STRATEGIES TO IMPROVE ARTIFICIAL INSEMINATION BY DONOR

SILKE JULIANE DYER
MBCHB (MUNICH, GERMANY) FCOG (SA)

Groote Schuur Hospital & University of Cape Town Medical School

A dissertation submitted to the University of Cape Town in fulfilment of the requirements for the degree of Master of Medicine (Obstetrics & Gynaecology) Part III

DECLARATION

I, Silke Dyer, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

I empower the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

.....
SIGNATURE

.....
DATE

ACKNOWLEDGEMENTS

There are a number of people who I wish to acknowledge for their support of this study.

Professor Zephne van der Spuy, Head of the Reproductive Medicine Unit and Head of the Department of Obstetrics and Gynaecology has been my tutor and mentor for many years. She has supported and encouraged me in my field of sub-specialisation. I wish to thank her for motivating me to initiate this project and for supervising it's progress and completion.

A special thank you to the staff of the Andrology Laboratory, Mr PJ Kempen, Mr Cedric North and Mrs Lisa White for their dedication to their work and their input into this project. They were available seven days a week and vigorously supervised "sperm quality".

Thank you also to the nursing staff of the Infertility Clinic for all the loving care given to the patients and for helping them through sad and happy times.

I am grateful to all my colleagues who have counselled and recruited patients and have helped me with the clinical management.

Professor Tim Dunne from the University of Cape Town assisted in the analysis of the data. His insightful statistical advice is gratefully acknowledged.

Mrs Rosemary Fynn deciphered my handwriting and typed the manuscript, for which I am most grateful.

Finally, I wish to thank my husband, Rob, for all his support of my academic career.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Artificial insemination with donor sperm is a widely accepted form of treatment for severe male factor infertility. The introduction of quarantined, cryopreserved semen and the associated reduction in cycle fecundity when compared to fresh semen necessitated the development of strategies to improve the performance of frozen sperm.

A prospective randomised clinical trail was undertaken in the Reproductive Medicine Unit at Groote Schuur Hospital to compare intrauterine insemination with intracervical insemination in a therapeutic donor insemination program with cryopreserved semen. The method of insemination was alternated in successive cycles in each patient after intitial randomised selection. Forty three patients underwent 61 intracervical insemination cycles and 48 intrauterine insemination cycles. Strict cycle control was exercised and the timing and frequency of insemination followed a specific protocol.

Eighteen clinical pregnancies occurred following eleven intrauterine insemination cycles (22.9% per cycle) and seven intracervical insemination cycles (11.5% per cycle). Treatment outcome was influenced by patient age, the severity of the male factor and endometriosis. Most pregnancies followed insemination with 15 to 25 million motile sperm. Sperm fecundity differed amongst donors.

The findings of our study and the current literature suggest that intrauterine insemination improves cycle fecundity in therapeutic donor insemination cycles with frozen donor sperm.

INTRODUCTION

While artificial insemination with donor sperm (AID) is recorded as far back as the 17th century, donor insemination has only become established in medical practice over the last four to five decades and is now an accepted treatment option for severe male factor infertility.

During this time the techniques of AID have undergone several changes, one of the most important being the shift from the use of fresh to frozen donor semen. With the establishment of sperm banks frozen donor semen was first used for practical reasons as the constant availability of donor sperm lead to a marked expansion of therapeutic donor insemination programs. Further advantages included the more accurate matching of donor and husband, improved anonymity and the availability of bacteriological culture results (especially for Neisseria Gonorrhea) prior to insemination. In addition sperm banks offered long term availability of semen, thus allowing for subsequent conception in the same recipient with the use of semen from the same donor (Hansen et al 1979, Sanger et al 1980).

This introduction of frozen semen into the clinical practice of therapeutic donor insemination initiated an intensive debate as to whether the advantages of use of frozen donor semen would outweigh what was considered the main disadvantage by many

practitioners, namely a substantially lower fecundability rate when compared with fresh semen (Shapiro 1991).

As the potential risk of HIV transmission with the use of fresh semen was appreciated, the use of frozen, screened and quarantined semen became mandatory. With the announcement by the American Fertility and Sterility Society in 1988 that the use of fresh semen for donor insemination was no longer warranted, attention had to focus on how pregnancy rates following AID with frozen semen could be improved.

The current study was undertaken to assess whether, as previously suggested, intrauterine insemination (IUI) would result in better pregnancy rates compared to conventional intracervical insemination (ICI) in women undergoing therapeutic insemination with frozen donor sperm (Urry et al 1988, Byrd et al 1990, Patton et al 1992, Hurd et al 1993, Williams et al 1995, Wainer et al 1995).

HISTORY:

The first recorded delivery of a child conceived by artificial insemination with sperm of the husband was in 1790. The insemination was done by John Hunter for male infertility secondary to hypospadias (Beck 1984). Artificial insemination with donor sperm on the other hand remained virtually unknown to the general public till 1945 when the first comprehensive account was published in the British Medical Journal (Barton et al 1945). The authors reported, what they considered to be few conclusions in a developing field. Cases of incurable male sterility and genetic reasons were considered indications for artificial insemination with donated semen. In the absence

of any organised collection of semen, the provision of semen was entirely in the physician's hands.

Complete anonymity was strongly recommended. Donors were considered suitable if they had at least two legitimate children, had no family history of genetic significance, "such as alcoholism, criminality, or tuberculosis", and were men of "intellectual attainment and endowed with good capacity for social adjustment". Semen parameters which were thought to reflect reasonably high fecundity included an ejaculate volume of at least 1 ml, with a sperm count exceeding 30 million sperm per ml with less than 15% abnormal forms. An arbitrary limit of 100 children for each donor was recommended. Insemination was performed according to temperature charts and cervical mucus changes and care was taken not to inject air or to allow semen to enter the uterine cavity. Infection was recognised as a potential risk of the procedure and concerns were voiced regarding the possible consequences of interference with semen which included miscarriage and a "deleterious" effect on the offspring. It was further recommended that storage and transport over long distance should be avoided and centrifugation was regarded with reserve.

The child was considered to be legitimate if the husband was registered as the father, although such registration was recognised to constitute an offence in Britain (Barton et al 1945). A commission, set up at the time to further evaluate the issue, remained highly critical and suggested that the practice should be made a criminal offence (Cox 1993). Irrespective of this, studies on human spermatozoa with the view to manipulate the natural act of conception progressed. Studies on cryosurvival of sperm date back

as far as 1866. Montegezza observed the survival of human spermatozoa after exposure to a temperature of -15° C. He speculated at the time - quite correctly - that in future frozen semen might be used in animal husbandry and even proposed that a man dying on the battlefield might conceive a legitimate child by his wife post humously (Bunge et al 1953). This vision started having practical potential when the introduction of a protective agent (glycerol) significantly improved post-thaw survival of human spermatozoa (Polge et al 1949). Bunge et al reported in 1953 the successful insemination of three women with frozen thawed semen. At the time of the report the recipients had missed between three and six menstrual periods and showed "presumptive signs of pregnancy". The semen had been pretreated with 10% glycerol prior to freezing in dry ice.

Freezing on dry ice at approximately - 86° C was found to be inadequately low and the subsequent thawing of semen specimens was generally accompanied by a substantial loss in sperm motility (Tyler 1973).

Sherman et al (1973) introduced the use of liquid nitrogen at a temperature of -196° C as a simple, efficient and clinically approved method for preserving human spermatozoa by freezing. Semen pretreated with 10% glycerol was frozen in glass ampoules, suspended in liquid nitrogen. After thawing in a room temperature water bath post-thaw cryosurvival was 70% with no further loss noted with increased storage time up to a period of one year. Four normal births were reported from the initial clinical application of this method.

Steinberger et al (1965) reported their preliminary experience with a human sperm bank in Pennsylvania. Thirteen pregnancies in twelve women resulted from inseminations with frozen donor sperm stored in liquid nitrogen for periods up to 6 months. He observed that post-thaw sperm motility did not appear to be related to the length of time specimens were stored.

Subsequent studies confirmed liquid nitrogen as superior to other refrigerants and glycerol as the best cryoprotective agent (Sherman et al 1973).

In 1979 a survey on national AID practice in the United States of America demonstrated that, while AID was widely practiced, it was with inconsistent standards. Frozen semen was used by 31.4% of responding physicians performing artificial insemination with donor sperm, but this group accounted for only 12.7% of total donor semen used. Furthermore 42.4% of users of frozen semen never stored semen for periods over three months. The information on the maximum number of pregnancies produced by a single donor was frequently unobtainable. Of those who answered, most restricted the use of a single donor to six pregnancies, however, there were reports where as many as 50 pregnancies per single donor were recorded. Only 30.4% of respondents kept permanent records of donors and 82.6% were opposed to legislation requiring such record keeping, apparently on the base of protecting donor anonymity. At the time of the survey only 14 American States had any laws concerning the status of children conceived by artificial insemination with donor sperm (Curie-Cohen et al 1979).

This survey prompted the promulgation of guidelines regarding therapeutic donor insemination by national boards such as the American Fertility Society and the American College of Obstetricians and Gynaecologists. The American Fertility Society (AFS) published their first guidelines for therapeutic donor insemination in 1980. Recommendations were drawn up for the selection and management of donors which included a medical and genetic history of the donor, sperm testing and limitations on multiple use of donors. Similarly recommendations were made with regards to selection and management of recipients giving consideration to indications, minimal evaluation of female fertility, techniques and timing of insemination as well as record keeping. General principles were stated regarding the technique of cryopreservation. It was hoped that these guidelines would assure the practitioner that his services fell within reasonable standards of care in an area devoid of specific legislation, and provide treatment with minimal risks for all parties involved including the child conceived through AID (Hulka 1981).

The growing prevalence of HIV and the recognition that fresh semen is a possible vehicle for HIV transmission led to concerns regarding the use of fresh semen for therapeutic donor insemination. In Australia four out of eight recipients of artificial insemination with semen from a symptomless carrier were found to have antibody to the virus (Stewart et al 1985, Peterson et al 1988). In December 1985 the American Association of Tissue Banks Task Force on the Role of Semen in Transmitting Human T - Lymphotrophic Virus - III Infection recommended the use of frozen semen as the optimal and safest means of testing for AIDS in semen donors (Sherman et al 1987). In May 1986 workers at the Center for Disease Control concluded that the use of fresh semen is clearly

hazardous and should be discouraged because it cannot be screened for certain sexually transmitted diseases, especially AIDS. The "window period of seroconversion" of up to 120 days, or possibly longer, during which time the HIV virus can be transmitted after the donor becomes infected and before seroconversion takes place, was considered the major risk of AIDS transmission with fresh semen (Peterson et al 1988). In 1986 the AFS revised their guidelines for the use of semen donor insemination to state that the use of fresh semen was warranted if the donor were initially seronegative for the AIDS virus, not a member of a high risk group, and if negative antibody titers for HIV virus were obtained at six monthly intervals. When frozen semen was used, it was recommended that the specimen be quarantined for 60 days and then the donor retested and found to be seronegative for HIV before the specimen was released.- The reason for a universal rejection of frozen in favour of fresh semen was apparently the perceived increase in cost and a 50 - 60% reduction in conception rates (American Fertility Society 1986, Sherman et al 1987).

In 1988 - in response to increasing concerns regarding the possible transmission of the HIV virus during donor insemination - the AFS revised its guidelines again and stated : "...that under present circumstances the use of fresh semen for donor insemination is no longer warranted and that all frozen specimens should be quarantined for 180 days and the donor retested and found to be seronegative for HIV before the specimen is released." And further more: "We recognize that there may be some decrease in pregnancy rates and /or length of time to conception when using frozen semen. We urge directed efforts be made to evaluate the true effectiveness of frozen semen and to improve cryopreservation techniques" (American Fertility Society 1988).

These guidelines brought a significant change to the management of therapeutic donor insemination and to the administration of donor programs.

FRESH VERSUS FROZEN SPERM :

At the time that the AFS published their guidelines in 1988 Peterson et al stated in an article, "A.I.D. and AIDS - too close for comfort", that it was general consensus that frozen sperm had a lower pregnancy rate per cycle than fresh semen, although some recent reports had suggested that there was no difference in conception rates (Peterson et al 1988). The authors considered a significant reduction in the number of motile sperm, with a reduction in longevity and potentially reduced fertilizing capacity to be objective evidence of cryopreservation damage. Other experimental findings on cryoinjury to spermatozoa included reduced motility, ultrastructural changes, biochemical alterations and reduced mucus penetration (Bromwich et al 1978, Bordson et al 1986, Sherman 1987, Jacobs and Ory 1989). Shapiro commented in an editorial in 1991 that frozen semen fecundability was about 50% of that which was obtainable with insemination of fresh semen (Shapiro 1991).

