.694	135.563	**138.258 (52.3%)**
Total	**41.084**	**11.656**	**252.733**	**264.390 (100%)**

Note: the addition of the total number of doses is made to the unit which explains the slight difference from the single addition from those figures here given to the thousand.

Table II. — Volume of international trade in frozen cattle semen, based on 'World statistics for artificial insemination in cattle' survey from 2002 (Thibier and Wagner).

Region	No. of doses imported	No. of doses exported
Africa	568.589	2.360
North America	1.366.100	13.564.500
South America	5.318.595	120.650
Far East	1.467.388	658.700
Near East	402.640	5.120
Europe	4.884.999	5.010.510
Total	**14.008.311**	**19.361.840**

Sperm sexing

Mainly determined by a specific breeding goal, it has always been the desire of cattle breeders to predict or determine the sex of the offspring before or as soon as possible following conception. When considering the possibility to determine the sex of offspring following conception, there are several methods described to sex embryos, which can be used in combination with Multiple Ovulation and Embryo Transfer (MOET) programmes. However, as stated by Seidel (2003), these are all time consuming, expensive and require biopsy of embryos which might possible cause damage and reduce pregnancy rates. In addition, these procedures are able to identify the sex of embryos, but do not predetermine sex, which means that half of the embryos produced are not of the desired sex. A possible alternative is to determine sex of the foetus during early pregnancy using ultrasonography (Müller and Wittkowski, 1986). This has already been successfully achieved in cattle and horses at 2-3 months of gestation. However, ethical problems can occur because the consequence of using this procedure is often that foeti with the undesired sex will have to be aborted. This implies that 2 or 3 months of gestation are lost and problems can occur with return to oestrus of aborted cows delaying the establishment of a subsequent pregnancy.

All these considerations forced scientists to concentrate on methods that predetermine sex, almost automatically leading to sperm sexing. Although over time, there has been a vast amount of papers published on different approaches to obtain this goal (for review: Garner and Seidel, 2008), most time and effort have been put in a method using flow cytometry and cell sorting after staining intact sperm with the membrane permeant bisbenzimidazole fluorescing DNA binding dye, Hoechst 33342 (Johnson and Welch, 1999). This technique is based on the difference in DNA content of X- and Y-chromosome-bearing sperm, which is approximately 3-5%, depending on the species studied (Moruzzi, 1979). Briefly, the sperm is stained with Hoechst 33342 after which the sperm cells are pumped into a stream in front of a laser beam at specific wavelengths (Garner and Seidel, 2008). The illuminated Hoechst 33342 stained sperm emit a very bright blue fluorescence which is measured using a photomultiplier tube. Subsequently, a high speed computer analyzes the relative fluorescence of the X- and Y-sperm populations as they flow in a fluidic stream. This stream is broken into individual droplets, many of which contain a sperm. The stained sperm is finally sorted by placing opposite charges on droplets containing X-sperm or Y-sperm. Positive and negative electrical fields will than sort out the different droplets into two different streams. A third stream of droplets that contain abnormal or no contents is not charged and discarded. This sperm sorting procedure is known as the Beltsville Sperm Sexing Technology and is patented by the USDA with Dr. Johnson as the inventor (US Patent #692958, 04/26/1991). Following optimization of the technique, AI of heifers with sexed sperm resulted in about 90% accuracy of sex of resulting offspring, although in some cases with reduced fertility (Seidel, 1999).

Although 90% is a pretty high accuracy, several hurdles need to be overcome before sperm sexing technology will become available for worldwide use. Apart from economical imperatives, which largely depend on local market conditions for meat and dairy production (Seidel, 2003), pure technical factors play an important role. The initial speed with which semen could be sexed and sorted was about 400,000 sperm/hour (Garner and Seidel, 2008), implying that it would take about 25 hours to sort one insemination dose of 10×10^6 sperm of each sex. Because sperm sexing can cause damage to the sperm (Garner, 2006; Garner and Suh, 2002), considerable debate is still going on about the minimal amount of sexed sperm needed for a successful

insemination (Garner and Seidel, 2008). While it has been determined that the insemination dose with nonsexed, frozen thawed sperm can be as low as 0.38×10^6 sperm to still obtain a NRR of 50% (Jondet, 1972), numerous other factors will determine the NRR, such as farm management, quality of sperm, production level and time of insemination, to name a few important ones. A study by DeJarnette et al. (2007) including data on 121 Holstein dairy herds, revealed an average conception rate of 44% following the use of sexed sperm. In 74% of these herds, the conception rates for sexed semen were at least 70% of those achieved with control semen.

To overcome the limitations imposed by the speed of analysis, alternative approaches were investigated, such as the use of sexed sperm for in vitro embryo production (Cran et al., 1993) and the use of alternative insemination techniques using lower numbers of sperm, such as deep intra-uterine insemination (see below). While the first strategy never became a financial success, because it also had to deal with additional drawbacks related to in vitro embryo production technology, the latter is still under investigation but slowly on the rise. As described by Gardner and Seidel (2008), several recent technical adaptations to the sperm sexing equipment have resulted in a considerable improvement in analyzing speed, which is now up to 20,000 sperm/second, sorting up to 6,000 sperm/second each of X- and Y-sperm at 90+% accuracy. In practical terms, this means that about 7 straws of sexed sperm with a sperm dose of 2×10^6 can be produced per hour.

Sexed sperm can be frozen using a conventional egg-yolk-Tris buffer (Schenk et al., 1999), so it can be collected and sorted at the same facility, which doesn't have to be the place where the actual inseminations take place. Sex sorted frozen sperm can be distributed, transported and subsequently offered to the farmers using the conventional distribution channels.

Given the fact that sperm is stained and subjected to laser light during the sexing process, a serious concern exists on the possible damage to sperm induced by this procedure. More specifically, sperm chromatin quality might be impaired by exposure to the Hoechst 33342 dye and the UV laser beam during flow sorting (Gardner and Seidel, 2008). However, the Sperm Chromatin Structure Assay (SCSA) revealed no obvious DNA damage with sex-sorted sperm (Garner et al., 2001), while genetic DNA damage can not yet completely be ruled out. Reassuringly, some large studies (over a 1,000 calves) are available on the characteristics of calves born following inseminations with sex-sorted sperm. These studies report no differences in gestation length, the occurrence of neonatal death, difficulty at calving, abortion rate (DeJarnette et al., 2007), birth and weaning weight or proportion of live births following AI with sexed semen compared to controls (Tubman et al., 2004).

In addition to cattle, sperm sexing technology has been applied to numerous other domesticated species including sheep (Hollingshead et al., 2004), rabbits (Johnson et al., 1989), horses (Buchanan et al., 2000), swine (Maxwell et al., 2004), cats (Pope et al., 2008) and dogs (Meyers et al., 2008).

Deep Intra Uterine Insemination

As mentioned above, the availability of sex sorted sperm in small quantities created the need for alternative insemination techniques. Whereas normal inseminations doses contain around 10 to 20×10^6 sperm, the concentration of straws containing sex sorted sperm usually is five to ten times less, around 2×10^6 sperm. In cattle, sperm is usually deposited into the uterine body when using conventional AI. This allows a strong 500-times reduction (Senger, 1993) of the number of spermatozoa needed compared to natural mating when the sperm is ejaculated into the vagina and the most caudal part of the cervix. However, in case of the use of sperm with limited commercial availability or sexed sperm, it has been proposed that deposition of sperm closer to the oviduct, at the utero-tubal junction (UTJ), could be an alternative to obtain acceptable pregnancy rates. Theoretically, the insemination dose as used for conventional insemination in cattle could be reduced by at least 100-fold if the sperm is deposited close to the UTJ (Hunter, 2001). This way, the loss of sperm through retrograde flow and phagocytosis can be minimized. In addition, sperm might survive for a longer time period in the sperm friendly environment of the isthmus (Suarez, 2001). In cattle, conventional insemination is performed under rectal guidance, with a stainless steel 'Cassou' insemination device. Already in the fifties, comparisons were made between the outcomes of insemination in the uterine body or deep intra-uterine insemination (IUI) in the uterine horns (Salisbury and VanDemark, 1951). In most of these studies, performed with high numbers of fresh semen, no differences between the two insemination techniques could be demonstrated. In most cases, a rigid insemination device was used for deposition of the sperm in the uterine horns. To enable deposition of semen near the UTJ, special insemination devices needed to be developed because a rigid device is not compatible with the curvatura of the uterine horns (Verberckmoes et al., 2004). Use of the 'Ghent' device for UTJ insemination with a flexible tip showed not to decrease pregnancy rates compared to conventional AI, even though a six fold

reduction in sperm dose was used (Verberckmoes et al., 2005).

Conclusions and future prospects

Although AI is the oldest technique in the spectrum of assisted reproductive techniques, it is still the most used one in livestock production as it is relatively simple, economical and easy to apply at the herd level to improve or introduce desired genetic traits. However, the choice between AI and natural mating will always depend on numerous factors related to production goals, market situation and farm management. While initially fresh semen was used, the development of freezing protocols and cryopreservation of sperm has introduced a huge trade activity with semen being transported intensively on international markets. While no substantial improvements have been made in the efficiency of cattle AI over the past decades, which is not only depending on sperm quality, several new developments in AI have been introduced. The ability to sex sperm and thus predetermine the sex of offspring before conception, has generated new interest in low dose and deep intra-uterine insemination protocols and equipment. The use of sexed semen has additional applications in embryo transfer programmes and in vitro embryo production. For some species, intracytoplasmatic sperm injection (ICSI) is already in use as the next step.

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History of human artificial insemination

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Abstract

Artificial insemination with homologous (AIH) or donor semen (AID) is nowadays a very popular treatment option for many subfertile women worldwide. The rationale for the use of artificial insemination is to increase gamete density at the site of fertilisation. The main reason for the renewed interest in artificial insemination is undoubtly the refinement of techniques for the preparation of washed motile spermatozoa. The sequence of events leading to today's common use of artificial insemination traces back to scientific studies and experimentation many centuries ago. Modern techniques used in human artificial insemination programmes are mostly adapted from the work on cattle by dairy farmers wishing to improve milk production by using artificial insemination with sperm of selected bulls with well chosen genetic traits. The history of artificial insemination is reviewed with particular interest to the most important hurdles and milestones.

Key words: Artificial insemination, assisted reproduction, history, human, intrauterine insemination, semen.

Introduction

The rationale behind artificial insemination is increasing the gamete density at the site of fertilization. Since many centuries different pioneers contributed to the history of artificial insemination, not only in humans but even more pronounced in farm animals. The primary reason for using this technique in farm animals is to speed up the rate of genetic improvement by increasing the productivity of food producing animals. This is accomplished by improving the selection differential wherein one highly selected male is mated with thousands of females. The AID industry was born.

For humans the situation is different: artificial insemination was originally developed to help couples to conceive in case of severe male factor subfertility of a physical or pschycological nature. Nowadays the process of AI is more commonly used in woman (lesbians or single women) with no male partner, in these cases the sperm is provided by a sperm donor. When donor sperm is used, the woman is the gestational and genetic mother of the child produced and the sperm donor is the genetic or biological father of the child.

Despite the extensive literature on the subject of artificial insemination with husband's semen, controversy remains about the effectiveness of this very popular treatment procedure, particularly in relation to IVF and ICSI (Cohlen 2005; Ombelet, 2005; Bensdorp *et al.,* 2007; Eshre Capri Workshop Group, 2009). Nevertheless, artificial insemination with husband's semen remains a widely used treatment option for many couples with unexplained infertility, cervical factor subfertility, physiologic or psychological sexual dysfunction and mild to moderate male subfertility.

Milestones in the history of artificial insemination

Unofficial history claims that the first attempts to artificially inseminate a woman were done by **Henry IV** (1425–1474), **King of Castile**, nicknamed *the Impotent*. In 1455, he married Princess



Fig. 1. — Picture of Antoni Van Leeuwenhoek (1632-1723)

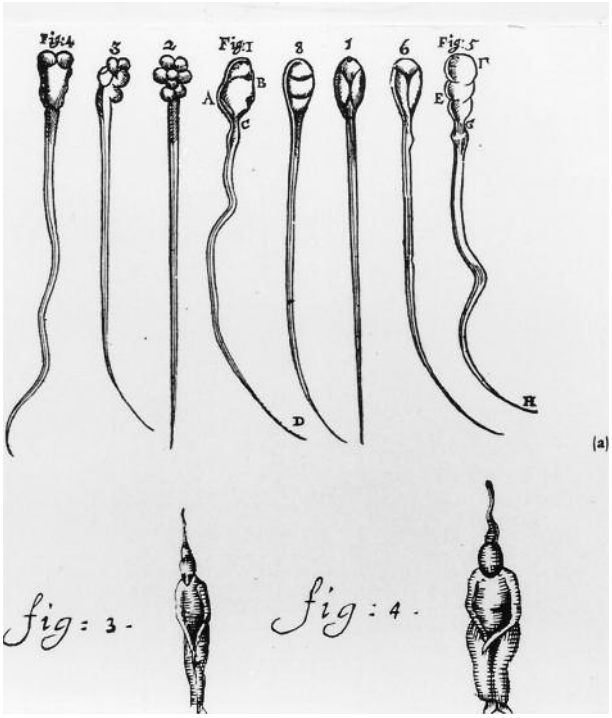

Fig. 2. — The 17th century conception of spermatozoa (A Van leeuwenhoek).

Juana, sister of Afonso V of Portugal. After six years of marriage she gave birth to a daughter, Joanna. Many contemporary historians and chroniclers assumed Henry was impotent. The possibility of artificial insemination was launched. Later on it was claimed that the princess was not the daughter of the king.



Fig. 3. — John Hunter (UK) wrote the first report of artificial insemination in medical literature in 1790.

Spermatozoa were first seen and described by **Antoni van Leeuwenhoek** and his assistant **Johannes Ham** in 1678. In a letter to William Bounker of the Royal Society the Royal Society of London (Phil.Trans. Vol. XII, nbr. 142, 1678) in which he showed a picture of sperm cells of the human and the dog. Van Leeuwenhoek described the spermatozoa as "zaaddiertjes" or "living animalcules in human semen ... less than a millionth the size of a coarse grain of sand and with thin, undulating transparent tails. He draws the conclusion that the tails must be operated by means of muscles, tendons and joints (Mol, 2006; Kremer, 1979). Van Leeuwenhoek did not study Latin, the scientific language of the day. Nevertheless, his paper amazed, and perhaps amused, the reigning King of England.

More than 100 years later, in 1784, the first artificial insemination in a dog was reported by the scientist **Lazzaro Spallanzani** (Italian physiologist, 1729-1799). This insemination resulted in the birth of three puppy's 62 days later (Belonoschkin, 1956; Zorgniotti, 1975).

The first documented application of AI in human was done in London in the 1770s by **John Hunter**, which has been called in medical history the "the founder of scientific surgery". A cloth merchant with severe hypospadias was advised to collect the semen (which escaped during coitus) in a warmed syringe and inject the sample into the vagina.

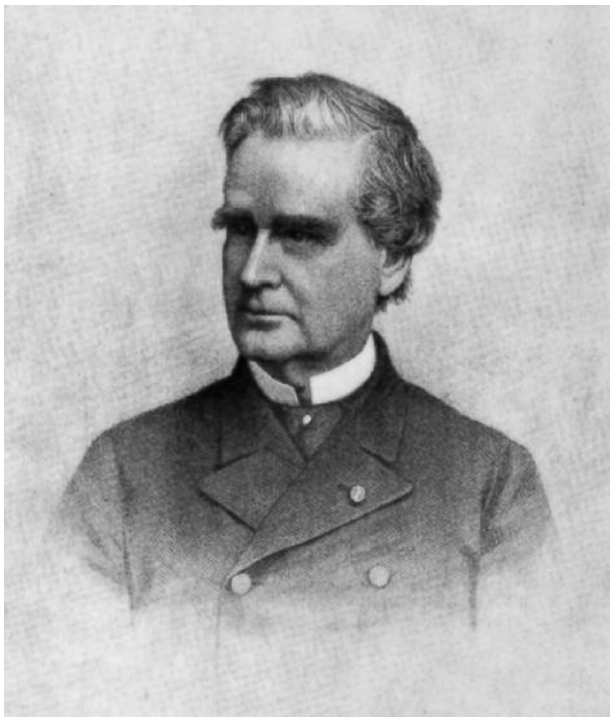

Fig. 4. — The first report of post-coital tests and the first description of 55 inseminations was done by JM Simms (US) in the 1850s (Source: South Med J, Lippincott, Williams & Wilkins 2004).

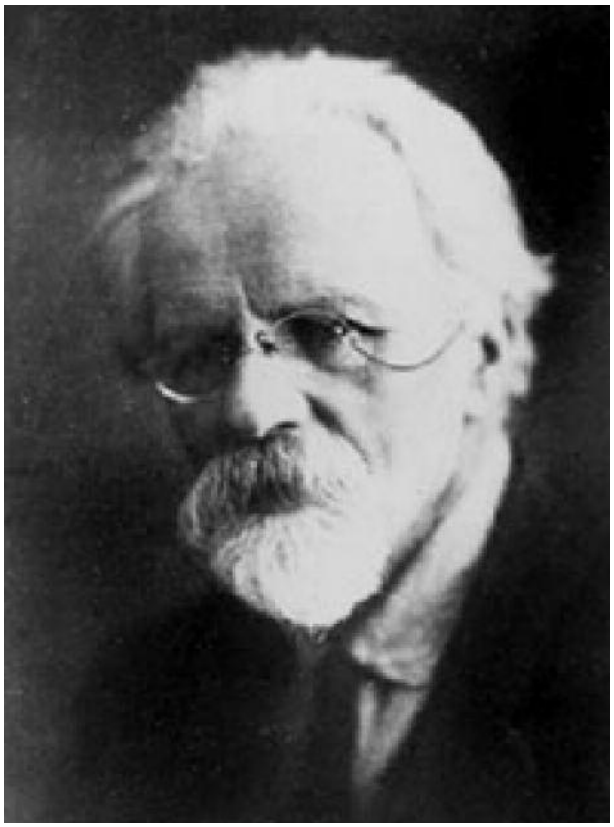

Fig. 5. — Ilya Ivanovich Ivanov (Russia) developed the methods of artificial insemination as we know them today in 1922.

J Marion Sims reported his findings of postcoital tests and 55 inseminations in the mid 1800s. Only one pregnancy occurred but this could be explained by the fact that he believed that ovulation occurred during menstruation. JM Simms was born in Lancaster County (USA) in 1813. In 1863 he began writing his innovative work *Clinical Notes on Uterine Surgery*, which was controversial but widely read. Its revolutionary approach to female diseases was refreshing, and its emphasis on treatment of sterility, including artificial insemination, was ahead of its time.

In 1899 the first attempts to develop practical methods for artificial insemination were described by **Ilya Ivanovich Ivanov** (Russia, 1870-1932). Although Ivanov studied artificial insemination in domestic farm animals, dogs, rabbits and poultry, he was the first to develop methods as we know today, also in human medicine. The work of Ivanov was taken over by **Milovanov**, another Russian scientist. He published his paper on "Artificial insemination in Russia" in the Journal of Heredity in 1938. The innovating work in Russia inspired **Eduard Sörensen** from Denmark to organize the first cooperative dairy AI organization in Denmark in 1933, followed by the introduction of the first AI co-operative in the US in 1938 by **EJ Perry**, a dairyman from New Jersey. In the US and other Western countries the number of AI cooperatives increased rapidly. Nowadays more than 90% of dairy cows are artificially inseminated in the Netherlands, Denmark and the United Kingdom.

Considering humans, only after the introduction and availability of donor sperm artificial insemination became very popular (AID). For many years homologous artificial inseminations were only indicated in cases of physiologic and psychologic dysfunction, such as retrograde ejaculation, vaginismus, hypospadias and impotence.

With the routine use of post-coital tests other indications were added such as hostile cervical mucus and immunologic causes with the presence of antispermatozoal antibodies in the cervical mucus.

The first reports on human artificial insemination originated from Guttmacher (1943), Stoughton (1948) and Kohlberg (1953a; 1953b). It was the real start of a new era in assisted reproduction.

Other important research discoveries in animal studies undoubtly influenced the development of artificial insemination, also in human. **Phillips and Lardy** (1939) were the first to use egg yolk to protect bull sperm cells from temperature shock upon cooling. This protection was explained by the effect of phospholipids and lipoproteins in the egg yolk. **Salisbury et al.** (1941) improved the media by using egg yolk with sodium citrate, permitting the

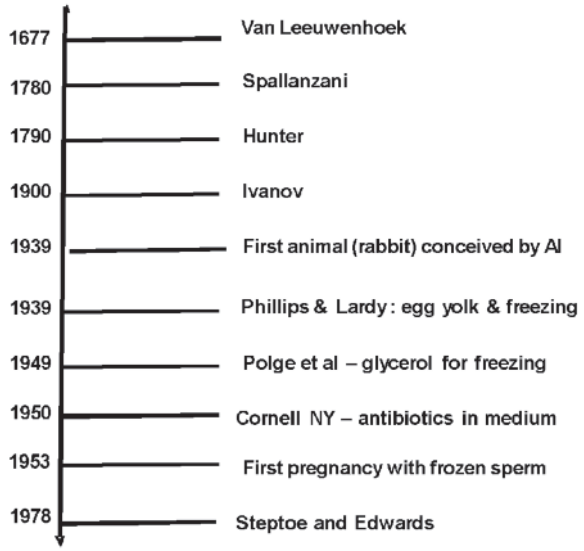

Fig. 6. — Most important milestones in the history of artificial insemination.

use of semen at 5° C for up to three days. **Polge and co-workers** (1949) were the first to freeze fowl and bull spermatozoa by using glycerol in the extender media. In 1950 Cornell University scientists (New York) discovered that antibiotics could be added to the sperm solution in artificial insemination processes. The so-called Cornell extender (**Foote and Bratton**, 1950) contained the antibiotic mixture of penicillin, streptomycin and polymyxim B and was used for many years as the standard. Antibiotics are presently still used for the protection against possible contamination.

In 1953 the first successful pregnancy from artificial insemination with frozen and thawed sperm was reported, a major breakthrough in history.

Considering all these new developments, it was not surprising that in the 1970s the sperm bank industry became very popular and commercialized, especially in the United States.

The IVF revolution

The main reason for the renewed interest in artificial insemination in human was undoubtly the introduction of in-vitro fertilisation (IVF) in 1978 by **Steptoe and Edwards**. Due to this new technique, methods for the preparation of washed motile spermatozoa were refined and examined. These washing procedures are necessary to remove prostaglandins, infectious agents and antigenic proteins. Another substantial advantage of these techniques is the removal of nonmotile spermatozoa, either leucocytes or immature germ cells. This may be an important factor in enhancing sperm quality by a decreased release of lymphokines and/or cytokines and a reduction in the formation of free oxygen radicals after sperm preparation. Most popular are the swim-up procedure, the discontinuous Percoll gradient method, the mini-Percoll (small volume) gradient technique and the use of Sephadex columns. The final result is a better sperm fertilising ability in vitro and in vivo (Aitken and Clarkson, 1987). As a consequence of these improved sperm selection techniques, the use of artificial insemination became very popular as a cost-effective first line treatment procedure in case of mild to moderate male factor infertility.

Legal, socio-cultural and religious considerations surrounding artificial insemination

The moral and social implications of artificial insemination were debated in both the medical and popular press in the United States since 1909, in Europe the debate started in the 1940s. The Catholic Church objected to all forms of artificial insemination, saying that it promoted the vice of onanism and ignored the religious importance of coitus. The main criticism was that AI with donor semen was a form of adultery ***promoting the vice of masturbation***. Other critics were concerned that AID could ***encourage eugenic government policies***.

Nevertheless, the demand for donor sperm increased tremendously. After the first successful pregnancy from frozen sperm, reported in 1953, the development of a thriving sperm-bank industry starting in the 1970s and the commercialization of AID became unavoidable. The growing number of AID‘s raised new concerns leading to new regulations. The following measurements became common practice: because of the possible transmission of sexually transmitted diseases, including HIV, by the use of fresh sperm screening for infections of donors and donations has become required by many local and national governments. The use of fresh semen samples almost disappeared. Another concern is the possibility to donate semen many times. In order to diminish the chances of unknowing marriage of biological siblings among AID children some government regulations tightly restrict the number of times a single donor’s semen may be used and/or restrict the number of children by a given donor.

Sociocultural concerns with biological paternity and the maintenance of the heterosexual, married couple as the basis of the family remains important in many countries. A lot of countries all over the world have not approved the use of AI with donor semen for single women and lesbian couples yet. Another point of debate is whether the donor has to be anonymous or non-anonymous, and when to

inform and what to tell AID children about their biological parentage, if non-anonymous donors are used. Is it possible and/or advisable to use sperm of relatives, such as brothers or the father? Whether or not to pay the donors is another point of discussion and recently more and more questions about sexing of sperm by DNA quantification using flow cytometry instrumentation.

Conclusion

Treatment with artificial insemination with husband's sperm seems to be a valuable first choice treatment before starting more invasive and more expensive techniques of assisted reproduction in many cases of human subfertility. It is a simple and non-invasive technique which can be performed without expensive infrastructure. laboratory infrastructure are mandatory. Many of the principle nowadays used in human artificial insemination are adapted from domestic animal studies, especially from cattle. The use of frozen/thawed donor samples and the renewed interest in sperm washing procedures due to the introduction of IVF were the most important milestones in the history of AI. The increasing demand of lesbians and single women for AI with donor semen is another challenge for the future in many countries worldwide. Many debates, socio-cultural and ethical, are to be expected in the near future, the use of sexing of sperm by DNA quantification will be one of them.

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Intrauterine insemination, what do we really know? A critical appraisal of the literature

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Abstract

Intrauterine insemination (IUI) is the first line treatment in couples with unexplained subfertility, cervical factor subfertility and male subfertility. To appraise the effectiveness of IUI for these three indications, we performed a systematic review and a comprehensive series of meta-analyses. We included Cochrane reviews and searched the literature for additional studies. Outcomes were live birth, ongoing pregnancy, clinical pregnancy and multiple pregnancy.

We were able to include 14 studies reporting on IUI for unexplained subfertility, two studies reporting on IUI for cervical factor subfertility and nine studies reporting on IUI in male subfertility.

In couples with unexplained subfertility, IUI without controlled ovarian hyperstimulation (COH) was associated with higher ongoing pregnancy rates than expectant management (relative risk (RR) 1.3, [95% CI 0.84 to 1.9]), whereas IUI with COH was more effective than IUI without COH (RR 1.8, [95% CI 1.2 to 2.7]). However, in couples with relatively good prospects for spontaneous pregnancy, there was no benefit from IUI with COH over expectant management. In couples with a cervical factor, IUI without COH was associated with higher pregnancy rates compared to expectant management (RR 1.6, [95% CI 0.87 to 3.1]), but addition of COH did not further improve the pregnancy rates (RR 1.0, [95% CI 0.59 to 1.8]). In couples with male subfertility, IUI was more effective than expectant management, although the limited power of the included studies hampers strong conclusions. In these couples, addition of COH also had no extra benefit (RR 0.92, [95% CI 0.46 to 1.8]). Studies comparing IUI and IVF were rare, limiting assessment of the strategy of IVF as first line treatment.

Despite the fact that IUI is one of the most frequently used treatments in reproductive medicine, our review shows that the number of studies assessing it's effectiveness is limited and that most of these studies had small sample sizes. This results in imprecise effect estimates, as demonstrated by the non significant effects and large confidence intervals. Also, many studies did not adhere to present quality standards for design, conduct and report of clinical trials.

Therefore, there is an urgent need for more RCTs in which IUI is compared to expectant management or IVF.

Key words: cervical factor, effectiveness/intrauterine insemination, male subfertility, subfertility, unexplained subfertility.

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Introduction

Subfertility, defined as one year of unprotected intercourse without conception, affects 10 to 15% of the couples who are trying to conceive. In 5% of subfertile couples a cervical factor is diagnosed, in 35% mild male subfertility, in 5% severe male subfertility and in 35% of the couples there is another cause for their subfertility. In 20% of the couples an explanation for their subfertility cannot be found (Collins and van Steirteghem, 2004; Dutch Society Guideline 2004).

In couples with unexplained subfertility, cervical factor subfertility and male subfertility intrauterine insemination (IUI) is often the first step in the treatment cascade. In an IUI cycle, semen of the partner is processed in the laboratory and thereafter inseminated in the uterine cavity at the time of ovulation. Treatment with IUI was first performed two centuries ago and has gained great popularity since (Schellen, 1960). IUI is easy to perform, inexpensive, and a minor burden to the couples. For these reasons it is probably the most frequently performed treatment in daily fertility practice.

But what do we really know? Recently, some authors have recommended the abolition of IUI with COH, because of its limited effectiveness and high multiple pregnancy rates (Fauser *et al.,* 2005). They argued that pregnancy rates after in-vitro-fertilisation (IVF) have steadily increased, and that the problem of multiple pregnancies after IVF can be controlled with elective single embryo transfer (Gerris *et al.,* 1999; Martikainen *et al.,* 2001; Gardner *et al.,* 2004; Thurin *et al.,* 2004; Lukassen *et al.,* 2005; van Montfoort *et al.,* 2005).

Decision-making on the place of IUI in reproductive medicine should be based on valid and precise effect estimates of IUI relative to expectant management and to other treatment modalities. Meta-analysis after systematic review of the available literature is the best method to obtain such estimates. So far, separate reviews on the effectiveness of IUI have been published in the Cochrane library for the indications unexplained subfertility, cervical factor subfertility and male subfertility (Helmerhorst *et al.,* 2006; Verhulst *et al.,* 2006; Bensdorp *et al.,* 2007). Here, we perform a comprehensive review of the literature, including the most recently published trials, to critically evaluate the effectiveness of IUI for all indications.

Methods

Search strategy

We summarized the Cochrane reviews on IUI for unexplained subfertility, cervical factor subfertility and male subfertility. To find additional studies on IUI for these indications, we searched the Cochrane Menstrual Disorders & Subfertility Group trials register, the Cochrane Central Register of Controlled Trials (both searched February 2007), MEDLINE (January 1966 to February 2007) and the website for the registration of controlled trials (controlled-trials.com). All electronic databases were searched using the following keywords: unexplained subfertility, cervical factor or male subfertility and intrauterine insemination or in-vitro fertilisation. We also searched the reference lists of selected trials. No restrictions were made concerning publication year or language.

Study inclusion criteria and data extraction

Studies were included if they reported on couples with unexplained subfertility, cervical factor subfertility and/or male subfertility. Subfertility was defined as trying to conceive for at least one year without establishing a pregnancy. Unexplained subfertility was defined as subfertility without any demonstrable cause after the basic fertility work-up. Cervical factor subfertility was defined as the absence of progressive motile spermatozoa in cervical mucus of good quality and normal semen parameters (Dutch Society Guideline 2000). Male subfertility was defined by semen quality below the standards of the World Health Organization (WHO), i.e. sperm concentration $< 20 \times 10^6$ per ml, and/or total motility $< 50\%$ and/or normal morphology $< 15\%$ and/or $> 50\%$ anti-sperm antibodies (WHO, 1999).

We included randomised trials that compared the effectiveness of IUI with or without COH to expectant management or IVF. We also included trials that assessed the additional value of COH in IUI by comparing IUI with COH to IUI without COH.

Outcomes were live birth, ongoing pregnancy, clinical pregnancy and multiple pregnancy. Live birth was defined as the delivery of at least one living child beyond 20 weeks. Ongoing pregnancy was defined as the presence of fetal cardiac activity at ultrasound at a gestational age of $\geq$ 12 weeks. Clinical pregnancy was defined as a pregnancy confirmed by a gestational sac on ultrasound. Studies only reporting on other types of pregnancy, such as biochemical pregnancies, were not included in the analysis.

For each trial, information on the quality of the trial was extracted based on a number of items: adequate randomisation, concealment of allocation, parallel design, exclusions after randomisation, and the intention-to-treat principle in the analysis. If a trial did not adhere to these standards, its validity

was deemed to be compromised, and the randomised trial was not included in the analysis.

Statistical analysis

Trial specific characteristics of all studies were tabulated. We extracted the data on an intention to treat basis. From crossover trials we only used data from the first phase, prior to crossover. Relative risks (RR) with 95% confidence intervals were calculated for all studies. Statistical homogeneity was assessed using forest plots, I^2 and c^2 test statistics. Clinical homogeneity was assessed by reviewing differences across trials in characteristics of randomised couples. In case homogeneity could not be rejected, pooled relative risks were calculated using fixed effects models for live birth rates, ongoing pregnancy rates, clinical pregnancy rates and multiple pregnancy rates where possible (Mantel and Haenszel, 1959). We did so, because the majority of studies reported only ongoing or clinical pregnancies. If statistical homogeneity was rejected, we performed a sensitivity analysis by pooling using a 'random effects'-method (Dersimonian, 1986). Review Manager-software (RevMan 4.2.7, Cochrane Collaboration, Oxford, United Kingdom) was used for the statistical analysis.

Results

Unexplained subfertility

We identified 14 trials, of which 11 had been included in the Cochrane review (Deaton *et al.,* 1990; Crosignani *et al.,* 1991; Murdoch *et al.,* 1991; Karlstrom *et al.,* 1993; Arici *et al.,* 1994; Chung *et al.,* 1995; Melis *et al.,* 1995; Arcaini *et al.,* 1996; Janko *et al.,* 1998; Guzick *et al.,* 1999; Goverde *et al.,* 2000; Agarwal and Mittal, 2004; Bhattacharya *et al.,* 2006; Steures *et al.,* 2006). The quality features of these 14 trials and their main characteristics are presented in Table I. The outcomes of the comparisons are summarized in Figure 1.

IUI without COH was associated with higher ongoing pregnancy rates than expectant management and timed intercourse with COH, but these effects were not statistically significant, with RRs of 1.3 [95% CI 0.84 to 1.9] and 1.5 [95% CI 0.95 to 2.3], respectively.

IUI with COH offered no benefit over expectant management (RR 1.0, [95% CI 0.67 to 1.5]). In line with the comparison IUI with COH versus expectant management, IUI with COH had also no beneficial effect over timed intercourse with COH (RR for clinical pregnancies: 1.1, [95% CI 0.90 to 1.5]).