The shift from fresh to frozen sperm and the AFS guidelines were criticised recently by Nachtigall et al (1994). According to this author there is little question that the exclusive use of frozen semen has lowered the effectiveness of the practice of donor inseminations while increasing the financial and emotional cost to the patient. The author calculated the risk of dying of AIDS after treatment with fresh donor insemination to be at least several orders of magnitude lower than, for instance, the risk

of pregnancy itself. It was concluded that neither safety nor efficacy needed to be sacrificed in the current practice of donor insemination by offering patients the choice of appropriately screened fresh or frozen sperm.

Whilst several authors agreed with the finding of reduced cycle fecundity with the use of frozen sperm (Silva et al 1989, Byrd et al 1990, Coulson et al 1996), some however found no change in the cumulative conception rates over 6 to 12 months (Iddenden et al 1985, Bordson et al 1986, Sherman 1987). In summary, the numbers of cycles required for pregnancy might be increased with the use of frozen sperm, however, the overall pregnancy rate per patient receiving either fresh or frozen semen was found to be similar. While the overall chance of conception might not be compromised with the use of frozen semen, it has to be recognised that the possible increased time to conception is likely to carry additional financial and emotional costs to the patient.

MAXIMIZING PREGNANCY RATES RESULTING FROM DONOR INSEMINATION WITH FROZEN SEMEN:

Since the use of cryopreserved, quarantined semen in donor insemination programs became mandatory the issue of whether frozen semen had equal fertilising potential as fresh semen lost its clinical relevance and turned into an academic question (Jacobs et al 1989, Barratt et al 1990). Modern technological improvements including the investigation and management of female infertility factors, better timing of insemination, insemination with adequate semen sample quality and better cryopreservation techniques were considered options to overcome the disadvantages and result in conception rates with frozen semen comparable to those obtained with fresh semen

(Jacobs et al 1989). All of these factors, as well as the frequency and mode of insemination have been widely explored and discussed in the literature. While agreement exists in many areas there is ongoing controversy in others.

THERAPEUTIC DONOR INSEMINATION AT GROOTE SCHUUR HOSPITAL:

In 1979 a therapeutic donor insemination program was initiated at Groote Schuur Hospital. At this time the legal aspect of this treatment was unclear, since there was no statute law and very little case law (Editorial 1978, Allen et al 1985). A Code of Practice with regard to therapeutic donor insemination was however compiled in 1981 and followed by the Human Tissue Act (1983), specifying regulations regarding the artificial fertilisation of persons and related matters. This Human Tissue Act governs the current practice of therapeutic donor insemination (Anon 1980, Anon 1991a). Therapeutic donor insemination has thus been practised for nearly 20 years at Groote Schuur Hospital.

The potential of HIV transmission with fresh semen in a therapeutic donor insemination program has to be of particular concern in South Africa. Since HIV is predominantly transmitted by heterosexual intercourse in this country, any screening for the so called "high risk groups" is likely to be ineffective. In all communities affected by a rapidly rising HIV incidence, the risk of HIV transmission during the so called window period has to be considered significant. In a recent antenatal survey in the public sector (1996) the incidence of HIV positive blood specimens ranged from 3.1% (Western Cape) to 25.1% (North Western Province / Gauteng). This comparatively low incidence in the

Western Cape shows variation within certain areas: Blood samples from Guguletu and Khayelitsha tested positive in 7.1% of samples compared to 1.3% of samples from other antenatal clinics (personal communication, A. Keen). The incidence is increasing and there is justified concern that the region may soon face an incidence similar to other regions in this country.

The use of fresh or frozen donor sperm and the importance of HIV screening is not stipulated in the current Human Tissue Act. An amendment to this Act is however currently proposed restricting the use of donor sperm and oocytes to the use of HIV screened, cryopreserved and quarantined gametes only.

In 1987 Groote Schuur Hospital's Andrology Laboratory opened the first sperm bank in South Africa and is currently the only sperm bank providing sperm for all ethnic groups.

Since 1991, all therapeutic donor inseminations have been undertaken with frozen semen, which is quarantined for a minimum of 6 months and only released once the donor tests repeatedly seronegative for HIV. Despite this complete change to frozen semen the actual cycle management however remained variable and at the discretion of the practicing doctors. Variables included therapeutic or empiric ovulation induction, midcycle ultrasound scanning for the assessment of endometrial thickness and follicular development, midcycle endocrine profiles (LH, progesterone and estradiol) and especially timing and frequency of the inseminations. Cycle fecundity was low.

This prospective study was undertaken at Groote Schuur Hospital to compare conventional intracervical insemination of frozen, thawed donor sperm with intrauterine insemination of frozen, thawed and washed donor sperm.

METHODS

PATIENT SELECTION:

From November 1993 to November 1996 patients presenting for artificial insemination by donor were recruited from the Groote Schuur Hospital Infertility Clinic for this study. The protocol was approved by the Ethics Committee of the University of Cape Town Medical School. Following counselling of the couple informed consent was obtained for the procedure of artificial insemination by donor and for participation in the study. Particular care was taken to explain to the couple that their decision whether or not to participate in this study would in no way negatively affect their management.

All couples underwent routine baseline infertility investigations. Ovulatory function was assessed by midluteal progesterone levels with more detailed investigations undertaken if considered appropriate. Patients were routinely screened for tubal patency by hysterosalpingogram (HSG). Some patients however underwent laparoscopy for pelvic assessment which was either part of the routine infertility assessment (patients referred by other hospitals or private practitioners) or done for a specific indication (eg. abnormal HSG or pelvic pain). Male partners, unless referred with an established diagnosis of azoospermia or severe subfertility, underwent two or three semen analyses. Retrograde ejaculation in men with azoospermia was excluded by assessing a post-ejaculatory urine sample for the presence of sperm. In individual cases patients were referred for specific assessment to a urologist working in our

infertility service. Both partners were screened for HIV (following informed consent). All women had VDRL and rubella immunity status assessed.

Patients were randomised to undergo either intrauterine insemination (IUI) or intracervical insemination (ICI) in their first treatment cycle. Thereafter, if pregnancy did not occur, the mode of insemination was alternated usually to a maximum of six treatment cycles. With this cross-over design the patient acted as her own control. It also offered the patient an even exposure to the chance of conception and hence facilitated recruitment to the study. Couples who did not conceive during this period of treatment were carefully counselled regarding the continuation of treatment as well as the availability of other options (ie. assisted reproductive techniques and adoption).

Irrespective of their ovulatory status most patients received some form of ovulation induction.Treatment was usually with clomiphene citrate, but exogenous gonadotrophins were used when considered indicated. A few patients (n = 6) underwent insemination in natural cycles. Particular attention was paid to cycle quality. This included ultrasonographic monitoring of follicular development and endometrial thickness with midcycle serum luteinising hormone (LH) levels and progesterone levels in all cycles. Assessment of midcycle estradiol levels became part of routine cycle monitoring in the latter part of this study (after the first 24 cycles).

DONOR SELECTION:

Donors were healthy volunteers with no personal or family history of congenital disorders. Prospective donors had a semen analysis performed using standard methods after three days of abstinence from ejaculation. Acceptance criteria were a count of over 120 million/ml with a motility of over 60%, forward progression of SFP 2+ and a normal morphology of at least 5%. Sperm morphology was assessed according to the Tygerberg strict criteria (Menkfeld et al 1993). Briefly, according to these criteria a semen sample with less than 5% normal morphology falls into the p-pattern category ("poor prognosis"). Semen samples with 5% to 14% normal morphology are judged to be sub fertile but fall into the g-pattern category ("good prognosis"). Semen samples with 15% or higher normal morphology are considered to be normal ie reflect optimal chance for conception. Donors were screened serologically for sexually transmitted disease, namely syphilis, hepatitis B and HIV. Bloodgroup status was documented. Semen samples were stored in liquid nitrogen as detailed below for a minimum period of six months. Thereafter sperm was released for usage provided that the donor tested repeatedly negative for HIV. Donors were matched to the receiving couple by physical characteristics and, if requested by donor or recipient, by religious faith. Anonymity was maintained between donors and recipients.

SPERM CRYOPRESERVATION, THAWING AND PREPARATION FOR INSEMINATION:

Semen specimens were produced by masturbation in a room adjacent to the laboratory. Specimens were allowed to liquify and an aliquot was taken for analysis. Semen samples were suspended in a cryoprotectant medium containing 50% egg yolk enrichment, 3% Sodium Citrate, 5% glucose and a final concentration of 9% glycerol.

These samples were rapidly frozen in liquid nitrogen vapour and subsequently immersed and stored in liquid nitrogen in marked plastic straws.

Prior to insemination the straw was left to thaw at room temperature. Sperm characteristics were recorded after thawing and - in the case of IUI cycles - after sperm wash. Unsatisfactory samples were discarded. Initially only semen samples containing approximately 10 million motile sperm were used for each insemination. A few months after the beginning of this study these requirements were raised to approximately 20 million motile sperm per single insemination. Records of sperm assessments were not kept initially (n = 12 cycles). Subsequently however both pre- and post-thaw motility and motiliy count (n = 98 cycles) as well as pre- and post-thaw morphology (n = 86 cycles) were recorded.

In case of intracervical insemination the frozen thawed semen sample was drawn up into a syringe. Following visualisation of the cervix with a Cusco Speculum the semen sample was placed pericervically via a catheter (Jelco sheath, Tomcat catheter or embryo transfer catheter).

For intrauterine insemination the frozen thawed specimen was suspended in sperm preparation medium (Medi-cult medium for assisted reproduction techniques). The sample was centrifuged at 1000 spm for ten minutes. Supernatant fluid was removed and the sperm pellet was resuspended in medium. After a second centrifugation the sperm pellet was resuspended in 0.5mls of medium and used for the insemination.

No attempt was made to recover a swim-up fraction for the insemination. The prepared sperm suspension was aspirated into an insulin syringe and following visualisation of the cervix an attached Tomcat or Embryo transfer catheter was gently passed through the cervical canal and the entire volume of the specimen deposited into the uterine cavity.

Following both methods of insemination the patient was asked to rest in the supine position for 20 minutes.

TIMING OF INSEMINATION:

Strict cycle control was exercised with regard to the timing and the frequency of the insemination. Once one to three follicles of 18 to 20 mm size were present on ultrasound scanning and in the absence of a spontaneous LH surge ovulation was triggered with 5000 IU human chorionic gonadotrophin (hCG). Administration of hCG was at times delayed by one or two days in the presence of low estradiol levels (less then 1000 pmol/l) or inadequate endometrial thickness (less than 8 mm). No cycle was, however, cancelled because of low estradiol levels or thin endometrium, since all cycles with at least one dominant follicle were inseminated.

Two inseminations were scheduled for each cycle, namely one insemination prior to and one insemination coinciding with ovulation. More specifically intracervical inseminations were performed between 12 to 16 hours and between 33 to 38 hours following hCG administration. Intrauterine inseminations were performed between 16 to 20 hours and between 36 to 40 hours following hCG administration. The rationale

for the somewhat earlier intracervical insemination was to allow time for sperm capacitation which, in the case of intrauterine insemination, was initiated by the sperm wash. In case of a spontaneous LH surge a single insemination was performed between 29 to 33 hours for ICI and between 34 to 38 hours for IUI following the presumed onset of the endogenous LH surge.

Midluteal serum progesterone concentrations were measured to confirm ovulation. Patients were asked to return to the clinic either with their next period for a repeat treatment cycle or, if menses did not occur for a pregnancy test. In case of a positive pregnancy test ultrasound confirmation of pregnancy was obtained at about 8 weeks gestation.

STATISTICAL ANALYSIS:

Pregnancy was the main end point measured. Only clinical pregnancies, that is pregnancies documented on ultrasound or confirmed histologically following a miscarriage or ectopic pregnancy were recognised (ie biochemical pregnancies were excluded).

Several other variables were analysed to determine their influence on the success rate of therapeutic donor insemination with frozen semen. Only inseminated cycles were included in the analysis of this study. Anovulatory cycles were included if insemination was performed.

Pertinent statistical analysis of the presented data is difficult because the data set is limited in size. Apart from the method of insemination - the main issue under investigation in this study - the eventual treatment outcome (ie. pregnant or non pregnant) is influenced by a series of events and given circumstances. These include male and female fertility factors, cycle quality, timing of insemination, quality of the semen sample and donor fecundity.

Each of these variables was analysed to explore whether they are either positively or negatively associated with cycle fecundity. This analysis was done through Pivot tables (multiway contingency tables) which are a standard option in the program of MS-Excel. These Pivot tables report frequencies for categories of one nominal or ordinal within the categories of another variable against categories of a third variable (usually treatment outcome), eg. does the mode of insemination in the presence or absence of female pelvic pathology appear to influence cycle fecundity.

Unfortunately this 3-way classification frequently led to small sub groups. While this data can be interpreted meaningfully from a clinical point of view - especially when considered in the context of our current knowledge (literature review) - the study sample size is not large enough to allow for the discernment of statistically significant evidence of associations which we might expect from clinical experience.

The cross-over design of this study, ie. the alternate use of intrauterine and intracervical insemination did not affect the Pivot table analysis in principle. Careful

analysis of data stratified by cycle number allows for suitable comparable conditional insights.

Other studies frequently use a χ^2 test for statistical analysis. This test however would be incorrectly applied for the analysis of the aggregated set of treatment cycles, since cycles within a patient are dependent upon each other (ie. the fact that conception failed to occur in the previous cycle may have an influence on the subsequent cycle of the same patient). For comparison with the literature we record the relevant χ^2 values, but insist upon their validity being dependent upon the implausible assumption of cycle independence.

The χ^2 test can be applied to the treatment outcome of women but our sample size was essentially too small to discuss statistical evidence in favour of the clinically preferred treatment.

RESULTS

Between November 1993 and November 1996 140 cycles with artificial insemination by donor were initiated in 47 patients. Of these 47 patients, one patient defaulted further treatment after cancellation of her first cycle. Three patients underwent five inseminations not according to the protocol since one patient withheld trial consent (two cycles) and technical difficulties made it impossible to adhere to the timing as stated in the protocol in the other two patients (three cycles). Of the remaining 135 cycles, 26 cycles were cancelled prior to insemination. Indications for cycle cancellation included inadequate folliculogenesis (n = 14), evidence of spontaneous LH surge not allowing for timely insemination (n = 4), multiple follicular development (n = 1) and cancellation on request of the patient due to transport problems or minor illness (n = 7). Forty three patients thus underwent 109 cycles of artificial insemination according to the trial protocol. The analysis and discussion of the results will be restricted to this study group.

PREGNANCY RATES:

Forty three patients underwent 109 insemination cycles - 61 intracervical insemination (ICI) cycles and 48 intrauterine insemination (IUI) cycles. Two IUI cycles had to be converted to ICI due to technical difficulties and were thus analysed as ICI cycles. Not all patients underwent both modes of insemination due to conception in the first treatment cycle, patient drop out or technical difficulties (Table 1).

Eighteen clinical pregnancies occurred following eleven IUI cycles and seven ICI cycles. The overall pregnancy rate (PR) was 16.5 % per cycle with a PR of 22.9 % per IUI cycle and a PR of 11.5% per ICI cycle, but the difference did not reach statistical significance (Table 1).

The PR per patient was 41.8% over a mean of 2.5 cycles. The PR per patient who underwent at least one cycle of IUI was 35.5% compared to a PR per patient who underwent at least one cycle of ICI of 17.9% (Table 1).

Table 1. Aggregated Treatment Outcome.

	ICI	IUI	Total
No. of Patients	39	31	43
No. of cycles	61	48	109
No. of Pregnancies	7	11	18
PR/cycle	11.5%*	22.9%*	16.5%
PR/patient in group	17.9%	35.5%	-
PR/patient	-	-	41.8%

* $\chi_1^2 = 2.550$ $p > 0.10$

Although all patients were encouraged to continue treatment until pregnancy occurred or six insemination cycles had been completed, several patients underwent fewer insemination cycles. Overall 23 patients were analysed over less than six cycles

without conceiving. Of these, three patients discontinued treatment following medical advice and five patients are still undergoing treatment. The remaining 15 patients discontinued treatment of their own accord. Thus nearly every third patient dropped out without achieving conception and prior to the completion of the recommended number of cycles.

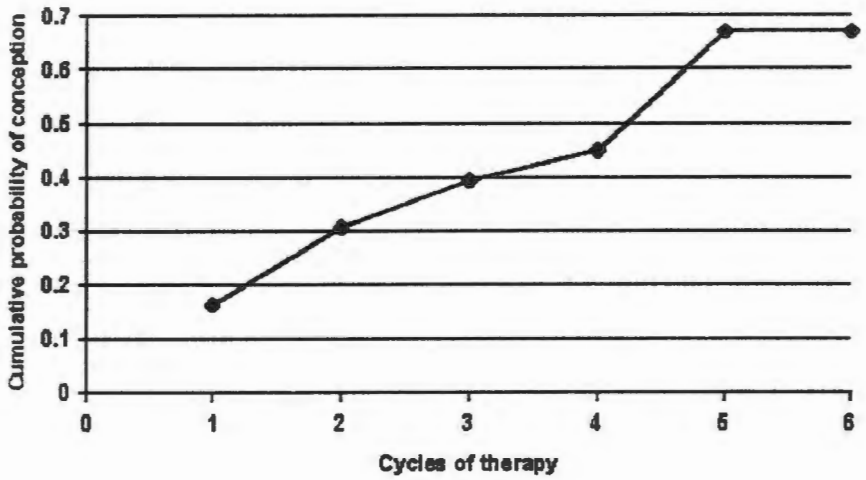
A lifetable analysis of the 43 couples shows that a cumulative PR of 66.7% is obtained after six insemination cycles when adjustment is made for those couples who underwent less than six insemination cycles (Table 2, Figure 1).

Table 2: Life Table Analysis of the Occurrence of Pregnancy in 43 Couples and 109 Insemination Cycles

Duration of treatment	Not Pregnant at start of interval	Pregnant during interval	x LTFU	* Prop.P in interval	** Prop.NP in interval	+ Cumul.P. pregnant	+ Cumul.P not pregnant
1. cycle	43	7	7	0.163	0.837	0.163	0.837
2. cycle	29	5	8	0.172	0.828	0.307	0.693
3. cycle	16	2	3	0.125	0.875	0.394	0.606
4. cycle	11	1	5	0.090	0.91	0.45	0.550
5. cycle	5	2	0	0.40	0.60	0.67	0.330
6. cycle	3	0	-	0.00	1.00	0.67	0.330

x LTFU: Lost to follow-up/dropout
* Prop.P.: Proportion pregnant
** Prop.NP.: Proportion not pregnant
\+ Cumul.P.: Cumulative proportion

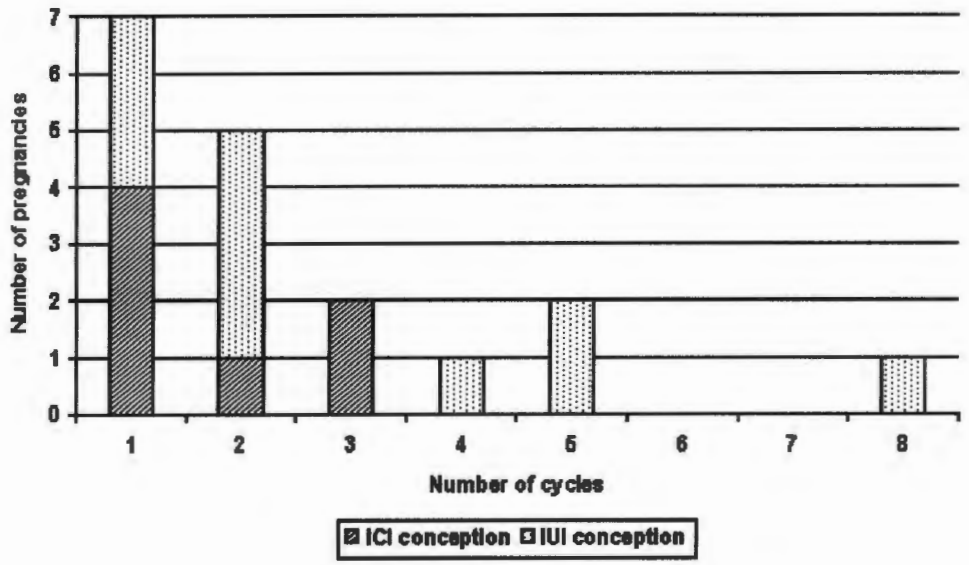
Figure 1. Cumulative Probability of Conception over Six Treatment Cycles.



Unfortunately numbers were too small to conduct a lifetable analysis on the occurrence of pregnancy following IUI or ICI cycles respectively. However, following the not altogether correct assumption that the chance of conception after each mode of insemination is stable over six cycles, the cumulative probability of conception is 75.3% after six cycles of IUI and 56.9% after the same number of ICI cycles.

Figure 2 shows the time to conception. Fourteen of the eighteen pregnancies occurred in the first three cycles. Fifty three ICI cycles and 35 IUI cycles resulted in seven pregnancies each. The overall PR per cycle over the first three cycles (15.9%) did not differ from the PR over the subsequent cycles (19%). However, all four conceptions requiring more than three insemination cycles followed intrauterine insemination cycles (n=13) with no pregnancy occurring after intracervical insemination cycles (n=8).

Figure 2. Time to Conception.



The 18 clinical pregnancies resulted in 14 livebirths, of which twelve were term deliveries. Two infants were born preterm due to severe GPH in one instance and intrauterine growth retardation in the other instance. The neonatal outcome was good in both infants. One patient was lost to follow up and the pregnancy outcome could not be established. Pregnancy failure in the remaining three patients was due to one first trimester miscarriage, one ectopic pregnancy and one unexplained stillbirth at term. The antenatal course of the patient delivering the stillborn infant was uneventful. The post mortem result on the stillborn infant showed an incidental finding of an atrial septal defect. The cause of the intrauterine demise could not be determined.

PATIENT CHARACTERISTICS:

1. FEMALE FACTORS:

1.1 AGE :

The women treated had a mean age of 29.9 years with a range of 22 to 39 years. Table 3 shows the PR per cycle and patient for three age categories, namely age 20 to 29 years (I), age 30 to 34 years (II) and age 35 years and older (III). The overall pregnancy rate was lower in the last age category. Both pregnancies in category III occurred in patients aged 35 years.

TABLE 3. Female Age and Treatment Outcome.

	I 20-29 years	II 30-34 years	III ≥ 35 years	TOTAL
Patients (n)	17	19	7	43
Cycles (n)	41	50	18	109
Pregnancies (n)	7	9	2	18
Cycles ICI	24	26	11	61
Pregnancies	4	3	0	7
Cycles IUI	17	24	7	48
Pregnancies	3	6	2	11
PR/cycle	17%	18%	11.1%	16.5%
PR/ICI cycle	16.6%	11.5%	0%	11.5%
PR/IUI cycle	17.6%	25%	28.5%	22.9%
PR/pt	41.2%	47.4%	28.6%	41.8%

The PR per mode of insemination has to be considered with reservation due to the small numbers involved. Although there is an interesting trend towards improved cycle outcome with IUI in the two older age groups (whilst no such trend is observed in category I) there is evidently an artefact relating to small numbers with the patients in category III undergoing IUI having the highest PR of any of the subgroups.

1.2 PARITY :

Thirty seven patients were nulliparous. The remaining six patients were primiparous with three pregnancies conceived in a previous relationship and three pregnancies conceived in the current relationship of which one followed previous artificial insemination by donor; ie. of the whole group only two couples had had a previous spontaneous conception. Seventeen of the 37 nulliparous patients and one of the six primiparous patients conceived.

1.3 OVULATORY FUNCTION :

In three patients ovulatory function was not assessed prior to the onset of treatment. Of the remaining 40 patients, 29 patients showed evidence of spontaneous ovulation, whilst eleven patients were classified as anovulatory following one or two midluteal progesterone assessments. All but one of these eleven patients had regular menstrual cycles.

As shown in Table 4, cycle fecundity did not differ between ovulatory and anovulatory patients.

Table 4. Ovulatory Function and Treatment Outcome.

	Ovulation	Anovulation
No. of patients	29	11
No. of cycles	66	30
No. of pregnancies	11	6
PR/cycle	16.6%*	20%*
PR/pt	37.9%**	54.5%**

* $\chi_1^2 = 0.157$ $p > 0.60$
** $\chi_1^2 = 0.9$ $p > 0.30$

1.4 PELVIC FACTOR

Thirty five patients were assessed as having a normal pelvis following hysterosalpingography (24 patients) or laparoscopy (11 patients).

In the remaining eight patients pelvic pathology was found. One patient had unilateral tubal blockage on hysterosalpingography. The patient conceived on her second cycle. The remaining seven patients had evidence of pelvic pathology on laparoscopy: One patient had minimal endometriosis with no tubal pathology, two patients had mild endometriosis with unilateral tubal block and three patients had severe endometriosis. One patient had bilateral tubal blockage without evidence of endometriosis and a unilateral neosalpingostomy was performed.

The diagnosis of pelvic pathology was established in five patients prior to initiation of

treatment, ie insemination. In the other three patients a diagnostic laparoscopy was performed after four and six failed insemination cycles. All three patients were found to have endometriosis. A previous hysterosalpingogram had been normal on all three patients.

The 35 patients with no evidence of pelvic pathology underwent 84 insemination cycles resulting in 15 pregnancies. Both patients with tubal disease only (ie. no evidence of endometriosis) conceived (following one IUI and one ICI cycle respectively). One of these two conceptions resulted in an ectopic pregnancy in the patient who had undergone unilateral neosalpingostomy.

Of the six patients with evidence of endometriosis only one patient with minimal endometriosis conceived following an IUI cycle. The PR for patients with endometriosis was thus lower when compared with patients with no evidence of pelvic pathology, but the difference did not reach statistical significance (Table 5).

Table 5. Pelvic Factor and Treatment Outcome.