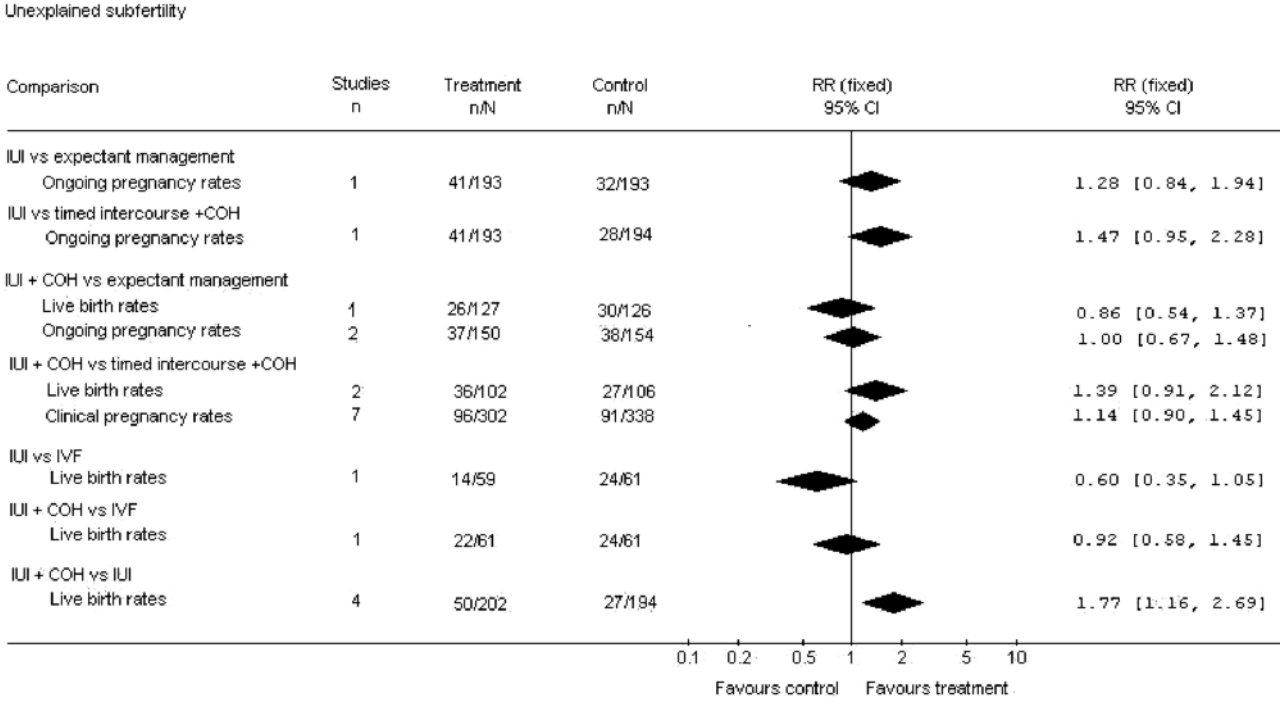

Fig. 1. — Summary of pregnancy results per couple for unexplained subfertility

Table I — Characteristics of randomised trials on the effectiveness of IUI in unexplained subfertility

Trial n = number of randomised couples w = number of withdrawn couples	**Participants**	**Intervention**	**Comparison**	**Main Outcomes**	**Quality features**
Agarwal 2004 n = 70 versus 70w = 26 versus 1	Unexplained subfertility Age: 29.5 versus 28.8 Duration subfertility: 4.9 versus 4.9 Basic fertility work up normal, semen normal, no previous treatment	IUI with COH 50-150 mg CC/day, day 3-7 Ovulation: 10000 IU hCG, ≤ 4 follicles of > 16 mm Timing 36-40 hr after hCG Duration 6 cycles max	TI + COH 50-150 mg CC/day, day 3-7 Ovulation: 10000 IU hCG, ≤ 4 follicles of > 16 mm Timing 36-40 hr after hCG Duration 6 cycles max	PR per couple and per cycle Pregnancy: US showing gestational sac	Randomisation with random number table, sealed envelopes Trial design: Parallel Single centre ITT- analysis: possible
Arcaini 1996 n = 36 versus 32 w = 14	Unexplained subfertility Age: 34.6 versus 33.4 Duration subfertility: 4.2 versus 3.9 Basic fertility work up normal, semen normal, previous treatment not stated	IUI + COH 100 mg CC/day, day 3-7 and 1-3 ampule hMG/day Ovulation: 10000 IU hCG, 2-6 follicles > 17 mm Timing 24 and 48 hr after hCG Duration 5 cycles max	TI+COH 100 mg CC/day, day 3-7 and 1-3 ampule hMG/day Ovulation: 10000 IU hCG, 2-6 follicles > 17 mm Timing 24 and 48 hr after hCG Duration 5 cycles max	PR per couple Multiple pregnancies Pregnancy: confirm by US	Randomisation unclear Trial design: Parallel Single centre ITT- analysis: yes
Arici 1994 n = 26 w = unclear	Unexplained and male subfertility Age: 33 (range 24-41) Duration subfertility: 3.5 (range 1-15) Unexplained: Basic fertility work up normal, semen normal, no previous treatment	IUI +COH 50 mg CC/day, day 5-9 Ovulation: 10000 IUI hCG, at least 1 follicle 18 mm Timing: 32 hr after hCG Cancel: unclear Duration 4 cycles max	IUI without COH - - Timing: On day of LH-surge and next day - Duration 4 cycles max	Live birth and PR per couple PR per 1 st cycle Multiple pregnancies Pregnancy: US showing gestational sac	Randomisation with random number table by computer in locked files Trial design: crossover (after 1 cycle) Single centre ITT- analysis: yes
Bhattacharya 2006 n = 193 versus 193 w = unclear	Unexplained subfertility Age: mean 31.7 Duration subfertility: median 30 months Unexplained Basic fertility work up normal, semen normal, previous treatment not stated	IUI without COH Ovulation: not stated Timing not stated Duration 6 months	Expectant management Duration 6 months	Ongoing pregnancy rate per couple Miscarriage rates per couple Multiple pregnancies	Randomisation and concealment not stated Trial design: parallel Multicentre: 5 centres ITT- analysis: yes
Bhattacharya 2006 n = 193 versus 194 w = unclear	Unexplained subfertility Age: mean 31.7 Duration subfertility: median 30 months Unexplained Basic fertility work up normal, semen normal, previous treatment not stated	IUI without COH Ovulation: not stated Timing not stated Duration 6 months	Timed intercourse with COH 50 mg CC/day, day 2-6 Ovulation not stated Timing not stated Duration 6 months	Ongoing pregnancy rate per couple Miscarriage rates per couple Multiple pregnancies	Randomisation and concealment not stated Trial design: parallel Multicentre: 5 centres ITT- analysis: yes

Chung 1995 n = 50 versus 50 w = 12	Unexplained subfertility Age: 31.8 versus 32.1 Duration subfertility: 4.7 versus 5.3 Basic fertility work up normal, semen 15 million motile per ejaculate, previous treatment not stated	IUI + COH 150 IU/day FSH and GnRH nasal spray from day 21 on Ovulation: 5000 IU hCG, < 4 follicles > 16 mm Timing: 36-48 hr after hCG Duration 3 cycles max	TI + COH 150 IU/day FSH and GnRH nasal spray from day 21 on Ovulation: 5000 IU hCG, < 4 follicles > 16 mm Timing: 24+ 48 hr after hCG Duration 3 cycles max	PR per couples and per cycle Total delivered Multiple pregnancies	Randomisation blocked randomisation scheme in sealed envelopes Trial design: Parallel Single centre ITT- analysis: possible
Crosignani 1991 n = unclear w = unclear Analysed= 90	Unexplained subfertility Age: < 38 years Duration subfertility: > 3 years Basic fertility work up normal, semen normal, previous treatment not stated	IUI + COH Stimulation not stated Ovulation: not described Timing: not described Duration 2 cycles max	TI + COH Stimulation not stated Ovulation: not described Timing: not described Duration 2 cycles max	PR per 1 st cycle and per cycle	Randomisation not clear Trial design: Crossover after 1 cycle Multi centre, 4 centres ITT- analysis: not possible
Deaton 1990 n = 67 w = 4 Analysed 51, of which 24 were unexplained	Unexplained subfertility & surgically treatment of endometriosis Age: 33 years Duration subfertility: 3.5 years Basic fertility work up normal, semen normal, previous treatment not stated	IUI + COH 50 mg CC/ day, day 5-9 Ovulation: 10000 IU hCGat least 1 follicle 18 mm Timing: 36 hr after hCG Cancel: unclear Duration 8 cycles max	TI without COH - - Timing: Based on urinary LH-surge or BBT Duration 8 cycles max	Ongoing PR Multiple pregnancies	Randomisation not clear Trial design: Crossover after 4 cycles Single centre ITT- analysis: not possible
Goverde 2000 n = 120 w = unclear Analysed 93	Unexplained and male subfertility Age: 31.7 versus 31.6 Duration subfertility: 4.2 versus 3.9 Basic fertility work up normal, semen > 20 million in ejaculate, previous treatment not stated	IUI + COH 75 IU/day FSH (starting dose) Ovulation: 10000 IU hCG, 1-3 follicles of 18 mm Timing: 40-42 hr after hCG Cancel > 3 follicles of 8 mm or > 6 follicles of 14 mm Duration 6 cycles max	IUI without COH - - Timing: 20-30 hr after LH surge Duration 6 cycles max	Live birth per couple	Randomisation computer generated randomisation schedule in sealed envelopes Trial design: Parallel Single centre ITT- analysis: yes
Goverde 2000 n = 120 w = unclear Analysed 77	Unexplained and male subfertility Age: 31.6 versus 32.1 Duration subfertility: 3.9 versus 4.5 Basic fertility work up normal, semen > 20 million in ejaculate, previous treatment not stated	IUI without COH - - Timing: 20-30 hr after LH surge Duration 6 cycles max	IVF 150-225 IU/day FSH with GnRH agonist Ovulation:10000 hCG, at least 1 follicle 18 mm, and minimum 3 follicle 16 mm Aspiration: 35 hr after hCG ET: 48-72 hr after retrievel, 2-3 embryo's Luteal phase: progesterone	Live birth per couple	Randomisation computer generated randomisation schedule in sealed envelopes Trial design: Parallel Single centre ITT- analysis: yes

Table I — Continuation

Trial n = number of randomised couples w = number of withdrawn couples	**Participants**	**Intervention**	**Comparison**	**Main Outcomes**	**Quality features**
Goverde 2000 n = 122 w = unclear Analysed 84	Unexplained and male subfertility Age: 31.7 versus 32.1 Duration subfertility: 4.2 versus 4.5 Basic fertility work up normal, semen > 20 million in ejaculate, previous treatment not stated	IUI + COH 75 IU/day FSH (starting dose) Ovulation: 1000 IU hCG, 1-3 follicles of 18 mm Timing: 40-42 hr after hCG Cancel > 3 follicles of 8 mm or > 6 follicles of 14 mm Duration 6 cycles max	IVF 150-225 IU/day FSH with GnRH agonist Ovulation:10000 hCG, at least 1 follicle 18 mm, and minimum 3 follicle 16 mm Aspiration: 35 hr after hCG ET: 48-72 hr after retrievel, 2-3 embryo's Luteal phase: progesterone	Live birth per couple	Randomisation computer generated randomisation schedule in sealed envelopes Trial design: Parallel Single centre ITT- analysis: yes
Guzick 1999 n = 932, of which 465 treated with IUI w = 72 Unexplained: 211	Unexplained subfertility & couples with stage I or II treated endometriosis or male subfertility Age: 32 versus 32 Duration subfertility: 3.5 versus 3.8 Basic fertility work up normal, semen normal, no previous treatment	IUI + COH 150 IU FSH/day, day 3-7 Ovulation: 10000 IU hCG, 2 follicles > 18 mm Timing: 36-40 hr after hCG Cancel: E2 > 3000pg/ml Duration 4 cycles max	IUI without COH - - Timing: Day after urinary LH-surge Duration 4 cycles max	Live birth and PR per couple Multiple pregnancies Pregnancy: two positive hCG tests. Confirmed by live birh	Randomisation computer generated permuted block in locked files Trial design: Parallel Multi centre, 10 clinics ITT- analysis: not possible
Janko 1998 n = 72 w = not stated	Unexplained subfertility Age: not stated Duration subfertility: > 3 years Basic fertility work up normal, semen normal not specified, previous treatment not stated	IUI + COH hMG (10 ampul per cycle) Ovulation: 10000 IU hCG Timing: not specified Cancel: not given Duration 3 cycles max	TI + COH hMG (10 ampul per cycle) Ovulation: 10000 IU hCG Timing: not specified Cancel: not given Duration 3 cycles max	PR per cycle	Randomisation not clear Trial design: Parallel Single centre ITT- analysis: possible

Karlstrom 1993 n = not clear w = not clear Analysed 79	Unexplained subfertility & minimal or mild endometriosis Age: 32 (range 21-38) Duration subfertility: 5 (range 2-14) Basic fertility work up normal, semen normal, no previous treatment	IUI + COH 1ste method: 150 IU hMG/ day Ovulation: 10000 IU hCG, at least 1 follicle 17 mm or LH-surge Timing: 36-41 hr after hCG or 24 hr after LH-surge 2nd method: 100 mg CC/day for 5 days Timing: 20-28 hr after LH-surge Cancel: according to serum E2 Duration 1 cycle max	TI + COH 1ste method: 150 IU hMG/ day Ovulation: 10000 IU hCG, at least 1 follicle 17 mm or LH-surge Timing: the two following nights after hCG injection 2nd method: 100 mg CC/day for 5 days Timing: day of LH-surge and day after Cancel: according to serum E2 Duration 1 cycle max	PR per cycle	Randomisation not clear Trial design: Parallel Single centre ITT- analysis: not possible
Melis 1995 n = 108 w = 5 Analysed 103	Unexplained subfertility & mild male subfertility Age: 33.1 years Duration subfertility: 4.3 years Basic fertility work up normal, semen normal, previous treatment in all couples	IUI + COH 3 amp FSH/ day Ovulation: 10000 IU hCG, at least 2 follicles 16 mm Timing: 30-36 hr after hCG Cancel: E2 > 1500pg/ml Duration 3 cycles max	TI + COH 3 amp FSH/ day Ovulation: 10000 IU hCG,at least 2 follicles 16 mm Timing: 12 hr after hCG Cancel: E2 > 1500pg/ml Duration 3 cycles max	Live birth and PR per couple Multiple pregnancies Pregnancy: fetal heart activity on US	Randomisation computer generated random number list, sealed envelopes Trial design: Parallel Single centre ITT- analysis: possible
Murdoch 1991 n = 20 versus 19 w = 5	Unexplained subfertility Age: 33.1 versus 30.5 Duration subfertility: 5.1 versus 5.7 Basic fertility work up normal, semen normal, no previous treatment	IUI + COH 75 IU hMG/ day and 200 µg buserelin 4/day intranasal Ovulation: 5000 IU hCG, < 4 follicles of > 16 mm Timing: 30-36 hr after hCG Cancel: > 4 dominant follicles Duration 3 cycles max	IUI without COH - - Timing: IUI on alternate days until ovulation confirmed on US Duration 3 cycles max	Live birth and PR per couple PR per cycle Multiple pregnancies Pregnancy: fetal heart activity on US	Randomisation: random number sequence, sealed envelopes Trial design: Parallel Single centre ITT- analysis: yes
Steures 2006 n= 127 versus 126	Unexplained subfertility Age: 33 versus 33 Duration subfertility: 2.0 versus 1.9 Basic fertility work up normal, one sided tubal pathology was allowed, semen normal, no previous treatment	IUI + COH Mean 75 IU FSH/ day (range 37-150), minority used 50-150 CC/day for 5 days Ovulation: 5000 or 10000 IU hCG, at least 1 follicle 16 mm Timing: 36-40 hr after hCG Cancel: 3 follicles at least 16 mm, or 5 follicles at least 12 mm Duration 6 months, mean 4 cycles	Coitus without COH - - Timing: - Duration 6 months	Live birth and ongoing PR per couple Multiple pregnancies Ongoing pregnancy: fetal heart activity on US at gestation of 12 weeks	Randomisation computer generated in balanced blocks, sealed envelopes Trial design: Parallel Multi centre, 26 centres ITT- analysis: yes

One study compared IUI versus IVF. It did not show a significant beneficial effect with a RR for IUI without COH compared to IVF: 0.60, [95% CI 0.35 to 1.1] and a RR for IUI with COH compared to IVF: 0.92, [95% CI 0.58 to 1.5].

The comparison IUI with COH versus IUI without COH was made in four studies and showed that IUI with COH was significantly more effective than IUI without COH (RR: 1.8, [95% CI 1.2 to 2.7]).

Nine of these 14 studies reported on multiple pregnancies. The multiple pregnancy rate varied between 3% and 30%, with wide confidence intervals of the effect. The RR for the comparison IUI with COH versus expectant management was 1.3 [95% CI 0.25 to 6.7] and for the comparison IUI with COH versus timed intercourse with COH 1.1 [95% CI 0.44 to 2.7]. In the comparison of IUI with COH versus IUI without COH only one out of the three pregnancies in the group allocated to IUI with COH was a multiple pregnancy.

Cervical factor subfertility

In the Cochrane review, five RCTs were reported (Glazener *et al.,* 1987; Martinez *et al.,* 1990; Kirby *et al.,* 1991; Check *et al.,* 1995; Goverde *et al.,* 2000). These five studies compared IUI without COH with timed intercourse. The quality of these five trials was rather poor as none of these studies used an appropriate method of randomisation or had adequate concealment of allocation. All five trials had a crossover design, and only one study reported separately on the pre-crossover data. Since we included studies with parallel design or pre-crossover data in the meta-analysis, we could only evaluate this study. In this study 80% of the cycles were stimulated despite the aim to investigate the effectiveness of IUI without COH versus timed intercourse without COH. Separate data on the stimulated and none stimulated cycles were not available and therefore we had to exclude this study from the analysis.

Since the publication of the Cochane review two new studies have been published (Steures *et al.,* 2007a; 2007b). The quality of these trials and their main characteristics are presented in Table II. The outcomes of these studies are summarized in Figure 2.

The first study compared IUI with expectant management and included couples with an isolated cervical factor. In this study, treatment with IUI for 6 months was compared to expectant management for 6 months. Treatment with IUI started with three attempts of IUI without COH, followed by IUI with COH if these attempts failed. IUI was associated with higher pregnancy rates, albeit not statistically significant (RR live birth: 1.6, [95% CI 0.87 to 3.1], and RR ongoing pregnancy: 1.6, [95% CI 0.91 to 2.8]).

The second study compared IUI with COH to IUI without COH in couples with an abnormal PCT, due to a cervical factor or a male factor. In the couples with a cervical factor additional COH had no beneficial effect (RR: 1.0, [95% CI 0.59 to 1.8]). Two multiple pregnancies occurred in the IUI with COH group and one in the IUI without COH group.

Male subfertility

We were able to identify 18 trials, of which 17 had been included in the Cochrane review (Kerin *et al.,* 1984; Kerin *et al.,* 1987; Ho *et al.,* 1989; te Velde *et al.,* 1989; Martinez *et al.,* 1990; Kirby *et al.,* 1991; Ho *et al.,* 1992; Crosignani and Walters, 1994; Nan *et al.,* 1994; Aribarg and Sukcharoen, 1995; Arici *et al.,* 1995; Melis *et al.,* 1995; Gregoriou *et al.,* 1996; Cohlen *et al.,* 1998; Jaroudi *et al.,* 1998; Guzick *et*

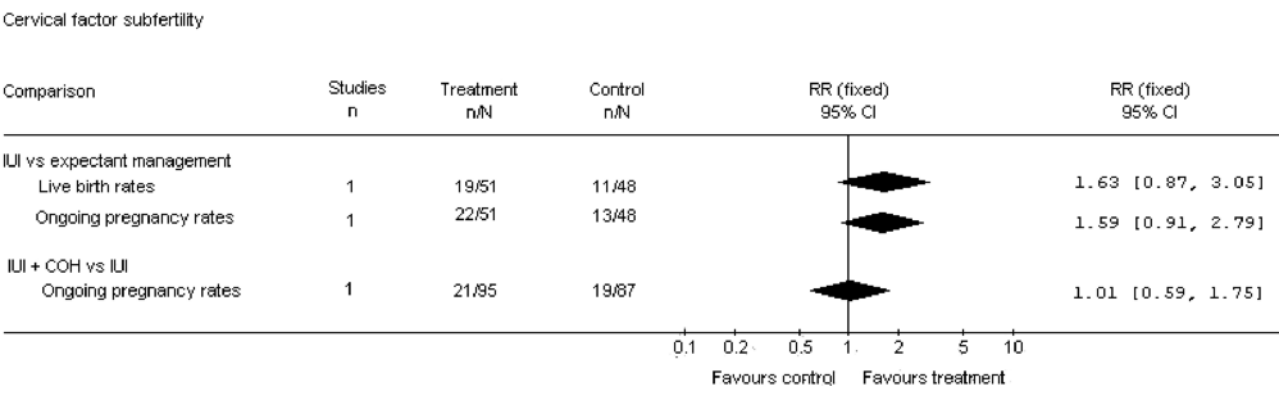

Fig. 2. — Summary of pregnancy results per couple for cervical factor subfertility

Table II. — Characteristics of randomised trials on the effectiveness of IUI in cervical factor subfertility

Trial n = number of randomised couples w = number of withdrawn couples	**Participants**	**Intervention**	**Comparison**	**Main Outcomes**	**Quality features**
Steures 2007 n = 52 versus 49 w = 1 versus 1 Analysed= 51 versus 48	Isolated cervical factor taking the prognosis into account Negative PCT: a well-timed non-progressive PCT, i.e. absence of spermatozoa moving in a straight direction and at a functional speed Age: 30 years Duration of subfertility: 1.7 years Basic fertility work up normal, semen normal, no previous treatment, in each group two women with one-sided tubal occlusion	IUI without COH Timing: 20-30 hr after LH-surge or 36-40 hr after hCG hCG when follicle at least 16 mm Duration: 6 months, mean 4 cycles After 3 failed cycles IUI with COH was given Mean 75 IU FSH/ day (range 37-150) Ovulation: 5000 or 10000 IU hCG, at least 1 follicle 16 mm Timing: 36-40 hr after hCG Cancel: 3 follicles at least 16 mm, or 5 follicles at least 12 mm	Coitus without COH - - Timing: - Duration 6 months	Live birth and ongoing PR per couple Multiple pregnancies Ongoing pregnancy: fetal heart activity on US at gestation of 12 weeks	Randomisation computer generated in balanced blocks, sealed envelopes Trial design: Parallel Multi centre, 17 centres ITT- analysis: yes
Steures 2007 n = 136 versus 136 w = 4 versus 3 Analysed = 132 versus 133, of which 95 versus 87 had TMC > 10 million	Abnormal PCT due to cervical factor or male factor, taking the prognosis into account Negative PCT: a well-timed non-progressive PCT, i.e. absence of spermatozoa moving in a straight direction and at a functional speed Age: 33 versus 33 years Duration of subfertility: 2.5 versus 2.7 years Basic fertility work up normal, prognosis of a spontaneous pregnancy < 30%, no previous treatment, with one-sided tubal occlusion 8 versus 9 women	IUI with COH Mean 75 IU FSH/ day (range 37-150), minority used 50-150 CC/day for 5 days Ovulation: 5000 or 10000 IU hCG, at least 1 follicle 16 mm Timing: 36-40 hr after hCG Cancel: 3 follicles at least 16 mm, or 5 follicles at least 12 mm Duration: max 3 cycles	IUI without COH Timing: 20-30 hr after LH-surge or 36-40 hr after hCG hCG when follicle at least 16 mm Duration: max 3 cycles	Live birth and ongoing PR per couple Multiple pregnancies Ongoing pregnancy: fetal heart activity on US at gestation of 12 weeks	Randomisation computer generated in balanced blocks, sealed envelopes Trial design: Parallel Multi centre, 24 centres ITT- analysis: yes

al., 1999; Goverde *et al.,* 2000). Nine of these studies had no parallel design or pre-crossover data. We could therefore only evaluate nine of the 18 studies in the current review. The quality of these nine trials and their main characteristics are presented in Table 3. The outcomes of the comparisons are summarized in Figure 3.

The effectiveness of IUI without COH was only investigated in one study in which IUI without COH was almost five times more effective than timed intercourse. However, due to the small numbers the confidence interval is very wide (RR 4.5, 95% CI 0.52 to 39).

One study found IUI with COH equally effective as timed intercourse with COH in terms of live birth (RR: 0.91, [95% CI 0.39 to 2.1]). For the outcome clinical pregnancies two more studies could be included. The summary estimate indicated a beneficial effect of IUI with COH but this was not statistically significant (RR: 1.4, [95% CI 0.79 to 2.4]).

Only one randomised trial compared IUI with or without COH with IVF, which was already discussed in the section on unexplained subfertility. We found no significant beneficial effect of any of the treatment modalities (RR for IUI without COH compared to IVF: 1.2, [95% CI 0.59 to 2.4] and RR for IUI with COH compared to IVF: 1.1, [95% CI 0.52 to 2.3]). Most trials about IUI in male subfertility compared IUI with COH to IUI without COH. COH had no additional effect in these couples (RR live birth: 0.92, [95% CI 0.46 to 1.8], RR ongoing pregnancies 1.2, [95% CI 0.88 to 1.8]). Effects on multiple pregnancies could not been extracted from the study reports.

Discussion

Treatment of subfertile couples with IUI has been applied for over 60 years. In many countries, IUI is the first line treatment in couples with unexplained subfertility, cervical factor subfertility and male subfertility. Our comprehensive systematic review showed that the number of randomised trials assessing the effectiveness of IUI is limited and that most of these studies had small sample sizes. Overall, this results in imprecise effect estimates, as demonstrated by relatively large confidence intervals. Also, many studies did not adhere to present quality standards for designing, conducting and reporting randomised clinical trials. Only one comparison was significant in this meta-analysis: IUI with COH improved pregnancy rates compared to IUI without COH in unexplained subfertility. However, this data is of limited clinical value as we do not know whether IUI without COH is of any benefit compared to expectant management.

An explanation for the limited evidence of the effectiveness of IUI could be that IUI was already widely performed before evidence based medicine was introduced. The term "evidence based" appeared in the medical literature for the first time in 1993 (Guyatt and Nishikawa). The Cochrane Collaboration, developing systematic reviews of the effectiveness of interventions in health care, was founded in 1993. It may be possible that the appealing rationale of IUI (bringing the gametes closer together) and the lack of competition from alternative treatment options limited the challenge posed by the principle of evidence-based medicine to existing beliefs in the effectiveness of IUI.

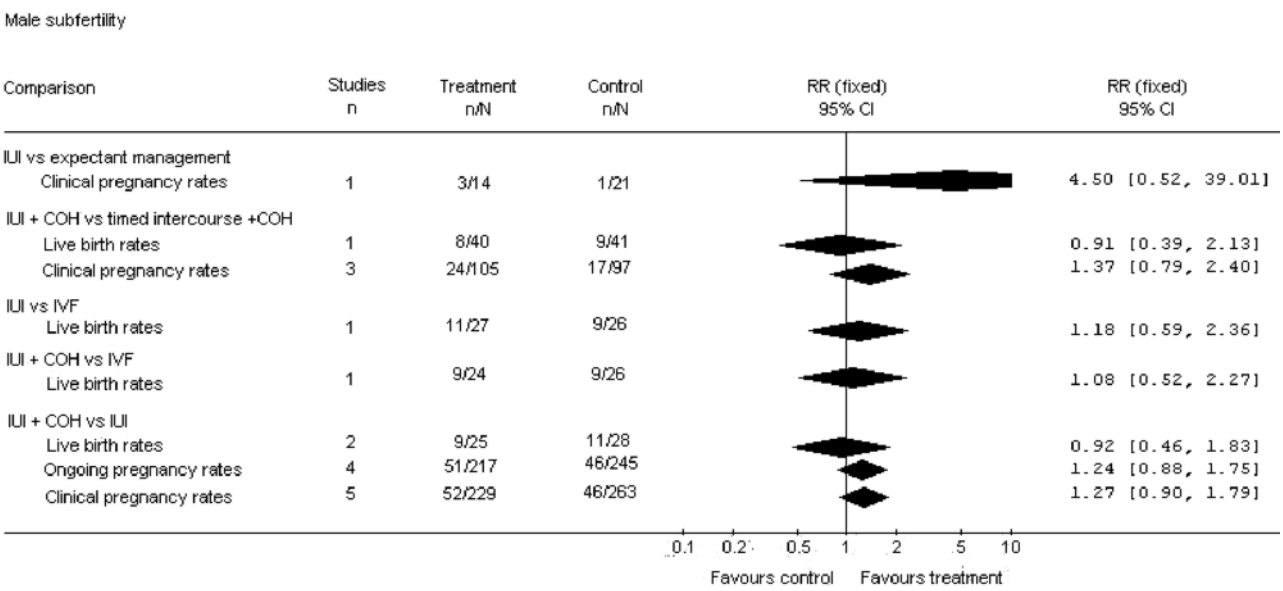

Fig. 3. — Summary of pregnancy results per couple for male subfertility

Table III. — Characteristics of randomised trials on the effectiveness of IUI in male subfertility

Trial n = number of randomised couples w = number of withdrawn couples	**Participants**	**Intervention**	**Comparison**	**Main Outcomes**	**Quality features**
Arici 1994 n = 75 w = 19 Analysed 56 Male sub: 26	Unexplained and male subfertility Male subfertility: concentration < 20 million/ml and/or motility < 50% and/or morphology < 50% (WHO 1987) Age: 33 (range 24-41) Duration subfertility: 3.5 (range 1-15) no previous treatment	IUI +COH 50 mg CC/day, day 5-9 Ovulation: 10000 IUI hCG, at least 1 follicle 18 mm Timing: 32 hr after hCG Cancel: unclear Duration 4 cycles max	IUI without COH - - Timing: On day of LH-surge and next day - Duration 4 cycles max	Live birth and PR per couple PR per 1 st cycle Multiple pregnancies Pregnancy: US showing gestational sac	Randomisation with random number table by computer in locked files Trial design: crossover (after 1 cycle) Single centre ITT- analysis: yes
Cohlen 1998 n = 74,36 versus 38 w = 0	Male subfertility: concentration < 20 million/ml and/or motility < 40% and/or morphology < 40% Age: 30.7 (range 24-39) Duration subfertility: 3.1 (range 2-9) previous treatment not stated	IUI +COH 75-150 IU hMG/day Ovulation: 5000 IU hCG leading follicle at least 18 mm or LH surge Timing: 38-40 hr after hCG Cancel: ≥ 4 follicles of ≥ 18 mm, or E2 level exceedd 1635 pg/ml Duration 6 cycles max	IUI without COH - - Timing: 26 hr after onset of LH-surge (≥ 24 IU/l) - Duration 6 cycles max	PR per couple in first cycle Multiple pregnancies Pregnancy: hCG urine tst and confirmation by US at gestation of 6-7 weeks	Randomisation not stated, allocation by sealed envelopes Trial design: Crossover, alternating cycles Single centre ITT- analysis: possible
Goverde 2000 n = 24 versus 27 w = unclear Analysed 50	Male and unexplained subfertility Male subfertility: 3 of 5 semen analyses TMC < 20 million progressive motile sperm in ejaculate and minimum of 1 million Age: 31.7 versus 31.6 Duration subfertility: 4.2 versus 3.9 previous treatment not stated	IUI + COH 75 IU/day FSH (starting dose) Ovulation: 10000 IU hCG, 1-3 follicles of 18 mm Timing: 40-42 hr after hCG Cancel > 3 follicles of 8 mm or > 6 follicles of 14 mm Duration 6 cycles max	IUI without COH - - Timing: 20-30 hr after LH surge Duration 6 cycles max	Live birth per couple	Randomisation computer generated randomisation schedule in sealed envelopes Trial design: Parallel Single centre ITT- analysis: yes

Table III — Continuation

Trial n = number of randomised couples w = number of withdrawn couples	**Participants**	**Intervention**	**Comparison**	**Main Outcomes**	**Quality features**
Goverde 2000 n = 27 versus 26 w = unclear Analysed 52	Male and unexplained subfertility Male subfertility: 3 of 5 semen analyses TMC < 20 million progressive motile sperm in ejaculate and minimum of 1 million Age: 31.7 versus 31.6 Duration subfertility: 4.2 versus 3.9 previous treatment not stated	IUI without COH - - Timing: 20-30 hr after LH surge Duration 6 cycles max	IVF 150-225 IU/day FSH with GnRH agonist Ovulation:10000 hCG, at least 1 follicle 18 mm, and minimum 3 follicle 16 mm Aspiration: 35 hr after hCG ET: 48-72 hr after retrievel, 2-3 embryo's Luteal phase: progesterone	Live birth per couple	Randomisation computer generated randomisation schedule in sealed envelopes Trial design: Parallel Single centre ITT- analysis: yes
Goverde 2000 n = 24 versus 26 w = unclear Analysed 50	Male and unexplained subfertility Male subfertility: 3 of 5 semen analyses TMC < 20 million progressive motile sperm in ejaculate and minimum of 1 million Age: 31.7 versus 31.6 Duration subfertility: 4.2 versus 3.9 previous treatment not stated	IUI + COH 75 IU/day FSH (starting dose) Ovulation: 1000 IU hCG, 1-3 follicles of 18 mm Timing: 40-42 hr after hCG Cancel > 3 follicles of 8 mm or > 6 follicles of 14 mm Duration 6 cycles max	IVF 150-225 IU/day FSH with GnRH agonist Ovulation:10000 hCG, at least 1 follicle 18 mm, and minimum 3 follicle 16 mm Aspiration: 35 hr after hCG ET: 48-72 hr after retrievel, 2-3 embryo's Luteal phase: progesterone	Live birth per couple	Randomisation computer generated randomisation schedule in sealed envelopes Trial design: Parallel Single centre ITT- analysis: yes
Gregoriou 1996 n = 62, 31 versus 31	Male subfertility: 3 samples concentration < 20 million/mL and/or motility < 30% and/or norm. morphology < 40% Age: 30.5 years Duration subfertility: 5.8 years previous treatment not stated	IUI + COH Stimulation: 75 IU hMG/day with adjustment (150 IU hMG/max) Ovulation: 10000 hCG Timing: 36-40 hr after hCG Cancel: ovarian hyperstimulation, not specified Duration 6 cycles max	TI + COH Stimulation: 75 IU hMG/day with adjustment (150 IU hMG/max) Ovulation: 10000 hCG Timing: 36-40 hr after hCG Cancel: ovarian hyperstimulation, not specified Duration 6 cycles max	PR per cycle couple Pregnancy: hCG urine tst and confirmation by US	Randomisation not clear Trial design: Crossover after 3 cycles Single centre ITT- analysis: possible

Guzick 1999 n = 932, of which 154 male factor and treated with IUI w = unclear	Unexplained subfertility & male subfertility Male subfertility: concentration < 20 million/ml and/or motility < 50% Age: 32 versus 32 Duration subfertility: 3.5 versus 3.8 no previous treatment	IUI + COH 150 IU FSH/day, day 3-7 Ovulation: 10000 IU hCG, 2 follicles > 18 mm Timing: 36-40 hr after hCG Cancel: E2 > 3000pg/ml Duration 4 cycles max	IUI without COH - - Timing: Day after urinary LH-surge Duration 4 cycles max	Live birth and PR per couple Multiple pregnancies Pregnancy: two positive hCG tests. Confirmed by live birh	Randomisation computer generated permuted block in locked files Trial design: Parallel Multi centre, 10 clinics ITT- analysis: unclear
Kerin 1984 n = 14 versus 21	Male subfertility: (in 3 samples, at least 2 abnormalities): concentration < 40 million/mL; motility < 45% and morphology < 40% Age: not stated Duration subfertility: ≥ 3 years previous treatment not stated Male & unexplained subfertility	IUI without COH Timing: day after LH-rise Duration 9 cycles max	TI without COH Timing: no timing or by LH tests, on day of LH-rise Duration 9 cycles max	Pregnancy per couples Pregnancy not stated	Randomisation not clear Trial design: Crossover alternating cycles Single centre ITT- analysis: possible
Melis 1995 n = 92 w = 11 analysed= 81 41 versus 40	Male subfertility: (2 samples): concentration 10-20 million/mL and/or progressive motility 15-25% and/or total motility 30-50 % and/or norm. morphology 30-50 % Age: 34.2 years Duration subfertility: 4.3 years previous treatment in all couples	IUI + COH 225 FSH/ day Ovulation: 10000 IU hCG,at least 2 follicles 16 mm Timing: 30-36 hr after hCG Cancel: E2 > 1500 pg/ml Duration 3 cycles max	TI + COH 225 FSH/ day Ovulation: 10000 IU hCG,at least 2 follicles 16 mm Timing: 12 hr after hCG Cancel: E2 > 1500pg/ml Duration 3 cycles max	Live birth and PR per couple Multiple pregnancies Pregnancy: fetal heart activity on US	Randomisation computer generated random number list, sealed envelopes Trial design: Parallel Single centre ITT- analysis: possible
Nan 1994 n = 76 first cycle: 34 versus 25	Male subfertility: (in 4 samples): concentration < 20 million/mL and/or motility < 40% and/or morphology < 40% Age: 32 years (range: 24-39) Duration subfertility: 4.5 years (2-10) previous treatment not stated	IUI + COH 150 IU hMG/day Ovulation: 10000 IU hCG Timing: 38-40 hr after hCG unless LH surge: 26 hr after detecting LH surge Cancel: > 4 follicles ≥ 18 mm or E2 > 6000 pmol/L Duration 6 cycles max	TI + COH 150 IU hMG/day Ovulation: 10000 IU hCG Timing: 24-28 hr after hCG unless LH surge: same evening as detection LH surge Cancel: > 4 follicles ≥ 18 mm or E2 > 6000 pmol/L Duration 6 cycles max	Live birth and PR per couple PR per cycle Multiple pregnancies Pregnancy: fetal heart activity on US	Randomisation: random number sequence, sealed envelopes Trial design: Crossover, alternating cycles Single centre ITT- analysis: not possible

Table III — Continuation

Trial n = number of randomised couples w = number of withdrawn couples	**Participants**	**Intervention**	**Comparison**	**Main Outcomes**	**Quality features**
Steures 2007 n = 136 versus 136 w = 4 versus 3 Analysed= 132 versus 133, of which 37 versus 46 had TMC < 10 million	Abnormal PCT due to cervical factor or male factor, taking the prognosis into account Male subfertility: TMC < 10 million Age: 33 versus 33 years Duration of subfertility: 2.5 vs 2.7 prognosis of a spontaneous pregnancy < 30%, no previous treatment, with one-sided tubal occlusion 8 versus 9 women	IUI with COH Mean 75 IU FSH/ day (range 37-150), minority used 50-150 CC/day for 5 days Ovulation: 5000 or 10000 IU hCG, at least 1 follicle 16 mm Timing: 36-40 hr after hCG Cancel: 3 follicles at least 16 mm, or 5 follicles at least 12 mm Duration: max 3 cycles	IUI without COH Timing: 20-30 hr after LH-surge or 36-40 hr after hCG hCG when follicle at least 16 mm Duration: max 3 cycles Live birth and ongoing PR per couple Multiple pregnancies	Ongoing pregnancy: fetal heart activity on US at gestation of 12 weeks	Randomisation computer generated in balanced blocks, sealed envelopes Trial design: Parallel Multi centre, 24 centres ITT- analysis: yes

IUI has been incorporated into clinical guidelines from 1996 onwards. Since then IUI was also officially established as the treatment of first choice. In view of the limited evidence, it is surprising that current clinical guidelines make rather firm recommendations about the use of IUI. The guideline made by The National Collaborating Centre for Women's and Children's Health, for example, stipulates that "Couples with mild male subfertility and unexplained subfertility should be offered IUI". On the issue of ovarian hyperstimulation it recommends that "IUI in male subfertility should not be offered with COH". Elsewhere it states that "in unexplained subfertility IUI without COH should be offered, even though this is associated with lower pregnancy rates than IUI with COH, because of the risk of multiple pregnancy after IUI with COH" (Nice Guideline, 2003).

Others authors have suggested that the risk of multiple pregnancies after IUI should encourage its replacement by IVF with single embryo transfer[4]. Still others proclaim that there is no place for the use of gonadotrophins in IUI treatment in unexplained and male subfertility (Goverde *et al.,* 2005).

This comprehensive review shows that there is very little data to ground the firmness of these recommendations, either in favour or against the use of IUI. More good quality trials with sufficient sample sizes are urgently needed to remedy the disturbing lack of evidence. Such studies should compare IUI with expectant management and IUI with IVF, with or without single embryo transfer.

So far, most studies included couples with a great variety in baseline characteristics. This makes a fair comparison between studies and extrapolation to daily practice a real challenge. We feel that, to make studies more generalizable, the prognosis of the couples should be taken into account in designing and analyzing trials. Incorporating the undeniable variability in prognosis provides us with the opportunity to compare and summarize study results in a more meaningful manner. Doing so may enable us to distinguish those couples who may benefit from treatment from those who are unlikely to.

Couples with an intermediate to good prognosis of a treatment independent pregnancy may be encouraged to participate in trials comparing IUI to expectant management, while couples with a poor prognosis are invited to participate in trials comparing IUI to IVF with or without single embryo transfer.

In conclusion, the evidence on the effectiveness of intrauterine insemination, the most frequently used treatment in subfertility, is surprisingly scarce and does not allow us to provide firm recommendations. Only two conclusions can be drawn; IUI with COH improves pregnancy rates over IUI without COH in unexplained subfertility and IUI with COH has not been shown to be effective in couples with unexplained subfertility and a good prognosis. Quality studies comparing IUI to expectant management and to IVF are urgently needed.

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Cost-effectiveness of IVF compared to other infertility treatments; A United States perspective

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Key words: Cost-effectiveness, IVF, IUI, insemination, infertility.

The relative cost-effectiveness of infertility treatments is an important issue. Couples desire infertility treatments that are not only efficacious- allowing for a short time to pregnancy, but also affordable, since infertility care is often not covered by insurance policies in the United States. Although the costs of treatment are often an individual's expense, insurance companies and society in general are frequently burdened the high costs of caring for premature infants that can result from multiple gestations induced by infertility treatments. Therefore, cost-effectiveness studies are important not only to the individual, but also to the health care industry and society in general.

In some instances, there is little debate about the preferred treatment for an infertile couple. For example, in cases of severe tubal disease, the low pregnancy rates after surgery makes IVF the clear treatment of choice from a cost-effectiveness standpoint (Van Voorhis *et al.*, 1997). Similarly, in a couple presenting with infertility and anovulation, oral ovulation induction medications are effective and inexpensive, making this the logical first line therapy. However, in some cases, the most cost-effective strategy to infertility care is not as clear. Couples with unexplained infertility or mild to moderate male factor infertility are the groups that have been most often studied in cost-effectiveness studies comparing IVF to intrauterine inseminations (IUI).

There are several important limitations when evaluating the relative cost-effectiveness of IVF and IUI. There have been relatively few randomized trials comparing these two treatments which limits the confidence we have in effectiveness comparisons, since most are based on retrospective cohort studies. On the cost side, many studies have reported infertility treatment charges as a surrogate for the actual cost of providing care. Charges can be artificial and don't necessarily reflect the true costs of providing a service. In addition, the outcome reported by many studies is the cost/delivery of at least one child. This outcome may not include the costs of caring for premature infants- a cost that can be substantial with the high rates of multiple gestations seen with infertility care. Finally, cost-effectiveness studies may be based on the outcomes and charges from a few centers limiting the more general applicability of the findings.

We have an extensive database that records the outcomes and costs of all infertility treatment cycles performed at our center, including both stimulated and unstimulated IUI cycles as well as IVF cycles. In a series of studies, we have previously compared the relative cost-effectiveness of common infertility treatments prescribed by reproductive endocrinologists for patients with all types of infertility. We found that IUI, whether done in a natural cycle, a clomiphene citrate-stimulated cycle or a gonadotropin-stimulated cycle, was significantly more cost-effective than one IVF cycle (Table 1). Although IUI cycles had lower delivery rates per cycle, ranging from 5–17% versus 28% per cycle for IVF at that time, the charges accumulated by IUI cycles were substantially less than those with IVF leading to a better cost-effectiveness ratio when expressed as a cost per delivery. In fact, the cost per delivery with IVF was three to fourfold higher than that with IUI (Van Voorhis *et al.*, 1997, 1998). We also found that

Table 1. — Relative cost-effectiveness of various infertility treatments at one academic medical center (Van Voorhis *et al.*, 1997)

Procedure	No. of couples	No. of procedures	No. (%) of deliveries	Multiple birth rate (%)	Cost per delivery ($)
IUI	54	103	6 (5.8)	0	8,674
CC-IUI	91	188	12 (6.3)	8.3	7,808
HMG-IUI	52	80	14 (17.5)	21.0	10,282
ART	136	155	43 (27.7)	30.0	37,028

all infertility treatments had a worsening cost-effectiveness ratio as women aged and, for women over the age of 40, the use of donor oocytes was significantly more cost-effective than doing IVF with a woman's own oocytes (Van Voorhis *et al.*, 1997, 1998). The use of cryopreserved embryos is a highly cost-effective treatment option for those couples who have excess embryos cryopreserved (Van Voorhis *et al.*, 1995). Finally, we reported that in couples where the male partner had extremely low sperm counts, as defined by the average total motile sperm count in the ejaculate from at least two samples of less than 10 million sperm, a lower pregnancy rate was achieved with IUI leading to worsened cost-effectiveness ratios (Van Voorhis *et al.*, 1997).

This finding led us to further study IUI cost-effectiveness using a retrospective cohort study from our center (Van Voorhis *et al.*, 2001). Our objective was to identify factors that affected pregnancy rates after IUI and IVF and then study the cost-effectiveness of these treatments based on these parameters. We were most interested in defining the effect of male factor infertility on cost-effectiveness of infertility treatments. We found that the best predictor of delivery following IUI was the average total motile sperm count (TMSC: calculated as seminal fluid volume X sperm concentration X motility percentage, from at least 2 samples) in the ejaculate. If the average TMSC was less than 10 million, we had very low delivery rates (less than 2%) with IUI. If the TMSC was between 10 and 30 million, IUI delivery rates were in excess of 5%/cycle but there was no additional value to hyperstimulation of the woman. If the TMSC was greater than 30 million, then good delivery rates were achieved with IUI and even higher pregnancy rates were seen with ovarian stimulation using either clomid or gonadotropins. Because IVF pregnancy rates were not affected by the TMSC, IVF was found to be more cost-effective than IUI for couples in which the male had a TMSC < 10 million. Above this threshold, IUI was more cost-effective than IVF. Thus, depending on the prognosis for an individual, the relative cost-effectiveness for various infertility treatments can differ (Van Voorhis BJ *et al.*, 2001). A number of other investigators have likewise found a cut-off value below which IUI is unlikely to be successful (reviewed in 5). Most studies have looked at the TMSC in the inseminant following processing for IUI rather than in the ejaculate as in our studies. These studies have identified TMSC cut-offs between .8 to 5 million (Van Weert *et al.*, 2004). When utilizing these values, there is poor sensitivity for predicting who will conceive but a high specificity predicting failure to conceive with IUI. However, the exact cut off likely needs to be based on the individual lab and sperm prep technique used.

There have been several prospective randomized trials comparing stimulated IUI treatments with IVF and both have concluded that IUI in general is more cost-effective than IVF (Goverde *et al.*, 2000, Karande *et al.*, 1999). However, findings from cost-effectiveness analyses are best used to decide allocation of resources for large populations of people. For the individual, clinical conditions and circumstances can influence the best course of treatment, even from a cost-effectiveness standpoint.

Time to conception is of importance to many couples both to alleviate the suffering and disappointment that comes from being infertile and to avoid the negative effects of aging on their reproductive potential. This factor was recently addressed in a prospective randomized trial termed the Fast Track and Standard Treatment trial (FASTT) trial (Reindollar *et al.*, 2009). This study enrolled women between the ages of 21 and 39 who had unexplained infertility and a normal ovarian reserve. In addition, severe male factor infertility was excluded as sperm concentrations of greater than 15 million motile sperm in the ejaculate were required for entry. The outcomes of the trial were both timed to pregnancy as well as cost-effectiveness of treatment. For this study cost-effectiveness was calculated by summing all insurance charges divided by the number of couples delivering at least one child. Charges were calculated from the time of randomization through hospital discharge of both the mother and baby or until one year after the protocol if pregnancy was not achieved. In the study, 243 women were randomized to a "conventional" treatment protocol which consisted of clomiphene citrate at 100 mg for 5 days

plus IUI for three cycles followed by FSH (150 IU/day) stimulated cycles plus IUI for three cycles followed by up to six IVF cycles. 256 couples were randomized to a "fast track" arm of the study consisting of clomiphene citrate at 100 mg for 5 days plus IUI for three cycles followed immediately by up to six IVF cycles. The "fast track" arm of the study had a significantly shorter time to pregnancy (a median time to pregnancy difference of 3 months) than the "conventional" treatment algorithm. The number of couples ultimately conceiving a pregnancy was the same in both arms but the charges per delivery were lower in the accelerated arm of the study. The authors concluded that, after use of clomid and IUI for couples with unexplained infertility, going directly to IVF was more cost-effective than using FSH-IUI cycles before IVF (Reindollar *et al.*, 2009). Couples in the accelerated arm were pregnant faster with fewer treatment cycles and with less cost. Importantly, there were equivalent rates of multiple gestations in the two arms of this study.

One limitation of most cost-effectiveness studies in infertility is the omission of the high costs of multiple gestations (Callahan *et al.*, 1994). Many analyses end with delivery and do not account for the high cost of neonatal care for prematurely born infants, often secondary to multiple gestations. This is a critical issue because it has been recently estimated that in the United States alone, the cost of preterm infants born after IVF therapy is nearly $1 billion/year. It is difficult to know where to draw the line for health care costs of infants born from infertility treatments. In the short term, these infants are very expensive to society but, in the long-term, they will often serve as productive, contributing members of society. Nevertheless, the multiple gestation issue is important and should be addressed in cost-effectiveness studies.

As we look to the future, it is clear that there may be shifting paradigms as to the most cost-effective treatment strategy for infertile couples. In recent years, there has been a marked increase in pregnancy rates with IVF, in general. Similar increases have not been achieved with IUI treatments. Indeed several recent studies modeling outcomes and costs have concluded that moving directly to IVF is more cost-effective than starting with IUI cycles for unexplained and mild male factor infertility (Bhatti *et al.*, 2008; Pashayan *et al.*, 2006). Particularly if the singleton delivery rate per cycle can be improved, perhaps with single embryo transfer, IVF may become the favored first line treatment for most causes of infertility. However, at this time, the balance of published studies still favor starting with a more conservative treatment regimen before moving to IVF for the treatment of unexplained infertility.

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To inseminate or not, that is the question. A reflection of existing evidence

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Abstract

A short review stating levels of evidence is given regarding the indications for IUI and the cost-effectiveness of (stimulated) IUI compared with that of IVF. It is concluded that IUI in natural cycles should be applied in couples with cervical hostility, might be applied in couples with a moderate semen factor and should not be applied in couples with unexplained subfertility. Intrauterine insemination in cycles with MOH should be applied in couples with unexplained subfertility and might be applied in couples with a mild semen factor. Furthermore there still seems to be a place for IUI in natural and stimulated cycles comparing cost-effectiveness with IVF. However, cumulative pregnancy rates after mild IVF combined with single transfer of frozen and thawed embryos is increasing and therefore future RCTs are mandatory comparing modern IVF with IUI.

Key words: IUI, IVF, cost-effectiveness, indications.

Introduction

When eye-balling the recent literature there seems to be a discord between those who still believe in the cost-effectiveness of intrauterine insemination (IUI) and those who do not (ESHRE Capri workshop Group, 2009). On the one hand there is existing evidence that IUI should not be started too soon (Steures *et al.*, 2006a) and on the other hand the success rates of IVF and ICSI seem rising. However, IUI is save, simple, non-invasive and relatively inexpensive. In developing countries IUI seems to gain popularity whereas in developed countries it is still one of the most frequently applied treatment options to enhance the probability of conception for several indications. What is nowadays the place of IUI in the wide range of treatment options?

Indications for IUI

The three classical indications for IUI are: (1) Cervical Hostility, (2) Male Subfertility and (3) Unexplained subfertility including mild endometriosis. In couples with anovulation, ovulation induction without IUI seems to be the treatment option of first choice while in coupes with sexual dysfunction high intravaginal or intracervical insemination with unprepared semen could be applied.

In many (older) trials IUI has been compared with so-called timed intercourse. Discussion has been raised that timed intercourse might impair the probability of conception compared with unrestricted intercourse. A direct trial comparing both options does not exists. Gathering indirect evidence and performing a meta-analysis Snick and colleagues showed no significant difference between timed intercourse or expectant management as a control group in RCTs dealing with IUI (level of evidence (LOE) 1a) (Snick *et al.*, 2008). It would have been rather surprising when the authors would have detected a significant difference between these two control options: all couples defined as subfertile have probably performed unrestricted intercourse for several years before entering a trial of IUI.

In case of a cervical factor IUI in natural cycles has been proven effective (LOE 1b)(Steures *et al.*, 2007). The publication by Steures et al. showed that IUI significantly increased ongoing pregnancy rates

compared with unrestricted intercourse with a number needed to treat of 6 (Steures *et al.*, 2007). However, cervical hostility is a rare diagnosis. When the postcoital test is timed and performed adequately, and a male factor is excluded, only few couples will suffer from cervical hostility. This is one of the reason why many clinics stopped performing postcoital testing. On the other hand, the postcoital test has an strong prognostic value in predicting treatment-independent conception and should therefore remain part of the fertility work-up (Hunault *et al.*, 2002).

In case of a male factor most of the evidence from RCTs is somewhat older and the characteristics of these trials do not fulfill the strict criteria of the Cochrane Collaboration. For years IUI seemed to be the treatment of first choice in case of a moderate to mild male factor based upon several RCTs (Cohlen *et al.*, 1995). However, the latest Cochrane review clearly states that there is insufficient data for robust conclusions (LOE 1) (Bensdorp *et al.*, 2007). For daily practice IUI in natural cycles is recommended when at least 0.8 – 5.0 million motile sperm can be inseminated after sperm processing (LOE 1) (van Weert *et al.*, 2004). In case of a mild semen defect (average total motile sperm count above 10 million before sperm processing) IUI in cycles with mild ovarian hyperstimulation is recommended (LOE 1b) (Cohlen *et al.*, 1998).

In couples with unexplained subfertility IUI in natural cycles should not be applied. Compared with intercourse IUI does not increase the probability of conception (Bhattacharya *et al.*, 2008; Verhulst *et al.*, 2006). This seems very plausible because without a cervical factor nor impaired sperm or tubal function sufficient motile sperm reach the site of fertilization every month. The combination of IUI with mild ovarian hyperstimulation (MOH) does improves live birth rates and it is therefore advised to apply this combination as first-line treatment option in couples with unexplained subfertility (LOE 1) (Verhulst *et al.*, 2006). The thin line between enhancing the probability of conception by increasing the number of available oocytes and minimizing the risk for achieving a multiple pregnancy should be walked with great care. Adjusting the individual dosage of FSH to strive after two oocytes seems to be the optimal strategy (LOE 1) (van Rumste *et al.*, 2008). Close ultrasound monitoring of all follicles larger than 10 mm in combination with strict cancellation criteria has been proven in daily practice to minimize the risks for twin pregnancies below 10% and triplets below 1-2% (Steures *et al.*, 2006b).

In conclusion IUI in natural cycles should be applied in couples with cervical hostility, might be applied in couples with a moderate semen factor and should not be applied in couples with unexplained subfertility. Intrauterine insemination in cycles with MOH should be applied in couples with unexplained subfertility and might be applied in couples with a mild semen factor.

IUI compared with IVF

Should we compare the cost-effectiveness of (MOH) IUI with that of IVF? In many countries there is not a question of choice because both treatment modalities are reimbursed or IVF is much too expensive to be applied at all. When reimbursed, the optimal strategy in couples with mild male subfertility or unexplained subfertility seems to be a number of 3 to 9 cycles of MOH/IUI followed by IVF in those couples that did not conceive. Cumulative ongoing pregnancy rates after MOH/IUI in these couples varies among clinics but might be as high as 40% (LOE 2b) (Custers *et al.*, 2008).

When resources are limited or couples pay for their own treatment the question of the most cost-effective treatment option arises. A systematic search of the literature reveals limited evidence only. This might be explained by the fact that cost-effectiveness is an issue of recent growing interest. The perfect multicenter, adequately powered, randomized trial that compares the cost-effectiveness of MOH/IUI with IVF including direct and indirect costs, obstetrical and neonatal costs but also benefits for society has to be performed yet (a nice example of the complexity of such a study is described by Fiddelers *et al.* (2009)).

In 1997 and 2001 van Voorhis and colleagues performed two retrospective cohort studies in which they compared the cost effectiveness of (stimulated) IUI with IVF or IVF-ICSI (LOE 2b) (Van Voorhis *et al.*, 2001;Van Voorhis *et al.*, 1997). In couples with unexplained or mild male subfertility they concluded MOH/IUI to be more cost-effective than IVF and they recommended that IUI should be applied before starting IVF. The direct costs per delivery after (stimulated) IUI varied between $ 7,800 and $ 10,300 whereas after IVF is was $ 37,000. In couples with an average total motile sperm count below 10 million IVF/ICSI seemed more cost-effective than IUI. By modeling several treatment pathways Philips and coworkers concluded that in case of unexplained or moderate male subfertility MOH/IUI is the most cost-effective treatment option in the UK (LOE 2b) (Philips *et al.*, 2000). In 1999 Karande *et al.* published the first randomized trial to compare the cost-effectiveness of two treatment strategies: either couples received IVF or a standard infertility treatment algorithm (Karande *et al.*, 1999). Again it was advised not to start IVF immediately although indirect costs nor obstetrical or neonatal cost were

included (LOE 1b). In 2000 Goverde *et al.* published one of the first RCTs comparing the cost-effectiveness of (MOH) IUI with IVF in couples with mild male or unexplained subfertility (Goverde *et al.*, 2000). Their conclusion was clear: IUI was a more cost-effective treatment than IVF (costs per pregnancy resulting in at least one live birth: $ 4,511-5,710 for IUI vs $ 14,679 for IVF) (LOE 1b). Critics stated that the drop-out rate in the IVF group was rather high (42%) and pregnancy rates per IVF attempt were too low (12,2%). Recently Reindollar *et al.* compared two treatment strategies in a randomized trial: CC/IUI followed by FSH/IUI and IVF compared with CC/IUI followed by IVF directly (Reindollar *et al.*, 2009). They concluded that the accelerated track was the most cost-effective although confidence intervals were wide and crossed the zero (average charges per delivery were $ 9,800 lower 95% CI, $ 25,100 lower to $ 3,900 higher) (LOE 1b). Furthermore, even after three failed attempts of CC/IUI pregnancy rates per treatment cycle after FSH/IUI were non-significantly higher compared with CC/IUI (9.8% versus 7.6%). This underlines the conclusion by Cantineau *et al.* that in IUI cycles gonadotropins are more effective compared with CC (LOE 1) (Cantineau *et al.*, 2007).

While the success rates of IVF seem to have increased over the last decades, pregnancy rates after (stimulated) IUI remain more or less the same (Nyboe *et al.*, 2009). The ESHRE 2005 report stated a pregnancy rate per IVF aspiration of 26.9% and a pregnancy rate per IUI attempt of 12.6%. Multiple pregnancy rates still differ largely between both treatments: 21,8% after IVF and 12,1% after IUI. With these general results in mind and the fact that 1 IVF/ICSI cycle is approximately three times as expensive as 1 MOH/IUI cycle regarding direct costs, there still seems to be a place for (MOH) IUI in Europe. But will we still embrace this opinion in 10 years from now?

With an European tendency towards single embryo transfer in combination with increasing number of transfer cycles of frozen and thawed embryos, a mild IVF stimulation protocol aiming at lesser follicles (Verberg *et al.*, 2009) or even IVF in natural cycles (Pelinck *et al.*, 2002) new RCTs are mandatory to compare cumulative live birth rates between these modern IVF protocols and (stimulated) IUI. These RCTs should be adequately powered, multi-centered, performed in well defined populations of subfertile couples with a moderate to poor prognosis regarding spontaneous chance of conception. Furthermore, these trials should focus on live birth rates as primary outcome but also on side-effects, multiple pregnancies, patients discomfort and drop-out rate, direct and indirect costs (including obstetrical and neonatal costs) in relation to the number of healthy infants born. At this moment such a large RCT is performed in the Netherlands (Bensdorp *et al.*, 2009).

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Is the fallopian tube better than the uterus? Evidence on intrauterine insemination versus fallopian sperm perfusion

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Abstract

Fallopian tube sperm perfusion (FSP) is in short comprised of mild ovarian stimulation with the aim of maturing 2 follicles, ovulation induction and insemination using a large volume (4 ml) of inseminate. Perfusion studies demonstrated that with this large volume, the inseminate will fill the uterine cavity, flow through the fallopian tubes and some of it will end up in the peritoneal space. The rational being that this maximizes the chances the gametes will meet and fertilization occur.

We initiated a prospective randomized study that showed that FSP gave higher pregnancy rates compared to conventional intrauterine insemination (IUI), OR: 4.1, (1.2-13.4 95% Confidence interval).

In our hands, FSP seems to give the best results in couples with unexplained infertility.

In the years 1990 to 2002, 1005 inseminations with husband's semen were carried resulting in 141 pregnancies and 112 deliveries. In the same period, 1200 inseminations with frozen donor semen were carried out giving 333 pregnancies and 226 deliveries.

Several studies from other research groups have tried to elucidate whether FSP or IUI will give the highest pregnancy rates in the treatment of non tubal/unexplained infertility. Most of these studies are rather small. A complicating factor is that in these studies, different protocols, different utensils and different catheters for performing FSP have been used. This may well have influenced the outcome of the studies and contributed to the fact that currently there is no consensus on whether FSP or IUI is to be advocated.

Key words: Insemination, IUI, FSP, unexplained infertility, non-tubal infertility.

Introduction

Intrauterine insemination has been used for many years to treat infertile couples. Collecting data from a large number of European clinical in the late 80-ties, suggested that there was a great variation in indications, techniques and results (Sunde *et al.*, 1988). Even today, the indications for performing insemination and the way the procedure should be performed are debated.

Conventionally, intrauterine insemination is performed using a relative small volume of inseminate (~0.5 ml). In the end of the 80-ties we wanted to investigate whether the conventional method for intrauterine insemination could be improved by increasing the volume of the inseminate.

Development of the Fallopian tube perfusion

Our aim was to maximize the likelihood of sperm and egg to meet. We should ensure that sperm cells were present not only in the uterus, but in the fallopian tubes and even in the peritoneal space.

Volumetric study

Six patients admitted to the hospital for sterilization volunteered to take part in a volumetric study while

under anesthesia. The man age of the patients was 36 years and the investigation was carried out at the approximate time of ovulation. An IV-pump operating at 0.5 ml/minute was connected to a Frydman catheter (Frydman embryo replacement catheter CCD Paris). The perfusion volume was estimated by measuring the time from start of perfusion until flow of the perfusate out of the distal end of at least one fallopian tube. The mean perfusion volume was 1, 8 ml, ranging from 0.8-2.5 ml based on this data, we chose an insemination volume of 4 ml.

Semen preparation

A classical swim-up technique was normally used. Briefly, the semen was diluted in culture media, centrifuged at 800 g for 10 min. The pellet was resuspended in culture media and centrifuged again: One ml of culture media was carefully placed above the final pellet and incubated for 30 min at 37°C. Motile spermatozoa were then aspirated form the top of the culture medium. For classical IUI, 10-50 million sperm cells were resuspended un 0.5 ml culture media, for FSP the sperm cells were resuspended in 4 ml media. When frozen semen was used, the semen was thawed at the day of insemination and washed three times by centrifugation (800 g, 10 min) and resuspended in culture media. The final pellet was resuspended in 4 ml of culture media. Except for the very first inseminations, we used commercially available culture media from MediCult Denmark.

Hormonal pretreatment

Ovarian stimulation was in the beginning achieved by administering Clomiphene citrate 50 mg daily from cycle day 4 to 8. Human menopausal gonadotrophin (hMG, Humegon Organon) 75 I.U. daily from cycle day 7 to 9. For some of the women, hMG administration was continued depending on the ovarian response. From 1995 and onwards we used recombinant 75 I.U. FSH (Puregon, Organon) instead of hMG. From cycle day 10, ovarian response was monitored by vaginal ultrasound (number and size of the growing follicles) and daily measurements of serum estradiol. Human chorionic gonadotrophin (hCG) (Pregnyl Organon) 5000 IU was administered when at least one follicle was 17 mm. We aimed at maturing a maximum of two follicles (> 15 mm) and considered a serum concentration of ~ 1000 pmol/l/follicle as optimal. We cancelled cycles where the serum estradiol per follicle was too low (< 500 pmol/l/follicle). Cycles with too many maturing follicles (> 4) or too high serum estradiol (> 5000 pmol/l) were either cancelled or converted to IVF-treatment.

Insemination

Insemination was performed 34-36 hours following administration of hCG. Patients were premedicated by administering 10 mg Diazepam 1 hour before insemination. Insemination was performed with the patient in the lithotomy position. The vagina and the cervix were rinsed with sterile saline. For FSP, a 5 ml plastic syringe was filled with 4 ml of the inseminate. A catheter (Frydman embryo transfer catheter, CCD France) was trough the cervical canal and into the upper part of the uterine cavity. The insemination was performed rather slowly (~1 ml/minute). An Allis clamp was placed on the cervix to prevent reflux. The Allis clamp was kept in place for some minutes after insemination.

Treatment with FSP; Results and Discussion

First experience

A total of 139 couples with different infertility diagnosis were offered in total 239 treatment cycles, Pregnancy rates per cycle or per couple varied considerably between infertility diagnosis and the best result was obtained with couples having unexplained infertility (Kahn *et al.*, 1992a). We did not get very encouraging result in couples where the male has reduced semen quality and in general we do not offer couples where the male have reduced semen quality FSP (Kahn *et al.*, 1992a).

Other research groups have on the other hand demonstrated adequate pregnancy rates even with fairly low semen quality (Strandell *et al.*, 2003).

FSP or IUI?

A prospective randomized multicenter study was initiated in order to evaluate whether FSP gave better pregnancy rates that traditional IUI. Couples with unexplained infertility were included in the study Unexplained infertility was defined as:

Female parameters: Gynaecological examination should not reveal abnormalities and the woman should have regular menstrual periods (24-33 days). Ovulation should be evident by at least two biphasic basal body temperature charts band normal serum progesterone concentrations in the luteal phase. Serum levels of SHBG, testosterone, FSH, LH prolactin and TSH had to be in the normal range. A laparoscopic investigation must show normal fallopian tubes and peritoneal conditions. Post-coital test at the time of ovulation must show normal conditions.

Male parameters: Normal semen quality (> 20 million spermatozoa ml, > 50% motile motility grade 3 (scale 1-4), 50% morphologically normal spermatozoa, no detectable anti-sperm anti-

Table 1. — Fallopian tube sperm perfusion (FSP) or Intrauterine Insemination (IUI)?

Reference	Odds ratio of pregnancy (FSP vs. IUI) OR (95% confidence interval)
Kahn *et al.* 1993a*	4.0 (1,2 – 13,4)
Trout & Kemman (1999)	1.8 (1.2 – 2.9)
Cohrane 2004 (Cantineau *et al.* 2004)	1.9 (1.2 – 2.8)
Cohrane 2009(Cantineau *et al.* 2009)	1.2 (0.8 – 1.7)
Cohrane 2009**	2.0 (1.3 – 3.2)

*First study, not a Meta-analysis.
**excluding studies using a Foley catheter.

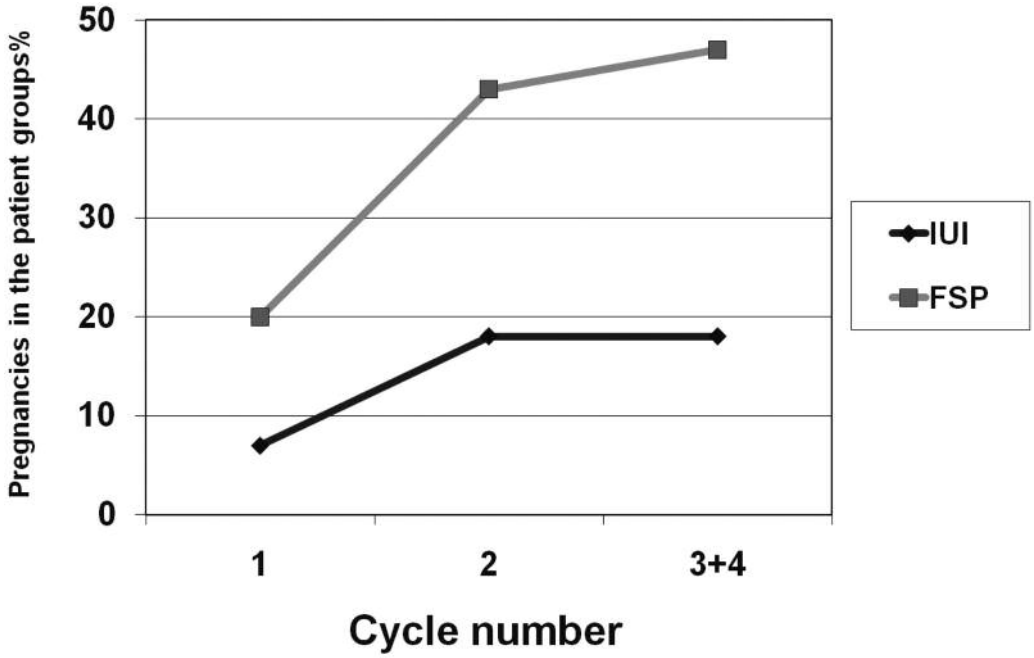

Fig. 1. — Cumulative pregnancy rate in the patient groups randomised either to intrauterine insemination (IUI) or to fallopian tube sperm perfusion (FSP).
Adapted from Kahn *et al.*, 1993a including unpublished data. No pregnancies occurred in the 5th or 6th treatment cycle.

bodies and a normal sperm penetration test. The couples were offered up to 4 treatment cycles.

Outcome in unexplained infertility

We observed a significant higher pregnancy rate both per cycle (26, 9 vs. 9, 8%) and per woman treated (46, 7 vs. 17, 9%) The difference between the groups were statistically significant ($p < 0.05$ χ^2 test, OR 4.0 (1.2 – 13.4) (Table 1 and Fig. 1) (Kahn *et al.*, 1993a).

Several other randomized studies have been done by other clinics with varying results inspiring research groups to do meta-analysis to decide whether the accumulated data were in favor of FSP or not. Trout and Kemman (1999) published in 1999 the first meta-analysis and this was followed in 2004 by a Cohrane analysis (Cantineau *et al.*, 2004). Both these studies concluded that FSP was more efficient that classical IUI (Table 1). The NICE clinical guidelines published in UK in 2004 then advocated the use of FSP in cases of unexplained infertility (NICE guidelines February 2004). Recently a new Cohrane analysis was performed including more studies, suggesting that FSP does not give higher pregnancy rates that classical IUI (Cantineau *et al.*, 2009). One of the new studies included in the recent Cohrane analysis is using a Foley catheter for performing FSP and obtained much better results with IUI than FSP (Biacchiardi *et al.*, 2004). It is a concern that Foley catheters seem to be associated with lower pregnancy rates in FSP (Cantineau *et al.*, 2004, 2009) Computing the Odds ratio of pregnancies omitting the studies using the Foley catheter give an OR of 2,0 (1.3 – 3.2, 95% confidence interval) in favor of FSP (Table 1).

FSP was our standard protocol for the treatment on unexplained infertility from 1990 to 2002. As a

Table 2. — Results obtained with FSP with fresh husbands semen (all indications) in the period 1990 to 2002.

	Numbers	Percentages
Number of started cycles	1340	
Number of cancellations	221*	16.4% of started cycles
Number of insemination	1005	
Number of pregnancies'	142	14.1% of inseminations
Number of deliveries	112	11.1% of inseminations
Number of multiple deliveries	6**	5.4% of births

*95 of these cycles converted to IVF-cycles.
**5 sets of twin one set of triplets.

routine, the patients were offered up to 3 cycles with FSP prior to entering the IVF-program. In this period 1340 cycles were started and 1005 inseminations done resulting in 112 deliveries (Table 2). In 2002, the reimbursement system changed allowing partial reimbursement of drug cost for up to three treatment cycles regardless of type of treatment. This change in the reimbursement system meant that patients rather would spend their "allotted three cycles" on IVF rather than on FSP.

Donor insemination

We tested whether FSP also could give good clinical results using frozen/thawed donor semen. In the first two treatment cycles, we observed a pregnancy rate of 34% and 25% respectively. The outcome was judged to be so favourable that FSP became the standard insemination protocol using donor semen. (Kahn *et al.*, 1992b). In the years 1990 to 2002, we performed in total 1200 donor inseminations using frozen semen with an average pregnancy rate per insemination of 27.8% (Table 3). ICSI was not allowed in Norway until 1996 and ICSI in combination with testicular or epididymal sperm was not allowed until 2004. The use of donor sperm in combination with IVF or ICSI was not allowed until 2004. Semen donors were anonymous in Norway until 2005 when a new law was passed banning anonymous donor insemination. At that time we stopped offering donor insemination.

Number of treatment cycles

We observed a clear relationship between the treatment cycle number and pregnancy rates (Fig. 2). A clear drop in pregnancy rates were observed after the first two treatment cycles and the pregnancy rates in cycles 4 and more were very low. We therefore routinely did not offer more that 3 FSP cycles in cases of unexplained infertility and rarely more that 4 cycles in cases where donor semen is used (Kahn *et al.*, 1992b, 1993b).

Variants of FSP

Since the seminal papers of Kahn *et al.* where published, several variants of FSP has been developed using different form of catheters, clamps and devices to perform the insemination itself including "The FAST System©" (Fanchin *et al.*, 1995), "The DNB speculum" (Mamas *et al.*, 1995) and a different device for blocking reflux (Ricci *et al.*, 2001).

Despite the fact that several randomized studies and ensuing meta-analysis presented data suggesting that FSP is more efficient than standard IUI, the method has not replaced the standard IUI-protocol. FSP is not more complicated to perform than IUI, it is well tolerated by the patients and there are not more side-effects or complications that IUI. A complicating factor in the evaluation of FSP is the fact that several and perhaps less efficient variants FSP have been developed by other research groups

Table 3. — Results obtained with FSP with frozen/thawed donor semen in the period 1990 to 2002.

	Numbers	Percentages
Number of started cycles	1316	
Number of cancellations	116	8.8% of started cycles
Number of insemination	1200	
Number of pregnancies'	333	27.8% of inseminations
Number of deliveries	226	18.8% of inseminations
Number of twin deliveries	36	15.9% of births

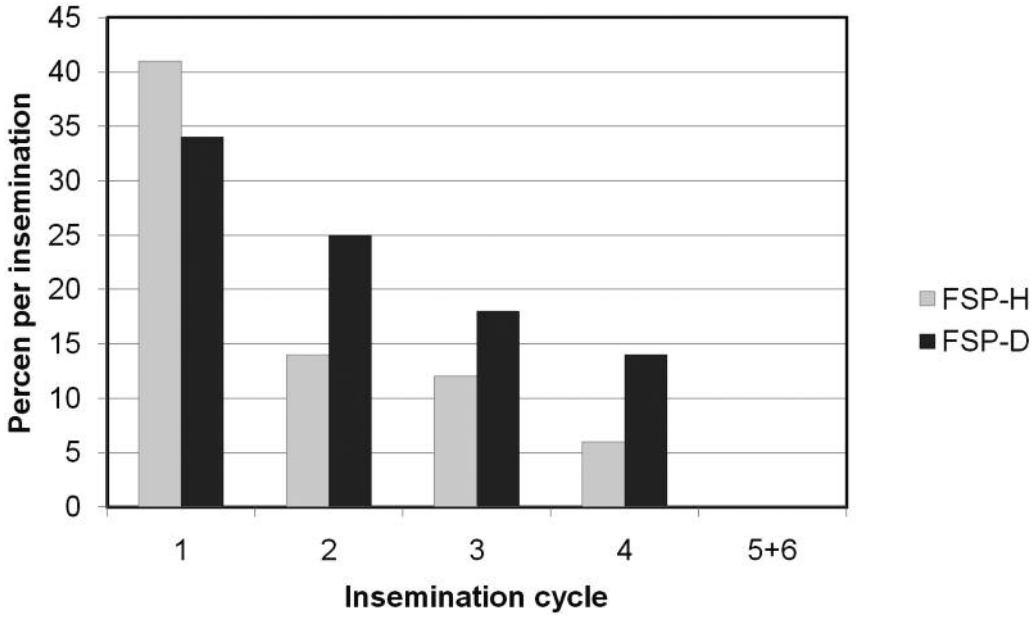

Fig. 2. — Pregnancy rate per insemination versus treatment cycle number, in inseminations with husband's semen (FSP-H) or with frozen donor semen (FSP-H).
Adapted from Kahn *et al.*, 1992b and 1993 b.

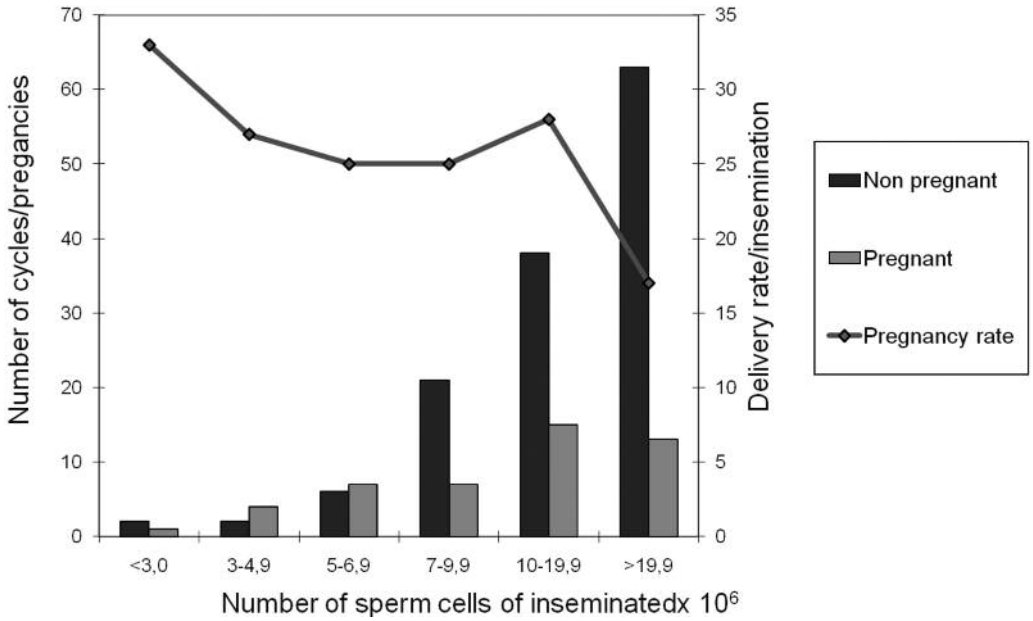

Fig. 3. — Fallopian tube sperm perfusion and male factor. Number of inseminations and number of pregnancies according to the total number of spermatozoa in the inseminate.
Adapted from Strandell *et al.*, 2003.

(Cantineau *et al.*, 2009). One puzzling finding is that two studies using the Foley catheter both reports that IUI clearly gives better results that FSP (Biacchiardi *et al.*, 2004; Nuojua-Huttunen, 1997). This is in contrast to most other published studies where pregnancy rates obtained with FSP was at least as good as, if not better, that the pregnancy rates obtained with IUI. Whether this observation warrants a clear advice not to use the Foley catheter for FSP is still unclear (Cantineau *et al.*, 2004, 2009).

Summary

Several published studies have tried to elucidate whether FSP or IUI is gives highest pregnancy rates in the treatment of non tubal/unexplained infertility. Most of these studies are rather small. A complicating factor is that, in these studies, different protocols, different utensils and different catheters for performing FSP have been used. This may well have influenced the outcome of the studies and

contributed to the fact that currently there is no consensus on whether FSP or IUI is to be advocated. A meta-analysis excluding studies using the Foley catheter for insemination gives however a significant higher pregnancy rate using FSP compared to standard IUI.

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The effect of age on the outcome of intrauterine insemination: A review

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Abstract

Aim: Postponing childbearing has led to an increasing demand for treatments due to age-related subfertility. While intrauterine insemination (IUI) is widely used for different indications, here too the age of women in need of this treatment is increasing. Success rates after IUI in relationship to age are often expressed per cycle, but for patient counselling, cumulative delivery rates are to be preferred. We reviewed the literature to assess the impact of age on IUI, and the role of ovarian hyperstimulation in improving the success rates.
Methods: A review of the literature was performed using Medline. We used the UK National Health Service levels of evidence (and grades of recommendation) to categorise all studies.
Results: Overall, controlled ovarian stimulation (COH) resulted in significantly higher live-birth rates when compared to IUI in natural cycle. Since age was not always taken into consideration in many studies, differences among them may account for inconsistent conclusions. For older women aged > 37 years, natural cycle IUI resulted in a significantly higher pregnancy rate and live birth rate than IUI with COH. In women aged 37 and younger, COH resulted in a significantly higher pregnancy rate and live-birth rate compared to natural cycle IUI ($p = 0.025$).
Conclusions: Above the age of 37, succes rates decline, but despite this fact, women up to 40 years, may be encouraged to continue IUI treatment, in case of donor insemination even up to 42 years of age. Overall, superovulation with IUI yields better results than unstimulated IUI, but not in women over 37 years who may rather benefit from natural cycle IUI. When the female partner is older than 35 years, a synergistic adverse effect of paternal age has been reported.

Key words: Male age, female age, succes rates, clomiphene citrate, gonadotrophins, natural cycle.