	Normal Pelvis	Endometriosis
No. of patients	35	6
No. of cycles	84	18
No. of pregnancies	15	1
PR/cycle	17.8%*	5.5%*
PR/pt	42.8%**	16.6%**

* $\chi_1^2 = 1.696$ $p > 0.15$
** $\chi_1^2 = 1.476$ $p > 0.20$

2. MALE FACTOR :

Thirty five male partners were diagnosed as having azoospermia including one paraplegic patient (TB spine). Of the remaining eight patients, five were found to have fertilisation failure on previous IVF cycles, one patient had a severely abnormal semen sample following reversal of vasectomy and two patients were referred by the andrologist with severe oligoteratozoospermia with the view to donor insemination. These eight patients underwent 20 insemination cycles without success (Table 6).

TABLE 6. Male Factor Infertility and Treatment Outcome.

	Azoospermia	Severe Oligoteratozoospermia
No. of Patients	35	8
No. of Cycles: Total	89	20
ICI	49	12
IUI	40	8
No. of Pregnancies: Total	18	0
ICI	7	-
IUI	11	-
PR/cycle	20.2%	-
PR/ICI cycle	14.3%*	-
PR/IUI cycle	27.5%*	-
PR/patient	51.4%	-

* $\chi_1^2 = 0.898$ $p > 0.30$

The 35 couples with azoospermia had 89 insemination cycles with a PR of 20.2% per cycle, 51.4% per patient and a cumulative probability of conception of 74% after six insemination cycles. Within the group of patients with azoospermia the PR was 14.3% for ICI cycles and 27.5% for IUI cycles. The difference, however, fails to reach statistical significance (Table 6).

3. COMBINED INFERTILITY FACTORS:

Five female patients had more than one infertility factor, namely a combination of anovulation and pelvic pathology. This group includes the one patient with an abnormal HSG and no laparoscopy. Sixteen insemination cycles resulted in three pregnancies, an outcome in keeping with the overall PR.

Of the eight patients with a severe male factor (as opposed to azoospermia) two female partners were found to have (severe) endometriosis. No obvious female factor was present in the remaining six female partners of whom three, however, did not have a laparoscopy.

CYCLE CHARACTERISTICS:

1. OVULATION INDUCTION:

All but six cycles involved some form of ovulation induction. This was in the form of clomiphene citrate - usually at a dosage of 25 to 50 mg and not higher than 100 mg - for five days at the beginning of the cycle. In 15 cycles clomiphene citrate was used in combination with exogenous gonadotrophins (Humegon/Pergonal), the usual dose

being 75 to 150 IU on alternate days. Four cycles were stimulated with gonadotrophins only at a dosage of 75 to 150 IU on alternate days. No patient received concomitant GNRH- agonist treatment.

Table 7 shows the outcome of IUI and ICI cycles as well as the overall outcome of cycles with regards to the three forms of ovulation induction and natural cycles.

Table 7. Cycle Outcome and Ovulation Induction.

	ICI			IUI			TOTAL		
	Cycle (n)	Pregnant (n)	PR (%)	Cycle (n)	Pregnant (n)	PR (%)	Cycle (n)	Pregnant (n)	PR (%)
Clomiphene Citrate	52	5	9.6%*	32	6	18.7%*	84	11	13%•
Clomiphene + Gonadotrophins	6	1	16.6%**	9	2	22.2%**	15	3	20%•
Gonadotrophins only	1	0	-	3	2	-	4	2	(50%)
Natural cycles	2	1	-	4	1		6	2	(33.3%)

* $\chi_1^2 = 1.452$ $p > 0.20$
** $\chi_1^2 = 1.974$ $p > 0.20$
• $\chi_1^2 = 1.013$ $p > 0.30$

The small numbers of gonadotrophin cycles and natural cycles makes the comparison difficult and statistical analysis was restricted to the clomiphene citrate only and the clomiphene citrate plus gonadotrophin cycles. The PR appears to be higher in

clomiphene citrate plus gonadotrophin cycles when compared to clomiphene citrate only cycles but the difference did not reach statistical significance ($p>0.30$).

Furthermore, for both these forms of ovulation induction, IUI cycles had a slightly higher PR when compared to ICI cycles but again the difference did not reach statistical significance ($p>0.20$).

2. NUMBER OF FOLLICLES:

In the majority of cycles (66.9%) a single large follicle (18 mm or above) was present at the time of hCG administration. This followed the assumption of follicular growth of 2mm per day, since the last ultrasound scan was not always performed on the day of hCG administration. There was no significant evidence of association over cycles between number of follicles and treatment outcome. Three out of the seven cycles with three large follicles resulted in pregnancies (Table 8).

Table 8. Number of Follicles and Cycle Outcome.

	F1*	F2	F3
No. of cycles	73	29	7
No. of pregnancies	10	5	3
PR/cycle	13.6%**	17.2%**	(42.8%)

*F1: One large follicle
F2: Two large follicles
F3: Three large follicles

** $\chi_1^2 = 1.270$ $p > 0.25$

Table 9 shows the effect of ovulation induction on the number of follicles. Given the dosages used, clomiphene citrate in combination with gonadotrophins appeared to have the highest chance of resulting in multiple follicular development but the differences were not statistically significant ($p > 0.10$).

Table 9. Ovulation Induction and Follicular Development.

	F1*	F2	F3
Clomiphene Citrate cycles (n)	57	22	5
Clomiphene + Gonadotrophin cycles (n)	7	7	1
Gonadotrophin only cycles (n)	4	0	0
Natural cycles (n)	5	0	1
TOTAL	73	29	7

*F1: One large follicle
F2: Two large follicles
F3: Three large follicles

3. ENDOMETRIAL THICKNESS :

In Table 10 the association between endometrial thickness and cycle fecundity is demonstrated.

Table 10. Endometrial Thickness and Cycle Fecundity.

	Endometrial Thickness		
	< 8mm	≥ 8mm	Total
Cycles (n)	41	54	95
Pregnancies (n)	7	8	15
PR/ cycle (%)	17.0%*	14.8%*	15.8%

* $\chi_1^2 = 0.089$ $p > 0.75$

Only cycles in which an ultrasound scan of the endometrial thickness was available on the day of or on the day prior to hCG administration were taken into account for this analysis (n = 95). This was done on the assumption that endometrial thickness does not change significantly on ultrasound scan over a period of 24 hours. Endometrial thickness was considered as "adequate" at 8 mm or above. Cycles were divided into those with "adequate" and those with "inadequate" endometrial thickness. The cycle fecundity did not differ between the two groups ($p > 0.75$).

The association between endometrial thickness and ovulation inducing agents is shown in Table 11.

Table 11. Ovulation Induction and Endometrial Thickness.

	Endometrial Thickness		
	< 8mm	≥ 8mm	Total
Clomiphene Citrate cycles (n)	35	41	76
Clomiphene Gonadotrophin cycles (n)	5	5	10
Gonadotrophin only cycles (n)	-	4	4
Natural cycles (n)	1	4	5

Only approximately 50% of clomiphene citrate cycles (with or without additional use of gonadotrophins) resulted in "adequate" endometrial thickness, compared to eight out of nine non-clomiphene citrate cycles. This negative association over cycles between clomiphene citrate treatment and endometrial thickness is statistically significant ($p < 0.05$).

4. ESTRADIOL LEVELS :

Only cycles with estradiol levels on the day of hCG administration were taken into account for this analysis (n = 77).

The mean estradiol level on the day of hCG administration was 1540 pmol/l, with a range of 301 pmol/l to 5433 pmol/l. There was no difference in the mean estradiol levels between conception cycles (1495 pmol/l, range 660 to 4242 pmol/l) and non conception cycles (1551 pmol/l, range 301 to 5433 pmol/l).

If the outcome of cycles is divided - somewhat arbitrarily - into two categories, namely cycles with "adequate" estradiol levels (1000 pmol/l or above) and "inadequate" estradiol levels (less than 1000 pmol/l), about one third of all cycles showed estradiol levels below 1000 pmol/l at the time of hCG administration with no effect on the PR (Table 12).

Table 12. Estradiol Level and Cycle fecundity.

	Estradiol Level		
	< 1000 pmol/l	≥ 1000 pmol/l	Total
No. of cycles	30	49	79
No. of pregnancies	8	7	15
PR/cycle	26.6%*	14.3%*	18.9%

* $\chi_1^2 = 1.894$ $p > 0.15$

As demonstrated in Table 13 the various forms of ovulation induction carried a similar likelihood of inducing cycles with estradiol levels below 1000 pmol/l ($p > 0.20$).

TABLE 13. Ovulation Induction and Estradiol level.

	Estradiol Level		
	< 1000 pmol/l	≥ 1000 pmol/l	Total
Clomiphene Citrate cycles (n)	21	39	60
Clomiphene + Gonadotrophin cycles (n)	3	8	11
Gonadotrophin only cycles (n)	2	1	3
Natural cycles (n)	4	1	5

As expected, cycles with multiple follicular development were associated with higher estradiol levels (Table 14).

Table 14. Follicular Development and Estradiol level.

	Estradiol Level		
	< 1000 pmol/l	≥ 1000 pmol/l	Total
No. of cycles :			
F1*	28	27	55
F2	1	18	19
F3	1	4	5

*F1 : One Large follicles
F2 : Two large follicles
F3 : Three large follicles

Half of all cycles with single follicular development were associated with estradiol levels below 1000 pmol/l and all but two of the 24 cycles with multiple follicular development were associated with estradiol levels of 1000 pmol/l or above. This association between estradiol levels and multiple follicular development is statistically significant ($p < 0.001$).

SPERM CHARACTERISTICS:

Prior to insemination semen samples were assessed with regards to total number of motile sperm, sperm motility and sperm morphology after thawing as well as sperm motility and morphology after sperm wash in cases of IUI. While occasionally sperm motility and/or morphology was either increased or decreased after sperm wash, no such changes were noted in the majority of samples after washing. This was expected since sperm specimens were merely washed and resuspended in culture medium and no attempts were made at sperm selection.

The total number of motile sperm ranged from 6.8 million to 36.8 million per sample inseminated. The association of number of motile sperm inseminated to cycle fecundity is complicated by the fact that two inseminations were performed in most cycles. Whilst this was done in order to assure an adequate number of motile sperm at the site of fertilisation over an extended period of time, it is possible that the two inseminations lead to some additive effect with regard to the total number of motile sperm. The degree of this additive effect is impossible to estimate. It is furthermore likely to vary, firstly depending on a donor and /or specimen specific post-thaw sperm longevity and

secondly depending on the actual time interval between the two inseminations. This time interval could range from 16 hours to 24 hours in case of IUI and from 17 hours to 26 hours in case of ICI (as stipulated in the treatment protocol). The association of treatment outcome and total number of motile sperm inseminated for single insemination cycles (n =18) - where no additive effect could occur - and double insemination cycles (n = 69) is demonstrated in Table 15.

Table 15. Motility Count and Treatment Outcome.

	Total no. Motile Sperm			
	$<15 \times 10^6$	$15\text{-}20 \times 10^6$	$21\text{-}25 \times 10^6$	$>25 \times 10^6$
Single insemination:				
cycles (n)	7	6	3	2
pregnancies (n)	-	3	-	1
Double insemination:				
cycles (n)	26	25	14	7
pregnancies (n)	3	5	3	-
Total:				
cycles (n)	33	31	17	9
pregnancies (n)	3	8	3	1
PR/cycle	9%	25.8%	17.6%	11%

No records on the motility count were available in 12 cycles. A further seven cycles were excluded from the analysis since the motility count of the two inseminations fell into two different categories (eg. above and below 20 million motile sperm). Most pregnancies occurred with semen samples of 15 to 25 million motile sperm, both in single and in double insemination cycles. Statistically, however, there was no

significant evidence of a positive association over cycles between the total number of motile sperm inseminated and treatment outcome ($p > 0.30$).

Post-thaw motility ranged from 35% to 65%. There was no significant difference between the post-thaw motility of ICI samples compared to the post-thaw and wash motility of IUI samples. Pregnancies occurred from a sperm motility of 35% onwards (Table 16). Increased motility did not correlate to increased cycle fecundity. Nineteen cycles were excluded from the analysis. In eight cycles post-thaw motility had not been assessed and in eleven cycles the two inseminations fell on either side of the cut-off taken at a 50% post-thaw motility.

Table 16. Post-thaw Sperm Motility and Treatment Outcome.

	Post-thaw Motility	
	≤ 50%	>50%
No. of cycles	53	37
No. of Pregnancies	8	8
PR/cycle	15.1%*	21.6%*

* $\chi_1^2 = 0.635$ $p > 0.40$

The association between sperm morphology and treatment outcome is shown in Table 17.

TABLE 17. Sperm Morphology and Treatment Outcome.