Introduction

From the sixties onwards, the large-scale availability of reliable methods for contraception has greatly influenced the number of children born per family in Western societies (Broekmans *et al.*, 2007). In the developed world, postponement of marriage and childbearing resulted in unprecedented numbers of couples who desire pregnancy at a relatively later stage in life. This is a social trend, which has led to an increase in treatments for age-related female subfertility (te Velde *et al.*, 2002; Broekmans *et al.*, 2007). By postponing childbearing, a growing proportion of women attempting to conceive will fail in achieving this goal within a time frame of 12 months, a condition referred to as subfertility (Evers *et al.*, 2002). An increasing number of couples will depend on ART to achieve a pregnancy, solely on the basis of postponing childbearing. Unfortunately, there is an age-related decrease in success rates of ART treatments (van Noord-Zaadstra *et al.*, 1991).

Patients undergoing fertility treatment need to be clearly informed about their chances of having a baby. Therefore, it is important for both the candidate couples and the fertility specialists who treat them to know the probability for pregnancy and delivery after a defined number of treatment cycles. There have been many reports about the success rates per cycle, but cumulative delivery rates in different age groups for artificial insemination on large patient populations are scarce. Especially for older

Table 1. — Level of evidence and grade of recommendation.

Grade of recommendation	Level of evidence	
A	Level 1a:	Systematic review of randomised, controlled clinical trials (RCT)
	Level 1b:	Individual randomised controlled trial
	Level 1c:	All or none
B	Level 2a:	Systematic review of cohort studies
	Level 2b:	Individual cohort study (including low-quality RCT)
	Level 2c:	Outcomes research
	Level 3a:	Systematic review of case-control studies
	Level 3b:	Individual case-control study
C	Level 4:	Case series, poor quality cohort and case control studies and review of poor quality
D	Level 5:	Expert opinion without explicit critical apparaisal

patients, these data are very important. Therefore, the aim of this review is to overview the impact of age on the outcome of intrauterine insemination (IUI) and to analyze whether ovarian superovulation may have any benefit on the success rates.

Methods

Systematic review of the literature was performed on Medline for each topic. We used the UK National Health Service levels of evidence (and grades of recommendation) to categorise all studies (see Table 1).

Age effect on IUI using Husband's sperm

As shown in Table 2, our literature search revealed only one level 1 study (1b, grade A of recommendation) dealing with the age impact on IUI outcome: Goverde *et al.* (2000) wrote a prospective, randomized, parallel trial. The authors stated in their paper that women's age is the most important factor influencing the likelihood of pregnancy, whatever treatment is chosen (IUI or IVF) and that the indication, i.e. male factor or unexplained infertility, had no impact at all. A mean pregnancy rate per cycle as a function of women's age in fictional groups of 100 patients in each age category was calculated and more important, the authors investigated the cumulative delivery rates after six cycles of IUI.

All other studies retrieved on age impact on IUI were retrospective studies (level 2b of evidence and less). We will briefly discuss some of them but refer to Table 2 for specific details.

In the retrospective cohort study of Merviel *et al.* (2008) (level 2b), the woman's age significantly influenced the ongoing pregnancy rate per couple, i.e. 38.5% under 30 years old versus 12.5% for over 40 years old. Frederick *et al.* (1994) performed a retrospective case-control study (level 3a, grade B of recommendation) in which IUI results for women aged 40 years were compared with results obtained for younger women. Because of very poor success rates in women over 40 years of age, they suggested that IUI should not be used in women aged over 40 years and that a prompt recommendation for IVF should be made soon after the failure of a few attempted cycles of IUI combined with ovarian stimulation treatment.

Surprisingly, Ibérico *et al.* (2004) reported in their cross-sectional multivariate retrospective review (level 2b) lower pregnancy rates among women aged 30-39 years compared to women younger than 30 years of age, while no decline in pregnancy rates was found among women 40-43 years old. Overall, female age was not siginificantly associated with pregnancy rates (P = .18). These results are in contradiction with most trials published in the literature, so the authors suggest further research.

Other retrospective studies show similar pregnancy results (Nuojua-Huttunen *et al.* 1999 (level 2b), Bronte *et al.* 1999 (level 4), Belloc *et al.* 2008, (level 2b)). The number of patients was not reported in these papers and received therefore a grade C of recommendation. Bronte *et al.* (1999) reported an age-related difference in the pregnancy rate per cycle but the authors failed to provide cumulative delivery rates. Nuojua-Huttunen *et al.* (1999) and Belloc *et al.* (2008) had similar conclusions, and both achieved to report cumulative pregnancy rates. In 2002 a retrospective chart review (level 2b) was published to determine the age-related decline in live birth rates after insemination (Haebe *et al.*, 2002). The authors performed a retrospective analysis of 1117 IUI cycles in women age 40 years or more, resulting in an overall pregnancy rate of 19,4%. The

Table 2. — Overview of studies describing impact of age on success rates after IUI

Reference	Study sample (n)	Study design analysis	Level of evidence	Grade of recommendation	Number of IUI cycles (n = number of patients)	Pregnancy rates (%)	Delivery rates (%)	Cumulative delivery rates (%)	Miscarriage rates (%)	Results
IUI using husband's sperm										
Goverde *et al.* 2000	258 couples were treated either IUI in spontaneous cycle, either IUI after mild ovarian stimulation or IVF.	Prospective randomized parallel controlled trial	1b	A	693 cycles (n = 258)	7.4% per cycle (IUI alone) 8.7% per cycle (IUI with stimulation) (12.2% per cycle (IVF))	Not analysed	31% (IUI alone, max. 6 cycles) 37% (IUI with stimulation) (38% (IVF)	Not analysed	Couples with idiopathic or male subfertility have the same likelihood of successful pregnancy as IVF. The woman's age was the only factor that influenced a couple's chance of success.
Iberico *et al.* 2004	To identify predictors of pregnancy rate (PR) in 470 women	Cross-sectional, retrospective	2b	B	1010 (n = 470)	Overall 9.2% per cycle * 30-34 years (n = 537): 7.6% per cycle * 40-43 years (n = 49): 12.2% per cycle	Not analysed	Not analysed	Overall 11.8%	Female age was not significantly associated with PR in multivariate analysis (P = .18). The authors suggest further research.
Merviel *et al.* (2008),	Determination of predictive factors for pregnancy after IUI in 353 couples	Retrospective cohort trial	2b	B	1038 (n = 353)	* overall 14.7% ongoing PR per cycle	Not analysed	Ongoing pregnancy rate /couple (6cycles) * < = 30: 38.5% * 31-35: 31.7% * 36-40: 26.3% * > 40: 12.5%	* overall 20.3%	Women's age was the strongest predictor of success in all indications, with an ongoing pregnancy rate per couple of 38.5% for the under 30s and 12.5% for the over 40s (P < .000001).
Frederick *et al.*, 1994	Conception rate in 77 women who were > or = 40 years old and who received ovarian stimulation treatment and IUI	Retrospective case-control study	3b	B	210 (n = 77)	14% per patient and 5% per cycle	3.9% per patient and 1.4% per cycle	Not analysed	73% rate of miscarriage (cumulative)	This report compares IUI results for women > or = 40 years with those obtained previously for younger women, and shows the very poor success rate in women > 40 years of age.
Nuojua-Huttunen *et al.* (1999)	Analysis of factors affecting outcome after IUI treatment.	Retrospective cohort	2b	C	811 (n = unknown, median female age was 32)	* overall: 12.6% per cycle * < 40 years: 13.7% per cycle * > 40 years: 4.1% per cycle	* > 40 years (n = 98): 3.1%	* overall 70.6% cumulative pregnancy rate after six cycles.	* overall 23.5% spontaneous abortion * overall 5.9% ectopic pregnancy	Pregnancy rate in women < 40 years was significantly higher than in older women.
Haebe *et al.* 2002	Pregnancy rates and life birth rates per insemination cycles according to female age.	Retrospective cohort	2b	C	1117 (n = unknown)	Overall 19.4% per cycle	* overall 12.9% per IUI cycle * 40-42 years (n = 82) 9,8% per cycle * 43 years or more (n = 24) limited to 4.2%	Not analysed	Miscarriage rate per insemination (%) * < 25 y = 20.0 % * 25-29 y = 29.6% * 30-35 y = 35.5% * 36-39 y = 46.2 % * > = 40 y = 52.6 %	These results may demonstrate that IUI is an appropriate treatment for women 40-42 years old.

Belloc *et al.* (2008)	Effect of maternal and paternal age on pregnancy and miscarriage rates after IUI (n= unknown)	Retrospective	2b	C	10034 (n = unknown)	* <30 y: 14.4% per cycle * 30-34 y: 14.5% per cycle * 35-37 y: 12.6% per cycle * 38-41 y: 10% per cycle * > 42 y: 8.9% per cycle	Not analysed	Overall 64% cumulative pregnancy rates after 6 cycles.	* <30 y: 11.1% per cycle * 30-34y: 16.1% per cycle * 35-37 y: 21.6 % per cycle * 38-41 y: 28.3 % per cycle * > 42 y: 46.4 % per cycle	Pregnancy rate decrease with maternal and parental age.
Bronte et al (1999)	To determine which factors influence the effectiveness of IUI.	Retrospective	4	C	9963 (approx. 3200 patients)	* < 26 y: 18.9% per cycle * 26-30 y: 13.9% per cycle * 31-35 y: 12.4% per cycle * 36-40 y: 11.1% per cycle * 41-45 y: 4.7% per cycle, > 45 years old: 0.5% per cycle	Not analysed	Not analysed	Not reported	Overall pregnancy rates have increased from 5.8% per cycle in 1991 to 13.4% per cycle in 1996, during which time the average of patients undergoing intrauterine insemination has increased from 36.1 to 39.2 years.
Donor sperm										
De Brucker *et al.* (2009)	Analysis of cumulative delivery rates in different ag group after IUI with donor sperm (n = 1654)	Retrospective cohort trial	2b	B	6630	Not analysed	14 % per cycle	Expected cumulative delivery rates after 12 cycles: * 20-29 years: 87% * 30-34 years: 77% * 35-37 years: 76% * 38-39 years: 66% * 40-45 years: 52%	Miscarriage rate after six cycles * 20-29 years: 6.4% * 30-34 years: 8.0% * 35-37 years: 10.4% * 38-39 years: 20.8% * 40-45 years: 31.9%	Acceptable cumulative delivery rates have been reached up until 42 years of age.
Botchan *et al.* (2001)	Results of 18 years of IUI with donor sperm in 1001 women	Retrospective cohort trial	2b	B	6139	12.6% per cycle	Not analysed	Cumulative PR calculated for 3, 6 and 12 months were 36, 53 and 75%. CPR according to age were: (i) * < 37 years: 44, 61 and 82% *37-40 years: 24, 44 and 61% * > 40 years: 13, 22 and 50%	Miscarriage rate (undefined number of cycles per age group) * <37 years: 14.5%, * 37-40 years: 22.3% * > 40 years: 42.9%	Age was found to be a major determinant for success rates. Satisfying cumulative pregnancy rates have been reached even in women older than 40.
Schwartz *et al.*,1982	Report on 2193 nulliparous women who were receiving IUI with donor sperm.	Retrospective cohort trial	2b	B	n = 2193	Mean pregnancy rate per cycle. * < 25 y: 11% * 26-30 y: 10.5% * 31-35 y: 9.1% * > 35 y: 6.5%	Not analysed	Cumulative pregnancy rates after 12 cycles. * < 25 y: 73% * 26-30 y: 74% * 31-35 y: 61% * > 35 y: 54%	Not analysed	Cumulative pregnancy rates after 12 cycles revealed acceptable success rates in all 4 age groups.

number of patients included was not reported. These results may demonstrate that IUI is an appropriate treatment for women 40-42 years old.

Nearly all level 2, 3 and level 4 studies report results as pregnancy rate per cycle. The only level 1 study (Goverde *et al.*, 2000) expressed results as cumulative delivery rates. Overall, a sharp decline of IUI success rate after the age of 40 is observed in almost all studies, irrespective of their quality.

Age effect on IUI using donor sperm

In the literature only limited data are available on the age effect on intrauterine insemination with donor sperm. Only few retrospective studies have been published. In total we found 4 studies assessing the age effect on IUI success rates when donor sperm is used. Providing an accurate answer about the success rates for older women remains thus hampered by a lack of power associated to the small numbers included and the limited number of deliveries included.

De Brucker *et al.* (2009) report on the largest retrospective cohort analysis (level 2b of evidence, level B of recommendation) to calculate cumulative delivery rates after 6630 donor insemination cycles in 1654 women. Overall, a delivery rate of 14% per cycle with an expected cumulative delivery of 77% after 12 cycles was reached. Subgroup analysis in the oldest subgroup (40-45 years; $n = 223$ patients) revealed an expected cumulative delivery after 12 cycles of 55% for women aged 40, 58% for those aged 41, 32% for those aged 42 and 20% for those over 43 years. Dropout analysis in the latter subgroup showed that only one patient discontinued treatment because of medical reasons. In contrast to age, neither indication nor ovarian stimulation protocol had any significant effect on the delivery rates.

Botchan *et al.* (2001) and Schwartz *et al.* (1982), both level 2b of evidence, reported results only on the cumulative pregnancy rates but not on the cumulative delivery rates. A statistically significant, age-related decline in pregnancy rates was found ($P < 0.0001$) in Botchan *et al.* As shown in Table 2, these results were more pronounced when subdividing the population into three groups: age < 37, 37-40 and > 40 years. Schwartz D *et al.* (1982) reported on 2193 women undergoing IUI in the period 1973-1980 at the Centres d'Etude et de Conservation du Sperme Humain (CECOS). The women were divided into 4 age subgroups. Overall, their study shows that a decrease in pregnancy rate per cycle as a function of a woman's age is present and significant after 30 years of age but only relevant after 35 years.

The three level 2b studies show satisfactory success rates in agreement with the large study from De Brucker *et al.* (2009) and we should advise our patients to continue IUI treatment up to the age of 42.

Has controlled ovarian superovulation any benefit to counteract the age effect?

In the literature, two systematic reviews (Cohlen *et al.*, 2005, Hughes *et al.*, 1997) indicate a benefit for superovulation in combination with IUI in patients with unexplained infertility.

Two level 1b studies were published drawing the same conclusion as mentioned in both systematic reviews (Guzick *et al.*, 1999; Goverde *et al.*, 2000,). Studies dealing with IUI in combination with superovulation and the impact of age are scarce and only of level 2b or less. An overview of the results of these studies can be found in Table 3.

Merviel *et al.* (2008) determined pregnancy rates and ongoing pregnancy rates in 353 couples in a retrospective cohort trial (level 2b, Table 3). All cycles were combined with gonadotrophic ovarian stimulation. Acceptable succes rates have been shown in all age groups. Harris *et al.* (2009) (level 2b) performed a small retrospective study in women aged > 38 years to determine the succes rates in gonadotrophin-induced controlled ovarian stimulation (COH) in conjunction with IUI (COH/IUI). Poor prognosis was demonstrated in these older women. *Corsan et al.* (1996) (level 3b) reviewed retrospectively data, from their ovulation induction with IUI programme for patients aged > = 40 years. In women aged > = 40 years, ovulation induction with IUI is most likely to result in successfull pregnancy in women 40-42 years of age. Women > = 43 years should consider other alternatives such as adoption or egg donation.

Although COH in combination with IUI has been shown to result in significantly higher pregnancy rate compared to unstimulated (natural cycle) IUI in some papers (Guzick *et al.*, 1999, level 4 of evidence, retrospective case serie), Kalu *et al.* (2007), level 2b of evidence study, wanted to assess the effect of age on each. Age is an important confounding factor that may affect the outcome of stimulated or unstimulated IUI. This important factor was not always taken into account. In their retrospective cohort paper on 1759 IUI cycles, women age 37 and younger, COH resulted in significantly higher pregnancy rate and live-birth rate compared to natural cycle IUI. However for older women age > 37 years, natural cycle resulted in a significantly higher pregnancy rate and live birth rate than IUI with COH. This difference was even more significant when COH was performed with CC. Thus older women may benefit from natural cycle IUI. See table 3 for

Table 3. — Superovulation in older women. Overview of all level 1, level 2 and level 3 of evidence studies

Reference	Study sample(no.)	Study design analysis	Level of evidence	Grade of recommendation	Number of IUI cycles	Pregnancy rates (%)	Delivery rates (%)	Cumulative delivery rates (%)	Miscarriage rates (%)	Results
Level 1 of evidence										
Goverde *et al.* 2000	258 couples were treated either IUI in spontaneous cycle, either IUI after mild overian stimulation or IVF.	Prospective randomised parallel controlled trial.	1b	A	693	7.4% per cycle (IUI alone) 8.7% per cycle (IUI with stimulation) (12.2% per cycle (IVF))	Not analysed	31% (IUI alone, max. 6 cycles) 37% (IUI with stimulation) (38% (IVF))	Not analysed	Couples with idiopathic or male subfertility have the same likelihood of successful pregnancy as IVF. The woman's age was the only factor that influenced a couple's chance of success. IUI with stimulation gave better success rates than IUI alone.
Guzick *et al.* 1999	932 couples were randomly assigned to receive intracervical insemination, IUI, superovulation and intracervical insemination, or superovulation and IUI. Treatment continued for four cycles un- less pregnancy was achieved.	Prospective randomized controlled trial.	1b	A	4676	* Intracervical insemination: 10% PR/couple * IUI: 18% PR/couple * Superovulation and intracervical insemination: 19% PR/couple * Superovulation and IUI: 33% PR/couple	Not analysed	Not analysed	Not analyse	Among infertile couples, treatment with induction of superovulation and IUI is 3 times as likely to result in pregnancy as intracervical insemination and twice as likely to result in pregnancy as with either superovulation and intracervical insemination or IUI alone.
Level 2 and level 3 of evidence										
Merviel *et al.* (2008),	Determination of predictive factors for pregnancy after IUI in 353 couples	Retrospective cohort trial	2b	B	1038 (n=353)	* overall 14.7% ongoing PR per cycle	Not analysed	Ongoing pregnancy rate /couple (6cycles) < = 30: 38.5% 31-35: 31.7% 36-40: 26.3% > 40: 12.5%	* overall 20.3%	Women's age was the strongest predictor of success in all indications, with an ongoing pregnancy rate per couple of 38.5% for the under 30s and 12.5% for the over 40s (P < .000001).
Corsan *et al.* (1996)	Analysis of ovulation induction in women > 40 (n = 168)in comparison with an randomly selected group of patients < 40 (210 cycles, n = 180)	Retrospective case-control	3b	C	679	* women 40, 41 and 42 y: 13.3%, 7.9% and 4.8% pregnancy rate per cycle * women < 40 y: 17.95 % PR/cycle	* women 40, 41 and 42 y: 9.6%, 5.2% and 2.4% delivery rate per cycle * women above 43: no delivery rates were reported	Not analysed	* women > 40 y: 34.4% MR/cycle. * women <40 y: 17.14% MR/cycle	When utilized in women aged > = 40 years, ovulation induction with IUI is most likely to result in successfull pregnancy in women 40-42 years of age.

Harris *et al.*, 2009	To determine the success rates and utility of controlled ovarian hyperstimulation in conjunction with intrauterine insemination (COH/IUI) cycles in 130 women aged 38-39 years versus women >/= 40 years old. 1-3 cycles per patient were performed.	Retrospective cohort	2b	B	262 (n = 130) (*38-39 years (n = 42.6%)) (*>= 40 years (n = 57.4%))	* 38-39 (42.6%) years: 9,0 % per cycle * >= 40 (57.4%) years: 7,8% per cycle	* 38-39 (42.6%) years: 6.1 % per cycle * > = 40 (57.4%) years: 2.0% per cycle	Cumulative live birth rates after 3 cycles. * 38-39 years: 12.3% * > = 40 years: 4.1%	Overall 23.5% per cycle	The efficacy of COH/IUI cycles significantly decreases with age, but women aged 38-39 years had reasonable success during the first two cycles. However, for women aged >/= 40 years, no benefit after a single cycle of COH/IUI was observed. Women aged >/= 40 years should be considered for in vitro fertilization after one failed COH/IUI cycle.
Kalu *et al.*, 2007	Analysis of the outcome of IUI with COH and IUI in natural cycle(unstimulated) in women age < 37 and women age > 37.	Retrospective , cohort	2b	B	1759	In women age 37 and younger, COH resulted in a significantly higher pregnancy rate (13.0% vs 6.5%) compared to natural cycle IUI (p = 0.025) * For older women age > 37 years, natural cycle IUI, resulted in a significantly higher pregnancy rate (12.0% vs 8.5%) than IUI with COH (p = 0.0037).	In women age 37 and younger, COH resulted in a significantly higher live-birth rate (10.7% vs 5.2%) compared to natural cycle IUI (p = 0.045). * For older women age >37 years, natural cycle IUI, resulted in a significantly higher live-birth rate (7.5% vs 3.5%) than IUI with COH (p = 0.0037).	Not analysed	Not analysed	Older women (> 37 y) have a better outcome from unstimulated IUI cycle.

detailed results of this study. Why natural cycle IUI is associated with better outcome in older women is not entirely clear. As the ovarian pool diminishes with age, a dominant follicle selected naturally may be the best in the cohort, hence increasing the likelihood of fertilisation and implantation. COH may encourage the recruitment of suboptimal follicles that may otherwise not have developed.

In addition, a study of Brzechffa *et al.* (1997), level 2b, retrospective cohort trial, examined the influence of female and male patient age and human menopausal gonadotrophin (hMG) on clinical pregnancy rates and live birth rates. This study demonstrated a critical threshold of hMG requirements beyond which pregnancy did not occur. No pregnancies occured in treatment cycles requiring > 25 ampoules (1875 IU) of menotrophins, independent of female partner age. Furthermore in treatment cycles in which female partner was aged > 40 years no pregnancies occurred when > 20 ampoules (1500 IU) were necessary to achieve follicular maturity. It has to be mentioned that a combination of clomiphene citrate with hMG or FSH (with equivalent results to the use of gonadotropin alone) results in a lower total dose of gonadotropins (Dickey *et al.*, 1993).

And what about male ageing and IUI outcome?

It is well known that virtually no children are born to mothers aged more than 45 years. More and more men of older age will father children with younger partners. (Kühnert and Nieschlag, 2004). The discrepancy in the reproductive span between males and females is astonishing and reduced fertility and higher reproductive risks associated with advancing maternal age prompt the question whether advanced paternal age too is associated with compromised fertility and such increasing risks. A review on this topic was recently written for the Royal College of Obstetricians and Gynaecologists (Tournaye, 2009).

In contrast to female fertility, male fertility is maintained until very late in life, and, in addition to anecdotal reports, it has been documented scientifically up to an age of 94 years (Seymour *et al.*, 1935). Age-dependent decrease of fertility in couples are usually attributed to female ageing, make studies on a male age effect difficult. Besides female age, further confounders, such as reduced coital frequency, an increasing incidence of erectile dysfunction and smoking habits, have to be considered. For natural conception, paternal age has no profound effect whenever the female partner is young. Time to pregnancy is longer than compared to younger men. However, when the female partner too is of age, then a synergistic adverse effect of paternal age has been reported in a large retrospective multicentre study (de la Rochebrochard *et al.*, 2003).

Only limited data are available about male aging and IUI success rates. By analyzing data from intrauterine artificial insemination with the husband's spermatozoa, Mathieu *et al.* (1995), retrospective (multivariate analysis) concluded that paternal age over 35 years was an important predictive factor of success, after controlling for maternal age. Brzechffa and Buyalos (1997), analyzed in a retrospective cohort trial (level 2b) 184 patients to assess the male impact of male ageing on IUI combined with hMG. The clinical pregnancy rate was significantly lower in males aged 40 years or older compared with males aged less than 40 years whose female partners were 35 years or more (16.3% versus 6.5%, P = 0,02). No differences were observed in the clinical pregnancy rates in treatment cycles in which males aged 40 years or older were compared with males aged less than 40 years whose female partners were aged less than 35 years (P = 0,81).

Belloc *et al.* (2008) (level 2 b study) found that paternal age led to a decrease in the pregnancy rate from 12.3% before 30 years of age to 9,3% after 42 years of age (P < 0,0001) and a doubling of the miscarriage rate from 13.7% before 30 years of age, to 32.4% after 45 years of age, and this independently of women's age. The impact of increased age on necrozoospermia and sperm-DNA structure is a probable direct cause of this paternal effect, but further research about this topic is needed.

Conclusion

A female age effect on the success rates after IUI is evident: bove the age of 37, success rates decline, but despite this fact, women up to 40 years, may be encouraged to continue IUI treatment, in case of donor insemination even up to 42 years of age. The adjuvant role of superovulation to counteract the adverse age-effect on IUI results remains unclear since age was not always taken into consideration in many studies and methodological differences among them may account for inconsistent conclusions. When the female partner is older than 35 years, a possible synergistic adverse effect of paternal age too has been reported.

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Improving intrauterine insemination pregnancy outcomes by enhancing sperm motility with platelet activating factor

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Abstract

Ten to 15% of reproductive age couples in the United States are not able to achieve a successful pregnancy and are considered infertile. Infertility affects men and women equally. Male fertility requires the production of an adequate number of morphologically normal spermatozoa with sufficient motility and the ability to undergo hyperactivation, capacitation, and the acrosome reaction in order to penetrate the oocyte's cumulus oophorus and bind to the zona pellucida for fertilization. Defects in any of these necessary events will lead to infertility. A number of endogenous factors are implicated in the regulation of spermatozoan fertility potential, including platelet-activating factor (PAF). PAF clearly plays a significant role in reproductive physiology. It influences ovulation, fertilization, preimplantation embryo development, implantation, and parturition. Exogenous PAF has been used to promote sperm motility, sperm capacitation and the acrosome reaction. Finally, the addition of PAF to sperm wash for use in an intrauterine insemination program results in significantly higher pregnancy rates.

Key words: Sperm, platelet activating factor, sperm wash, intrauterine insemination, pregnancy.

In many fertility centers drug induced ovulation induction (OI) followed by intrauterine insemination (IUI) has become standard therapy for non-tubal factor related infertility. As the initial treatment for infertile couples, IUI is commonly used and is a cost effective procedure. The success of IUI is dependent on the ovulation induction regimen as well as seminal parameters. Cumulative pregnancy rates for three cycles have approached those of a single IVF treatment when exogenous gonadotropins are combined with IUI.

Spermatozoa prior to any assisted reproductive technique (e.g. intrauterine insemination) must be first processed (e.g. density/gradient wash or swim-up). This processing of spermatozoa insures that the most normal motile population is used to help facilitate conception. For IUI procedures, semen washing is important as it removes prostaglandins, debris and white blood cells while also reducing the number of non-motile and morphologically abnormal sperm cells. Additionally, removal of seminal plasma also benefits the sperm by enhancing capacitating conditions-resulting in the enhancement of sperm motility. A number of additives which improve sperm motility have been investigated, including but not limited: cAMP, human Follicular fluid, xanthine, caffeine, pentoxifyline and platelet activating factor (PAF). This review will focus only on PAF, primarily due to the author's experience, but also in that an advantage that PAF is a natural substance and is non-toxic as is many other motility stimulators (Henkel and Schill, 2003).

Platelet-activating factor (PAF; 1-O-alkyl-2-Oacetyl-sn-glycero-3-phosphorylcholine) has attracted increasing interest as one of the more important signaling mediators since it was first discovered more than 30 years ago on the discovery that PAF mediated rabbit platelet aggregation in immunoglobulin E–stimulated basophils (Benveniste *et al.*, 1992). Since then, numerous investigators have demonstrated that PAF is a unique signaling phospholipid that has pleiotropic biologic properties in addition to platelet activation (Hanahan, 1986; Braquet *et al.*, 1987). PAF clearly plays a significant

role in reproductive physiology. It influences ovulation, fertilization, preimplantation embryo development, implantation, and parturition (Harper, 1989). PAF exists endogenously as a mixture of molecular species with structural variants of the alkyl moiety, with the C-16 species predominating in human sperm (Sanwick *et al.*, 1992). Although the exact mechanisms of PAF action remain unclear, the importance of PAF for normal reproductive function is clear. Ultimately, PAF may provide us with a biomarker for normal sperm function.

Phospholipase A2 is present in human sperm. It is calcium dependent and catalyzes the formation of 1-alkyl-2-lyso-sn-glycero-3-phosphocholine (lyso-PAF, which is biologically inactive) from alkyl-acylglycerophosphocholine, an inert structural cellular membrane component (Bennet *et al.*, 1987). Lyso-PAF is then acetylated by acetyl transferase using acetyl-CoA as an acetate donor to form PAF. Lyso-PAF may also be acetylated by a CoA-independent arachidonyltransacylase to form alkyl-acyl-glycerophosphocholine. Acetylhydrolase is the primary enzyme responsible for inactivating PAF by the removal of the acetate group from the sn-2 position, resulting in the reformation of lyso-PAF. Acetyltransferase and acetylhydrolase are both present in mammalian sperm and seminal fluid (Gujarit *et al.*, 1987). Consequently, both the enzymes necessary for PAF activation and deactivation are present in sperm. Acetylhydrolase may itself act as a sperm decapacitation factor (Letendre *et al.*, 1992). In fact, the data suggest that the elimination of acetylhydrolase during normal capacitation promotes PAF synthesis, which results in increased sperm motility and improved sperm–egg interactions (Hellstrom *et al.*, 1991; Roudebush *et al.*, 1990; Angle *et al.*, 1993; Roudebush *et al.*, 1993). PAF may indeed be a biomarker for capacitation, and PAF-hydrolase as a decapacitation factor (Zhu *et al.*, 2004).

PAF is localized to the sperm and is not present in seminal secretions (Kumar *et al.*, 1988; Parks *et al.*, 1990). PAF is found in the sperm of many mammalian species including the rabbit, mouse, bull, and boar (Kuzan *et al.*, 1990; Kumar *et al.*, 1988; Parks *et al.*, 1990; Mook *et al.*, 1998). PAF and its receptor are also present in various primate species (Roudebush *et al.*, 1998; Roudebush *et al.*, 2001; Roudebush *et al.*, 2002). Finally, PAF is present in human sperm (Minhas *et al.*, 1991).

PAF metabolism is affected by androgens, estrogens, and progesterone (Mguruma *et al.*, 1993; Ohshihe *et al.*, 1994). Androgenic hormones play an important role in male fertility and are decreased significantly during the nonbreeding season. PAF concentrations in rhesus monkey *(Macaca mulatto)* sperm are directly correlated with sperm motility and forward progression (Roudebush *et al.*, 2002). The concentration of PAF in human sperm originally was found to be inversely related to sperm quality (Angle *et al.*, 1991). However, Roudebush and Purnell (2002) reported that the PAF content in human sperm, processed for use for in vitro fertilization (IVF), has a positive correlation with motility indices and pregnancy rates.

The addition of PAF to human sperm increases sperm motility, enhances sperm penetration of cervical mucus, and improves sperm penetration assay results (Naz and Minhas, 1995). To fertilize an oocyte successfully, sperm must be able to penetrate the outer layers investing the oocyte, including the cumulus cells and the zona pellucida. Many investigators have examined the effect of exogenous PAF on human sperm motility (Ricker *et al.*, 1989; Jarvi *et al.*, 1993; Krausz *et al.*, 1994). Exposure of sperm to PAF significantly improves sperm motility. Incubation of human sperm with PAF causes an increase in the number of spermatozoa that undergo the acrosome reaction (Karusz *et al.*, 1994; Lee *et al.*, 1997). PAF-treated spermatozoa fertilized oocytes at a higher rate than those treated with lyso-PAF (Fukuda *et al.*, 1994). A higher rate of blastocyst formation was also noted in embryos that resulted from PAF-treated spermatozoa. The formation of the pronuclei reportedly took place at a faster rate in oocytes that were injected with sperm, which were induced to undergo the acrosome reaction as compared with spermatozoa in which the acrosome was intact (Fukuda *et al.*, 1994).

Human sperm prepared for intrauterine insemination using a sperm wash medium supplanted with PAF results in significantly increased pregnancy rates (Roudebush *et al.*, 2004; Fig. 1). This finding has been subsequently verified by others and thus confirms our original results demonstrating the effectiveness of exogenous PAF supplementation in an IUI program (Grigoriou *et al.*, 2005; Fig. 1). It should be noted that in all studies the significant improvement in IUI pregnancy rates could only be observed in patients presenting with a normal semen analysis (Baka *et al.*, 2009). Treatment of sperm in male factor patients with PAF showed an increase in pregnancy rates, albeit not statistically significant. It is possible that sperm in these individuals are incapable of responding to the exogenous PAF due to poor PAF receptor levels and, or faulty PAF receptors in the sperm. We have recently reported on the presence and distribution of the PAF receptor in human sperm and our preliminary data demonstrate that distribution of the receptor is significantly altered in abnormal sperm (Roudebush *et al.*, 2000; Purnell and Roudebush, 2001). We have also

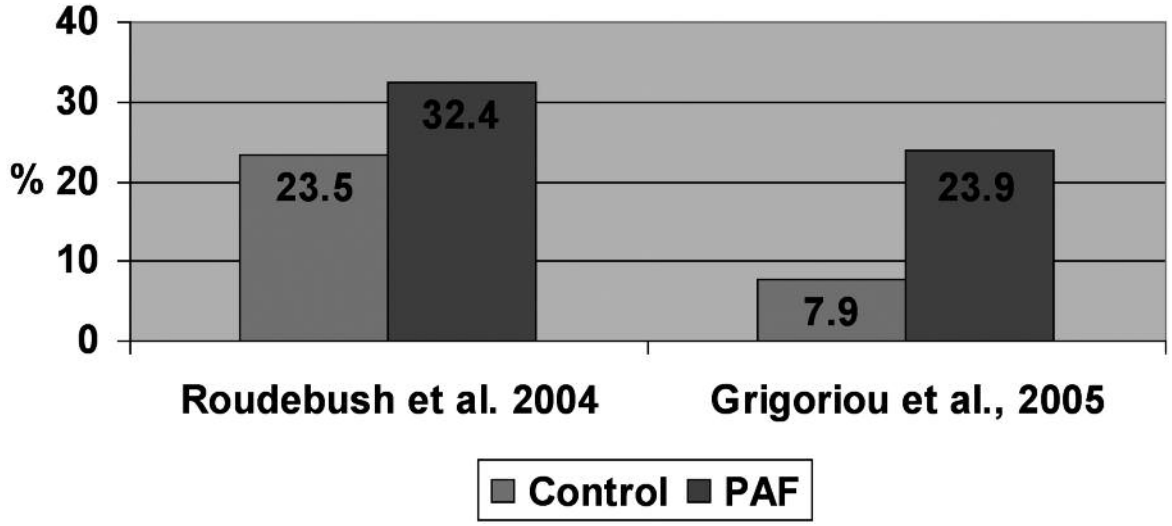

Fig. 1. — PAF IUI Pregnancy Rates

discovered that PAF receptor mRNA expression differs significantly between motile (high content) and nonmotile (low content) sperm (Purnell and Roudebush, 2001). The PAF plays a significant role in sperm function and may affect sperm motility and fertilization through a receptor- mediated control of intracellular calcium. The PAF has been shown to augment sperm capacitation (Sengoku *et al.*, 1993).

Additional clinical studies are warranted to further establish the use of PAF therapy for patient's undergoing IUI therapy for infertility treatment. In particular, larger numbers of male factor infertility patients will determine the significance of PAF–IUI therapy for these individuals. To summarize, exposure of sperm to PAF can significantly increase IUI pregnancy rates

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The rough guide to insemination: cross-border travelling for donor semen due to different regulations

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Abstract

Donor insemination is one of the main treatments for which patients cross borders. The present paper presents the date from a Belgian and a European study. Sperm donation in Belgium is mainly performed for single and lesbian women coming from France. The other main host country for sperm donation, Denmark, has a much more diverse group of visitors from Sweden, Norway and Germany. 74% of all Swedish women leaving the country, went abroad for semen donation, compared to 57% from France and 51% from Norway. In all other countries, the proportion was much lower. For all nationalities (apart from the Netherlands) the majority of the patients indicated that they went abroad because of legal restrictions in their home country. This is unlikely to change and travelling to obtain donor insemination will continue in the future.

Keywords: Cross-border reproductive care, donor semen, ethics, law, reproductive tourism.

Introduction

Cross-border reproductive care is a multifaceted phenomenon (Pennings, 2004). It refers to movements by candidate service recipients from one institution, jurisdiction or country where treatment is not available for them to another institution, jurisdiction or country where they can obtain the treatment they desire. These movements have raised many ethical questions: is it acceptable that rich people buy their way out of the legal restrictions of their country? Are people's rights violated when they are forced into reproductive exile? Can standards of safety and efficiency be maintained when patients travel? What is the responsibility of the different doctors involved? (ESHRE Task Force on Ethics and Law, 2008).

Different kinds of cross-border movements can be distinguished by looking at the moving parts: the material (gametes or embryos), the recipients and the donors. The 'standard' meaning, most in accordance with normal tourism, is when patients move to other countries to obtain the treatment they cannot get in their home country. The data presented in this article are mainly focussed on the travelling patients but I start with a short discussion on the two other forms because I believe they are equally important.

Different forms of crossing borders

Given the alternatives (mainly shipping of sperm itself), movements by sperm donors are not evident. When donors cross borders, they most likely have an additional reason for travelling. Nevertheless, several examples can be found that reveal the differentiation and complexity of the phenomenon. In the United Kingdom, the percentage of overseas sperm donors among all sperm donors was 12% in 2005, 24% in 2006, 12% in 2007 and 17% in 2008 (Human Fertilisation & Embryology Authority, 2009). It is unclear how this percentage can be explained. Are these British citizens of foreign origin or are these foreign students, immigrants or what? According to the Human Fertilisation & Embryology Authority document, foreign refers to the country of residence. This seems to suggest that these are temporary visitors. In India, foreign donors of fair skin are much in demand because parents want a fair child. Especially exchange students studying medicine and

engineering are recruited as commercial donors (Bakshi, 2008). An Australian clinic made a fairly drastic step by trying to recruit foreign donors with a package worth more than US$5000 (including a return flight, accommodation for a fortnight and a daily allowance (Anonymous, 2003). This also gives a whole new meaning to travel expenses.

The most important form of movement is the import and export of sperm. Sperm can be easily transported from one country to another. Import and export of semen is regulated by national laws. Import of anonymous sperm is limited or banned in the United Kingdom, the Netherlands, Norway, Sweden, Austria, Switzerland, New Zealand and Australia precisely because of the donor anonymity. One would expect countries to impose the same rules and restrictions on imported semen as they do on locally obtained semen, including rules of anonymity and payment of donors. Most of the sperm originates from Denmark where, for unknown reasons, many more sperm donors are found than in the other European countries. Although part of the explanation is the payment of the donor, this certainly cannot explain the difference between Denmark and the rest of Europe. In 2005, Ole Schou, director of Cryos International Sperm Bank, stated that about 10,000 units per year (approximately 70%) of donor semen from Cryos is exported to other European Union countries (Schou, 2005). In a 2009 newspaper article, it is reported that by then Cryos exports 85% of the 20,000 sperm donation to about 400 fertility clinics in 60 countries (Anonymous A, 2009). The largest buyers of sperm are clinics in Ireland, Belgium and Finland (Pavia, 2006). Every sample shipped across borders is one patient less who has to make the reverse journey. This certainly is a much more patient-friendly way than asking or forcing patients to travel. Taking into account that there are more large sperm banks in Europe who also ship sperm across the world, this is an important part of the total phenomenon of cross-border reproductive care.

The shipping of semen from one country to another is not limited to Europe. In Canada, for instance, there are at the moment 33 semen donors on a population of 33 million inhabitants (Anonymous B, 2009). With a recruitment rate around 1% of the donor candidates, it is extremely difficult to maintain a sufficient number of donors (Bradley *et al.*, 2005; Said and Del Valle, 2008). This shortage of candidates is mainly explained by the prohibition on payment introduced by the Assisted Human Reproduction Act of 2004. The law foresaw a transition period (that is extended until today) during which sperm could be imported. It is estimated that about 80% of children born in Canada by donor semen have an American donor (Bissessar, 2005). Interestingly, this means that these donors were paid for their donation. The advantage in terms of abidance by the ethical rule of non-remuneration is difficult to see.

Sperm donation in Belgium

Belgium is the only country in the world for which (almost) complete and reliable data exist regarding incoming patients for medically assisted reproduction (Pennings *et al.*, 2009). The majority of foreign patients seeking treatment in Belgium are French women for sperm donation. This group largely consists of lesbian couples and single women. These women have no access to medically assisted reproduction in France since treatment is restricted to heterosexual stable (co-habiting for at least 2 years) couples (Pennings, 1997). Of all patients coming from France to Belgium for assisted reproduction, 73% came for sperm donation. When we turn the picture around and focus on all foreign patients coming to Belgium for sperm donation, the French women make up 80% of all requests.

The data show a clear correlation between legal prohibitions in the home country of the patients and the number of patients who look for a solution abroad. These prohibitions may not directly concern the patients or their characteristics but may target certain guidelines that have an impact on the number of donors and/or sperm banks. The data presented above on Canada illustrate this point. When countries abolish donor anonymity or make payment for donors illegal, this has an impact on the number of candidates (Pennings, 2001). Moreover, more stringent requirements for donor screening (testing, quality standards etc.) and semen storage and distribution make things much harder for sperm banks (Yee, 2009). As a result, only a fraction of the candidates are retained. In the Newcastle clinic in the United Kingdom, 3.6% of all candidates finally are allowed to donate (Paul *et al.*, 2006). This proportion of lost and/or excluded candidates is typical of many sperm banks in the United Kingdom (British Fertility Society, 2008). This leads to higher costs, more investment of time and personnel and decreased efficiency. The cost per donor was estimated as high as £ 2700 (3000 €) per donor (British Fertility Society, 2008). Not surprisingly, many sperm banks in Canada decided that it was no longer worthwhile and voluntarily closed their doors. A very similar evolution took place in the Netherlands. The number of donors decreased by more than 70% and the number of sperm banks by 50% in the last 15 years (Janssens *et al.*, 2006). This was a consequence of the long-lasting debate (15 years) on donor

anonymity eventually resulting in the Law on donor data of 2004 that abolished donor anonymity. The effect could immediately be seen in Belgian clinics, especially those located near the Dutch border (Ombelet, 2007). The diminished offer on the Dutch side combined with the conviction of some Dutch couples that donor identifiability was not acceptable for them led to a steep increase in patients going to Belgium where anonymity is maintained. Between 2004 and 2005, the number of Dutch patients going to Belgium for donor insemination almost doubled from 57 to 99 patients (Pennings *et al.*, 2009).

European data

In 2008, the European IVF Monitoring (EIM) and the Task Force on Ethics and Law of the European Society of Human Reproduction and Embryology (ESHRE) started a study to collect data on cross-border reproductive care. The Task Force on Cross-border Reproductive Care, coordinated by Françoise Shenfield, designed 2 questionnaires that were distributed in centres of 6 European countries: Belgium, Czech Republic, Denmark, Switzerland, Slovenia and Spain. The centres that agreed to participate handed the patient form to all women coming from abroad for infertility treatment during one calendar month. The patient form contained socio-demographic characteristics (age, marital status, sexual orientation, patient's and partner's education), the main reasons for crossing the border, the type of treatment sought, the information received by the patients, the reimbursement situation and the degree of support received from the doctor at home. The patients were asked to indicate the reasons for travelling (more than one reason could be given). They were offered the following options: the treatment you want is not legal in your home country; you cannot obtain treatment because of age, unmarried, single, sexual orientation etc.; treatment is not easily accessible because of long waiting list, distance to the centre, cost etc.; you expect better quality and/or outcome in this centre than in your home country; you want anonymous / known / identity release donation of sperm / egg / embryo; and you had a previous treatment failure at home. The survey was conducted between October 2008 and March 2009. The patient questionnaire contained no patient or centre identification. The study was approved by ethics committees, according to the rules of each specific collaborating country.

The general data can be found in the article of the Task Force (Shenfield *et al.*, 2010). The data presented here focus on the use of donor semen and were not analysed in the general paper.

Results

For the whole study, we received 1230 forms from 46 clinics participating in the 6 countries: 29.7% from Belgium, 20.5% from the Czech republic, 12.5%
from Denmark, 16.3% from Switzerland, 15.7% from Spain, and 5.3% from Slovenia. Patients came from 49 countries, among which four countries were particularly represented, with more than 100 forms each: Italy (31.8%), Germany (14.8%), the Netherlands (12.1%) and France (8.7%). More than 50 forms were returned by patients from Norway (5.5%), the UK (4.3%) and Sweden (4.3%). Civil status varied according to the countries of residence. In total, 69.9% of women were married, 24.0% cohabiting and 6.1% single. Most Italian women were married (82.0%) whilst 50% of French women and 34.9% of Dutch women were cohabiting. In Sweden 43.4% were single (Shenfield *et al.*, in press).

The number of patients (N = 270) coming for donor insemination includes those who mentioned this in the questionnaire and the single women and lesbian couples who have no other way of treatment. Most patients came from 6 countries (see Table 3).

The majority of the women arriving in Belgium for donor insemination came from France (Fig. 1). This confirms the broad picture in the Belgian study (Pennings *et al.*, 2009). The second largest group were the Dutch patients followed by the Italians.

When one analyses the patients going to Denmark, there are 2 almost equally large groups, i.e., the Swedish and the Norwegian patients (Fig. 2).

Table 1. — Percentage of patients from a certain country for donation.

Home country	Number of forms	Semen	Oocyte	Embryo
Italy	391	15.6%	17.9%	2.3%
Germany	177	13.6%	42.4%	6.2%
The Netherlands	149	15.4%	8.1%	0.7%
France	107	57.0%	20.6%	5.6%
Norway	67	50.7%	1.5%	1.5%
United Kingdom	53	17.0%	60.4%	11.3%
Sweden	53	73.6%	5,7%	1.9%

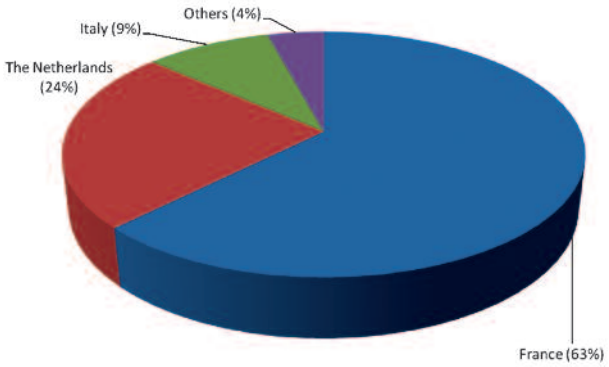

Fig. 1. — Home country of patients going to Belgium for sperm donation.

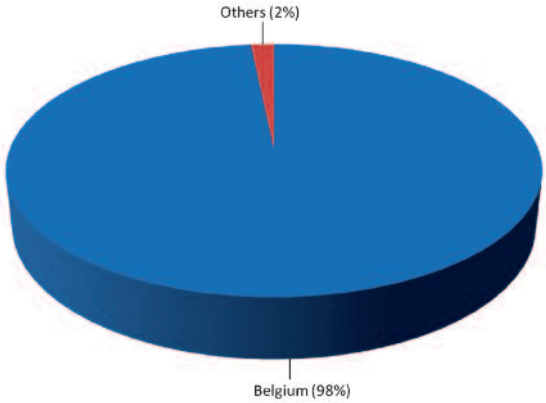

Fig. 3. — Host country of patients coming from France for sperm donation.

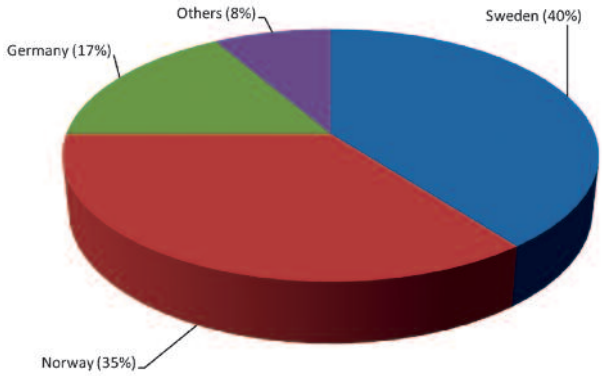

Fig. 2. — Home country of patients going to Denmark for sperm donation.

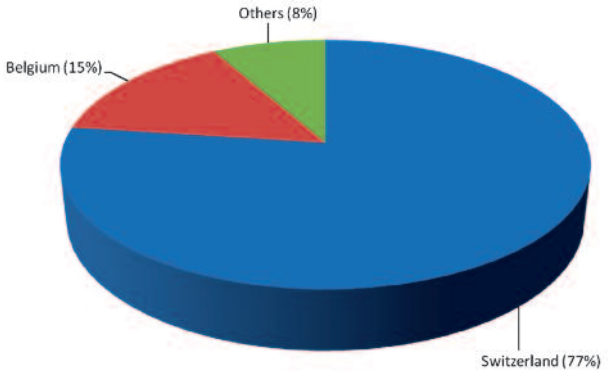

Fig. 4. — Host country of patients coming from Italy for sperm donation.

When we switch perspective and look at the distribution of patients of a certain nationality going abroad, we see that the French women almost exclusively go to Belgium for donor semen (Fig. 3).

Patients from Italy on the contrary travel mostly to Switzerland and then to Belgium for donor sperm (Fig. 4).

Fairly large difference can be found for the donation of gametes (semen and oocytes) and embryos (Table 1). Patients from France, Norway and Sweden travel considerably more for donor insemination than patients from the other countries. In the case of Sweden, almost 3 out of every 4 patients go for this type of treatment.

Regarding the distribution of the treatment type for which people travel to a certain country, it is clear, even with the limited data that were obtained from these countries, that Spain and Czech Republic were very popular for oocyte donation (Table 2). Belgium, Switzerland and Denmark, on the contrary, were more appreciated for donor insemination.

Table 2. — Percentage of patients travelling to a host country for donation.

Host country	Number of forms	Semen	Oocyte	Embryo
Belgium	365	26.3%	6.3%	0.3%
Czech Republic	251	9.2%	50.6%	12.0%
Switzerland	201	23.4%	1.0%	0.5%
Spain	193	4.1%	62.2%	4.7%
Denmark	154	61.7%	1.3%	0.6%
Slovenia	65	1.5%	0.0%	0.0%
Total	**1229**			

Table 3. — Reasons for travelling according to the home country of the patients.

Home country	Number of forms	Not legal in home country	Cannot obtain treatment (age,)	Treatment not accessible (list, …)	Expect better quality	Anonymous donation	Previous failure
Italy	61	95%	5%	0%	26%	44%	16%
France	61	69%	30%	7%	13%	54%	5%
Sweden	39	54%	28%	13%	15%	21%	3%
Norway	34	65%	41%	0%	6%	24%	3%
Germany	24	67%	33%	8%	17%	13%	21%
The Netherlands	23	17%	13%	13%	26%	35%	9%

Table 3 gives the reasons why people go abroad when they go for donor insemination. Apart from the women from the Netherlands, the majority travel because of legal restrictions. The second most important reason is that they cannot obtain treatment at home because of a personal characteristic (too old, ...). Respondents could tick more than one reason and there probably was an overlap between these two reasons since in some countries the law excludes some groups from treatment on the basis of personal characteristics. A number of patients indicate that they went for anonymous donation but it is unclear to what extent this means that anonymity was their reason for travelling. Some countries, like France, have donor anonymity imposed by law and so French women do not have to travel to obtain anonymous sperm.

Discussion

The results of the ESHRE study should be taken with the necessary caution. When the data of the host countries are mentioned, one should take into account that for 3 countries (Spain, Czech Republic and Switzerland), only a small number of centres agreed to participate. The response rate for the 3 other host countries was 50% for Belgium (9/18), 100% for Slovenia (3/3), and 88% for Denmark (21/24). This implies that especially the data for the first 3 countries may show a distorted picture. For the home countries, it is obvious that if a major country of destination was not included or was badly represented, a large group of patients may have been missed. Anecdotal evidence indicates that Spain is very popular with Italians but the data are very limited. Nevertheless, this study is a starting point that should be amended by more detailed and more representative studies.

Patients preferentially travel to neighbouring countries (Shenfield *et al.*, in press). Nevertheless, much depends on what they need or want and where they can obtain it. In a questionnaire among American IVF clinics, it was found that patients sometimes travelled large distances to obtain donor insemination. This survey, performed in 2008, showed that 88 patients came from Canada, 44 from Europe and 73 from Latin America (Hughes and DeJean, 2010). One possible explanation for making this long journey could be that the recipients wanted to choose the donor on the basis of an extended donor profile. This possibility is only offered in the US or in commercial sperm banks.

Conclusion

Most patients who travel abroad looking for donor sperm cannot obtain this treatment at home due to legal restrictions. Single women and lesbian couples are denied access to assisted reproduction in many European countries. This is unlikely to change in the near future and so travelling for donor semen will continue in the future. It is a shame to Europe that, at a time when discrimination on the basis of sexual orientation is condemned as a violation of human rights, so many lesbian couples still have to travel to obtain elsewhere what they should be able to obtain at home.

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Risk factors for higher order multiples following stimulated intrauterine insemination

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Abstract

Higher Order Multiple Pregnancies (HOMP) are defined as pregnancies with three or more foetuses. Higher Order Multiple Births (HOMB) are defined as deliveries of three or more babies.
Over the last decades, there has been a four to eight fold increase of higher order multiple births. This increase can mainly be attributed to fertility treatments, but the exact attribution for stimulated intrauterine insemination (IUI COH) is not known, since IUI treatments are not always recorded. It is generally accepted that multiple gestations are high risk pregnancies. They are associated with maternal, obstetric, and perinatal complications, and neonatal and infant mortality In addition, they are associated with increased financial and negative psychological consequences. These risks and consequences are higher for HOMPS than for twins.
This overview summarizes briefly how to prevent higher order multiple pregnancies in IUI COH. In order to prevent HOMP, prediction models can be used to distinguish patients with a good prognosis for a spontaneous pregnancy. In this patient group expectant management needs to be considered. With mild stimulation protocols, and monitoring, particularly in young women and especially in their first cycles, most high order multiples can easily be prevented. Pregnancy rates can be increased by immobilisation following IUI, without a further increasing risk of HOMP. When primary prevention fails, cancelling of cycles is a low impact option for secondary prevention. Remedies such as aspiration of supernumerary follicles, or converting to IVF or should be considered as a last resort. This applies especially for Multifoetal Pregnancy Reduction (MFPR).

Key words: intrauterine insemination, higher order multiples, prevention.

Introduction

Higher Order Multiple Pregnancies (HOMP) are defined as pregnancies with three or more foetuses. Higher Order Multiple Births (HOMB) are defined as deliveries of three or more babies.

The frequency of HOMBS after natural conception is described by Hellin's law; if twin births occur once in N births, then triplet births occur once in N^2 births and quadruplet births occur once in N^3 births. (Fellman and Eriksson, 2009). Spontaneous twin births occur at a rate of 1 in 50 to 1 in 100 births (1% to 2%). Thus a spontaneous HOMB can be expected to occur in 0.01% to 0.025% of all deliveries.

Over the last decades there has been a four to eight fold increase in higher order multiple births exceeding the numbers predicted by Hellin's law by far. (Blickstein and Keith, 2003; Martin and Park, 1999). This explosive growth is mainly attributed to fertility treatments (Keith and Oleszczuk, 1999). The sparse data in the literature suggests that non IVF ovarian stimulation is responsible for 35-50% of all multiple pregnancies (Levene *et al.*, 1992; Bergh *et al.*, 1999; Tur *et al.*, 2001). Unfortunately, data on an increase of the higher order multiple births caused by intrauterine insemination with ovarian hyperstimulation are lacking.

It is generally accepted that multiple gestations are high risk pregnancies. They are associated with maternal, obstetric, and perinatal complications, and neonatal and infant mortality (Albrecht and Tomich, 1996; Luke and Brown, 2008). In addition, they are

associated with increased financial and negative psychological consequences (Ombelet *et al.*, 2005; Roca de *et al.*, 2009). These risks and consequences are higher for HOMPS than for twins (Seoud *et al.*, 1992; Gleicher *et al.*, 1995; Evans *et al.*, 1992).

Prevention of multiple pregnancy has become important, and at the European Society of Human Reproduction and Embryology consensus meeting in 2002 it was agreed that the preferred outcome of ART should be the birth of one healthy child, and that twin, and thus higher order multiple pregnancy should be considered a complication (Land and Evers, 2003).

For IVF and ICSI, various studies have demonstrated that elective SET is an effective treatment to prevent multiple pregnancies and this has resulted in a consistent trend of transferring fewer embryo's leading to a decrease of the number of HOMB (Gerris *et al.*, 1999; Martikainen *et al.*, 2001; Gardner *et al.*, 2004; Thurin *et al.*, 2004; Lukassen *et al.*, 2005; van Montfoort *et al.*, 2005).

For intra-uterine insemination in a stimulated cycle (IUI COH), the methods of prevention of multiple pregnancies are more intricate. After COH, the higher number of released oocytes increases the chance of a pregnancy thus withholding COH will decrease pregnancy rates (Nyboe *et al.*, 2009; Verhulst *et al.*, 2006).

In this short overview we focus on 1) the increased incidence of multiple pregnancies following intra uterine insemination in a stimulated cycle (IUI COH); 2) on the maternal and neonatal risks of triplets and higher order pregnancies; 3) and on how these IUI induced HOMPs can be prevented.

IUI and the incidence of multiple pregnancy

IUI has been a common treatment since 1962 for a broad range of subfertility diagnoses such as unexplained subfertility, mild male subfertility, mild endometriosis, and cervical factor (Cohen, 1962).

Cumulative pregnancy rates with IUI COH vary between 20 and 30% after 6 cycles, depending on the cause and duration of subfertility (Ombelet *et al.*, 2005; Allen *et al.*, 1985; Ombelet *et al.*, 2005). Addition of ovarian hyperstimulation increases the pregnancy rates in couples with unexplained subfertility (OR of 2.07, 95% CI 1.22-3.50) (Verhulst *et al.*, 2006). In couples with mild male subfertility IUI COH is generally applied, but there is little evidence of its effectiveness (Bensdorp *et al.*, 2007).

As IUI is an intermediate step of low to moderate complexity before applying more sophisticated assisted reproductive technologies (ART) such as IVF with or without ICSI (Oehninger, 2001), it is in many countries more accessible than ART. Minor adjustments to current IUI practice, such as immobilization for 15 minutes after randomisation might increase pregnancy rates, without further increasing the risk of multiple pregnancies (Custers *et al.*, 2009).

Unfortunately, many of the countries performing IUI do not have national registries or reports that depict IUI numbers and success (Nyboe *et al.*, 2009).

In Europe, where countries are increasingly reporting IUI data, the number of cycles performed is still increasing. In 2001, 15 countries performed 52.939 IUI cycles, in 2004 19 countries performed almost twice as many cycles. Pregnancy rates per insemination have remained well above 12%. Twin rates and triplet rates were 10% and 1% respectively in 2005.(Nyboe *et al.*, 2009).

Although IUI data are not reported separately, US data from 2004, show that ovulation induction outside of IVF and other ART was estimated to be responsible for 28.912 twins and 1654 higher order multiples, of which 371 were quadruplet and even higher orders. These represent 22% of all twin and 40% of all triplet and 71% of all quadruplet and higher order babies born that year (CDC, 2008; Dickey, 2007).

It can be expected that the actual triplet conception rate following IUI is about twice as high as reported, since 50% of triplet and higher order pregnancies undergo spontaneous reduction to lower orders during the first trimester (Dickey and Pyrzak, 2002). Furthermore, in ongoing triplet and higher order pregnancies foetal reductions are often being performed thus reducing the HOMP and HOMB numbers. Without these costly foetal reductions, the proportion of triplet deliveries would have been higher, although it is unclear to what extent since national foetal reduction rates are not being registered (Nyboe *et al.*, 2009).

Maternal and neonatal risks of higher order multiple pregnancy

Women carrying HOMPS are at an increased risk of diabetes, pregnancy induced hypertension, preeclampsia, and delivery through a caesarean section as well as post partum haemorrhage.These risks are increased by an additional 20-50% in comparison to twin pregnancies, except for caesarean section, which is increased by 300-500% (Luke and Brown, 2008; Wen *et al.*, 2004). The largest cohort of triplets described a total of 198, and 94% were delivered by caesarean section (Newman *et al.*, 1989).

Neonatal complications associated with HOMPS are predominantly related to premature delivery. Prematurely delivered children are prone to suffer from respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular bleeding and pneumonia (Seoud *et al.*, 1992). Recent US data from 2006

supplied by 426 fertility clinics show that after 138.198 cycles of ART 95% of triplets or more, compared to 63% of twins were born preterm (CDC, 2008). There is no reason to assume that HOMPS after IUI treatment would behave differently. The risk of a very preterm delivery (birth at < 29 weeks of gestation) is increased for triplets and quadruplets compared with twins by a factor 4 and 8 respectively (Luke and Brown, 2008).

Growth retardation is another risk factor for children delivered from multiple pregnancies, and is increased by 42 to 57%. Low birth weight infants are more at risk for short and long term disability such as cerebral palsy, mental retardation and limited motor and cognitive skills (Collins and Bleyl, 1990; Seoud *et al.*, 1992). US data show that low birth weight (< 2500 gram) was found in 90% of the triplets and higher order multiples, compared with 57% of the twins, and 8% of the singleton pregnancies (CDC, 2008). Mortality rates are 6 times higher for HOMP when compared to singletons (Alexander *et al.*, 2005).

Financial consequences

Higher order pregnancies are expensive; medical costs, obstetric costs and perinatal costs are substantially higher than in singleton or twin pregnancies.

Medical costs include medication, as well as hospital admission and sometimes even emergency surgery, often to delay preterm labour. Obstetric costs are 4 to 5 times higher for triplets, and 7 fold for quadruplets compared to singletons (Mugford, 1995). Perinatal costs are enormous; and mostly associated with prematurity and dysmaturity (Petrou and Henderson, 2003; Ombelet *et al.*, 2005).

Admission costs to the neonatal intensive care unit are high and result in a two to four fold increase in cost per child born. It has been estimated that the costs of an infant with Extremely Low Birth Weight (ELBW) which is defined as < 1000 g, vary between £ 30.000 and £ 40.000 depending on the country of treatment (some 40.000 to 60,000 Euro at 2005 exchange rates) (Ombelet *et al.*, 2005). Additionally, the long-term costs due to handicaps like spastic cerebral palsy are substantial, and subsequently a burden for society (Mugford, 1995).

Primary prevention

There are several ways to prevent HOMPS after IUI-COH:

Selection of patients

Patients should be carefully selected. Prediction models can be used to do this. Several models have been published which estimate a couples' chance of conceiving naturally. Hunault's model is a combination of the three models of Eimers, Snick and Collins, and predicts a couples chances of natural conception leading to live birth within 1 year (Hunault *et al.*, 2004). This model includes independent patient characteristics like age of the woman, referral status, duration of subfertility, whether subfertility is primary or secondary, and sperm quality. From 2002 to 2004 this model has been calibrated and externally validated in a subfertile population in 38 centres in the Netherlands. In this cohort study, the model was used to predict the chance of spontaneous pregnancy in 3021 couples. Calibration of the prediction model was almost perfect; of the couples who had a calculated chance of > 40% of conceiving, the cumulative pregnancy rate without treatment was 46% (van der Steeg *et al.*, 2007).

As pregnancy can be predicted, this model was then used in a randomised controlled trial, also in the Netherlands. In 253 couples with unexplained or mild male subfertility and an intermediate prognosis (30-40% probability of spontaneous ongoing pregnancy within 12 months) no differences in pregnancy rates were found between expectant management for six months and IUI COH for six months, demonstrating that expectant management should be advocated for this patient group (Steures *et al.*, 2006).

Use of low dose stimulation protocols

An effective method to prevent twins and HOMPS in IUI, even in an unselected population, is abiding by low dose stimulation protocols. Already in 1970, no multiple pregnancies or HOMB were found after a starting dosage of 75 IU HMG compared to a 13% twinning rate and 4.8% HOMB when stimulating with 150 IU of HMG (Thompson and Hansen, 1970).

In a retrospective Italian study, of 1259 cycles with a low starting dose of 50 IU good pregnancy rates (15.8-21.9%) were obtained with 10% twins, without any higher order pregnancies (Ragni *et al.*, 2006). Approximately 5% of cycles had to be cancelled because of hyperresponse (predefined as three or more follicles ≥ 16 mm, and/or five or more follicles ≥ 11 mm).

In a review including ten studies that followed a low dose protocol administering ≤ 75 IU FSH clinical pregnancy rates per cycle varied from 9% to 20%, with a mean clinical pregnancy rate of 11% per cycle. Triplets and higher order pregnancy rates were kept to a minimum and varied between 0 and 2.4%. When cancellation criteria were applied in addition

to the low dose protocol, the mean clinical pregnancy rate was 10% per cycle, and the HOMP frequency was further reduced to 0.3% (Ragni *et al.*, 2006).

Cycle monitoring

In a recent meta-analysis of 14 studies with 11.599 IUI cycles multifollicular growth was associated with increased pregnancy rates in IUI with COH. Absolute pregnancy rates were 8.4% for monofollicular growth, and 15% for multifollicular growth. In cycles with 3 to 4 follicles the multiple pregnancy rate increased without a substantial gain in overall pregnancy rate (van Rumste *et al.*, 2008).

In addition to the number of developing follicles, various authors have found a relation between multifollicular growth, age of the woman, estradiol levels and HOMP rates. In 3347 stimulated IUI cycles the incidence of triplets and higher order pregnancies was significantly related to the total number of follicles ≥ 7 mm and E2 concentration ≥ 1385 pg/mL (CHL assay). No HOMP occurred when there were fewer than 10 total follicles ≥ 7 mm. The rate of HOMP was 3.8% if there were fewer than 15 follicles ≥ 7 mm and E2 concentration was below the earlier mentioned threshold. If there were more than 15 follicles ≥ 7 mm and E2 concentration was above the threshold, the HOMP rate reported was 22.2%. (Gleicher *et al.*, 2000).

These results were confirmed in a Spanish study albeit with slightly different thresholds. In 1878 stimulated IUI cycles the HOMP rate was 3.3% if the woman's age was above 33 years, and the E2 concentration was above the equivalent of 1051 pg/mL (CHL assay) and the number of follicles ≥ 10 mm was fewer than six. For the younger women (< 33 years) who had six or more follicles ≥ 10, with the E2 concentration above the previously mentioned threshold, the rate of triplets and higher order pregnancies was 22.0% (Tur *et al.*, 2001).

Findings from the most recent study on this subject concur; for women of 38 years and older, no triplets and higher order pregnancies occurred in 4062 cycles of stimulated IUI. For the slightly younger women (33-37 years) the HOMP rate was 16.7%, and for the even younger ones (< 32 yrs) the HOMP rate was 23.1% when there were seven or more follicles ≥ 10 mm present with an E2 concentration ≥ 1000 pg/mL by CHL (Dickey *et al.*, 2005).

Also, as women are more prone to conceive in their first treatment cycles, it is in these cycles that they are at risk for HOMP. This is clearly demonstrated by figure 1. Four large retrospective studies show that practically no HOMP occurred after the third cycle (Dickey *et al.*, 2001; Dickey *et al.*, 2005; van Rumste *et al.*, 2006; Custers *et al.*, 2007).

Secondary prevention

Cancellation of cycles

Cancellation is the most frequently applied strategy in the secondary prevention of HOMPs during IUI cycles, but international guidelines show different

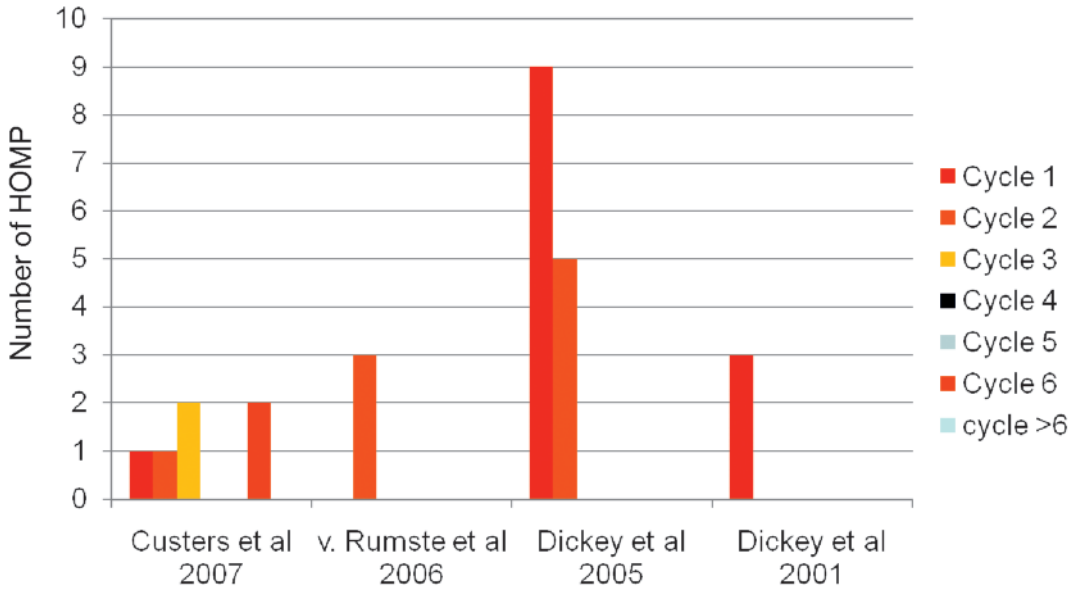

Fig. 1. — The number of HOMP each cycle in 4 different retrospective studies

cut off points.The American college of Obstetrics and Gynaecology (ACOG) recommend cancelling cycles or withholding hCG when more than three follicles are > 15 mm and the Royal College of Obstetrics and Gynaecologists sets this threshold at > 16 mm. Yet there is no consistent evidence to support either of these precautionary measures. In Europe only three other countries have issued IUI guidelines (Denmark, France, the Netherlands and England and Wales) and the ESHRE Capri Workshop has considered them to be of sufficient quality for use in clinical practice, even if their recommendations and references differ considerably. The varying indications for stimulated IUI in the Danish, French, English and Dutch guidelines are mainly due to the difference in selection of evidence (Haagen *et al.*, 2006; Nyboe *et al.*, 2009; 2009).

Aspiration of supernumerary follicles

An alternative secondary prevention is transvaginal ultrasound-guided aspiration of supernumerary ovarian follicles. This technique was first described in 1984 to increase the efficacy and the safety of IUI with gonadotropins (Hazout *et al.*, 1984). One of the few, small cohort studies of 26 IUI cycles performed found that aspiration of supernumerary is a valid approach to avoid multiple pregnancies without affecting pregnancy rate. (Albano *et al.*, 2001).

Inn 257 of 571 COH IUI cycles aspiration was performed of four or more follicles >- 14 mm before administrating hCG. Pregnancies occurred in approximately 21%, with 8% twinning rate and triplets of 1.7% (De Geyter *et al.*, 1996).

Coasting

Whether coasting, a possible third option for secondary prevention, is effective in IUI remains a matter of debate. Whereas gonadotropin stimulation can be discontinued in IVF cycles and hCG administration can be delayed to avoid ovarian hyperstimulation syndrome (OHSS), it is uncertain that applying the same technique to IUI cycles to prevent multiple pregnancies is effective. Trials that have investigated this technique for IUI are sparse and of poor quality (Urman *et al.*, 1992; Ulug *et al.*, 2004).

Conversion to IVF

The fourth option, conversion to IVF, is an invasive strategy to reduce HOMP. For the patient the follicle aspiration is unanticipated, as are the concomitant higher costs. In countries where only a limited number of IVF cycles are reimbursed by the insurance companies this clearly is an issue, and therefore seems not a viable option.

Multifoetal pregnancy reduction

Finally multifoetal pregnancy reduction (MFPR), was introduced in 1978 to selectively eliminate a foetus affected by a genetic order in a twin pregnancy (Aberg *et al.*, 1978). Nowadays it is also carried out to improve the chances of survival and health of the remaining foetus in twin and high order multiple pregnancies. This is the most invasive technique to prevent HOMP in an IUI programme, morally questionable, but may reduce associated risks. As the risk for adverse perinatal and maternal outcomes increases with a greater number of foetuses within the uterus, patients and doctors are faced with dilemmas. The high risk pregnancy can be continued, terminated entirely, or the number of foetuses can be reduced in an attempt to decrease the maternal and perinatal morbidity. Risks associated with MFPR are pregnancy loss, and prematurity. Pregnancies reduced to twins do not seem to have worse outcomes than conceived twin gestations. (Evans *et al.* 1992, 1994, 2004).

Conclusion

Triplets and higher order pregnancies remain a major problem in artificial insemination. To prevent HOMP, prediction models can be used to distinguish patients with a good prognosis for a spontaneous pregnancy. In this patient group expectant management needs to be considered. With mild stimulation protocols, and monitoring, particularly in young women and especially in their first cycles, most high order multiples can easily be prevented. Pregnancy rates can be increased by immobilisation following IUI, without further increasing the risk of HOMP. When primary prevention fails, cancelling of cycles is a low impact option for secondary prevention. Remedies such as aspiration of supernumerary follicles, or converting to IVF or MFPR should be considered as a last resort.

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Bank your future: Insemination and semen cryopreservation

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Abstract

Semen cryopreservation has become an important part of the routine work of most Andrology or Assisted Reproductive laboratories. There are two main reasons for cryopreservation of semen, the one is for the use in donor semen related procedures and the second for the purpose of fertility preservation in males, also known as autoconservation, for many different reasons. These two main areas can further be subdivided into several categories or uses depending on their clinical application.

In this article the emphasis is on storage of semen for autoconservation uses only, with reference to reasons for autoconservation of semen, the effects of cryopreservation on semen parameters and sperm functions and the use(fullness) of cryopreserved semen in practice.

The wide range for autoconservation of semen samples includes *inter alia* the following; conservation of fertility in male cancer patients, treatment of male and/or female subfertility, back-up for ART procedures, fertility preservation before vasectomy or other reasons/procedures and storage of separated X and Y bearing spermatozoa.

It is concluded that semen cryopreservation is an essential tool in ART. IUI with cryopreserved, non-donor, semen is mainly used for cancer patients. Although the request rates for cancer patients and infertility treatment is low, if used pregnancy rates are compatible with other IUI procedures. Despite the low usage rate of stored semen samples the cryopreservation of semen samples in these cases is extremely important given the therapeutic or physiological effect by the knowledge that in worst case scenarios due to the unavailability of fresh semen samples a cryopreserved semen sample is available.

Key words: IUI, semen parameters, autoconservation, cryopreservation, donor semen

Introduction

Semen cryopreservation has become an important part of the routine work of most Andrology or Assisted Reproductive laboratories (Nijs and Ombelet, 2001). There are two main reasons for cryopreservation of semen, the one is for the use in donor semen related procedures and the second for the purpose of fertility preservation in males, also known as autoconservation, for many different reasons (Björndahl *et al.,* 2010). These two main areas can further be subdivided into several categories or uses depending on their clinical application.

For donor cryopreservation semen is obtained from healthy men, or donors, who are presumed to be fertile i.e. capable of impregnation. These donors may be recruited by the clinic or sperm bank and their spermatozoa are then used anonymously, although the contemporary trend is now that the identity of the donor should be made know to the recipients if they so wish. Donor spermatozoa may be used for IUI, IVF or ICSI for a wide range of indications, for instance for an ART procedure of the partner of an infertile man, with no live spermatozoa or elongated spermatids and thus unsuitable for ICSI, where treatment with the male partners semen has failed or treatment became too costly. To prevent transmission of a genetic inherited disorder in heterosexual couples or to prevent foetal haemolytic anaemia in cases of blood group incompatibility or recurrent abortions or for the insemination of single

women or lesbian couples wishing to conceive (Björndahl *et al.,* 2010; WHO, 2010). In this article the emphasis will be on storage of semen for autoconservation uses only, with reference to reasons for autoconservation of semen, the effects of cryopreservation on semen parameters and sperm functions and the use(fullness) of cryopreserved semen in practice.

1 Reasons for autoconservation of semen

The wide range for autoconservation of semen samples includes *inter alia* the following; conservation of fertility in male cancer patients, treatment of male and/or female subfertility, back-up for ART procedures, fertility preservation before vasectomy or other reasons/procedures and storage of separated X and Y bearing spermatozoa.

1.1 Conservation of fertility in male cancer patients

Semen cryopreservation for male cancer patients before chemotherapy, radiotherapy or orchiectomy in cases of cancer or autoimmune diseases, is apart from donor semen cryopreservation one of the most important reasons for autoconservation of semen (Nijs and Ombelet, 2001). This is a very important option for cancer patients were semen can be obtained by masturbation. Other, but less used options available for cancer patients and especially young boys, where it is difficult to obtain semen by masturbation, are testicular tissue banking and spermatogonial stem cell banking with the intention that there will be the necessary progress in research on in vitro maturation of these cells to obtain elongated spermatids or mature spermatozoa.

For patients to undergo cancer therapy with alkylating agents or radiotherapy, the semen must be collected before the patient commences with any therapy in order to decrease the risk of mutagenesis in the spermatozoa. Semen storage before a potential sterilizing procedure often has significant physiological value because of the hope of future paternity.

1.1.1 Effect of cancer treatment on semen parameters and male fertility

It is well known that the kind of cancer and the kind of treatment i.e., chemotherapy with cytotoxic agents or radiotherapy may have different effects on spermatogenesis and eventually on the fertility status of the male. The effect may be permanent or temporally, but it is seldom found that the male will recover to his full fertility potential (Meseguer *et al.,* 2006; Schmidt *et al.,* 2004).