	Sperm Morphology (% Normal)		
	≤ 4%	5-14%	≥ 15%
No. of cycles	2	59	24
No. of pregnancies	-	12	5
PR/cycle	-	20.3% *	20.8%*

* $\chi_1^2 = 0.014$ $p > 0.90$

Twenty four cycles were excluded since no assessment of sperm morphology was present in 23 cycles and the two inseminations in the other cycle had a sperm morphology on either side of the cut-off point (15%). Only two cycles, at the beginning of the study, were inseminated with a morphology falling into the p-pattern category (less than 4% normal sperm) according to the strict assessment of sperm morphology. No pregnancy occurred. Subsequently, samples were considered unsatisfactory for insemination if the morphology fell into the p-pattern category. There was no apparent difference between the g-pattern category (5 - 14% normal looking sperm) and the "normal" category of at least 15% normal sperm morphology with regards to cycle fecundity.

DONOR RELATED CYCLE FECUNDITY:

For the 109 insemination cycles semen samples from 17 donors were used from all ethnic groups in South Africa. Thirteen donors were used for less than five insemination cycles. Given the overall PR of 16.1% per cycle, these numbers are too small to assess donor related cycle fecundity.

Of the remaining four donors, three donors had a cycle fecundity in keeping with the average PR and one donor had a cycle fecundity of nearly twice the average PR (Table 18). These differences in donor fecundity did not reach statistical significance ($p > 0.45$).

Table 18. Donor Specific Cycle Fecundity.

	Cycles (n)	Pregnancies (n)	PR/cycle (%)
Donor 1	12	2	16.6%
Donor 2	15	2	13.3%
Donor 3	17	5	29.4%
Donor 4	33	4	12.1%

The two pregnancies from donor 1 and donor 2 both followed IUI cycles. The five pregnancies in donor 3 followed two ICI and three IUI cycles and the four pregnancies in donor 4 followed two ICI and two IUI cycles.

DISCUSSION

The mandated use of quarantined, cryopreserved semen in clinics practicing therapeutic insemination stimulated the development of strategies to improve the performance of frozen sperm.

The current study was designed in a prospective randomized fashion to assess the optimal route of insemination in therapeutic donor insemination cycles with frozen sperm. The overall PR of 16.1% per cycle falls into the upper range reported in the literature (Bromwich et al 1978, Bordson et al 1986, Hummel et al 1989, Wong et al 1989, Federation CECOS 1989, Subak et al 1992, Coulson et al 1996). Cycle fecundity after IUI (22.9%) was persistently higher when compared to ICI (11.5%).

The optimal route of insemination has been assessed in several other studies, both randomised and non - randomised. Bordson et al (1986) compared the fecundability of fresh and frozen semen in therapeutic donor insemination cycles. 120 patients underwent 401 insemination cycles. Inseminations were performed after spontaneous LH surge or following hCG administration either once or on two consecutive days. Inseminations were administered high in the cervix with unwashed semen or intrauterine with washed semen. The indication for the use of either intracervical or intrauterine insemination was not specified. Seventeen pregnancies followed 165 insemination cycles using frozen semen only, resulting in an overall cycle fecundity of 10.3%. No difference in PR was noted between the methods of insemination.

Subak et al (1992) assessed fresh versus frozen sperm in a prospective randomized trial. Fifty seven women underwent 198 treatment cycles, randomized to receive either fresh or frozen sperm. A single insemination was performed 12 - 36 hours after the LH surge with either cervical cap insemination or intrauterine insemination. Indications for intrauterine insemination included poor cervical mucus, female serum immunobead antisperm antibodies and high dose clomiphene citrate ovulation induction (at least 100 mg/day). Nine conceptions took place in 96 insemination cycles with frozen sperm resulting in a cycle fecundity of 9.4%. Again no difference in the PR between cervical cap insemination and intrauterine insemination with frozen donor sperm was recorded.

Urry et al (1988) compared pregnancy rates with intrauterine versus intracervical inseminations prospectively and concluded that significantly higher pregnancy rates could be obtained with intrauterine insemination. A weakness of this study however is that both AIH (artificial insemination with husbands sperm) and AID cycles were included and that for the latter both fresh and frozen sperm was used.

In 1990 Patton et al reported the results of the first controlled, prospective trial comparing fecundity after intrauterine or intracervical insemination with fresh donor sperm.Twenty six women underwent 54 insemination cycles. Patients were randomly allocated to either IUI or ICI, with the alternate mode of insemination performed in the subsequent cycle should pregnancy not occur. A single insemination was performed on the day after detection of the urinary LH surge. The cycle fecundity following IUI with a swim-up preparation of motile sperm was 14.8% and comparable to a cycle fecundity of 18.5 % following ICI with whole semen. Subsequent studies on the optimal

method of insemination were undertaken, as in this study, with the use of frozen donor sperm only.

Byrd et al published in 1990 the results of a prospective randomized study of intrauterine and intracervical insemination using frozen donor sperm. A total of 154 patients were randomised into alternating treatment cycles and underwent 238 cycles of IUI and 229 cycles of ICI. Inseminations were performed on the day of the LH peak as monitored by twice daily urinary LH assays and the following morning whenever possible. The overall PR per treatment cycle was significantly higher following IUI (9.7%) when compared to ICI (3.9%).

These findings were confirmed by Patton et al (1992). Sixty nine patients underwent 161 donor insemination cycles with frozen sperm. The mode of insemination was again alternated in consecutive cycles and a single insemination performed on the day after LH surge. The monthly fecundity for ICI cycles was 5.1% compared with 23% for IUI cycles. By life table analysis, the PR for IUI was significantly higher than for ICI.

Hurd et al (1993) compared the efficacy of ICI, IUI and a combination of intratubal (ITI) and intrauterine insemination for donor insemination with frozen sperm. Forty one patients underwent 166 insemination cycles. Single inseminations were performed on the day after urinary LH surge. Cycle fecundity for IUI (18.6%) was significantly higher than for either ICI (3.8%) or for ITI/IUI (7.3%). Using life table analysis, the cumulative PR for IUI was significantly higher compared to the other methods of insemination. The authors concluded that IUI appeared to be a relatively simple and

cost effective technique for optimising cycle fecundity with cryopreserved sperm samples.

Williams et al (1995) confirmed the above findings. Forty two women undergoing 141 inseminations of frozen donor sperm were alternately inseminated using either IUI or cervical cap insemination. A single insemination was performed on the day after the urinary LH surge. Between ten million (for IUI) and 40 million motile sperm (for ICI) were inseminated. The clinical PR was significantly higher in the IUI group (6.4%) when compared with the cervical cap insemination group (5.9%).

In a study of similar size (43 patients, 156 insemination cycles) Wainer et al (1995) concluded that intrauterine insemination was the method of choice. Following routine ovulation induction with human menopausal gonadotrophin two inseminations were undertaken in the ICI group (12 ± 4 hrs and 38 ± 4 hrs after hCG injection) and one insemination in the IUI group (38 ± 4 hrs after hCG injection). All cycles received luteal support with vaginal micronised progesterone pessaries. The PR per cycle and the cumulative probability of conception was 19.4% and 75.4% in the IUI group compared to 6.7% and 35% in the ICI group. This difference was statistically significant.

In a non randomised study of intrauterine insemination of cryopreserved donor sperm Silva et al (1989) reported a monthly fecundity rate of 24% and an ongoing viable PR of 18%. This conception rate was considered to be higher than that generally reported for cryopreserved specimens and higher than most results reported for fresh specimens.

Royere et al (1993) however found no difference in cycle fecundity when comparing IUI and ICI. Twenty five patients underwent 100 AID cycles with alternate mode of insemination after previously having failed to conceive in six ICI cycles. A single insemination was performed on the day of LH surge or hCG injection for ICI cycles. In IUI cycles a single insemination was performed 10-20 hours after spontaneous LH surge or 35-40 hours after hCG administration. Forty nine IUI and 51 ICI cycles resulted in seven pregnancies each. It might however be argued, that the study population represented a selection of "subfertile" recipients, since the previous six ICI insemination cycles had failed to result in a pregnancy and that this degree of subfertility was not overcome by altering the mode of insemination.

Depypere et al (1994) published a retrospective study on methods to increase the success rate of artificial insemination with donor semen. 248 patients underwent 1608 insemination cycles. The method of insemination was not randomised. Patients either underwent cervical cap insemination combined with intrauterine insemination timed by vaginal echography and daily blood examination or intracervical insemination timed by basal body temperature. The authors concluded that intrauterine insemination did not enhance the success rate, but that the use of human menopausal gonadotrophin treatment (in combination with intrauterine insemination) probably exerted a favourable effect.

The American College of Obstetrics and Gynaecology (ACOG) has published updated Committee opinions with regards to maximising pregnancy rates resulting from donor insemination with frozen sperm. This recommendation stated in 1991 and 1993 that intracervical insemination was preferred to intrauterine insemination in the presence of

good quality mucus. This recommendation was changed in 1994 to state that whilst a variety of protocols for performing inseminations existed, no one procedure was superior to the others (Anon 1991b, Anon 1993, Anon 1994).

A summary of this literature review is shown in Table 19.

Table 19. Summary of Studies Comparing ICI with Donor Sperm and IUI with Donor Sperm.

Author	Year	No. of Patients	No. of cycles	sperm	PR/cycle ICI	PR/cycle IUI	Mode of insemination
Bordson	1986	120	401	Fresh/frozen	10.3%	10.3%	non randomised
Subak	1992	57	198	Fresh/frozen	9.4%	9.4%	non randomised
Patton	1990	26	54	Fresh	18.5%	14.8%	randomised
Byrd	1990	154	467	Frozen	3.9%	9.7%	randomised
Patton	1992	69	161	Frozen	5.1%	23%	randomised
Hurd	1993	41	166	Frozen	3.8%	18.6%	randomised
Royere*	1993	25	100	Frozen	13.7%	14.2%	randomised
Depypere	1994	248	1608	Frozen	12.4%	16.5% **	non randomised
Williams	1995	42	141	Frozen	5.9%	16.4%	randomised
Wainer	1995	43	156	Frozen	6.75%	19.4%	randomised
GSH	1996	43	109	Frozen	11.5%	22.9%	randomised

* after 6 failed ICI cycles
** ICI combined with IUI

Interestingly, the first three studies, using either fresh or fresh and frozen sperm, demonstrate no difference in cycle fecundity between IUI and ICI. Of the eight studies using frozen donor sperm six indicate improved cycle fecundity with IUI when compared to ICI. This is in keeping with our own finding. In all studies but ours this

difference was reported as statistically significant either with regard to the monthly conception rate or to the cumulative probability of conception after six cycles. Two studies disagree with this finding. Both these studies have certain limitations. Royere et al (1993) only included patients who had previously undergone six failed ICI cycles. The study by Depypere et al (1994) was retrospective and not randomised.

In conclusion the findings of our own study and the current literature suggest IUI improves cycle fecundity in therapeutic donor insemination cycles with frozen donor sperm.

Before a recommendation can be made to routinely undertake intrauterine insemination for artificial insemination with cryopreserved donor sperm, advantages and disadvantages have to be carefully considered.

The obvious advantage is the anticipated higher cycle fecundity resulting in a higher number of patients conceiving over a shorter period of time. This might be of particular importance where patients are unlikely to persist with treatment for a number of cycles. The disadvantages are the additional laboratory requirements and the additional costs. In the context of South Africa the laboratory requirements may be the more important disadvantage. With intracervical insemination frozen donor sperm can be sent to any practitioner registered to do AID and the procedure be performed without any laboratory procedures, possibly only with the help of ultrasonographic cycle monitoring. It is however our experience that artificial insemination with donor sperm

is usually performed by specialists in urban areas with ready access to laboratory facilities.

Secondly, the process of sperm washing will result in extra costs, mainly due to extra laboratory time and, to a lesser extend, due to extra equipment cost such as the culture medium.These costs however have to be balanced against higher cycle fecundity and shorter time to conception if intrauterine insemination is performed.

Our patients underwent an average of 2.5 insemination cycles. All patients were carefully counselled to undergo at least six insemination cycles. Despite this, nearly every third patient dropped out without achieving a pregnancy and prior to completion of the recommended treatment. Patient dropout is considered by several other authors as being one of the commonest causes of treatment failure occurring with a similar frequency to ours (Quinlivan 1979, Bergquist et al 1982, Hummel and Talbert 1989, Williams et al 1995).

Persistency is thus recognised as one of the most important factors in the success with donor insemination. There remains however some controversy as to what represents adequate persistency. This controversy centres around the question as to whether cycle fecundity decreases over time. Several studies indicate that the great majority of patients who conceive do so within six cycles and a decline in cycle fecundity is frequently observed already after three cycles (Trounson et al 1981, Bordson et al 1986, Federation CECOS 1989, Patton et al 1992, Subak et al 1992). Other authors report no decrease in cycle fecundity for up to twelve cycles and more (Schoysman and

Schoysman-Deboeck 1976, Bromwich et al 1978, David et al 1980a, Bergquist et al 1982, Hummel and Talbert 1989). In our own study 14 of the 18 pregnancies occurred within the first three cycles. This, together with the experience that several patients drop out after a relatively short course of treatment supports our current policy of recommending six treatment cycles as a realistic length of treatment.

Fourteen out of the 18 pregnancies resulted in the live birth of normal babies. This is in keeping with several literature reports indicating that the chance of a successful pregnancy outcome is not affected by artificial insemination with frozen donor sperm. The frequency of spontaneous miscarriage and congenital abnormalities is reported to be either lower or within the range of spontaneous conceptions, probably because of the rigorous screening donors undergo (Tyler 1973, Sherman 1973, Schoysman and Schoysman-Deboeck 1976, Quinlivan 1979, Byrd et al 1990).

Whilst the above data indicate that intrauterine insemination cycles will result in higher pregnancy rates when compared to intracervical insemination cycles, the eventual treatment outcome may be influenced by several other variables. These variables include male and female fertility factors, cycle quality and sperm parameters.

The mean age of female patients in this study was 29.9 years with no difference between pregnant and non pregnant patients. The PR in the age category 35 years and older was however lower compared to patients in the two younger age categories. No patient over the age of 35 years conceived in this study. Our data suggest a possible trend towards improved cycle fecundity in the two older age categories (30-34 years

and 35 years and older) with the use of IUI rather than ICI. No such trend was observed in the age group 20 to 29 years. It might be that young, highly fertile women achieve an adequate PR following ICI, which cannot be further improved with IUI. IUI on the other hand may overcome an element of female age related decline in fertility in the older age group. Unfortunately our numbers are too small for statistical analysis.

Interestingly Wainer et al (1995) report a similar finding. While female age over 30 years was an important factor associated with treatment failure for ICI, the age related decline in cycle fecundity was of less significance for IUI. Again, however, the numbers were too small for a statistical conclusion. Further analysis of this observation in a larger study would be of interest.

The age related decline in female fertility is well documented and observed in most studies on therapeutic donor insemination. The decline in cycle fecundity with increasing maternal age develops clinical relevance somewhere between the age of 30 to 35 years (Bergquist et al 1982, Emperaire et al 1982, Kovacs et al 1988, Federation CECOS 1989, Wong et al 1989, Shenfield et al 1993, Meyer et al 1996). Byrd et al (1990) divided his patients into four separate categories (20 to 25, 26 to 30, 31 to 35 and 36 to 42 years) and observed that the PR following IUI decreased with increasing age of the women (from 33.3% to 5.3%). Whether a similar effect was observed for ICI cycles is not documented. Furthermore, once women with known infertility factors were eliminated from this analysis, the PR between the respective age groups was no longer statistically significant.

This observation was confirmed by Barratt et al (1990) who observed that most studies which demonstrate an overwhelming decrease in cycle fecundity with increasing age can be criticised for not controlling for other fertility factors.

In our study parity did not affect cycle outcome. The majority of our patients were nulliparous and the remainders were primiparous. This is in keeping with other literature reports, in which cycle fecundity was not altered by the parity of the recipient (Bergquist et al 1982, Shenfield et al 1993, Meyer et al 1996).

Ovulatory function also had no impact on cycle fecundity in our study. This might firstly be due to the fact the majority of anovulatory patients probably had minor ovulatory dysfunction, since they reported regular menstrual cycles. Secondly, this ovulatory dysfunction could have been adequately corrected by the ovulation inducing agents that were routinely used in the majority of cycles and by the close attention paid to cycle monitoring. These results are in keeping with the findings of Hammond et al (1986). In his study of 226 women undergoing therapeutic donor insemination with fresh and frozen semen ovulatory dysfunction was a relatively frequent finding (36% of patients). This however did not affect the probability of conception in patients who were treated for this ovulatory dysfunction. Similarly, Williams et al (1995) in a study of 42 women undergoing 141 cycles of donor insemination found that patients with corrected ovulation had the same PR compared to patients with spontaneous ovulation. Several other authors however found reduced fertility in patients with documented ovulatory disorders (Smith et al 1981, Albrecht et al 1982, Chauhan et al 1989, Wong et al 1989, Byrd et al 1990). Barratt et al (1990) concluded that whilst treatment of ovulatory

disorders certainly improved the probability of conception, the fertility of donor recipients with ovulatory dysfunction was still lower than in patients without any infertility factors.

Similarly, reduced cycle fecundity in women with evidence of pelvic pathology has been documented (Albrecht et al 1982, Bordson et al 1986, Chauhan et al 1989), with endometriosis having a particular negative impact (Hammond et al 1986). Byrd et al (1990) found women with endometriosis to have the lowest pregnancy rates (6.1% per cycle following IUI and 2.2% per cycle following ICI). However, as pointed out by Barratt et al (1990), in most studies reporting a negative association between female fertility and endometriosis, the incidence of endometriosis in those patients that conceived was unknown.

The reported incidence of pelvic pathology varies widely. Barratt et al (1990) reported the incidence of endometriosis in fertile and infertile women to range between 2.5% - 5% and 20% - 50% respectively. Aiman (1982) found pelvic pathology to account for 2% - 72% of conception failures in therapeutic donor insemination programs.

All patients in our study underwent evaluation of tubal patency prior to the initiation of treatment. In the majority of patients (n = 25) this was by hysterosalpingography only. The remaining 18 patients underwent laparoscopy. Pelvic pathology was found in a

total of eight patients, of whom all but one had been investigated by laparoscopy. Six patients had endometriosis. Pregnancy rates per cycle and patient were much lower in the endometriosis groups than in patients with no apparent pelvic pathology. As discussed above this conclusion is however limited by the fact that the majority of both pregnant and non pregnant patients was never investigated for the presence or absence of endometriosis.

Somewhat surprisingly both patients with evidence of tubal disease without endometriosis conceived. This however resulted in the only ectopic pregnancy in this study - in a patient who conceived following tubal surgery (neosalpingostomy). In the other patient the diagnosis of unilateral tubal blockage was made on hysterosalpingogram only.

The role of routine assessment of tubal patency prior to therapeutic donor insemination has been debated in the literature. Whilst undertaken commonly as part of the initial evaluation of the prospective AID patient the value, especially of routine hysterosalpingography has been challenged. Nash et al (1978), in a retrospective study of 89 consecutive patients undergoing AID firstly found a low incidence of significantly abnormal findings on hysterosalpingogram and secondly a failure of these findings to correlate with pregnancy outcome.

Similarly Stovall et al (1992) in his study of 208 women, undergoing 1460 cycles of therapeutic donor insemination, found the routine use of pre-treatment hysterosalpingography in asymptomatic women not indicated. The presence of significant pathology (bilateral tubal occlusion, hydrosalpingx or salpingitis isthmica nodosa) was extremely low (six cases) and the presence of minor pathology (uterine filling defect and/or unilateral tubal blockage) did not affect cycle fecundity. Furthermore a substantial false positive rate with regards to bilateral tubal blockage was observed.

It is our own experience that bilateral (proximal) tubal occlusion on HSG can be caused by tubal spasm and thus represent a false positive finding. We still maintain that routine evaluation of tubal patency, regardless of the presence of symptoms or risk factors, is indicated in the majority of our patients. Tubal infertility is recognised as one of the leading causes of infertility in Africa and the incidence is high among patients presenting to Groote Schuur Hospital Infertility Clinic. Several epidemiological risk factors such as early onset of sexual intercourse, multiple sexual partners, high risk sexual partners, high incidence of sexually transmitted diseases and inadequate treatment of pelvic inflammatory disease contribute to this. Patients are frequently unaware of these risk factors. Screening by history or symptoms only is thus likely to be inadequate in most instances. The studies by Stovall et al (1992) and Nash et al (1978) were both undertaken in the United States of America. Whether the study population was considered to be of high or low risk with regards to tubal infertility was not specified.

The severity of male factor infertility had a significant influence on the probability of conception in this study. No pregnancy occurred in eight women with partners who were found to have severe oligoteratozoospermia as opposed to azoospermia. Conversely, women with partners, diagnosed azoospermic, had a higher chance of conception when compared to the overall cycle fecundity in this study.

This effect of male factor infertility on fertility rates has been well documented in the literature (Emperaire et al 1982, Albrecht et al 1982, Hammond et al 1986, Chauhan et al 1989, Barratt et al 1990, Wainer et al 1995). These findings underscore a different degree or fertility in the female population. Women of high fertility with partners who are oligoteratozoospermic are likely to conceive spontaneously (ie their fertility "overcomes" the relative male factor) and thus do not present for infertility treatment. Consequently, women whose partners are oligoteratoozpermic and who have not conceived spontaneously represent a selection of patients of reduced overall fertility. No such selection mechanism occurs in partners of azoospermic men, who thus represent the full range of female fertility potential.

In our own study two women of partners with severe oligoteratozoospermia were found to have severe endometriosis after four and six failed insemination cycles respectively. No obvious female factor was present in the remaining six women, of whom three however never had a laparoscopy.

Following the analysis of our data it has become policy in our unit to initially counsel patients very carefully on their chance of conception with regard to the severity of the

male factor inferility. Secondly we counsel all women whose partners are not azoospermic to undergo pre-insemination laparoscopy and hysteroscopy - regardless of an apparently normal hysterosalpingogram .

Only a few patients presented with more than one infertility factor in our study and this did not affect the probability of conception. Barratt et al (1990) found the presence of combined infertility factors to have a major additive, negative impact on cycle fecundity. Byrd et al (1990) found that patients with two or more infertility factors had a reduced chance of conception when undergoing intracervical inseminations. No such reduction in fecundity was specified for the same patients undergoing intrauterine inseminations.

There are several explanations as to why we did not find this negative additive effect of combined fertility factors expected from clinical experience. Firstly, the numbers may be too small and many women never underwent a laparoscopy. Secondly, since the presence of ovulatory dysfunction in itself had no negative effect on cycle fecundity in this study it is unlikely to exert such an effect in combination with another infertility factor. Lastly, it is possible in view of the findings by Byrd et al (1990) that the insemination technique, namely intrauterine insemination, may have overcome this negative additive effect of combined infertility factors. Our own numbers in this study are too small to allow an analysis of the impact of combined infertility factors on intrauterine versus intracervical insemination.

The effect of the timing and the frequency of the insemination in therapeutic donor insemination programs has been the focus of many studies; especially since the introduction of frozen donor sperm. The window of opportunity for fertilisation in humans is narrow, probably 12 to 18 hours after ovulation. This restriction is dictated by the ovum, with fresh sperm having a much longer functional post-ejaculatory life (Shapiro 1991). Consequently, either a single insemination at expected time of ovulation or repeated inseminations at 48 hour intervals around the presumed day of ovulation was considered to result in adequate cycle fecundity when fresh donor semen was used. Since the reduced fertilising potential of frozen sperm has been at least partly attributed to a diminished post- thaw survival and diminished lifespan of frozen-thawed spermatozoa, either more frequent or better timed inseminations were considered one option to improve cycle fecundity (Saarenen et al 1986, Jacobs and Ory 1989, Shapiro 1991). The required degree of accuracy with regards to the timing and the optimal frequency of inseminations has however remained controversial.

According to most authors a single insemination with frozen, thawed donor sperm is best performed on the day of or the day after the LH surge (Matthews et al 1979, Odem et al 1991, Meyer et al 1996, Coulson et al 1996) with some reports suggesting a somewhat higher cycle fecundity for insemination performed on the day after the LH surge rather than on the day prior to it (Smith et al 1981, Meyer et al 1996). Different protocols exist with regard to the methods used to detect ovulation. While ultrasonographic evidence of follicular growth and subsequent follicular rupture may be considered the gold standard (Barratt et al 1990, Brook et al 1994), other, less accurate scheduling methods such as monitoring urinary or serum LH levels, basal body

temperature charts and cervical mucus assessment have been found equally successful with regard to the probability of conception (Trounson et al 1981, Kossoy et al 1989, Barratt et al 1990, Brook et al 1994).

Controversy also exists with regard to the optimal number of inseminations performed in a cycle. Some authors consider two or more inseminations necessary for optimal cycle fecundity while others find no benefit from repeated inseminations (Matthews et al 1979, Saarenen et al 1986, Kossoy et al 1989, Boyers et al 1991, Meyer et al 1996). David et al (1980b) in his report on semen characteristics in 1489 cycles of frozen donor insemination found the role of multiple inseminations variable, its influence being least for good quality ejaculates (> 10 million sperm, > 50% motility). If all poor semen samples were eliminated from insemination programs, multiple inseminations in a cycle would be less justified according to the author.

It is thus evident from the literature that it is extremely difficult to dissect out the individual importance of insemination timing, insemination frequency and sperm quality.

Introducing yet another variable, namely the mode of insemination, may furthermore affect the timing and frequency of inseminations. Byrd et al (1990) in their report on intrauterine versus intracervical insemination using frozen donor sperm pointed out that following intrauterine insemination the sperm may have a more finite period in which to fertilise the egg, since colonisation of the cervical crypts with slow release of sperm over time may not occur. Following 467 insemination cycles in 154 patients the authors

concluded that the highest cycle fecundity was achieved with intracervical insemination performed 0 to10 hours post natural LH surge (as assessed by urinary LH- level monitoring) and intrauterine inseminations performed somewhat later, namely 10 to 20 hours post natural LH surge. While the PR in the cycles with two intrauterine inseminations was significantly higher when compared to cycles with a single intrauterine insemination (11.6% vs 7.0%), no such difference was present in ICI cycles.