Agarwal and Allamaneni (2005) reported that the mean semen parameters of 205 adolescent cancer patients were severely reduced compared to the semen parameters of a control group. The mean volumes were 1.6 ml and 3.0 ml, respectively. The mean spermatozoa concentrations per ml were 50.6 million and 84.5 million and sperm motility 45.1% and 68.5%, respectively. In a group of 83 males with testicular cancer and a controle group the mean spermatozoa concentrations were 15.0 million and 48.0 million per ml semen, respectively. In a group of males with leukemia the mean sperm concentration per ml semen was 19.5 million compared to 106.0 million of a control group, respectively and the percentage of motile spermatozoa 45.0% and 64.0%, respectively.

Simons *et al*. (2005) found that one year post radiotherapy treatment 16% of men who were initially diagnosed with normozoospermia presented with oligozoospermia and 20% were still azoospermia. However, spermatogenesis in patients who received cancer treatment continued to improve over the next 5 years. Schmidt *et al.* (2007) reported that in a group of males diagnosed with cancer 91.2% of them had fathered a child before treatment, while after treatment only 67.1% were successful to father a child, with a so-called cumulative pregnancy index eventually reaching a pregnancy rate of 85%.

1.1.2 Availability and use of cryopreservation services for cancer patients

In the literature a couple of surveys are available reporting on the availability of semen cryopreservation services and the actual use once the semen samples have been stored. Heath and Stern (2006) reported on a survey done in Australia and New Zealand among paediatric oncology centres. From the 13 questionnaires send out to different centres, 12 centres responded. All 12 centres offered semen cryopreservation but only 9 of the 12 centres offered counselling as a routine procedure. Rofeim and Gilbert (2004) did a survey among oncologists in the USA. Of the responders 91% agreed that semen conservation procedures should be offered but only 27% offered the service sometimes while 10% always offered the service.

1.2 Treatment of male and female subfertility

In men with poor semen samples cryopreservation of their semen with the specific aim to use these cryopreserved samples as an IUI treatment option is not practiced on a wide scale, and only a single article in this regard could be found (Aboulghar *et al.,* 1991). The treatment described by Aboulghar *et al*. (1991) entitled that the subfertile males provided semen samples on a regular basis for cryopreservation. When a sufficient number of samples were stored the best cryopreserved sample of a male was

used for IUI together with a fresh sample. The average pregnancy rates per cycle and the cumulative pregnancy rate in the treatment group was 9.1% (19/73) and 27.1%, respectively. In a control group where only fresh semen samples were used the mean pregnancy rate per cycle was 4.1% and the cumulative pregnancy rate was 13% (9/77). Overall, pooling of multiple abnormal semen samples for AIH has not been proven to be useful.

1.3 Back-up for assisted reproductive procedures and non-cancer fertility preservation

Men may store spermatozoa for treatment of their partner by IUI, IVF or ICSI for many different reasons such as difficulty in collecting semen on the day of the procedure, erectile dysfunction, psychological problems or absence on the day of the ART procedure.

With the introduction of ICSI the possibility for the use of cryopreserved semen became even more widespread as only a few spermatozoa are required for this procedure and the high survival rate in terms of total motile spermatozoa as needed for IUI is not necessary. Men with severe oligozoospermia or intermittent presence of motile spermatozoa in their semen can now use cryopreservation as back-up for ICSI (Bourne *et al.,* 1995). Another widely used application of cryopreservation is the freezing of material obtained by testicular or epididymal biopsies i.e., MESE or TESE. As only a single spermatozoon is needed in ICSI for each oocyte, cryopreservation of any live sperm is worthwhile and all patients, including pubertal adolescents (< 15 years old) boys, as well as from men suffering from spinal cord injuries should be offered the possibility of storage of their spermatozoa (Kamischke *et al.,* 2004).

Fertility preservation for non-cancer reasons are also done for a wide variety of reasons. One of the more frequent applications is before the performance of a vasectomy as insurance against a change in the desire for a child within an existing partner/marital situation or in case of a new partner/marriage commitment where a desire for a new or extended family may occur. In these cases the cryopreservation is performed as insurance for procreation. Other reasons for this kind of fertility insurance is in cases where the husband is performing active duty in a dangerous occupation such as the military forces on condition that it is performed in countries where posthumous procreation is acceptable.

Other reasons may be in cases were a progressive loss of semen quality may be suspected for instance in cases of y-chromosome microdeletions, transient azoospermia or oligozoospermia (Björndahl *et al.,* 2010). Cryopreservation can also be used in cases where it may be suspected that successful treatment of infertility that may not persist, for instance where surgery for genital tract obstruction was performed or after treatment with gonadotrophins for men who are diagnosed with hypothalamo-pituitiary hypogonadism (WHO, 2010). In all these cases and especially in cases where the main reason for cryopreservation is for fertility preservation or male or female infertility treatment, enough specimens should be stored to provide for ten or more insemination cycles to ensure a good chance of a pregnancy.

1.4 Minimising transmission of infectious diseases.

The advent of HIV and the possibility of transmission of the virus via semen or assisted reproductive procedures in the early 1980's required the introduction of a quarantine period for donor semen and further testing of the donor before the stored semen could be used. More recently an alternative method was published where in cases of HIV positive semen a special preparation procedure was employed (Loskutoff *et al.,* 2005). After semen preparation through washing and multiple gradient centrifugation in a special double barrel test tube the resultant spermatozoa from men carrying the HIV diseases can then be cryopreserved for use in ART. Now men with HIV controlled by antiretroviral therapy may store semen samples with undetectable viral load for IUI, IVF or ICSI to attempt conception while reducing the risk of transmission of the HIV to the female partner.

1.5 Separation of X and Y bearing spermatozoa

Semen cryopreservation and IUI has also been used for the purpose of sex selection (Karabinus, 2009). The main reason for this procedure was for the reducing of X-linked genetic disorders or family balancing. Sperm were stained with Hoechst 33342, sorted by flow cytometry, and then used for IUI or cryopreserved for subsequent use for IUI, IVF or ICSI. Fluorescence in situ hybridization (FISH) analysis was used to determined the post-sort enrichment of X- and Y-bearing sperm. The sperm separations performed were in 74.9% of cases for X-sort and in 25.1% for Y-sort. Post-sort purity averaged 87.9% for X-sort and 73.4% for Y-sort. A total of 3629 IUI cycles were performed with a pregnancy rate of 15.6% and a miscarriage rate of 15.7%. Overall X-sort, after IUI, resulted in 92.0% female and Y-sort in 81.5% male babies born. The first outcome from this clinical trail, running from 1994 to 2007, was reported by Fugger *et al*. in 1998.

2 Effects of cryopreservation on semen parameters and sperm functions

It is well known that cryopreservation of semen does have a negative effect on semen parameters and sperm function, especially on sperm motility. On average only 50% of the motile spermatozoa will survive freezing and thawing (Keel and Weber, 1993) with a corresponding reduction in vitality while damage of sperm structure and function also occurs. Alternations in acrosome structure as well as shrinkage of sperm nuclei and cytoplasmic membranes with loss of plasma membrane integrity and the concurrent reduction of intact acrosomes and consequently a reduction in (pro)acrosin activity has been observed (Nijs *et al.,* 2009). Due to the addition of the cryoprotectant there is also a reduction in the sperm concentration. The effect of cryopreservation on semen parameters of cancer patients is in the same order. Menkveld (unpublished data) observed that in 64 patients the mean sperm concentration was reduced from 39.4 ± 28.7 mill/ml (range, 0.1-100.0 mill/ml) before freezing to 18.4 ± 13.5 mill/ml (4.5-40.0 mill/ml) after freezing and thawing. The mean progressive motility (a + b) was reduced from 45.4 ± 13.5% (0.0-60.0%) before freezing to 23.3 ± 12.1% (0.0-40.0%) after freezing and thawing.

With regard to the effect on long term sperm storage on sperm functions and structure Edelstein *et al*. (2008) investigated the effect of storage time on sperm DNA integrity using the TUNEL assay. Semen samples from 14 men were cryopreserved for a short period (1 to 5 years) and from 16 men semen samples were stored for a long term of 9 to 13 years. Edelstein *et al*. (2008) found that in the short term storage group the semen samples contained an average of 31% of spermatozoa with DNA fragmentation compared to the 37% sperm DNA fragmentation of the long term of storage group. The conclusion was that long term storage did not significantly increased sperm DNA fragmentation. Unfortunately they did not presented data on sperm DNA damage before cryopreservation was performed. However, Zribi *et al*. (2010) found that cryopreservation had deleterious effects on sperm DNA by inducing DNA fragmentation and oxidation but could not give an explanation for the underlying mechanism for such damage. Nijs *et al*. (2009) found that cryopreservation had no negative effect on the results of the sperm-hyaluronan binding assay as an average of 68.5% sperm binding was found before freezing and 71.3% after freezing and thawing.

3 Utilisation of cryopreserved semen in practice

Only a small percentage of males who cryopreserved their semen samples will eventually use the stored semen samples. The usage of cryopreserved semen varies between 30% to less than 10% of the stored samples (Simons *et al.,* 2005) but in many centres it may be as low as 5% or less (Chung *et al.,* 2004). In our own experience we found that only 4.7% (3/64) of our patients used their cryopreserved semen samples. In total 3 IUI, 2 ICSI and 1 GIFT procedures were performed (Menkveld, unpublished data).

3.1 Use of cryopreserved semen/sperm in cancer patients

In early reports cryopreservation of semen for cancer patients with the intention for the performance of artificial insemination was thought to be pointless if motility was <40% and sperm concentration was < 20 million spermatozoa per mL, due to unacceptable low chances for conception. Even if the semen quality before cryopreservation adhered to the above mentioned criteria pregnancy rates were very poor. Therefore, as a result even today many oncologists consider semen cryopreservation for cancer patients as insufficient and not worthwhile to perform. With the introduction of IVF and especially ICSI this situation changed drastically as only a few motile spermatozoa are needed for these procedures (Tournaye *et al.,* 2004).

Ragni *et al*. (2003) reported that over a 15 year period, involving 776 men with malignant diseases, 5.2% (36/686) of these men used their cryopreserved samples for ART with cumulative pregnancy rates after 4, 8 and 12 years of 4.5%, 8.7% and 11.8%, respectively. Magelssen *et al.,* (2005) reported their experience with the use of cryopreserved semen from cancer patients over a twenty year period. They reported that 7% (29/422) of men used their cryopreserved semen samples at least once or sometimes more than once for assisted reproductive procedure in order to try to achieve fatherhood. Of these men 55.1% (16/29) achieved a pregnancy, while 17% (67/393) of men achieved a pregnancy without use of their cryopreserved semen samples. In a group of men who did not cryopreserve their semen before cancer treatment, 21% (205/966) achieved a pregnancy. Hourvitz *et al*. (2008) reported on the results of 118 couples who underwent 169 IVF cycles using cryopreserved pre-cancer treatment sperm. The observed clinical pregnancy rate was 56.8% per retrieval with a total of 96 pregnancies, resulting in 126 children born and 11 spontaneous abortions. Semen samples from males presenting with prostate cancer showed the poorest semen parameters before cryopreservation and also achieved the lowest pregnancy rates. Van Casteren *et al*. (2008) reported on the use of 749 cryopreserved semen samples from 557 men over an average follow-up period of 7 years. Over this period, 42 patients requested the

use of their cryopreserved semen samples. In a group of 37 patients where the results were available, 32 IVF cycles were performed, 53 ICSI cycles, 9 cryo-embryo transfers and 7 IUI's, resulting in 8, 16, 2 and 1 pregnancies, indicating that pregnancy rates for IVF and ICSI's were significantly higher than those for IUI. Over the follow-up period 7.5% of the patients used their cryopreserved semen resulting in a live birth of 49%. Crha *et al*. (2009) performed semen cryopreservation for 619 cancer patients over an 11 year period from 1995 to 2006. In this period of time 32 (5.2%) of the patients sought infertility treatment and cryopreserved semen samples were used for 28 couples who underwent a total of 44 ICSI cycles, resulting in 13 pregnancies.

Time of storage

Semen stored under appropriate conditions will show no deterioration of sperm quality with time and children have been born following fertilization using semen stored for over 28 years (Clark *et al.,* 2006; Feldschuh *et al.,* 2005). In one couple two pregnancies resulted after IUI treatments performed with cryopreserved semen samples stored for 21 and 28 years, respectively (Feldschuch *et al.,* 2005).

Pregnancy rates with stored semen

Best pregnancy rates with artificial insemination performed with cryopreserved donor semen are no different from those achieved with fresh semen and pregnancy rates range from 15% to 25% per month, or cycle, of treatment. However, pregnancy rates of 7-12% per month are more common. Rates are often related to post-thaw sperm quality, timing of insemination and particularly influenced by recipient factors such as age of the women and tubal or uterine disorders (Le Lannou and Lansac, 1993).

Evidence suggests that there is no increase in abnormal pregnancy outcomes after the use of cryopreserved semen for IUI. The rates for spontaneous abortion of 13%, major birth defects of about 1% and the sex ration of 51:49, males:females, are similar to those of spontaneous pregnancies (Lansac and Royere, 2001).

Conclusion

Semen cryopreservation is an essential tool in ART. IUI with cryopreserved, non-donor, semen is mainly used for cancer patients. Although the request rate is low, if used pregnancy rates are compatible with other IUI procedures. The storage of semen from cancer patients should be offered, after sufficient consultations, to all cancer patients before cancer treatment because it is a mental booster and if not offered legal action can be taken against the treating institution. If semen is stored for the treatment of a subfertile couple in cases were the male presents with poor semen parameters, the frozen samples are mainly used for IVF and especially ICSI procedures. The question is very often asked if it is worthwhile to do outo(cryo)conservation of semen due to the fact that the frozen samples are not used on a great scale. The answer is that the therapeutic or physiological effect given by the knowledge that in case of worst scenario cryopreserved semen is available is a sufficient reason for performing this procedure.

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Men not included: A review of single and lesbian mother DI families: mother-child relationships en child development

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Abstract

The number of single and lesbian mothers who want a child by means of donor insemination (DI) increased rapidly during the past twenty years. In this article a brief overview is presented of empirical findings with regard to family relationships and child development in these non traditional families.
Findings of single DI mothers remain preliminary but no adverse effects have been identified among the still very young children. There is a larger body of knowledge with regard to lesbian DI families. Overall, no differences were found compared with heterosexual two parent families with regard to family functioning and child development. The co-mother appeared to be more involved in child rearing than fathers.
In both groups children were aware of their donor origin in an early developmental stage. Although they did not consider the donor as part of their family, the majority of children requested (non)identifying donor information.
Based on the findings so far, single and lesbian women should be accepted in DI programs and the use of identity registered donors is advisable. Further large scale follow up studies are still needed.

Key words: Single and lesbian DI mothers / family relationships and child development /donor information.

Introduction

Donor Insemination (DI) was introduced more than 100 years ago, it is therefore one of the oldest techniques in reproductive medicine. During the nineteen seventies DI practice became widely available in most Western European countries but was only practiced within a strictly confidential doctor-patient context where the doctor guaranteed the anonymity of the donor and advised patients to keep the matter a closely guarded secret. Donors were selected for their similarity with the infertile man in order to hide the use of gametes as good as possible.

It was in the eighties that lesbian couples and single women made their first appearances in Fertility Centers. Their demands introduced a number of new challenges in DI practice. Programs were meant to treat heterosexual couples but these women wanted DI not because of a fertility problem of their husband but because of the absence of such a male partner. The majority intended to inform the child about the use of a sperm donor and many requested (non) identifying donor information (Brewaeys *et al.*, 1993). Their demand made perfectly clear that in fact, donor insemination was not a medical fertility treatment but just an alternative road to parenthood with possibly more psychological consequences than previously thought of.

It was the growing social tolerance within the western social context that stimulated these non-traditional requests. During the seventies of the previous century societal values and attitudes with regard to marriage, sexuality, contraception and child bearing changed rapidly. Economic independence of women increased, gender roles became less dichotomist and a marriage was not necessarily lifelong. Single mother families were no longer an exception and for the first time some, mainly highly educated women, started a family on their own. As a result of the gay liberation movement "homosexual orientation" was officially excluded as a psychopathology from the International Diagnostic and

Statistical Manual of Mental Disorders. Homosexuality was to be regarded as normal sexual behavior. Social tolerance towards lesbian and gay people increased tremendously and lesbian mothers became more and more visible. Consequently, a diversity of non traditional families arised.

In what follows, the most prominent features of both family types are highlighted and a brief overview is presented of the most relevant empirical findings with regard to family relationships and child development.

Similarities and differences between single and lesbian mother families

The most controversial feature of these non traditional families is *the absence of a father*. In western culture the fundamental conviction prevails that a father is essential for the healthy psychological development of a child. Children growing up without a male parental figure would be at risk for identity confusion. Fathers are believed to be of particular importance in the development of a stable gender identity. Several psychological theories have addressed this issue. The most prominent one is Psychoanalytic theory. Central is the resolution of the oedipal conflict in which the child relinquishes the symbiotic relationship with the mother and identifies with the father (Chodorow, 1978). Empirical evidence for this theory is restricted to a number of clinical case descriptions.

In both family types the absent father is replaced by a *sperm donor*. Thus all children will be unknown with half of their genetic make-up. In contrast with heterosexual DI families where there is a father figure present in the family, children of lesbian and single mothers are expected to ask question about this father absence in an early developmental stage. The majority is aware of the donor conception before the age of 5 (Brewaeys *et al.* 1995). This enables us to study the donor concept of the children involved: if informed about their donor conception, what will they want to know about the sperm donor?

Although they have the absence of a father in common, two essential differences remain between lesbian and single mothers. Lesbian women applying for DI intend to raise their child together with their female partner. In contrast with single mothers, their child will grow up with two parents instead of one. Sharing educational tasks with a partner is different from doing it alone. Being a single parent might induce extra stress for mother and child.

On the other hand, single mothers appear to be mainly heterosexual, thus their children will not have to deal with their mothers' homosexuality and with potential homophobic reactions. There is now abundant evidence that social stigmatization affects quality of life negatively and impairs social development.

Empirical follow up studies

Single mother families

Since the number of single mother families has dramatically increased in western society, data of several population studies have become available (Hetherington and Clingempeel, 1992; Ringback *et al.*, 2003). The great majority of these families were single parents after divorce and children had a known father. Results are unanimous. Mothers perceive more parental stress, have more psychological and physical health problems. When the children are compared with children raised in two parent families they appear to have more problems on all variables involved: physical health, psychopathology, addiction, cognitive development, socio-emotional development and the ability to engage in intimate relationships.

There are however a number of crucial differences between the families mentioned above and mothers who have chosen to become a single parent by means of DI. Most children in the previously meant studies experienced parental separation. As it is well known that parental conflict and divorce have a negative impact on children, these data tell us little about children of mothers who were single by choice. Moreover the majority of single mother families had a low socio-economic status and are very often confronted with poverty, both factors that influence the family climate negatively.

Single mothers by choice

Who are those single women who want a child by means of a sperm donor and what do we know about their children? Studies remain sparse, samples are small and not always representative. Most children studied were younger than 5 years. Consequently, crucial questions about the quality of life of adolescents and adults have not yet been addressed.

Motives and demographic features. All interviewed women were very much aware of their biological clock which did not allow any further delay of their child project. Their mean age when applying at the fertility clinic appeared to be higher than 35 years in all studies (Leiblum *et al.*, 1995; Klock *et al.*, 1996; Murrey and Golombok 2005). Most of these women went through a period of grief for not having found the appropriate partner at the appropriate moment. So, becoming a single DI mother was not their first choice. When looking at their educational and professional status, results were

unanimous, the majority of these women belonged to a socio economic privileged group, had a high professional status and were financially independent.

Psychological, relational and social characteristics. The majority of studies reported that mothers were psychologically healthy and could rely on a supportive social and family network. Moreover, single mothers felt more satisfied with being a mother than a control group (Leiblum *et al.*, 1995; Klock *et al.*, 1996; Murray and Golombok, 2005). Only one Belgian study, describing characteristics of applying single women, labelled 20% of the group as being psychologically unstable (Baetens *et al.*, 1995). However, diagnosis was based on clinically impression without the use of standardized instruments. All studies reported that these women had meaningful *partner relationships* in the past. What these findings do not tell us is why they failed to develop long lasting partner relationships. Did some of these women lack the abilities to develop intimate relationships or is it the changing social context that makes it hard for some well educated women to find the right man?

Family relationships and the psychological development of children. When compared with two headed families, no major differences were found in the quality of parent child relationships. However the children studied were mostly of a pre-school age (Golombok *et al.*, 1997; Murray and Golombok 2005). Two studies investigated 7 year old children (Flaks *et al.*, 1995; MacCallum and Golombok, 2004). MacCallum and colleagues reported more interaction between mother and child in the single families, but also more disputes in disciplinary interactions. Chan and colleagues failed to find any difference between single and two headed DI families with regard to mother-child interaction.

No difference was found in the psychological development of the children (social, emotional and behavioural development) compared with two headed families. The only difference found in one study of the 7 year old children was that children of fatherless families perceived themselves to be less cognitively and physically competent than their peers in father-present families (MacCallum and Golombok 2004).

Lesbian mother families

There is these days a considerable body of knowledge on lesbian mothers and their children. The design and research questions changed over the years and a brief overview will be presented. In the early eighties of previous century a number of custody cases withdraw children from their lesbian mothers assuming that they could not be fitted parents. These women were divorced and came out as homosexuals after their divorce. So, a number of researchers developed studies in which these mothers and their children were compared with divorced heterosexual single mothers (Golombok *et al*, 1983; Green *et al.*, 1986). When the first lesbian couples started to apply at fertility clinics for Donor Insemination, new questions were raised. In the previous lesbian families, all children had a known father since their mother divorced after having lived for a number of years in a heterosexual family. Little was known about the psychological effect of being fatherless right from birth. New studies emerged. This time lesbian mothers were compared with heterosexual DI families in which a father was present (Brewaeys *et al.*, 1997; Chan *et al.*, 1998). When it appeared from previous studies that all these young children were doing fine, there was a need for long term follow up studies. Indeed, young children do not have the cognitive and emotional abilities to fully understand the special features of their family. And it is only during adolescence that they fly out in the often homophobic world. A number of longitudinal studies investigated adolescents and adults. They interviewed the children themselves about having a lesbian mother and being donor conceived (Golombok and Tasker 1996; VanFraussen *et al.*, 2003; Garttrell *et al.*, 2005).

Despite the differences in research designs, numbers of participants and used instruments, findings of a large body of studies were strikingly unanimous.

With regard to the *development of family relationships* during childhood and adolescence, lesbian mothers did not differ in the quality of the parent-child interaction compared with heterosexual DI and Naturally Conceived families. They were equally emotional involved and equally disciplining the child. Grandparents did accept these children as their offspring and were equally involved than grandparents in the heterosexual families.

However, a number of interesting differences with the heterosexual family did appear. The co-mother, the biological mother's lesbian partner was more involved in all aspects of child rearing than the heterosexual father. And this was particularly so during childhood. Furthermore, educational tasks were more equally divided between lesbian mothers than between mother and father in the heterosexual families (Brewaeys *et al.*, 1997). When adolescent children themselves were interviewed about their family relationships, it appeared that they were equally attached to both mothers. Moreover, children of lesbian mothers communicated more about emotional issues with the co-mother than children in the heterosexual families with the father (Vanfraussen *et al.*, 2003).

The *psychological development* of children raised by lesbian mothers was similar in all studies.

Children were well adjusted and showed normal emotional and behavioral development compared with heterosexual controls. A few studies investigated gender role development during childhood. Results failed to find any difference between a child raised in a lesbian family compared with a heterosexual family. One study reported on the sexual identity of adults raised in a lesbian family and again there were no indications that these adults developed a homosexual orientation (Golombok and Tasker 1996). Social development and the quality and number of contacts with peers was also within the normal range (Vanfraussen *et al.*, 2003).

Social stigmatization. All these findings are pretty reassuring and one wonders whether children experience any homophobic reaction in their social surroundings. A number of studies investigated this issue in detail. In the study of Vanfraussen *et al.* it was found that children became more secretive about their non-traditional family when growing older. Children appeared to be more selective to whom they disclosed their two mother family unit. And although peer teasing did not occur more frequently, if they were teased it was about themselves being homosexual or similar issues (Vanfraussen *et al.*, 2001; Vanfraussen *et al.*, 2003). In the US Study by Gartrell (2005) and colleagues nearly half had experienced some form of homophobia and those who did, reported more psychological distress.

Children's donor concept

A number of studies investigated the donor concept of the children raised in lesbian mother families (Vanfraussen *et al.*, 2001; Vanfraussen *et al.*, 2003; Gartrell *et al.*, 2005; Scheib *et al.*, 2005). Children of lesbian and single mothers are informed about the use of a donor in an early developmental stage and this is in sharp contrast with the majority of heterosexual parents. Even though children of the former families and heterosexual DI families cannot be compared (the latter have a father), findings about their donor concept remain relevant. If children are aware of their donor origin, what is then his role in their donor and family concept.

In our own longitudinal study of lesbian families we interviewed the children themselves. When they were 5 years old, they could all tell the interviewer a story in which it appeared very clearly that they had two mothers and no father. In their concept the donor was reduced to seeds (no person) and remained absent in their representation of their family. Children had no or little awareness that their family was different (Brewaeys *et al.*, 1995). The question remained what these children would think of their donor origin once they had reached a more advanced stage of cognitive and social development. In the second interview, when children were between 8 and 18 years old, It was obvious that both mothers were still considered as the only parents and none of the children regarded the donor as a family member. (Vanfraussen *et al.*, 2003). When children were between 8-18 years of age, the question was asked what they wanted to know about the donor. Please keep in mind that all were conceived by means of an anonymous donor and children were aware of that. Forty one percent of the boys and 10% of the girls said that, if they had the opportunity, they would have liked to meet the donor. Their major motive was curiosity about physical and personality characteristics and about the existence of half siblings. Nine percent of the boys and 32% of the girls were happy with non identifying information and 50% of the boys and 58% of the girls did not need any information at all (Vanfraussen *et al.*, 2001).

Similar result were found in two American studies (Gartrell *et al.*, 2005 and Scheib *et al.*, 2005). Whether or not children were conceived by an anonymous or identifiable donor, seemed to make only a small difference in their curiosity (Gartrell *et al.*, 2005). Note worthy is the high number of searchers found in the study by Joanna Scheib and colleagues. Here all donors were identity registered and the number of adolescents who wished to meet the donor in future was up to 80%.

Discussion

In this article a brief overview was presented of the most relevant empirical findings with regard to family relationships and child development in single en lesbian mother families.

The sparse studies investigating single women applying for DI outline a picture of last chance mothers not wanting to wait any longer for an appropriate partner. Overall these women were highly educated, financially independent, had a supportive social network and did not rule out men in their future lives. Although minor differences had been identified between single mother and two parent families, no major negative effect was found on family relationships and child development. Findings remain preliminary and the majority of children involved were of pre-school age. Questions about emotional, social and identity development during adolescence and adulthood remain unanswered. Taking into account the trend in Western Society in which a growing number of women remain single and tend to delay their child wish, applications at Fertility centres are growing continually, reason enough to set up large scale longitudinal studies.

In contrast with the former group, there is a larger body of evidence about lesbian mother families and no adverse effect of lesbian motherhood could be identified. Family relationships were stable and co-mothers were more involved in child rearing than fathers. Children were doing well and there were no signs of an atypical gender role development or homosexual orientation. An issue needing more attention however is the effect of social stigmatization on the family. One should realize that the families studied so far were privileged: mothers were white, well educated and living in a tolerant social environment. During the last twenty years, motherhood is no longer a taboo among the majority of lesbian women in western society. Lesbian women applying at Fertility Centers these days are younger and come from a wider social and educational background. Replication studies of these new emerging group are still needed.

The majority of children who are informed in an early developmental stage about their donor conception are curious about certain donor characteristics and a respectable number wants to meet him. It appears that their main motive is to find a better understanding of themselves and develop a stable sense of identity. What we don't know is if the absence of a father figure influenced their curiosity. In the study by Vanfraussen and colleagues (2003) boys outnumbered girls in their wish to meet the donor. These findings are in sharp contrast with results of adoption research which unanimously finds that girls are much more curious than boys about their birth parents. The fact that there is no father figure to identify with in the lesbian families, might have caused the gender difference.

In conclusion, in the past twenty years single and lesbian mother families became more and more visible, social acceptance increased and a variety of non traditional families arised. No adverse effects for the children have yet been identified. In my opinion, these women should be included in DI programs. Just like all other DI applicants they fare well with appropriate counseling that addresses their specific characteristics and needs. Taking into account that the majority of their children wishes (identifying) donor information, only identity registered donors should be used.

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Preconception sex selection for non-medical and intermediate reasons: ethical reflections

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Abstract

Sex selection for non-medical reasons is forbidden in many countries. Focusing on preconception sex selection, the authors first observe that it is unclear what should count as a 'medical reason' in this context and argue for the existence of 'intermediate reasons' that do not fit well within the rigid distinction between 'medical'and 'non-medical'. The article further provides a critical review of the arguments for the prohibition of sex selection for non-medical reasons and finds that none of these are conclusive. The authors conclude that the ban should be reconsidered, but also that existing societal concerns about possible harmful effects should be taken seriously. Measures to this effect may include limiting the practice to couples who already have at least one child of the sex opposite to that which they now want to select ('family balancing'). Finally, a difficult set of questions is raised by concerns about the reliability and unproven (long-term) safety of the only technology (flow cytometry) proven to work.

Key words: Preconception sex selection, non-medical reasons, flow cytometry, ethics, regulation.

1. Introduction

Since ancient times, prospective parents have tried to influence the sex of their future children. Aristotle, for example, advised those wanting a boy to have intercourse when the wind is in the north. Other people have assumed that special diets, the timing of intercourse in relation to ovulation, binding up one of the testicles during intercourse, or the position during intercourse, might facilitate sex selection. Apparently, every folk culture has its own strategies. Modern science provides people with new, more effective, methods for sex selection. This is widely (though not universally) seen as a welcome development insofar as sex selection for medical reasons is concerned. However, sex selection for non-medical reasons has been the subject of recurrent ethical and public policy debate in many countries. The latest round in this debate was fuelled by the public consultation held in the United Kingdom on the initiative of the Human Fertilisation and Embryology Authority (HFEA) in 2002, and the HFEA's subsequent decision to continue to limit the use of sex selection techniques in licensed centres to cases 'in which there is a clear and overriding medical benefit' (HFEA, 2002; HFEA, 2003). This is now also given a further legal underpinning in the amended British Human Fertilisation and Embryology (HFE) Act of 2008. A recent overview (Darnovsky, 2009) shows that a similar restrictive stance is adopted in all 36 countries with laws and policies on sex selection, including China, India, Turkey, Australia, Canada, and 25 European countries (among which are also Belgium and the Netherlands). In the same vein, article 14 of the European Convention on Human Rights and Biomedicine (Oviedo Convention) of 1997 states that 'techniques of medically assisted reproduction shall not be allowed for the purpose of choosing a future child's sex, except where serious hereditary sex-related disease is to be avoided'. Some countries (in Europe: Austria and Switzerland) go even further and prohibit sex selection for any reason.

Although it may seem that the sheer weight of this prohibitive consensus between jurisdictions, at least

in Europe, has brought the debate about allowing sex selection for non-medical reasons to a closure, and although it is clear (as again confirmed in the consultation held in the UK) that among the public there are strong moral feelings against more liberal policies, we will argue that there are still good reasons for questioning the arguments behind this international consensus.

The focus of this paper is on medical methods for sex selection - not on the 'do it yourself' methods. Some further distinctions and specifications are important. A second distinction concerns the *timing* of sex selection. Sex selection can be performed at three stages:

– before conception/fertilization, where one can make a selection at the level of the sperm using sperm sorting. This strategy is called preconception sex selection, primary sex selection or sex pre-selection;

– just after fertilization, by means of in vitro fertilization (IVF) combined with preimplantation genetic diagnosis (PGD), aiming at a selective transfer of an embryo of the desired sex. This procedure is called preimplantation - or secondary sex selection.

– during pregnancy, where people might use prenatal diagnosis - chorionic villus sampling (CVS), amniocentesis or even non-invasive prenatal diagnosis (NIPD) (Newson, 2008; De Jong, 2009; Wright, 2009) - to decide about continuation or selective termination of the pregnancy in view of the sex of the foetus. This is post-implantation, prenatal or tertiary sex selection.

A third distinction concerns the *reasons* for sex selection. The literature generally makes a distinction between medical and non-medical reasons. Medical reasons are often defined in terms of preventing the conception or birth of a child suffering from a sex-linked disorder, like Duchenne muscular dystrophy or haemophilia. Non-medical reasons are rather diverse; people may just prefer a child of one sex over the other, either for personal reasons or for socio-cultural reasons, or they may want to 'balance' their family if they have one or more children of one sex and would like to have a further child of the other sex. But obviously, a third category of possible reasons should be added, which can be regarded as intermediate between medical and non-medical reasons (De Wert, 1993; De Wert, 2005). Think of males affected with an X-linked recessive disorder, like haemophilia. Some of these patients would prefer to conceive boys only, because sons (inheriting the 'non-affected' Y chromosome from their father) will not carry the mutation, whereas all daughters would be (in principle healthy, but) obligate carriers of the mutation. What, then, if one of these male patients (and their partners) were to request sex selection in order to avoid the conception or birth of a carrier-daughter? In such cases, the reason for choosing sex selection is not to avoid the birth of a child with a serious disease, but to avoid the birth of a child that later in her life may have to face difficult reproductive decisions herself, given her 1 in 4 risk of having children affected by her father's disease.

As our contribution was part of a congress on "Artificial Insemination", this paper regards the ethics of *preconception* sex selection. More in particular, this paper focuses on the ethics of preconception sex selection for *non-medical* and *intermediate* reasons, as preconception sex selection for *medical* reasons is widely considered to be acceptable from a moral point of view. In the next paragraph, we will first summarize the normative views of some relevant committees and some (inter-) national legal regulations (section 2). Next, we will provide an ethical evaluation of the main arguments in the debate (section 3). The technology presently available for preconception sex selection will be sketched thereafter, with particular attention to its efficacy and safety (section 4). Finally, we will draw some conclusions and present some recommendations (section 5).

2. Preconception sex selection: highlights from the debate

To give an impression of the reception of preconception sex selection for non-medical reasons this section contains a brief, chronological overview of relevant recommendations of ethics committees, advisory bodies and of some (inter-)national regulations. Clearly, the overview is not exhaustive - but it is, we think, representative.

President's Commission (1983). This American Commission argued that sex selection for non-medical reasons reflects a morally objectionable attitude to the future child, an attitude which, taken to an extreme, treats the child as an artifact and the reproductive process as a chance to design children according to parental standards of excellence (President's Commission, 1983).

The Warnock Report (1984). The British 'Warnock Committee' mentioned possible negative social effects of sex selection for non-medical reasons, like effects on the ratio of males to females. These considerations made the Committee dubious about the use of sex selection techniques on a wide scale, but because of the difficulty of predicting the outcome of any such trend the Committee has not found it possible to make any positive recommendations on this issue. Nevertheless, the Committee considered that

the question of the acceptability of sex selection should be kept under review (Warnock report, 1984).

The Glover Report (1989). According to this report, submitted to the European Commission, it could be argued that there is nothing intrinsically wrong with sex selection for social reasons. But the Committee is 'impressed by the dangers of an unbalanced sex ratio, and inclined to the view that the motives behind the choice of sex will often be ones society would do better to discourage than to satisfy. So we think that sex selection should be strongly discouraged. It would be desirable for clinics to be banned from providing pre-conceptual techniques as a service.' (Glover report, 1989).

The (German) 'Embryo Protection Act' (Gesetz, 1991). This piece of legislation contains a special section on inappropriate sex selection ('mißbrauchliche Geschlechtswahl'): 'Any person who fertilizes a human egg with a sperm cell which has been specifically chosen because of its sex chromosome is guilty of an offence ... This is not the case if the sperm has been specified by a physician in order to protect the child from possible severe, sex linked diseases ...' (Embryonenschutzgesetz, 1991).

The Law Reform Commission (1992). This Canadian Commission states: 'To eliminate the possibility of eugenic practices, the selection of gametes and embryos with specific qualities should be prohibited, except where the objective is to prevent the transmission of serious genetic diseases.' Selection of sex for non-medical reasons is considered to be unacceptable, as 'such practices ... disrupt the demographic and social balance between the sexes for future generations, and could have a tremendous impact on these 'made-to-measure' children. It therefore seems appropriate to limit individual freedoms in the name of respect for human dignity.' (Law Reform Commission, 1992).

Health Council of the Netherlands, Standing Committee Medical Ethics and Health Law (1995). According to this committee, there is no cause to prohibit preconception sex selection in the Netherlands. Governments ought, in principle, to respect the reproductive freedom of parents - any exception to this basic principle can only be justified if the interests of others are harmed. After a review of the scientific evidence, the Committee concluded that 'there is no reason to suppose that this is the case'. However, the Committee did emphasize the provisional nature of this conclusion concerning social implications; it cannot be excluded that the availability of opportunities for sex selection as part of family planning might lead to parents finding the gender of their children to be more important than now appears the case. Developments in this area should, therefore, be monitored very critically (Health Council, 1995).

American Society for Reproductive Medicine, Ethics Committee (2001). This Committee concluded that if trials show that the techniques of preconception gender selection are safe and effective, the most prudent approach at present for the nonmedical use of these techniques would be to use them only with the aim of establishing gender variety in a family. If the social, psychological, and demographic effects of those uses have been found acceptable, then other nonmedical uses of preconception sex selection might be considered (Ethics Committee of the ASRM, 2001)

Dutch and Belgian Embryos Acts (2002, 2003). The Dutch Embryos Act prohibits 'performing procedures with gametes or embryos with a view to selecting the sex of a potential child'. An exception to this prohibition is made for cases in which 'there is a risk of a serious sex-linked hereditary disease in the child and the procedures are intended to prevent that disease'(Embryowet, 2002). The Explanatory Memorandum states that this ban was based on the argument that sex selection for non-medical reasons 'reduces children to mere objects of the wishes and preferences of their parents' (Embryowet, 2000). The Belgian Embryos Act contains a similar provision. This forbids 'conducting research or treatments aimed at sex selection, with the exception of selection aimed at preventing sex-linked diseases' (Embryowet, 2003). According to a commentary by Hansen *et al*., the main motive for not allowing preconception sex selection was the conclusion of the Belgian National Consultative Bioethics Committee in its brief advisory report of 1997, that the then only available method for preconception sex selection ('gradient method') to be combined with artificial insemination, was unreliable (Hansen *et al.,* 2004). A later report from this Committee contained a more comprehensive discussion of arguments both for and against allowing preconception sex selection for non-medical reasons, but without this leading to a consensus view or policy advice.