In our study all patients underwent ultrasonographic monitoring of follicular growth together with serum LH and progesterone monitoring. In order to allow accurate timing of inseminations ovulation was triggered with 5000 IU hCG in all cycles, unless there was evidence of a spontaneous LH surge. Timing and frequency of insemination was strictly according to protocol. In all cycles two inseminations were performed unless there was evidence of a spontaneous LH surge, in which case only a single insemination was performed. Inseminations were timed to occur prior to and at expected time of ovulation. Both timing and frequency of insemination were thus eliminated as a variable and the overall satisfactory cycle fecundity confirms this insemination protocol as being effective.

Cycle quality, as measured in this study by number of follicles as well as serum estradiol levels and endometrial thickness at the time of ovulation and/or triggering of ovulation did not obviously affect the probability of conception. This finding has to be viewed with caution. The analysis of estradiol levels and endometrial thickness are limited by the fact, that this information is not available for all cycles. Furthermore,

with regards to number of follicles, most cycles showed single follicular development and numbers were too small to allow comparison with multiple follicular development.

It has to be emphasised in this context that while nearly all cycles underwent some form of ovulation induction, this was done entirely with the view of inducing follicular growth in anovulatory patients, facilitating the timing of insemination in ovulatory patients and improving aspects of previously failed cycles ie estradiol levels and or endometrial thickness. To date it has not been our policy in therapeutic donor insemination cycles to purposefully induce superovulation - a policy reflected in the infrequent and low dose use of gonadotrophins. The more frequent use or higher dose of gonadotrophins in patients coming for therapeutic donor inseminations to our clinic is complicated by the significant expense of this medication, which can easily become the most expensive aspect in the entire procedure of artifical insemination with donor sperm and beyond the means of our patients.

The role of multiple follicular development ("superovulation") using gonadotrophin stimulation in intrauterine insemination cycles has been a topic of considerable interest in the literature. This has mainly been in the context of artificial insemination with husband's sperm and little is reported with regard to superovulation in a therapeutic donor insemination program. Depypere et al (1994) reported improved cycle fecundity with the use of human menopausal gonadotrophin and IUI in therapeutic donor insemination, but did not correlate this finding to the number of follicles induced.

We have introduced the measurement of endometrial thickness at the time of ultrasonographic assessment of follicular development as part of our routine cycle monitoring. We consider this as additive information when assessing cycle quality, but it has never been an indication to cancel a therapeutic donor insemination cycle. The data in this study did not show a correlation between endometrial thickness and cycle fecundity. This can be either due to a type II error or due to the fact that the endometrium is receptive irrespective of the measured thickness.

Endometrial development is obviously of paramount importance for implantation. It is however difficult to assess whether or not adequate endometrial development has taken place. One way of assessing endometrial development has been the ultrasonographic imaging of both endometrial thickness and endometrial pattern. This has been discussed widely in the literature but its usefulness is still controversial. In the current literature review of therapeutic insemination with donor sperm little is written about the role of endometrial development. Irons et al (1992, 1994) evaluated the use of vaginal ultrasound in donor insemination. Only women with leading follicles >18 mm and an endometrial thickness >9mm were inseminated and the authors concluded that vaginal ultrasonography helped to optimise the timing of the insemination. The endometrial thickness was not correlated to cycle fecundity. Li et al (1993) in an interesting study of endometrial morphology in women who failed to conceive in a donor insemination program found evidence of endometrial defects as assessed by endometrial biopsy in 12 out of 26 women. Ultrasonographic assessment of the endometrium was however not part of this study.

Despite obvious shortcomings semen analysis remains the most common objective measurement of the fertility potential of the male (Arumugam et al 1992). Irrespective of the potential effect of cryopreservation damage, frozen thawed semen samples are usually assessed by the same sperm characteristics as fresh ejaculates. In this study the total number of motile sperm inseminated correlated best with cycle fecundity, with most pregnancies occurring following insemination of 15 to 20 million motile sperm. This optimal range of total number of motile sperm inseminated did not differ between ICI and IUI cycles and there was no difference between cycles inseminated once or twice. Although the numbers are too small for firm conclusions, this lends support to the rationale of performing two inseminations per cycle with view of providing a high number of motile sperm over an extended period of time rather than to achieve an additive effect with regard to the total number of motile sperm. This finding also reflects a possible "ceiling effect" with regard to sperm quality and cycle fecundity: In the lower ranges of semen quality there appears to be a correlation to cycle fecundity, but no such effect is observed with semen samples over and above a certain level of "quality ".

Post-thaw motility did not correlate with cycle fecundity. No difference was found in treatment outcome between semen samples of 5 to 14% normal morphology (g-pattern) when compared to samples of over 15% normal morphology. Semen samples of less than 5% normal morphology were considered unsatisfactory and essentially not used for insemination.

It is generally accepted in the literature that semen quality does affect cycle fecundity and that differences in semen quality may be a significant source of variability in AID success rates (Federation CECOS 1989). What however constitutes "optimal semen quality" remains controversial and different authors report on different "minimal requirements" with regards to semen samples inseminated.

Recommendations range from a minimum of 5 million to 50 million motile sperm per inseminated semen sample and from a post-thaw motility of 30% to 65% and more (Emperaire et al 1982, Peterson et al 1988, Federation CECOS 1989, Moghissi 1990, Hogerzeil et al 1991). Matthews et al (1979) in a study of 133 patients undergoing intracervical insemination of frozen, thawed donor sperm found that while pregnancies did occur with semen samples containing 1-10 million motile sperm (PR of 1:19 cycles) the frequency of conception was substantially improved when samples containing a minimum of 25 million motile sperm were used (PR of 1:7.5 cycles).

David et al (1980b) in a report on the success of AID and semen characteristics (1489 cycles) found an increasing success as numbers of sperm inseminated increased to 10 million - with no further significant increase thereafter. Both these findings are very similar to our own of an optimal range between 15 and 25 million motile sperm.

Post-thaw motility is frequently regarded as another semen characteristic of critical importance, more so possibly than the number of motile sperm inseminated (David et al 1980b, Federation CECOS 1989). Improved cycle fecundity has been reported with

samples containing at least 40 - 50 % post-thaw motility compared to samples of lower post-thaw motility (David et al 1980b, Federation CECOS 1989, Boysen et al 1991).

Byrd et al (1990) showed a similar correlation between post-thaw motility and cycle fecundity following intrauterine insemination of frozen, thawed and washed donor sperm. Cycle fecundity was 5.5% with semen samples of less than 30% post-thaw motility, 15.4% for samples containing 30-50% post-thaw motility and rose to 27.2% with samples showing over 50% post-thaw motility. On the other hand Wong et al (1989) in a study of 984 treatment cycles found no correlation between post-thaw sperm motility in a range of 20% to over 70% and treatment outcome. Similarily, in our own study insemination with semen samples of above or below 50% post-thaw motility did not influence cycle fecundity.

Minimal sperm requirements may vary depending on the mode of insemination. Byrd et al (1990) reported the pregnancy success for intrauterine and intracervical inseminations to depend highly on the number of motile sperm inseminated. According to the authors the minimum number of motile sperm required for optimal cycle fecundity is between 6 and 15 million for IUI cycles and between 50 and 100 million for ICI cycles. Subak et al (1992) found no significant association between number of motile sperm and cycle fecundity for either mode of insemination. Semen samples contained an average of $22.7 \pm 1.2 \times 10^6$ motile sperm for intracervical insemination and an average of $5.6 \pm 1.1 \times 10^6$ motile sperm (following semen wash and swimup) for intrauterine inseminations. In a study by Silva et al (1989) on intrauterine insemination with cryopreserved donor sperm the monthly conception rate did not differ when either 12

or 24 million motile sperm were inseminated. Patton et al (1992), using similar semen samples for both intracervical insemination (49.2 x 10^6 ± motile sperm) and intrauterine insemination (43 x 10^6 ± 23), found no difference in the average number of motile sperm inseminated in women who conceived compared to women who did not. He recommended that, while the optimal number of sperm for therapeutic donor insemination is unknown, semen samples should contain at least 20 x 10^6 motile sperm per insemination (with a single insemination performed per cycle), a recommendation very much in keeping with our own observations.

Compared to an extensive analysis of the standard semen characteristics of frozen donor sperm in the literature the fertility potential of the individual donor has received relatively little attention. Barratt et al (1990) pointed out that the major problem in the selection of donors is the inability to accurately determine the fertilising capacity of the spermatozoa. The traditional approach of using donors with semen characteristics above certain standards is limited by the controversy of the predictive value of semen parameters with regards to fertility. These limitations may furthermore be aggravated by cryopreservation damage which might not readily be reflected in the standard assessment of sperm characteristics.

Byrd et al (1990) noted on completion of his study of 154 patients undergoing 467 cycles of frozen donor insemination that the fecundity of donors differed irrespective of post-thaw sperm parameters. This is in keeping with our own results in which individual donor fecundity differed regardless of similar fresh and post-thaw semen parameters.

Recently computer assisted semen analysis (CASA) has been reported as being of significant value in predicting the fertility potential of cryopreserved donor sperm (Byrd et al 1990, Marshburn et al 1992, MacLeod and Irvine 1995). Post-thaw sperm linearity, straight-line velocity, curvilinear velocity, sperm head morphometry and lateral head displacement have been found to correlate highly with conception when conventional semen analysis failed to find distinguishing features between fertile and non fertile semen samples.

CONCLUSIONS

In this study intrauterine insemination consistently resulted in higher pregnancy rates when compared to intracervical insemination. While statistically significant differences were not demonstrated, there is evidence in the literature supporting our findings.

Patients in the older age group and patients requiring more than three treatment cycles to conception seemed to benefit in particular from intrauterine insemination. The element of female subfertility in women with partners with severe oligoteratozoospermia was not overcome by intrauterine insemination. Cycle fecundity was reduced in patients with endometriosis.

A decision to routinely undertake intrauterine insemination for artificial insemination has to take advantages (higher cycle fecundity, shorter time to conception) and disadvantages (laboratory requirements, cost) into account.

At present it is probably more appropriate to individualise the role of intrauterine insemination. Women in the upper range of fertility are likely to conceive regardless of the mode of insemination. These patients could be treated with intracervical insemination over two to three cycles, especially where the facilities for intrauterine insemination are not available. After three failed cycles the routine performance of intrauterine insemination is probably justified. Women in the lower range of fertilty - ie women with pelvic pathology or women with partners with severe

oligoteratozoospermia as opposed to azoospermia - should be carefully counselled regarding their chance of conception. Assisted reproductive techniques (including the option of intracytoplasmic sperm injection) might be a more appropriate treatment option for many of these couples.

We recommend insemination with semen samples containing approximatly 20 million motile sperm irrespective of the method of insemination.

Careful cycle monitoring together with a strict protocol with regard to the timing and frequency of the insemination are likely to play an important part in the success of a therapeutic donor insemination program. This success may be further increased in future if investigations become available to identify highly fertile donors.

REFERENCES

1. Aiman J. Factors affecting the success of donor insemination. *Fertil Steril* 1982; 37(1): 94-99.

2. Albrecht BH, Cramer D, Schiff I. Factors influencing the success of artificial insemination. *Fertil Steril* 1982; 37(6): 792-797.

3. Allen DG, Alperstein A, Tsalacopoulos G. Artificial insemination by donor at Groote Schuur Hospital. *SAMJ* 1985; 67: 284-287.

4. American Fertility Society. New guidelines for the use of semen donor insemination:1986. *Fertil Steril* 1986; 46(4): 95 S-99 S.

5. American Fertility Society. Revised new guidelines for the use of semen donor insemination. *Fertil Steril* 1988; 49 (2): 211.

6. Anonymous. Suggested code of Practice for Artificial Insemination by Donor. *SAMJ* 1980; November 8: 781-783.

7. Anonymous. Human Tissue Act,1983. *Government Gazette* 1991a; 13228: 19-30.

8. Anonymous. Maximising pregnancy rates resulting from donor insemination with frozen semen. *Int J Gynaecol Obstet* 1991b; 36: 255 - 256.

9. Anonymous. Maximising pregnancy rates resulting from donor insemination with frozen semen. *Int J Gynaecol Obstet* 1993; 40: 80 - 81.

10. Author Anonymous. Maximising pregnancy rates resulting from donor insemination with frozen semen. Int J Gynaecol Obstet 1994; 44: 95 - 296.

11. Arumugam K, Omar SZ. The use of Semen Analysis in Predicting Fertility Outcome. *Aust NZ J Obstet Gynecol* 1992; 32(2): 154-151.

12. Barratt CLR., Chauhan M, Cooke ID. Donor insemination - a look to the future. *Fertil Steril* 1990; 54(3): 375-387.

13. Barton M, Walker K, Wiesher BP. Artificial insemination . *Br Med J* 1945; Jan 13:40-43.

14. Beck WW. Two hundred years of artificial insemination. *Fertil Steril* 1984; 41 (2): 193-195

15. Bergquist CA, Rock RA, Miller J, Guzick DS, Wentz AC, Jones GS. Artificial insemination with Fresh Donor Semen using the Cervical Cap Technique: A review of 278 cases. *Obstet Gynecol* 1982; 60(2):195-199.

16. Bordson BL, Ricci E, Dickey RP, Dunaway H, Taylor SN, Curole DN. Comparison of fecundability with fresh and frozen semen in therapeutic donor insemination. *Fertil Steril* 1986; 46(3): 466-469.

17. Boyers AI, Shenfield F, Valentine A. Semen and cervical mucus parameters and success in artificial insemination. *Hum Reprod* 1991; 6(8):1108-1114.

18. Bromwich P, Kilpatrick M, Newton JR. Artificial insemination with frozen stored donor semen. *Br J Obstet. & Gynaecol* 1978; 85(9): 641 - 644.

19. Brook PF, Barratt CLR, Cooke ID. The more accurate timing of insemination with regard to ovulation does not create a significant improvement in pregnancy rates in a donor insemination program. *Fertil Steril* 1994; 61(2): 308-313.

20. Bunge RG, Sherman JK. Fertilising capacity of frozen human spermatoza. *Nature* 1953; 172: 767-8.

21. Byrd W, Bradshaw K, Carr B, Edman C, Odom J, Ackerman G. A prospective randomized study of pregnancy rates following intrauterine and intracervical insemination using frozen donor sperm. *Fertil Steril* 1990; 53(3): 521-527.

22. Chauhan M, Barratt CLR, Cooke SMS, Cooke ID. Differences in the fertility of donor insemination recipients - a study to provide prognostic guidelines as to its success and outcome. *Fertil Steril* 1989; 51(5): 815-19.

23. Coulson C, McLaughlin EA, Harris S, Ford WCL, Hull MGR. Randomised controlled trial of cervical cap with intracervical reservoir versus standard intracervical injection to inseminate cryopreserved donor semen. *Hum Reprod* 1996; 11(1):84-87.

24. Cox J. Male Infertility: Artificial insemination by Donor . *Health visitor* 1993; 66(2): 54-55.

25. Currie-Cohen M, Luttrell L, Shapiro S. Current practice of artificial inseminaton by donor in the United States. *N Engl J Med* 1979; 300(11):585-590.

26. David G, Czyglik F, Mayaux MJ, Martin-Boyce A, Schwartz D. Artificial insemination with frozen sperm: Protocol, Method of Analysis and Results for 1188 women. *Br J Obstet Gynaecol* 1980a; 87: 1022-1028

27. David G, Czyglik F, Mayaux MJ, Schwartz D. The success of AID and semen characteristics: Study on 1489 cycles and 192 ejaculates. *Int J Androl* 1980b; 3: 613-619.

28. Depypere HT, Gordts S, Campo R, Comhaire F. Methods to increase the success rate of artificial insemination with donor semen. *Hum Reprod* 1994; 9(4): 661-663.

29. Editorial. Artificial Insemination by Donor. *SAMJ* 1978; June: 1006.

30. Emperaire J, Gauzere-Soumireu E, Audebert AJM. Female fertility and donor insemination. *Fertil Steril* 1982; 37(1) : 90-93.

31. Federation CECOS, Le Lannou D, Lansac J. Artificial procreation with frozen donor semen: experience of the French Federation CECOS. *Hum Reprod* 1989; 4(7): 757-761.

32. Hammond MG, Jordan S, Sloan CS. Factors affecting pregnancy rates in a donor insemination program using frozen semen. *Am J Obstet Gynecol* 1986; 155(3): 480-484.

33. Hansen BK, Nielsen NC, Rebbe H. Artificial insemination in Denmark by frozen donor semen supplied from a central bank. *Br J Obstet Gynaecol* 1979; 86: 384-386.

34. Hogerzeil HV, Stevenhagen C, Oosting HJ, Hamerlynck JV. Daily insemination with cryopreserved donor semen is more effective than alternate day insemination. *Int J Fertil* 1991; 35(5): 281-286.

35. Hulka JF. Donor insemination: Guidelines for uncharted territory. *Fertil Steril* 1981; 35(5): 500-501.

36. Hummel WP, Talbert LM. Current management of a donor insemination program. *Fertil Steril* 1989; 51(6): 919-930.

37. Hurd WW, Randolph JF, Ansbacher R, Menge AC, Ohl DA, Brown AL. Comparison of intracervical,intrauterine and intratubal techniques for donor insemination. *Fertil Steril* 1993; 59 (2): 339-342.

38. Iddenden DA, Sallam HV, Collins WP. A prospective randomised study comparing fresh semen and cryopreserved semen for Artificial Insemination by Donor. *Int J Fertil* 1985; 30(1): 54-56.

39. Irons DW, Singh MM. Vaginal ultrasound in donor insemination. *Br J Obstet Gynaecol* 1992; 99: 518-523.

40. Irons DW, Singh M. Evaluation of transvaginal sonography combined with a urinary luteinising hormone monitor in timing donor insemination. *Hum Reprod* 1994; 9(10):1859-1862.

41. Jacobs LA, Ory SJ. Changes in Artificial insemination regimens for male factor infertility. *Clin Obstet Gynecol* 1989; 32(3): 568-597.

42. Kossoy LR, Hill GA, Parker RA, Rogers BJ, Dalglish CS, Herbert GM,Wentz AC. Luteinizing hormone and ovulation timing in a therapeutic donor insemination program using frozen semen. *Am J Obstet Gynecol* 1989; 160(5): 1169-1172.

43. Kovacs G, Baker G, Burger H, De Kretser D, Lording D, Lee J. Artificial insemination with cryopreserved donor semen: a decade of experience. *Br J Obstet Gynecol* 1988; 95: 354-360.

44. Li TC, Klentzeris L, Barratt C,Warren MA, Cooke S, Cooke I.D. A study of endometrial morphology in women who failed to conceive in a donor insemination programme. *Br J Obstet Gynecol* 1993; 100: 935-938.

45. Macleod IC, Irvine DS. The predictive value of computer-assisted semen analysis in the context of a donor insemination programme. *Hum Reprod* 1995; 10(3): 580-586.

46. Marshburn PB, McIntire D, Carr BR, Byrd W. Spermatozoal characteristics from fresh and frozen donor semen and their correlation with fertility outcome after intrauterine insemination. *Fertil Steril* 1992; 58(1): 179-186.

47. Matthews CD, Broom TJ, Crawshaw KM, Hopkins RE, Kerin JFP, Svigos JM. The influence of insemination timing and semen characteristics on the efficiency of a donor insemination program. *Fertil Steril* 1979; 31(1): 45-47.

48. Menkveld R, Franken D, Kruger T. WHO criteria of normality for semen samples. *Int Society of Andrology* 1993; Newsletter (10).

49. Meyer WR, Smith PM, Clark MR, Cusmano LL, Fritz MA. Therapeutic cup insemination with cryopreserved donor sperm: prognostic value of cervical mucus score at insemination and the number of motile sperm at 24 hours. *Fertil Steril* 1996; 66(3): 435-439.

50. Moghissi KS. Reflections on the new guidelines for the use of semen donor insemination. *Fertil Steril* 1990; 53(3): 399-400.

51. Nachtigall RD. Donor insemination and human immuno deficiency virus: A risk/benefit analysis. *Am J Obstet Gynecol* 1994; 170(6): 1692-1698

52. Nash D, Haning RV, Shapiro SS. The value of hysterosalpingography prior to donor artificial insemination. *Fertil Steril* 1978; 31(4): 378-380.

53. Odem RR, Durso NM, Long AC, Pineda JA, Strickler RC, Gast MJ. Therapeutic donor insemination: A prospective randomized study of scheduling methods. *Fertil Steril* 1991; 55(5): 976-982.

54. Patton PE, Burry KA, Novy MJ, Wolf DP. A comparative evaluation of intracervical and intrauterine routes in donor therapeutic insemination. *Hum Reprod* 1990; 5(3): 263-265.

55. Patton PE, Burry KA, Thurmond A, Novy MJ, Wolf DP. Intrauterine insemination outperforms intracervical insemination in a randomized, controlled study with frozen,donor sperm. *Fertil Steril* 1992; 57 (3): 559-564.

56. Peterson EP, Alexander NJ, Moghissi KS. AID and AID's - too close for comfort. *Fertil Steril* 1988; 49(2): 209-210.

57. Polge C, Smith AU, Parkes AS. Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature* 1949; 164: 666.

58. Quinlivan WLG. Therapeutic donor insemination: Results and causes of non fertilisation. *Fertil Steril* 1979; 32(2):157-160.

59. Royere D, Ract V, Lanoue M, Lecomte C, Lansac J. Do intrauterine insemination with donor sperm improve the conception rate beyond six intracervical insemination cycles: A prospective randomised controlled study. *Hum Reprod* 1993;8, Suppl 1,Abstr 172: 60-61.

60. Saaranen M, Suhonen M, Saarikoski S. Ultrasound in the Timing of Artificial Insemination with Frozen Donor Semen. *Gynecol Obstet Invest* 1986; 22:140-144.

61. Sanger WG, Schwartz MB, Housel G. The use of frozen - thawed semen for therapeutic insemination (Donor). *Int J Fertil* 1980; 24(4): 267-269.

62. Schoysman R, Schoysman-Deboeck A. Results of donor insemination with frozen semen. *Prog Reprod Biol* 1976; 1: 252-258.

63. Shapiro S. Can timing improve therapeutic donor insemination fecundability? *Fertil Steril* 1991; 55(5): 869-871.

64. Shenfield F, Doyle P, Valentine A, Steele SJ, Tan SL. Effects of age, gravidity and male infertility status on cumulative conception rates following artificial * insemination with cryopreserved donor semen: Analysis of 2998 cycles of treatment in one centre over 10 years. *Hum Reprod* 1993; 8(1): 60-64.

65. Sherman JK. Synopsis of the use of frozen human semen since 1964: State of the art of human semen banking. *Fertil Steril* 1973; 24(5): 397-410.

66. Sherman JK. Frozen semen:efficiency in artificial insemination and advantage in testing for acquired immune deficiency syndrome. *Fertil Steril* 1987; 47(1): 19-21.

67. Silva PD, Neisch J, Schauberger CW. Intrauterine insemination of cryopreserved donor semen. *Fertil Steril* 1989; 52(2): 243-245.

68. Smith KD, Rodriquez-Rigau LJ, Steinberger E. The influence of ovulatory dysfunction and timing of insemination on the success of artificial insemination donor with fresh or cryopreserved semen. *Fertil Steril* 1981; 36(4): 496-502.

69. Steinberger E, Perloff WH. Preliminary experience with a human sperm bank. *Am J Obstet Gynecol* 1965; 92(4): 577-579.

70. Stewart GJ, Cunningham AL, Driscoll GL, Tyler JPP, Barr JA, Gold J. Transmission of human T- cell lymphotropic virus type III (HT LV-III) by artificial insemination by donor. *Lancet* 1985; 581-584.

71. Stovall DS, Christman GM, Hammond MG, Talbert LM. Abnormal findings on hysterosalpingography: Effects on fecundity in a donor insemination program using frozen semen. *Obstet Gynecol* 1992; 80(2): 249 - 252.

72. Subak L, Adamson GD, Boltz NL. Therapeutic Donor insemination: A prospective randomized trial of fresh versus frozen sperm . *Am J Obstet Gynecol* 1992; 166(6):1597-1606.

73. Trounson AO, Matthews CD, Kovacs GT, Spiers A, Steigrad SJ, Saunders DM, Jones WR, Fuller S. Artificial insemination by frozen donor semen: results of multicentre Australian experience. *Int J Androl* 1981; 4: 227-234.

74. Tyler ET. The Clinical use of frozen semen banks. *Fertil Steril* 1973; 24(5): 413-416.

75. Urry RL, Middleton RG, Jones K, Paulson M, Worley R, Keye W. Artificial insemination: a comparison of pregnancy rates with intrauterine versus cervical insemination and washed sperm versus serum swim-up sperm preparations. *Fertil Steril* 1988; 49(6): 1036 - 1038.

76. Wainer R, Merlet F, Ducot B, Bailly M, Tribalat S, LambrosoR. Prospective randomised comparison of intrauterine and intracervical insemination with donor spermatozoa. *Hum Reprod* 1995; 10(11): 2919-2922.

77. Williams DB, Moley KH, Cholewa C, Odem RR, Williand J, Gant MJ. Does intrauterine insemination offer an advantage to cervical cap insemination in a donor insemination program ? *Fertil Steril* 1995; 63(2): 295-298.

78. Wong AWY, Ho PC, Kwan M, Ma HK. Factors affecting the success of artificial insemination by frozen donor semen. *Int J Fertil* 1989; 34: 25-29.