HFEA (2003) and amended Human Fertilisation and Embryology Act (2008). In the Authority's view, the likely benefits of permitting sex selection for non-medical reasons in the UK are at best debatable and certainly not great enough to sustain a policy to which the great majority of the public are strongly opposed. Accordingly, the Authority decided that treatment services provided for the purpose of selecting the sex of children, by whatever means this is to be achieved, should be restricted under license to cases in which there is a clear and overriding medical justification (HFEA, 2003). In the amended British Human Fertilisation and Embryology (HFE) Act of 2008 this is defined as cases where there is a particular risk that a woman will give birth to a child

who will have or develop a 'gender-related' serious disease or other medical condition (HFE Act, 2008). The notion of a disease or condition being gender-related is further specified as either affecting only one sex or as affecting one sex significantly more than the other. Whereas the corresponding provisions in the German, Dutch and Belgian Embryos Acts can be read as referring only to monogenetic 'sex-linked diseases' (the German Act refers to 'Duchenne Muscular Dystrophy or equally serious sex-linked diseases'), the specification in the amended British Act explicitly also includes non-Mendelian disorders with unequal sex-incidence (Amor, 2008). Parents already having (more than one) children affected by disorders in this category, such as autism, are at a (significantly) higher risk that any further child will also be affected. Whether sex selection to avoid this risk would be legal in Britain may still depend on the interpretation of whether the disease in question would satisfy the requirement of seriousness. Interestingly, the Belgian Act does not require the disease in question to be of a serious or severe nature, whereas the German, Dutch and British Acts do. None of these Acts, nor any of the reports we are aware of, refer to sex selection for intermediate reasons.

On the basis of this short overview, the following conclusions can be drawn:

Firstly, there is wide approval, in principle, of the application of preconception sex selection for *medical* reasons, although both the precise definition of this category and the conditions imposed differ.

Secondly, opinions differ widely with regard to the moral acceptability of preconception sex selection for *non-medical* reasons.

– in many countries this is categorically forbidden, although the reports from ethical committees give a more varied picture.

– the ethical justification of the various positions regarding sex selection for non-medical reasons is often unclear. Indeed, some committees do not or hardly present any arguments in favor of their recommendations. The objections are very diverse and different committees use rather different objections.

Finally, sex selection for what we have called 'intermediate reasons' has not yet arrived on the agenda of societal debate and policy making. However, the often quite narrow definition of the category of non-prohibited medical reasons (in terms of avoiding the birth of a child with a sex-linked disease) and the rigid, binary distinction between medical and non-medical reasons, seems to rule out sex selection for intermediate reasons in most if not all jurisdictions with legislation on sex-selection.

In the next section, we will scrutinize the arguments for disallowing preconception sex selection for non-medical reasons. Where relevant, we will also discuss whether and how these arguments apply to the use of preconception sex selection for intermediate reasons.

3. Ethical analysis

For the sake of debate, we assume, for the moment, that preconception sex selection techniques are completely efficient and of proven safety. That this is not yet an established fact will be addressed in section 4. Several types of moral objections to preconception sex selection for non-medical reasons need to be discerned (Warren, 1985). On the one hand, so-called non-consequentialist or deontological objections refer to what those making such objections regard as morally problematic aspects of the act of sex selection for non-medical reasons taken in itself, that is to say: prior to or apart from any consequences such a choice may have. Consequentialist objections, on the other hand, refer to its presumed adverse consequences. Non-consequentialist objections include the following:

'Sex selection is unnatural'. Appealing to Nature as a moral norm is quite common in societal debates – but highly questionable. The argument that 'X is wrong because it is unnatural' can only succeed if there is an interpretation of the term (un)natural which enables us both to distinguish between natural and unnatural actions, and to understand what there is about the latter which is morally objectionable (Warren, 1985). It is doubtful whether there any such interpretations which are convincing. Interestingly, proponents of 'the argument from nature' are highly selective in their moral reasoning; they regularly object to sex selection for non-medical reasons in particular, but isn't sex selection for *medical* reasons likewise 'unnatural'? If valid, therefore, the argument would apply to all forms of sex selection, including medical and what we have called intermediate reasons. However, since it surely is an important part of human nature to be inventive, to intervene in nature, and thus to create culture, we must look elsewhere for criteria to ethically evaluate our actions.

'Sex selection for non-medical reasons has nothing to do with medicine. So physicians should refrain from this type of sex selection'. This objection implicitly refers to the traditional goals of medicine, namely the prevention of disease, curing the ill, and caring for sick people who can not be cured. Sex selection for non-medical reasons can not be subsumed under any of these traditional tasks of the physician, so there is no medical indication for

this practice (RCO, 1993). This criticism seems to mistakenly suggest that the domain of medicine and health care is surrounded by clear and fixed borders. For a start, one may ask how sex-selection for what we have called intermediate reasons would relate to the goals of medicine as traditionally understood. As in those cases sex selection will not change the health status of the child to be born, reasoning from a strict understanding of those goals would lead to the conclusion that sex selection for intermediate reasons is beyond the scope of medicine. After all, the prospect that a daughter may have to face difficult reproductive decisions later in her life is not in itself a medical condition. So understood, intermediate reasons seem closer to 'social' than to 'medical' reasons. On the other hand, one may reason that what makes those reproductive decisions difficult are health related considerations and that, indirectly, the request for sex selection would still be motivated by the wish to prevent gender-related diseases in one's offspring. From this perspective, a case can be made for regarding those intermediate reasons for sex selection as 'medical' in a wider sense of the word. The further question here is why an appeal to the bounds of medicine as concerned with health related issues would imply that sex selection for what are clearly non-medical reasons is not acceptable. In fact, there are various widely accepted medical solutions for non-medical problems. Classical examples are sterilisation for the purpose of family planning or cosmetic surgery. If these are acceptable practices, one may doubt as to whether the absence of a medical indication as such is a strong moral objection to assisting in preconception sex selection for non-medical reasons.

***'Sex selection for non-medical reasons is inherently sexist'*.** Sexism can be defined as the wrongful discrimination between individuals on grounds of sex. The current objection implies that sex selection for non-medical reasons is nothing but 'an expression of sex prejudice, reflecting an attitude that one sex is in general inferior.' (Glover Report, 1989) or even 'the original sexist sin' (Powledge, 1983). It is obvious that a sexist motivation for sex choice is morally wrong, as it stands in denial of the principle that all human beings have the same worth. This charge is clearly not applicable to sex selection for medical, nor for 'intermediate reasons', but what about sex selection for personal or cultural reasons? Do motives in the 'non-medical' category necessarily presuppose the view that the desired sex is superior or do they necessarily build on stereotyping views of differences between the sexes? If so, that would indeed amount to a strong argument for disallowing sex selection for such reasons. However, it is far from obvious that it is necessarily sexist for someone to want to select the sex of a child for a non-medical reason. Consider the following examples (Warren, 1985; Health Council, 1995). Firstly, in some subcultures in some countries, a son is an economic asset; a son will earn more money, and parents will have to provide their daughter(s) with a dowry, which might almost ruin their own family. Against the background of such conditions, son-preference is not necessarily a sign of sexism of the prospective parents – even though their preference would be related to sexism at the socio-cultural level. It may even be the case that couples prefer to have sons because they want to spare their daughters the plight of having to live in a sexist society. Of course, the fact that such choices may reinforce sexist structures and institutions is a relevant consideration where the possible social consequences of sex selection are concerned. However, this does not mean that those choices are inherently sexist themselves (Warren 1985). Secondly, what about parents wanting to have a 'mixed' or 'balanced' family? Are those who believe that family dynamics would be enhanced by having children of *both* sexes guilty of a sexist attitude? Think of couples who have one or more sons and prefer their next child to be a girl, or vice versa. Although the preference for a mixed family does not presuppose the view that one of the sexes is superior, it may still be informed by a sexist stereotyping of gender differences. Here again, the issue is that this would not necessarily be the case. The preference for a mixed family is perfectly compatible with respecting and welcoming children as individuals whose worth does not depend on whether they fulfill some preconceived image of what a boy or girl should be (Robertson, 1994; Health Council, 1995).

Obviously, these examples do not suffice to take away concerns that motives for sex selection may still be sexist in individual cases (cf. the concerns of the Glover-Committee, as summarized in section 2). However, the question is whether the fact that some applicants may have morally dubious motives should lead to a categorical rejection of sex-selection for non-medical reasons. An interesting analogy is that some applicants for sex selection for medical reasons (and the same would apply to what we have called intermediate reasons) may be motivated by discriminatory motives regarding people with disabilities. Most commentators and regulators do not think that this possibility should be taken as a reason for prohibiting sex selection aimed at avoiding the birth of a child with a gender-related disease.

'Sex selection for non-medical reasons is an invasion of human dignity'. Another objection

regards human dignity. Some critics, e.g. the (Canadian) Law Reform Commission (see section 2), suggest that non-medically motivated sex selection undermines human dignity. This was also the view of the Dutch government as laid down in the Explanatory Memorandum to the Embryos Act (quoted in section 2). In a related government document it is said that sex selection for non-medical reasons 'runs counter to the general understanding that children are more than a means to satisfying parental preferences' (Besluit Geslachtskeuze, 1998). In this connection, the government also referred to the reasoning behind the relevant prohibition in the European Convention on human rights and biomedicine. Implicit in this line of argument is the (anthropological version of the) categorical imperative of Immanuel Kant, which holds that a person should always be treated as an end in himself and never merely as a means to other ends (Kant, 1785; Sullivan, 1989). No doubt, there might be parents who prefer to have a child of a particular sex as mere material to optimally fit their parental gendered expectations. But, here again, non-medical reasons are not necessarily instrumentalising. People who opt for sex selection for non-medical reasons may very well respect their future child as an autonomous agent, as an end in him-/herself.

Consequentialist objections include the following:

'Sex selection for non-medical reasons will distort the sex ratio'. That sex selection for non-medical reasons may have adverse societal effects is shown by developments in some countries, including India (notably the Indian state Punjab), where this practice has resulted in a substantial increase of males (Patel, 2007). These 'surplus' males may not be able to find a mate and some commentators fear a (further) loss of status and rights of females by virtue of their being in the minority – although the reverse might be possible as well (Warren, 1985).

Critics sometimes assume that sex selection for non-medical reasons will likewise distort the sex ratio in Western countries (cf both the Glover Report and the Canadian Law Reform Commission as summarized in section 2). Whether this scenario is realistic, depends on sex selection both becoming widespread here and being used to select mainly for males. Recent surveys in Western countries (Canada, the USA, the UK, Germany and the Netherlands) reveal that most people prefer a more or less balanced family (Health Council of the Netherlands, 1995; Dahl, 2003; Dahl *et al.,* 2006). There seems to be no indication of a strong preference for either sex. Insofar as there still is a preference for boys, this seems to be a marginal phenomenon. Perhaps even more instructive than surveys are data from so-called 'gender clinics' offering sperm sorting and insemination to interested couples in several countries without prohibitive legislation. Apparently, a very large majority of applicants visiting gender clinics in the UK (prior to a recent tightening of licensing requirements; Laurance, 2008) and the USA were seeking preconception sex selection for family balancing (Dahl, 2003). However, this may not apply to applicants from some ethnic minorities. Of the more than 800 couples who visited the London gender clinic in the first two years of its existence, more than 60% were of Asian origin. The large majority of these couples wanted a son (Liu, 1995).

'Sex selection for non-medical reasons will have negative consequences for the psychosocial development of children'. This argument refers to possible harm that may result from the feeling of being conditionally wanted. However, the knowledge that one's parents have used sex selection is not enough to generate this feeling. This would seem to very much depend on whether and to what extent the way parents interact with their children is informed by stereotypical ideas about how girls or boys should behave. It has been observed that sex selection may also have the positive effect of reducing the number of children having to bear the burden of being wanted less because of their sex (Health Council, 1995). Without further data, the question whether allowing sex selecting for non-medical reasons would lead to exposing more children to harmful styles of parenting, remains speculative.

'Sex selection for non-medical reasons will affect the position of women in society'. The presupposition that in Western societies sex selection for social reasons will not significantly affect the sex ratio does not rule out that there may still be effects on the social position of women. It has been suggested that, given a preference for boys, men will profit more from the 'first-born advantage' than women. The scenario (not supported by the findings of more recent surveys in Western countries) that most people would want a boy as the first child is alarming to those who support the psychological theory that firstborns are advantaged because parents are apt to concentrate their efforts at providing a stimulating environment on them: 'with sex selection, boys will monopolize the eldest-child bonuses in addition to other male privileges.' (Holmes, 1985). The theory of the eldest-child bonuses, however, is controversial. In her overview, Warren concludes that the evidence available appears to support a sceptical view on the intrinsic importance of birth order (Warren, 1985). Anyway, even if it were evidence-

based, the current objection does not in itself imply that all forms of sex selection for non-medical reasons should be discouraged or banned. Indeed, it would only imply that selecting the sex of one's fist child should perhaps be prohibited. As such, the argument cannot serve as an objection against sex selection aimed at having a mixed family – the motive that the large majority of applicants of sex selection in gender clinics in Western countries seem to have (vide supra).

'Sex selection for non-medical reasons is a first step on a slippery slope towards morally unacceptable forms of reproductive selection'. This objection holds that allowing sex selection for non-medical reasons will inevitably result in permitting prospective parents to design what they consider to be the perfect child, to conceive a 'designer' baby, whether that involves high intelligence, high musical and/or athletic competencies, or any number of other special qualities. The former (American) President's Commission, for instance, argued that sex selection for non-medical reasons reflects a morally objectionable attitude to the future child, an attitude which, taken to an extreme, treats the child as an artifact and the reproductive process as a chance to design children according to parental standards of excellence (President's Commission, 1983; cf. section 2). As the latter is morally unacceptable, so the argument runs, we should not allow people to select the sex of their child, except for clear medical reasons. The slippery slope argument can likewise be brought to bear upon sex selection for what we have called intermediate reasons. One may argue that with this application, a crucial line is being crossed towards selecting children for characteristics unrelated to their own health. The idea is that after accepting this first step away from a strictly medical justification, it will be impossible not to end up also accepting selection of children for all kinds of non-health related traits. Even if sex selection for non-medical or intermediate reasons need not in itself be morally problematic, it will be impossible not to slide down towards reprehensible practices of commodification and instrumentalisation.

What to think of this objection? Let us first state that, from a scientific point of view, the feasibility of such reproductive selection or 'repro-designing' is strongly exaggerated in the media and in science fiction; in reality, the preferred characteristics are rather complex, co-determined by many genes and (mostly poorly understood) gene-environment interactions. This makes the reprogenetic creation of the prefect child highly unlikely (Human Genetics Commission, 2006). From an ethical point of view, the question is why and when (still futuristic) designing our progeny would be morally wrong. Some seem to argue that any type of reproductive enhancement (selecting for or designing special qualities) would undermine the autonomy of the future child and would, therefore, constitute an unacceptable violation of the dignity of the child (Habermas, 2001). Others may argue that such enhancement may be morally justified as long as the future child's autonomy is respected (Glover, 2006). Whatever we think of this debate, the current objection is problematic as it presumes that sex selection for non-medical (or indeed: intermediate) reasons will *automatically* lead to reproductive selection procedures or interventions that are intrinsically wrong. The presumed automatism is untenable: if selection for sex is morally acceptable, but selection for X, Y and Z is not, one may simply prohibit the latter and allow the former.

In summary: deontological arguments against allowing preconception sex selection for non-medical reasons (including intermediate ones) are either mistaken or unconvincing, whereas arguments referring to possible negative consequences are speculative at best. However, with regard to consequences, the analysis is not yet complete. So far, we have presumed that for couples wanting to select the sex of their children a reliable and safe sperm sorting technique was available that as such would not give rise to further moral concerns. Whether this is the case, is a question that we will now go on to consider.

4. Flow cytometry: the state of the art and its moral implications

Preconception sex selection involves sperm sorting, i.e. separating sperm cells that carry the X chromosome (gynogenic sperm) from sperm cells that carry the Y chromosome (androgenic sperm). The enriched sperm sample may ideally be used in artificial insemination (AI), which is less expensive and less invasive than IVF. While various methods of sperm sorting have been suggested, studied, and commercially offered, flow cytometry is currently the only option with a demonstrated efficacy. According to an overview of the HFEA, the pros of this technology include that it has a relatively high success rate and that sperm can indeed be used for AI rather than IVF, while its cons include that the technology is not completely reliable and that there are no conclusive studies yet confirming the safety for use in humans, although there are no contraindications from use in domestic cattle (HFEA, 2003). Obviously, both the reliability and safety of the technology used for preconception sex selection is morally relevant, as these relate to the interests of prospective parents applying for this technology, future children thus conceived

and society as a whole. In this section, we concentrate on the interests of the child.

Efficacy

In a recent paper, Karabinus (2009) provides the updated findings of the ongoing clinical trial performed at the Genetics & IVF Institute at Fairfax, USA. According to this study, the MicroSort sperm separation technology effectively increases the percentage of X- or Y-chromosome-bearing sperm in the sorted specimen. Consistent with this finding are the birth sex data: 92% of the babies were female after Xsort and 83.6% of babies were male after Ysort. These data provide evidence, so Karabinus concludes, of the efficacy of the method used for sex selection.

Although one may admit that this method has a relatively high success rate, its efficacy is clearly suboptimal. What happens if things go wrong, and the expected boy turns out to be a girl or vice versa? This is not a theoretical risk, at least for the moment: after using flow cytometry sorted sperm, in 8% of the cases where parents wish to have a girl they will have a boy and in approx. 16% of the cases where they would want to have a boy they will have a girl. One hopes the parents will adjust in such cases, recover from the surprise or shock, and will welcome and love their child for its own sake. But what if parents who have invested a lot in trying to select the sex of their child have difficulty in overcoming their disappointment? Of course, the imperfection of the technology for preconception sex selection is also relevant for its application for medical reasons. Where the use of flow-cytometry to avoid a the transmission of a serious sex-linked disease is concerned, a misdiagnosis rate of 8-16% rate may seem dramatic. However, this may still be acceptable if regarded as a preselection step to be combined with further selective procedures, either postconceptionally (through PGD and embryoselection), or indeed prenatally, through prenatal diagnosis and abortion. The combination of flow cytometry and PGD is explicitly envisaged by the HFEA as a conceivable option for 'patients at a significant risk of passing on a serious sex-linked genetic condition to their offspring' (HFEA, 2003). This would have as an advantage over direct PGD that a higher number of embryos of the desired sex will be available for postconception selection. Whether this step-wise approach should in principle also be available for couples at a higher risk of non-mendelian diseases with an unequal sex incidence (and perhaps also for sex selection for intermediate reasons) is a separate question that invokes considerations of proportionality in the context of the ethics of PGD and is outside the scope of this paper. The same holds for whether these considerations rule out the use of this combined approach where non-medical reasons are concerned. Even is this need not necessarily be the case, the combined approach will as a matter of fact be out of reach for most applicants wanting to select the sex of their offspring for non-medical reasons. This means that the suboptimal efficacy of flow cytometry remains a challenge given concerns about what it may mean for a child to be born to parents having gone so far in trying to have a child of the opposite sex.

Safety

Ever since flow cytometry has been proposed as a method for preconception sex selection, there have been concerns about its safety, mainly because it requires the staining of spermatozoa with a fluorescent dye (Hoechst 33342). However, the updated results from the MicroSort trial show that the malformation rate among babies conceived using this technology is similar to that of the general population. These data are now based on a total of 760 baby medical records (Karabinus, 2009). Reassuring outcomes were also reported in a recent review of the safety aspects of the same procedure in a large variety of mammalian species, based on data of around a million offspring (Garner, 2009). This review led to the conclusion that there is 'no apparent genotoxic effect from exposure of sperm to Hoechst 33342'. However, the same review also concluded that embryonic development is slower in sorted than in non-sorted sperm and that fertility rates are also lower. Moreover, there are no published data about cross-generational effects on health and fertility (Garner, 2009). Although there are different hypotheses for the effect on the lower fertility following insemination of mammalian females with sex sorted sperm (Grant, 2007), the more likely explanation seems to be that sperm may be damaged by the sorting process, in which the cells are exposed to a number of procedures that may be harmful, including (but not limited to) the fluorescent staining (Bermejo-Alvarez, 2008). Reports on specific effects refer to reduced motility and life-span of sperm. One report also found different expression patterns of developmentally important genes in bovine embryos that have been derived from sorted and non-sorted sperm (Morton, 2007). The clinical implications of these findings, however, are unknown for the moment.

5. Conclusions and recommendations

We conclude that the grounds for the present ban on preconception sex selection for non-medical reasons need reconsidering. First of all, the legislative

delineation of what counts as a 'medical reason' tends to be drawn too tightly. This is often defined in terms of avoiding a sex-linked disorder, which is usually understood as referring to a specific category of monogenetic diseases. This narrow definition is problematic, as it excludes sex selection aimed at avoiding the birth of a child with a non-mendelian disorder with an unequal sex incidence. In our view, parents at a higher risk of having a child with such a disorder should be allowed to use preconception sex selection, as is also acknowledged in the new British Act. The requirement that this should be a *serious* disease is too strict here, although it may be in place where postconceptional sex selection (via PGD) is at stake, as that would obviously change the proportionality of the procedure. Moreover, current regulations wrongly ignore the category of intermediate reasons: sex selection to avoid that one's offspring may have to face difficult reproductive decisions. We contend that like 'medical reasons' *stricto sensu* this is a morally unproblematic motive and that preconception sex selection for intermediate reasons should (in principle) be allowed.

With regard to what are clearly non-medical (personal, cultural) reasons things are more complex. As sex selection for non-medical reasons is not inherently sexist nor necessarily instrumentalising, and objections in terms of 'unnaturalness' or tensions with the traditional goals of medicine are unconvincing, reasons for a ban must be based on robust indications (rather than speculations) regarding harmful effects that allowing the procedure would have on children, women or society at large. Since, at least in a European context, no such indications have been identified, a categorical prohibition seems difficult to reconcile with the principle of respect for reproductive autonomy (Warren, 1985; Health Council 1995; Dahl, 2003; Robertson, 2004). However, this conclusion should not be read as a call for a 'laissez faire'-policy. Concerns about possible harmful effects of non-medical sex selection both on the psychosocial development of children and on society at large should be taken seriously (McMahon, 2004). The obvious response to this is indeed to allow preconception sex selection in a regulated setting aimed to strictly monitor its effects at all levels (Pennings, 2004). Whether this should include the condition that preconception sex selection may only be used to allow parents to enlarge their chances of having a mixed family (requiring that they already have at least one child of the other sex) is a matter for debate (Pennings, 1996). As has rightly been argued, it cannot be maintained that setting this condition would rule out the use of sex selection for sexist motives (including gender stereotyping) that may be harmful for the development of children in those families (Wilkinson, 2008). Even so, setting this condition might be a way of responding to societal concerns without making too large an inroad on the reproductive freedom of those who want to make use of preconception sex selection. After all, research has shown that the larger majority of those couples would want to do so with the aim of creating a mixed family.

Monitoring of effects should include long term follow-up of children born after preconception sex selection. In this connection, psychosocial research is needed to clarify issues such as possible pressures on the child to conform to gender stereotypes, effects on family dynamics and child development, uptake in and effects on ethnic communities, the position of women, etc.

Finally, a difficult set of questions is raised by concerns about the imperfect efficacy and unproven (long-term) safety of the technology. How should these concerns affect the debate over the acceptability of preconception sex selection for non-medical reasons? If the practice as such is acceptable in principle, is this still the case if the only technology – flow cytometry – that may presently work has these features? With regard to effects of failed sexing on the psychosocial development of the child: this is a further concern that we think should be closely monitored, but that as such is no less speculative than those listed before. Whether the fate of children in this situation would be worse than that of children of non-congruent sex whose parents have not attempted sex selection – cases of which would be prevented by allowing sex selection for non-medical reasons – is unclear (Health Council, 1995; Pennings, 2004). But what about possible (long term) health risks of flow cytometry? Although any remaining safety concerns are relevant for all clinical applications of the technology, accepting these is more difficult to justify where the procedure is carried out to satisfy a mere parental preference than where the aim is to avoid the birth of a child with a serious disease. We see two possible lines of reasoning here. Given the debate about the need for the field to proceed with greater care when introducing new reproductive technologies into clinical practice (Pennings *et al.,* 2007), one might argue that as long as safety concerns cannot be fully answered, flow cytometry may indeed only be used for medical reasons. The alternative is to say that this is too strict, given the extensive amount of reassuring data from the use of this same technology in cattle and other animals over more than 20 years, and that any remaining safety concerns should be addressed by careful monitoring rather than by imposing limitations on clinical use. We will not argue here for either of these positions. However, we do want to stress

that this issue should be on the agenda of the urgently needed reopening of the debate about preconception sex selection for non-medical reasons.

Why would this be urgent? Firstly, because unjustified limitations of reproductive freedom cannot be accepted in a liberal society (Dahl, 2004). But there is a second reason that is both morally relevant and of practical importance. In the coming years, new techniques allowing non-invasive prenatal diagnosis will become available for routine applications in early pregnancy (Wright, 2009). This will allow easy and risk-free testing for fetal aneuploidies, but also for fetal sex. In the light of the current ban on preconception sex selection for non-medical reasons, a possible consequence of this development may well be that interested couples will use information on fetal sex obtained from early fetal diagnosis for sex selective abortion (Newson, 2008; de Jong *et al.,* 2009). Not only would this amount to a form of sex selection that (unlike the use of a technique such as flow cytometry) is impossible to regulate and monitor, but also would the use of abortion as a means be morally more problematic than the forms of sex selection that are currently prohibited.

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Keep it simple: specific aspects of IUI in developing countries

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Introduction

Assisted reproduction (ART) is now an established therapy for the treatment of infertility in a multitude of clinical conditions. It embraces a wide scope of techniques including intrauterine insemination (IUI), in-vitro fertilization (IVF), gamete intra-fallopian transfer (GIFT), zygote intra-fallopian transfer (ZIFT) as well as intra-cytoplasmic sperm injection (ICSI). It is estimated that more than 3 million babies have now been born from ART. However, the great majority of those babies were born to couples living in developed countries (Ombelet, 2008; Ombelet *et al.*, 2008a). Various attempts are now being made to adapt these techniques for use in developing countries and bring this technology to the less fortunate (Sallam, 2008). This paper will discuss the specific aspects of one of these techniques, namely IUI, as it relates to developing countries and various methods to simplify it to maximize its benefits in these countries.

Developing countries

Two definitions of developing countries are currently being used: the World Bank definition and the United Nations Development Programme (UNDP) definition. The World Bank classifies countries according to their gross national income (GNI) per capita into low income, middle income (subdivided into lower middle and upper middle), or high income countries. Low income countries are those with a GNI of US$ 905 or less, lower middle income with a GNI of US$ 906-3595, upper middle income with a GNI of US$ 3596-11115, while high income countries are those with a GNI of US$ 11116 or more. Low income and middle income countries are sometimes referred to as developing countries. Although the use of the term is convenient for financial purposes, it does not necessarily reflect the development status of the country in question (Sallam, 2008).

Consequently, other measures for the classification of developing countries have been used by other international agencies. The UNDP takes into consideration three criteria for the evaluation of countries' development: the low income criterion, the human resources weakness criterion and the economic vulnerability criterion. Based on these criteria, the UNDP has compiled a list of the 50 least developed countries (LDC). Of these 34 are in Africa (Sallam, 2008).

Specific aspects of infertility in developing countries

Infertility in developing countries has its specific aspects and differs from infertility in the developed world in many ways. These include differences in its prevalence, its causes, the role played by infection as well as its effects and consequences. In addition, culture plays an important role in the causation, diagnosis and management of infertility in developing countries and scanty financial resources is an ever present factor affecting the provision of fertility services to these less fortunate patients.

Widespread differences exist in the prevalence of infertility in developing countries. In a study by Boivin *et al.*, the prevalence of infertility ranged from 3% of married couples in Shanghai in China to 25.7% in Chile (Boivin *et al.*, 2007). This confirms earlier findings that the prevalence of secondary infertility was 16% in North Africa, 23% in Asia, 40% in Latin America and 52% in Sub-Saharan Africa (Cates *et al.*, 1985). In Africa, Ericksen and Brunette found that the average prevalence for the whole continent was 14.9%, ranging from 9.8-12.2% in Eastern Africa to 16.7-21.4% in Southern Africa (Ericksen and Brunette, 1996).

There is also widespread variability in the causes of infertility in developing countries. In the study by Cates *et al.*, infertility was due to female causes in 25 to 37% and to male causes in 8 to 22% of couples. Both male and female causes were present in 21 to 38% of the couples. This variability has been blamed on various factors including variability in the sexually transmitted, infectious, and parasitic diseases, health care practices and policies as well as exposure to potentially toxic substances in the diet or the environment (Cates *et al.*, 1985). In particular, infection plays a disproportionately higher role in the causation of infertility in developing countries including gonorrhoea, Chlamydia and HIV (Ombelet *et al.*, 2008a).

In addition, infertility has serious effects on the life of women in developing countries including their social standing and psychological health. These effects include loss of social status, social isolation, marital instability, loss of social security, negative effects on gender identity as well as psychological consequences (guilt, depression, shame, grief and/or sense of worthlessness). In some communities, the stigma of infertility leads to violence against women (Ameh *et al.*, 2007). In some communities, it may even follow them to their graves, where traditional funeral services may be denied to them as they are considered witches or bringers of bad luck (Araoye, 2003; Ombelet *et al.*, 2008a). Other cultural factors affecting infertility in developing countries include the culture of son-preference, the bias against gamete donation and treatment of non-traditional families (Sen, 2003).

On top of these burdens, many obstacles to treating infertility in developing countries exist. These include lack of services, lack of know how, lack of access to services, acceptance of fate (curse from God), bad experience of neighbours, resorting to traditional healers as well as lack of finance (Nygren and Zegers-Hochschild, 2008). In fact developing countries, which form 84% of the global population and carry 90% of the global burden of disease, spend 12% of the global health spending compared to 88% spent by developed countries which form 16% of the world population and carry 10% of the global disease burden. In addition the public share of the total health spending is 29% in developing countries compared to 65% in developed countries (Schieber and Maeda, 1999).

Specific aspects of IUI in developing countries

IUI is the simpliest form of assisted reproduction and offers particular advantages to developing countries as the first line of ART management. These include the minimal equipment required, an easy technique to learn, being less invasive, less expensive with a reduced psychological burden on the couple. In addition, IUI has a good couple compliancy (low dropout rate), a low risk for OHSS and a low multiple pregnancy rate with natural cycles, clomiphene or low-dose HMG protocols (Ombelet *et al.*, 2008b).

However, in order to maximize the benefits of IUI in developing countries, some modifications have been suggested in order to render the technique cost effective under the circumstances of these countries. These include the use of simple stimulation protocols, simple monitoring techniques for timing IUI, simple methods for the evaluation of the semen sample, the use of simple sperm preparation techniques, simple insemination devices, simple insemination techniques and special precautions relating to HIV contamination.

1. Simple stimulation protocols

Various studies have shown that performing IUI with ovarian stimulation increases the pregnancy rates significantly compared to IUI in non-stimulated cycles. In a meta-analysis by Hughes, the odds ratio (OR) for clinical pregnancy in stimulated cycles versus non-stimulated cycles was 2.37 with 95% confidence interval (CI) of 1.43 to 3.90 (Hughes, 1997).

The success rate of IUI is also a function of the number of follicles developing in response to stimulation. In a recent meta-analysis by van Rumstke *et al.*, the absolute pregnancy rate was 8.4% for monofollicular and 15% for multifollicular growth. The pooled OR for pregnancy after two follicles as compared with monofollicular growth was 1.6 (99% CI = 1.3-2.0), whereas for three and four follicles, this was 2.0 and 2.0, respectively. However, the risk of multiple pregnancy increases also with the number of follicles. The pooled OR for multiple pregnancies after two follicles was 1.7 (99% CI = 0.8-3.6), whereas for three and four follicles this was 2.8 and 2.3, respectively (van Rumstke *et al.*, 2008).

Consequently, most infertility specialists perform IUI in stimulated cycles and it has generally been assumed that HMG stimulation can lead to higher pregnancy rates compared to clomiphene citrate (CC). In another non-controlled study on IUI in CC/HMG stimulated cycles, the clinical pregnancy rate was 12.6% per cycle and the multiple pregnancy rate was 13.7% (Nuojua-Huttunen *et al.*, 1999).

However, in a study by Ombelet *et al.* (1997), patients treated with CC and IUI (without HMG) achieved similar results with an overall cycle fecundity (CF) and baby take-home rate (BTH) of 14.6% and 9.9% respectively. The cumulative CF and BTH (per couple) after three cycles were 30.6% and

21.1% respectively. These findings were similar in all patients except when the insemination motile count was $< 1 \times 10^6$ with a morphology score of < 4% normal forms. There were only two twin pregnancies (2.5%) and no moderate or severe OHSS. These findings were confirmed in a cross-over study by Ecochard *et al.* (2000) who compared CC to HMG stimulation in IUI cycles and found no significant difference in clinical pregnancy rates between both regimens.

More recently, Dhaliwal *et al.* (2002) compared the conventional CC + HMG daily (from day 8) protocol to a minimal stimulation protocol of CC + one HMG injection (150 IU on day 9) in patients undergoing IUI. They found no significant difference in clinical pregnancy rate per cycle (19.12% versus 16.20%) between both regimens. The risk of miscarriage was significantly lower with minimal stimulation (5.7% versus 23%; $P < 0.05$). The multiple pregnancy rate was 5.13% and the incidence of OHSS was 3% in the conventional stimulation protocol versus none in the minimal stimulation protocol (Dhaliwal *et al.*, 2002). More importantly, the cost of the treatment cycle was US$ 63.50 for the minimal stimulation cycle versus US$ 216.50 in the conventional stimulation cycle. It is therefore clear that CC stimulation prior to IUI can be used safely and effectively in settings with low resources such as developing countries.

2. Simple monitoring techniques and timing of IUI

Various methods for monitoring ovarian stimulation and timing IUI have been proposed. These are based on the probabilities of conception models developed by various groups (Barrett and Marshall, 1969; Schwartz, 1980; Royston, 1982).

In 1982, we used serial ultrasound monitoring of folliculogenesis and performed the insemination when the leading follicle reached 18 mm in diameter. Using this technique we achieved a 12% pregnancy rate ?CF versus 6% in patients monitored with basal body temperature charts (Marinho *et al.*, 1982).

Subsequently, two other methods were used for timing IUI, namely serial measurement of urinary LH and triggering ovulation by the IM injection of 5000 – 10000 IU of HCG, with various claims of success. In 2007, Kosmas *et al.* performed a meta-analysis of seven prospective and retrospective studies and found that patients who received hCG before IUI demonstrated lower clinical-pregnancy rates than did women who had IUI after spontaneous ovulation (OR = 0.74; 95% CI = 0.57 to 0.96) (Kosmas *et al.*, 2007). In another study, there was no difference in clinical pregnancy rates when insemination was performed once at 24 hours or twice at 12 and 36 hours after HCG administration (Tonguc *et al.*, 2009).

Other methods for timing IUI have been proposed including the serial measurement of serum or salivary oestradiol or its metabolites (oestrone-3-glucuronide, oestradiol-16alpha-glucuronide and oestriol-16beta-glucuronide) in urine or in saliva (Branch *et al.*, 1982; Sallam, 1983; Tanabe *et al.*, 2001). However, these are more expensive and are only commercially available in some developed countries. Consequently, in a low resources setting, timing IUI with the use of relatively expensive and time-consuming methods does not increase the pregnancy rate over folliculometry or urinary LH monitoring of ovulation (Zreik *et al.*, 1999).

3. Evaluation of the semen sample

Traditionally, the evaluation of the semen sample consisted of the measurement of its volume, count, percentage of motile spermatozoa, of abnormal forms and an estimate of the white blood cells per high power field. Subsequently, evaluation of sperm morphology using strict criteria was introduced (Kruger *et al.*, 1987) as well as computer-assisted semen analysis to measure more sophisticated indices of sperm motility (Holt *et al.*, 1985; Vantman *et al.*, 1988). In addition a number of sperm function tests have been described with various claims of their efficiency in determining the fertilizing capacity of the sperm (Aitken, 2006).

In an attempt to determine the relative importance of the various criteria used in semen evaluation, we analyzed 11 sperm parameters, namely sperm count, eight sperm velocity parameters determined by computer-assisted semen analysis, sperm morphology score using strict criteria and the hypo-osmotic swelling (HOS) test. Data from 58 couples treated with IVF were analyzed retrospectively by simple linear regression and multiple stepwise regression analysis, taking the fertilization rate as the dependent factor. The mean sperm velocity and the strict sperm morphology assessment were the only parameters showing significant correlation with the fertilization rate. A minimum sperm velocity of 13 μm/sec and a minimum strict sperm morphology percentage of 2% were necessary to achieve fertilization in 50% of the oocytes. The results of the HOS test did not correlate with the fertilization rate (Sallam *et al.*, 2003). These results confirmed the earlier work by Menkveld *et al.* (2001) who, using ROC curves, reported cut-off values of 8% for the acrosome index, 45% for motility, and 4% normal spermatozoa for strict criteria.

Consequently, proper evaluation of the semen sample can be achieved by the simple but accurate

determination of the sperm count, sperm motility and sperm morphology using strict criteria, as resorting to complicated and more expensive semen evaluation techniques is not necessary for the majority of infertile couples. Training courses for laboratory technicians on semen evaluation are conducted regularly with help of WHO in various parts of the world in order to standardize the procedure and many of these courses are conducted in developing countries, particularly in Africa (Franken and Aneck-Hahn, 2008).

4. Simple sperm preparation techniques

Various sperm preparation techniques for IUI have been described. The most commonly used are sperm washing and centrifugation, the swim-up method as well as centrifugation on discontinuous Percoll, Ficoll or Nycodenz gradients (Gellert-Mortimer *et al.*, 1988; Bongso *et al.*, 1989; Van der Zwalmen *et al.*, 1991). Other less commonly used methods are glass bead column, swim-down, and sperm select systems (Fahmy, 2005).

A recent meta-analysis was conducted by Boomsma et al. Five RCTs, including 262 couples in total, were included in the meta-analysis. The reviewers found that no trials reported the primary outcome of live birth. There was no evidence of a difference between pregnancy rates (PR) for swim-up versus a gradient or wash and centrifugation technique (OR = 1.57, 95% CI = 0.74 to 3.32; OR = 0.41, 95% CI = 0.15 to 1.10, respectively), nor in the two studies comparing a gradient technique versus wash and centrifugation (OR = 1.76, 95% CI = 0.57 to 5.44). There was no evidence of a difference in the miscarriage rate (MR) in two studies comparing swim-up versus a gradient technique (OR = 0.13, 95% CI = 0.01 to 1.33) (Boomsma *et al.*, 2007). In summary, no method proved its superiority over the others.

Consequently, in low resource settings such as in developing countries, the simplest sperm preparation technique should be used. The swim-up method can therefore be used when the sperm count is adequate (> 5 million/ml), while the wash and centrifugation can be used when the count is low (< 5 million/ml). Gradient techniques should be reserved for infected samples.

5. Simple insemination devices

Different catheters have been used for IUI with various claims of success. It has been suggested that soft catheters may be less traumatic to the uterine cervix or to the endometirum. However, in a study by Miller *et al.* (2005), who compared the more rigid but cheaper Tomcat catheter to the softer and more expensive Cook catheter, there were no significant differences in clinical pregnancies between both catheters. These findings were later confirmed in a meta-analysis showing no significant differences in clinical pregnancy rate and live birth rate between both groups (OR = 0.96; 95% CI = 0.70 to 1.32 and OR = 0.82; 95% CI = 0.43 to 1.58, respectively) (Abou-Setta *et al.*, 2006).

6. Single versus double insemination

It has been suggested by different groups that performing IUI on two occasions during the same cycle may increase the chances of pregnancy. However, in a recent meta-analysis, involving 829 women from 6 randomized trials, there was no significant difference between the single and double IUI groups in the probability of clinical pregnancy (OR = 0.92; 95% CI = 0.58 to 1.45). The mean fecundity rate was 13.6% for double inseminations versus 14.4% for single inseminations (Polyzos *et al.*, 2009). This confirms the findings in an earlier Cochrane review which concluded that double intrauterine insemination showed no significant benefit over single intrauterine insemination (OR = 1.45; 95% = CI 0.78 to 2.70) (Cantineau *et al.*, 2003).

7. IUI in the presence of chronic viral diseases including HIV/AIDS

IUI for patients infected with chronic viral diseases presents a real challenge to the personnel involved in their treatment. HIV is endemic in many developing countries in Africa and Asia as is HBV and HCV. These viruses and others can be transmitted by IUI and present a health risk to the personnel dealing with the infected semen samples. Cross-contamination in tanks storing frozen semen samples is another hazard.

However, it has now been established that methods of preparing the sperm in which the seminal plasma is removed by washing and sperm are separated from the other cellular elements of the sperm (e.g. centrifugation on discontinuous gradients) can reduce the viral load up to an undetectable level (Semprini and Fiore, 2004). In a study by Bujan *et al.* (2007) 84 HIV-1 serodicordant couples underwent 294 IUIs. Spermatozoa from HIV-1 infected male partner were prepared and tested for HIV-1 using the sperm washing method. The pregnancy rate per cycle and baby take-home rate per couple were 18.0% and 52.4%, respectively. No female HIV-1 contamination occurred.

It has also been suggested that the systematic use of ICSI is the treatment of choice in these cases. In a recent study by Sauer *et al.*, 420 ICSI cycles were

performed in 181 HIV sero-discordant couples where the man was carrying the virus. The clinical and ongoing pregnancy rates per embryo transfer were 45% and 37%, respectively. No maternal or neonatal HIV infections or deaths occurred. In an attempt to further minimize the risks, Englert *et al.* (2004), suggested to perform systematic screening before IUI, to have a separate "infected laboratory", to adapt a set of safety procedures and to do HIV testing post preparation. The implementation of the latter policies in developing countries is obviously impractical.

Conclusions

IUI is a simple, affordable and effective treatment for infertility in indicated cases and is particularly suited to developing countries. Randomized trials have shown that IUI can be successfully performed in patients with limited resources using simple approaches. In those patients, clomiphne citrate stimulation should be prescribed and insemination timed with urinary LH monitoring or serial ultrasound folliculometry. The swim up technique should be used when the semen count is adequate and washing and centrifugation if the count is low. Gradient separation should be reserved for patients with pyospermia or virus infection. Rigid cheap catheters perform as well as softer and more expensive catheter, one insemination is as good as two and special strict precautions should be taken in communities with a high incidence of HIV/AIDS.

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SPERM ONLY PLEASE: Prevention of infections in an assisted reproduction laboratory in a developing country

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Abstract

The aim of the current paper is to discuss prevention of infections in a public sector laboratory-orientated assisted reproduction treatment (ART) setting in Gauteng, South Africa. The reproductive profile with reference to the screening for sexual transmitted infections, affordability of care and resources available, should determine the repertoire of fertility screening and ART scheduling. Alternative viewpoints on resource management of an ART laboratory in a public sector setting, semen decontamination for the human immunodeficiency virus type-1 (HIV-1) seropositive male and the subsequent choice of ART procedures, i.e. intra-uterine insemination (IUI) as a first-line treatment and intra-cytoplasmic sperm injection (ICSI) are addressed. Assistance for the safe reproduction of HIV-1 serodiscordant couples is globally acceptable, and should be moulded into an accessible ART program for developing countries.

Key words: Affordable, assisted reproduction, developing country, semen decontamination, HIV-1; infections

Introduction

The reproductive health profiles, affordability of and access to health services and resources will influence the reproductive wellbeing of a couple. Assisted reproduction technologies are in general perceived as expensive and childlessness as non-life threatening. Economics therefore dictated that infertility care is at times, not regarded as a public service health priority in developing countries. It is however important that current practices, experiences and facilities of a provider should be reviewed before cost-effective and safe fertility care for the human immunodeficiency virus type-1 (HIV-1) seropositive couple can be established (Frodsham *et al.,* 2006). Therefore, what is considered as "cost-effective" in a society, which carries a high burden of sexual transmitted diseases? The HIV/acquired immune deficiency syndrome (AIDS) epidemic also impacts on the procreation needs of a couple, since childlessness has an universal implication that transverses nationality, income status, physical conditions and gender. The Joint United Nations Program on HIV/AIDS (UNAIDS, 2008) estimated that in 2007, between 30 and 36 million people were living with HIV, with two thirds (67%) of all HIV-positive individuals residing in sub-Saharan Africa. Large variations in HIV prevalence also exist between the different regions within sub-Saharan Africa (Buvé, 2002). South Africa has the unenviable position to have the largest HIV epidemic globally, with an estimated 5.7 million of inhabitants living with HIV in 2007. The epidemic is defined as a hyper-endemic epidemic since 15% of the age group 15 to 49 are living with HIV (UNAIDS, 2008). A South African National Survey on the prevalence and incidence of HIV among more than 13,000 nationals in 2008, estimated that 50.8% of respondents aged 15+ years reported a history of HIV-testing in 2008, with more females (28.7%) participating in testing and knowing their status than their male counterparts (19.9%) (Shisana *et al*., 2008).

Experiences we have gained at the Reproductive and Endocrine Unit, which is situated in Gauteng, the most populous province of South Africa, indi-

cated that most individuals are knowledgeable of HIV/AIDS and the risks associated therewith, when seeking reproductive assistance. The unit is a tertiary infertility clinic with a diagnostic and therapeutic assisted reproduction laboratory, dealing with the low- and middle-class income population of the northern part of South Africa. It is the only public health care centre active in assisted reproductive technologies, in this part of the country. The aim of this paper is to discuss information relevant to laboratory-orientated services for couples in a public sector South African assisted reproduction treatment (ART) Unit, with reference to conditions where the male is HIV-1 positive.

Infection and contamination control in an ART laboratory

Infections within an ART laboratory can originate through health workers, samples from patients that harbour infectious conditions, the laboratory atmosphere, contaminated equipment and storage vessels, as well as materials and supplies utilized during procedures. The primary objective of any ART Unit should be to exclude all possible pathogens from all potential sources (Elder *et al*., 2005). By identifying the potential sources, protective measures can be taken to prevent transmission of pathogens. Unhygienic working conditions and waste disposal increases the risk that infections may be transmitted to personnel, patients, cultures, samples and equipment. Elder *et al*. (2005) recommended that a stringent infection control policy should refer to aspects of hygiene, disposal of clinical waste and protective measures including decontamination procedures. A question consequently arises, if best practice quality control frameworks and directives from developed countries should be used to guide emerging or future assisted reproduction laboratories in the developing world? Mortimer (2005) assessed the impact of the European Union Tissue and Cells Directive (2004) in an ART milieu, and is of the opinion that commonsense can; in some situations indicate an overload of good intentions. The author also recommended that particular circumstances, which could affect reproductive tissues, should be properly considered to ensure that technical regulations are realistic and based on objective evidence.

In some instances offices, rooms, wards or theatres are modified and adapted to serve as ART laboratories in developing countries, whereby air quality and humidity are the most difficult factors to regulate in our experiences. Contamination with spore-forming bacillus species, fungal spores (e.g. *Aspergillus* sp, zygomycetes or other saprophytic moulds) occur through crevices in the ceiling, and through air conditioning ducts and ventilation systems (Elder *et al*., 2005). Figure 1A(i) illustrates dust entering through gaps in the suspended ceiling and sticking to a textured wall in the diagnostic spermatology laboratory at Steve Biko Academic Hospital (Reproductive Biology Lab, Pretoria, South Africa) prior to renovations, and Figure 1A(ii) shows the modified sealed ceilings and filters installed in front of ventilation ducts after renovations. The intended clean room for embryo culture before and after constructions is depicted in Figures 1, pictures B(i) and (ii). All basins were removed due to the possibility of the introduction of waterborne pathogens in a clean room environment. Since *Pseudomonas* spp. were previously identified in tap water from adjacent laboratories and washrooms, we assess the water quality regularly and periodically clean the inside of all laboratory taps using a small bottle brush and a mild sodium hypochlorite solution. Male patients also receive bottled water (intended for irrigation) at our Unit, to wash with, prior to giving a semen sample.

Door closures, digital locks and security gates were installed to improve security and limit access to clean room areas during the upgrade of our laboratory. Monitors were placed strategically adjacent to the clean room areas to (i) observe e.g. follicular fluid screening or embryo selection, (ii) provide visual information in real-time during micro-manipulation training and (iii) decrease the need to enter sensitive areas. Embryo development is also computerized and images can be viewed by students in training, clinical staff and patients, either on the monitors and/or in a printed report format. All ill-fitting wooden pass-throughs, linking the clean rooms to adjacent areas, were replaced with one-way stainless steel and glass double-door pass-throughs (Figure 1B (i) and 1B(ii)). Sturdy washable overlapping blinds replaced flimsy material blinds, and electrical sockets were placed below all work surfaces, to provide a clutter free workspace which is easier to clean. A once off investment to obtain over-pressured separate laboratories for HIV-infected and non-infected patients with separate cryopreservation facilities provide a safe-environment for all our patients and staff. A dust-free laboratory environment is obtained through a high number of fresh air changes per hour, and the placement of in-line HEPA-filters in the ventilation system that functions separate from the hospital's system. Although the latter additions to the facility were costly and associated with developed countries modus operandi, it was well-worth the efforts. The investment in a basic air sampler (Biotest HYCON®, Dreieich, Germany) and indicator agar strips (Agar Strip TC - Total



Fig. 1. — Structural defects of facilities intended for (A) diagnostic and (B) therapeutic ART.
A(i): Illustrate dust seeping through crevices; suspended ceiling and textured wall paint;
A(ii): Modified ceiling and ventilation filter;
B(i) Prior to renovations: Drain inside sterile room area, wooden hatch, material blinds before window with incorrect placement of electrical sockets;
B(ii) After renovations: Drain removed, ceramic tiles placed, marble bench with stainless steel glass pass-through doors.

count; ref no 941105; Agar Strip YM – Yeast and moulds; ref no 941196; www.biotest.de), proved to be equally empowering. Sampling air using specific indicator agar strips, incubating the strips and learning to interpret and calculate the colony forming units (microbial count) resulted in a quick turn around time between testing and outcome. This interpretation led to lessened costs and provided a practical self-assessment tool to confirm the cleanliness of an area or instrument.

Most of the clinical and laboratory adaptations and control mechanisms to minimize viral transmissions during ART as described by Englert *et al*. (2004), Gilling-Smith *et al*. (2005) and Gilling-Smith (2006) have been adopted at our Unit over a period of four years. Entrée level embryologists receive ongoing formal training and are chaperoned for two years, with emphasize on risk-reduction and quality control procedures. An assisted reproduction laboratory is a complex structure geared towards the promotion of optimal cell survival and culture, not only for embryos, but also for viruses and bacteria (Englert e*t al*., 2004; Elder *et al*., 2005). Intrinsic laboratory factors such as temperature, gas/culture atmosphere, air quality and laboratory hygiene that directly affect gametes and embryos, must be monitored by laboratory staff, while the intake of known and unknown contaminated patient samples are governed mostly by clinical staff. Introduction of infectious conditions into the assisted reproduction laboratory can be prevented if the origin of the contaminant can be identified, contained or removed.

Screening for pathogens and treatment prior to ART

Tremendous improvements in the prevention and screening for sexual transmitted diseases (STDs)

have been made in the South Africa, probably due to HIV/AIDS awareness campaigns in recent times. In the public health sector patients are usually sectioned into groups based on their annual income and access to health insurances, whereby patients with a low income seeking ART, are most often subsidized. Economics clearly influence the choice and scope of sexual transmitted infections (STI) tests, selection of ART procedures and choice of a private or public ART sector in South Africa. Several authors have published data (Table I) on the outcome of intra-uterine insemination (IUI), *in vitro* fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) procedures for a large number of HIV-serodiscordant couples (males positive), without any documented case of sero-conversions during the time-periods of the surveys. These reports, from multiple investigators from developed countries, provide directives of the safety and outcome of the ART procedures for HIV-infected individuals in developing countries. ART centres should, however, always consider all risk reduction measures to safeguard all patients and staff when treating patients with known infections. Even if universal precautions are practised, zero risk does not exist (Gilling-Smith *et al*., 2005).

Microbes in semen

Bacteriospermia does not necessarily imply an infectious condition (Cottell *et al*., 2000) and can be due to contamination during semen collection. The practice of routine semen cultures in asymptomatic couples is therefore questionable. Four possible approaches to deal with bacterial contaminants in semen are (i) patients with a positive bacterial culture are treated with appropriate antibiotics prior to an assisted reproduction procedure, and re-evaluated to confirm a negative seminal bacterial culture, (ii) provision of guidelines (directives on sample collection) to male-patients to eliminate skin-contaminants, (iii) semen washing and embryo culture medium are supplemented with antibiotics, and the (iv) use of a physical mechanism to reduce microbes in the processed sperm sample, such as density gradient and a mechanical device e.g. the ProInsert™ (Nidacon, Sweden) (Huyser, 2008). Therefore, to pro-actively prevent the unnecessary prescription (costs and use) of anti-microbial medications to our patients, verbal information on laboratory procedures and guidelines on semen collection are provided to male patients in four indigenous languages.

Table I. — Summary of published ART outcome for HIV-1 serodiscordant couples (male positive) 2004-2010.

ART Procedure	Authors	Cycles	Couples	HIV-1 test of washed fraction		Pregnancy rate
				DNA	RNA	
IUI	Bujan *et al*., 2007*	2840	853	Yes**	Yes**	15.1%
	Manigart *et al*., 2006	68	25	Yes	Yes	14.7%
	Savasi *et al*., 2007	2400	581	No	Yes	19.0%
	Van Leeuwen *et al*., 2009	174	61	No	Yes	52.0%
Total		**5482**	**1520**			
IVF	Bujan *et al*., 2007*	107	76	Yes**	Yes**	29.0%
Total		**107**	**76**			
ICSI	Bujan *et al*., 2007*	394	262	Yes**	Yes**	30.6%
	Garrido *et al*., 2004	73	73	Yes	Yes	46.0%
	Manigart *et al*., 2006	62	20	Yes	Yes	45.0%
	Mencaglia *et al*., 2005	78	43	No	No	28.2%
	Sauer *et al*., 2009	420	181	No	No	46.0%
	Savasi *et al*., 2007	278	160	No	Yes	23.0%
Total		**1305**	**739**			
IVF/ICSI	Kashima *et al*., 2009	38	27	Yes	Yes	60.6%
	Prisant *et al*., 2010	44	28	Yes	Yes	18.2%
Total		**8**	**55**			
TOTAL		**6976**	**2390**			

*CREAThE – Centers for Reproductive Assistance Techniques to HIV couples in Europe
** Multicentre study between 8 centers; all centers except one, performed HIV-1 DNA and RNA validation of sperm samples

If needed, HIV-negative patients are issued with a non-spermicidal polyurethane condom (Male-FactorPak®, Apex Medical Technologies, California). A large retrospective study by Fourie *et al*. (2009) indicated that microbes were present in 53% of all semen samples (n=929) tested at the Pretoria Unit during 2007-2009. Micro-organisms commonly cultured in samples during diagnostic semen analyses were: coagulase negative staphylococci (27%), *Ureaplasma* spp. (11%), *Mycoplasma* spp. (5%), α-haemolytic streptococci (5%), *Escherichia coli* (5%), *Enterococcus faecalis* (4%), and *Enterobacter* spp. (3%). The positive cultures and the reported sensitivities, will then determine the antimicrobial-options available to the patient. Questionable is the infrequent practice at some ART Units, to only request microbial testing of semen and viral screening (HIV-1) of male patients judged to be at risk of infections. To assume on appearances and judgement of the females' blood pathology results that the male is negative, is a risky endeavour for all involved.

Sexual transmissible pathogens

Only a limited number of papers have been published on the prevalence of sexual transmitted diseases in South Africa. Pham-Kanter *et al*. (1996) provided an informative historical perspective on the disease burden of classical transmitted infections, and statistics on ulcerative infections, gonorrhoea, chlamydia and other organisms. A review by Johnson *et al*. (2005) updated the study by Pham-Kanter and colleagues (1996) to provide information on STI prevalence rates between 1985 and 2003 in South Africa. Bambra (1999) indicated that the most common STIs reported in Africa, besides HIV include chancroid, human papiloma virus, herpes simplex, trichomoniasis and candidiasis, while gonorrhoea, syphilis and chlamydia also contribute to damages of Fallopian tubes. Infectious conditions of the male reproductive tract may also impact on HIV-1 shedding and thus the HIV load in semen (Dejucq-Rainsford and Jégou, 2004; Kalichman *et al*., 2008). The origin, clinical presentation and transmission through semen of other sexual transmitted viruses, such as cytomegalovirus (CMV), hepatitis B, C, D (HBV, HCV, HDV) and human T-lymphotrophic virus (HTLV-I) have been reviewed elsewhere (Dejucq-Rainsford and Jégou, 2004; Elder *et al*., 2005). Sub-Saharan Africa is reported to have the highest HCV prevalence rate in the world, compared to European and North American populations. African countries with the highest HIV infections tend to have the lowest estimated HCV incidences (Madhava *et al*., 2002). The mode of hepatitis transmission and implications for infertile patients, as well as health workers have been described in detail by the Practice Committee of the American Society for Reproductive Medicine (ASRM, 2006).

(i) Screening for HIV in ART

Participation in an ART program implies agreement to disclose the HIV status to the participating partner (ESHRE Taskforce on Ethics and Law, 2004). Cost implications, as well as restricted access to pathology laboratories, could be reasons for the periodical screening of patients for selected blood borne viruses in some South African ART Units (Huyser, 2008). Although access to reliable HIV-1 testing for diagnostic and monitoring purposes is limited in Sub-Saharan Africa, most resource-constrained countries do have reference laboratories, especially in capital cities (Rouet and Rouzioux, 2007). A trade-off therefore exists between the cost for screening of sexual transmitted infections, indirectly assessing health risk indicators and the accommodation of patients in ART practices. Most South African ART centres screen patients who enter an ART program, using an automated enzyme-linked immunosorbent assay (ELISA) – i.e. HIV Combo testing for HIV-1 & 2 antibodies plus p24 (Abbott 4th Generation assay). Positive HIV ELISA results are then confirmed with a second HIV Combo ELISA (e.g. Roche Diagnostics) using the same blood sample. A positive result is then reported with the request for second blood sample to confirm the initial test (using ELISA), and to verify the origin of the sample (see testing strategy II, WHO, 2005). Branched-chain DNA test (bDNA) or polymerase chain reaction (PCR) or nucleic acid sequence-based amplification (NASBA) analyses, are usually requested to evaluate blood plasma viral load (BPVL). These commercial assays are also used to determine seminal plasma viral load (SPVL), as well as the efficacy of the decontamination procedure to obtain purified sperm samples after processing. For a review on current commercial methods to determine SPVL, with reference to performance advantages and limitations; see Chan and McNally (2008). These molecular validation techniques are labour-intensive as well as time consuming and especially expensive to patients with minimal or no health insurance. A retrospective survey in 2009 (N = 200 patients), indicated that less than 40% of patients that requested ART, have the benefit of health insurance. Extrapolation of costs to present day PCR analyses (confirmation of viral genome) at a private pathology laboratory in South Africa, indicated that HIV-1 analyses and CD4/8 amounts to a cost of approximately 90 €. Newer real-time technology (real-time

PCR) seems to offer excellent cost-benefits, have an expanded dynamic range, reduces the amount of manual handling and risk of contamination through automation (Chan and McNally, 2008) to benefit higher throughputs in resource-poor environments (Scott *et al.,* 2009). Comparing three real-time assays for HIV-1 subtype C VL quantification in South Africa, the latter-mentioned authors, are of opinion that the Abbott m2000 RealTi*m*e HIV-1 assay, will benefit a high throughput in the South African National Health Laboratory Service (to be implemented in mid 2010).

Blood and semen/purified sperm samples are usually couriered to pathology laboratories in major cities in South Africa for viral validations, using the previous-mentioned commercial assays. Patients are required to return for a follow-up appointment to obtain their results and to initiate treatment, since the results are usually available within 24-72 hours. A delay before treatment can result in further transmission, while a number of patients may prefer not to return and/or are unreachable for a follow-up appointment. Some patients may be apprehensive to return, or perhaps fear further cost escalations and therefore remain untreated. A test that provides immediate results will avert a delay between performing a test and obtaining the results (Ward and Weber, 2010). The WHO guidelines on HIV rapid testing and counselling services in resource-constrained settings indicated that most rapid tests are single tests, easy to perform, with a shelf-life of approximately 12 months, can be stored at 20-30°C, and cost between 0.40 – 1.20 US$ for a single test (WHO, 2004). Although the ELISA screening test is less expensive than the previously mentioned molecular assays for viral validation, it is still costly for most patients and requires an infra-structure and trained staff to perform and interpret results. The development of applicable rapid tests could play a significant role in future reproductive health screening and management of patients in low-resource settings.

(ii) HIV-1 validation in seminal plasma and purified sperm samples

It is debatable if an HIV/ART policy should be adopted based on the BPVL and/or SPVL of patients requesting ART in a resource-limited setting. An undetectable or low BPVL together with a repeatable, easy to perform, and cost-effective semen decontamination procedure is desirable in this environment. We initiated an experimental semen decontamination program at our Unit, to test the ability of a decontamination procedure (using a novel tube insert [ProInsert™] combined with density gradient centrifugation) to remove HIV-1, HCV, CMV and white blood cells from *in vitro* spiked semen samples (Loskutoff *et al*., 2005; Huyser *et al*., 2006). Subsequently we evaluated the effectiveness of the decontamination procedure to remove a variety of bacteria and yeast from *in vitro* spiked semen samples obtained from HIV-seronegative donors; and *in vivo* derived HIV-1 RNA as well as proviral DNA from semen of HIV-seropositive men (Huyser 2008; Fourie *et al*., 2009). Commercial validation assays were used to determine HIV-1 RNA VL (Cobas AmpliPrep-Cobas Taqman HIV-1 version 2, Roche Diagnostics, Indianapolis, USA; linear reportable range 20-10,000,000 copies/ml) and to evaluate proviral DNA qualitatively (Amplicor HIV-1 DNA, version 1.5 Roche diagnostics) in all neat semen samples from HIV-1 seropositive males and corresponding post-processed sperm aliquots. These validations were performed on all samples, by an independent private pathology laboratory. Evaluation of fifty semen samples indicated that 64% of the neat semen samples tested positive for HIV-1 RNA, DNA or both (26%, 20% and 18%, respectively). The decontamination method was effective in the removal of all HIV-1 proviral DNA and RNA (range 138 - 801,440 RNA copies/ml) in neat semen. Validation results were, however, usually received after 48 hours, which necessitates the cryopreservation of the purified sperm. Cryopreservation of the final sperm sample has major consequences for ART management of the HIV-serodiscordant couple (Leruez-Ville *et al*., 2002; Nakhuda and Sauer, 2007), especially in the developing world. Cryopreservation and the resultant sperm quality after thawing may negate a planned IUI procedure, and may necessitate the implementation of IVF procedures (Leruez-Ville *et al*., 2002). This implies that laboratory staff should be able to cryopreserve and store sperm samples in hermetically sealed ionomeric resin staws (CryoBioSystems, CBS™) (Tomlinson, 2005) within dedicated liquid nitrogen canisters. The volume of neat semen/sperm needed to perform the molecular viral validation analyses is also a limiting factor. Lereuz-Ville *et al*. (2002) mentioned that some ART groups proposed the use of selected spermatozoa without viral validation, where HIV detection in (previous) processed semen was negative. The authors cautioned that HIV RNA detection was most likely to be positive in sperm samples that originate from seminal plasma with a high VL (> 20,000 copies/ml). Seminal viral load will most definitely impact on the efficiency of sperm processing methods to obtain purified sperm samples. An upper limit of 1-5 $\times$ 10^5 RNA copies/ml in seminal plasma was indicated by Fiore *et al.* (2005), for discontinuous density gradient centrifu-

gation combined with a swim-up to be successful. The researchers quantified HIV-1 RNA using a NASBA assay with a lower detection limit of 80 copies/ml. Although South African health insurances often do not cover infertility treatment and do not subsidize HIV testing in neat semen or purified sperm samples, we are of opinion that SPVL quantification should be a starting point for any semen decontamination attempt. We therefore verify HIV-1 RNA and DNA in seminal plasma (see previous molecular assay specifications), and test the final sperm aliquot only if the validation test was positive in the neat semen sample.

Sperm processing techniques for the HIV-infected male

Assisted reproduction procedures for patients with blood borne viruses are generically geared towards risk-reduction to avoid horizontal transmission to both the male and female, and perinatal transmission to the infant. Minimal invasive procedures such as self insemination or intra-uterine insemination can be used when the *female is HIV-positive* (Gilling-Smith, 2006). The main objective when the *male partner is HIV-positive* is the removal of cell-free and cell-associated pathogens from sperm fractions, while optimal sperm quality is preserved (Semprini *et al*., 2004; Nakhuda and Sauer, 2007). This is obtained through sperm washing and decontamination techniques, using density gradients, swim-up steps and/or decontamination devices (Politch *et al*., 2004; Loskutoff *et al*., 2005). More than 60% of all neat semen samples from HIV-positive males processed at our Unit tested positive for HIV-1 RNA quantitative (range 138 - 801,440 RNA copies/ml), DNA qualitative or both RNA and DNA positive. Using the ProInsert™ without a final swim-up step, HIV-1 proviral DNA and RNA were not detected in any of the processed sperm samples within the detection range of the commercial PCR tests. The ProInsert™ facilitates precise density gradient layering, prevention of recontamination of the sperm pellet after centrifugation, and provides a bio-secure method for the operator. The term "sperm decontamination" is used (Huyser *et al*., 2006; Huyser, 2008; Fourie *et al*., 2009) since different interpretations of the term "sperm washing" exists. Semprini and Hollander (2007) refer to sperm washing as a three-step seminal processing method, i.e. density gradient centrifugation, washing of the sperm pellet and spontaneous migration of the spermatozoa; as was designed nearly two decades ago (Semprini *et al*., 1992). Persico *et al.* (2006) is of the opinion that it is improper

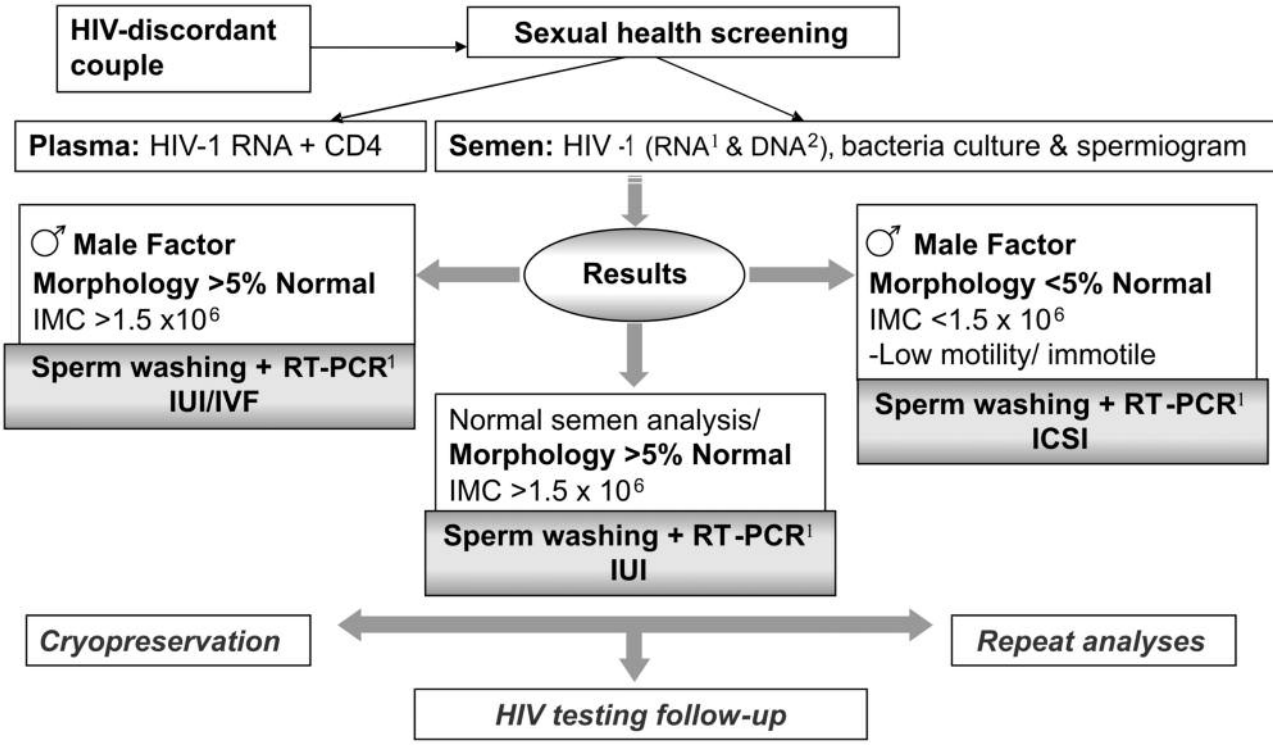

Fig. 2. — Flow chart for ART management of HIV positive males (adapted from Ombelet *et al*., 2003; Semprini and Hollander, 2007)
1. RNA – Quantitative (Cobas AmpliPrep - Cobas Taqman HIV-1 version 2, Roche Diagnostics, linear reportable range 20-10,000,000 copies/ml);
2. DNA qualitative (Amplicor HIV-1 DNA, version 1.5 Roche diagnostics).

to refer to sperm washing (Leruez-Ville *et al.,* 2002), if the final swim-up step of spermatozoa is not included during processing. Procedures such as centrifugation, washing as well as separation solutions, may vary in different laboratories. A balance must always be reached between removal of non-sperm cells from semen and maintaining sperm quantity and quality (Gilling-Smith, 2003), which have a direct impact on the choice and success of ART procedure for a couple. Repetitive washes can cause sperm damage and loss of sperm quality and quantity during repeated centrifugations (Gilling-Smith, 2003).

ART procedures

It is noted by Semprini and Hollander (2007) that until 1995 a large number of IUIs were performed for serodiscordant couples (males HIV-positive). During this period antiretroviral medication was not available and no PCR assays existed for viral validation of the processed sperm sample. Some authors advocate the use of a less expensive and labor-intensive IUI-procedure, compared to IVF-ICSI, in the absence of tubal pathology and a sperm sample of sufficient quality (motility and concentration) (Semprini and Hollander, 2007; Van Leeuwen *et al*., 2009). Others prefer to navigate or apply the IUI procedure as a special detour and rather perform IVF and/or employ the ICSI procedure (Mencaglia *et al*., 2005; Prisant *et al.,* 2010; Sauer *et al*., 2009; see Table I). Another possible avenue is the singular use of anti-retroviral pre-exposure prohylaxis (PrEP) prior to unprotected intercourse in carefully selected patients with suppressed VL (Van Leeuwen *et al*., 2009; see also http://www.creathe.org: Timed intercourse and PrEP). An overwhelming safety record of centers that offer ART to serodiscordant couples where the male is positive (Table I), aided our Unit to design a flow chart (Figure 2) to perform IUI as an inexpensive and safe first-line treatment (Ombelet *et al.,* 2003). Semprini and Hollander (2007) described the indications for ART procedures similarly, with the addition of IUI in spontaneous ovulation cycle or hormonal stimulated cycle, in combination with female age. The discourse by Bujan *et al*., (2009) on an article by Sauer *et al*., (2009) regarding the use of ICSI, and the reply of the latter-mentioned author, provide insight into the choice of a specific ART procedure for a serodiscordant couple where the male is HIV-positive.

Conclusion

An HIV-positive adult may not necessarily be infertile, but the practice of "safer sex" may precipitate in involuntary childlessness. Therefore, should ART treatment options for serodiscordant and concordant couples be based on viral status and/or presence of male and female factors in a resource-constraint setting? Also, is there any difference in the choice of ART procedure for HIV-infected individuals in a developed versus a developing country? In this regard best practice quality control frameworks and directives from developed countries can be used to guide emerging or future assisted reproduction laboratories in the developing world. Financial constraints and logistics might preclude the continuing monitoring of the plasma VL over time and secondly, the submission of seminal and purified semen samples for viral validations. As viral containment (e.g. use of antiretroviral medications or sperm processing techniques) become standardized globally, the requirement to perform a post-processing viral validation can not be compulsory. A robust and efficient simple semen decontamination procedure will certainly provide additional protection to overcome some of these hurdles within a low-resource setting. This does not imply that viral screening is to be omitted, but perhaps unattainable in certain scenarios in a developing country. We have monitored and evaluated the introduction of contaminants into the ART laboratory from all possible sources to develop and deploy a prevention-based strategy within the laboratory. On the other hand, an ART laboratory is part of a Unit and can not be viewed as sovereign in its actions and policies. If a Unit's patient population carries a high burden of STIs, and screening of these patients is not regularly performed or checked in a developing country, then the unprepared assisted reproduction laboratory can be a disaster waiting to happen.

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The Arusha project: Accessible infertility care in developing countries – a reasonable option?

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Steering Committee of the ESHRE Special Task Force on "Developing countries and infertility"

Possible questions for our debate corner

1. How can we reduce the stigma of infertility and childlessness in DC?
2. Does the value of children and the motive for parenthood differ between developed and developing countries, and if yes, what can we do about it?
3. Is it necessary and/or advisable to introduce accessable low-cost infertility services in DC?
4. Is it reasonable and feasible to incorporate infertility care in 'Reproductive Health Care Centres' and what will be the hurdles?
5. Which geographical areas should have priority in developing ART in resource-poor economies and what have to be the selection criteria for accessible IVF in DC?
6. Is access to acceptable perinatal care an unconditioned requirement for the delivery of infertility care in DC?
7. Is access to antiretroviral therapy an unconditioned requirement for the delivery of infertility care in HIV-infected couples?
8. Although the need for single embryo transfer (SET) is obvious, which may be the best algorithm to follow in developing countries. Who should have double embryo transfer (DET)?
9. Can endoscopic surgery (diagnostic and therapeutic) be implemented in a project of low cost infertility care in DC?
10. How can Public-Private partnership assist in the development of simplified ART to the advantage of developing countries? Private versus Public or Public-Private?

Infertility and developing countries (DC): frequently asked questions
(ART = assisted reproductive technologies)

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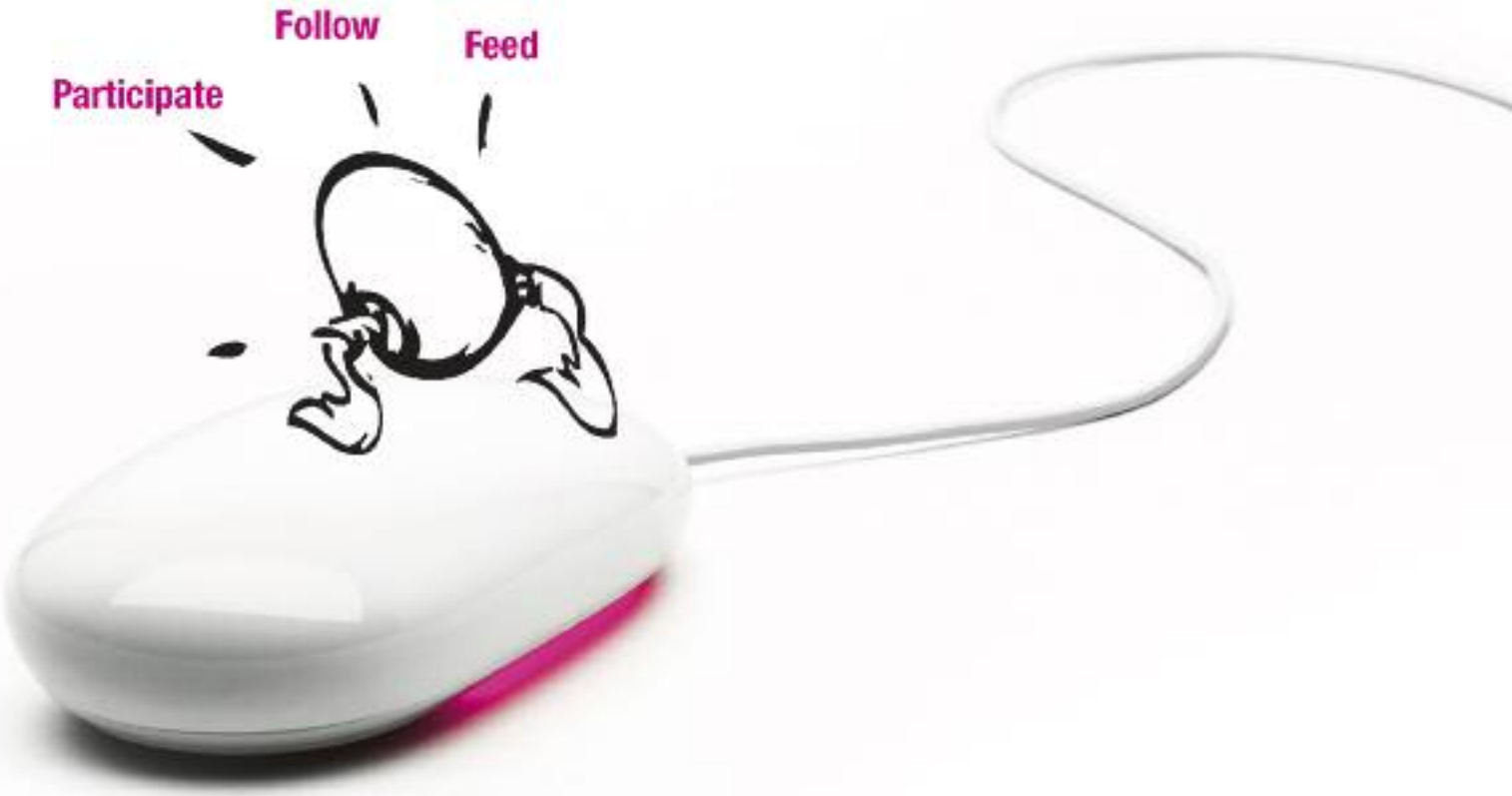

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