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TITLE

SPERM CRYOPRESERVATION AND ARTIFICIAL INSEMINATION

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CONTENTS

	Page
1. Introduction	3
2. History of Cryopreservation	5
3. Genetic Aspects	8
4. Quality Assessment of cryopreserved Spermatozoa	11
5. Applications of Cryosperma in Clinical Practice	22
6. Freezing Technique	33
7. Present state of AID in Cape Town	38
8. Legislation concerning AID in South Africa	46
9. Conclusions	49
10. Acknowledgements	62

## INTRODUCTION

The diagnosis and therapy of infertile couples is traditionally managed by the specialities of Gynaecology and Andrology. The latter is a subspeciality, which should combine the knowledge of urologists and gynaecologists in the treatment of sub or infertile men.

Unfortunately the treatment options for these patients are limited and unsatisfactory.

The methods of sperm conservation and artificial insemination have gained more significance over the past years as public opinion has become more enlightened regarding the problems caused by infertility and the hardships caused by the failure to comply with the powerful natural human urge to reproduce.

Sperm freezing has made it possible to conserve spermatozoa over long periods of time, without major loss of vitality and ability to fertilize. In 1954 the first sperm bank was founded in the USA by Sherman and Associates and it was soon accepted that sperm freezing and conservation with artificial insemination by donor or husbands sperm, was a practical method of infertility management for properly selected couples.

This thesis will review some important aspects of cryopreservation and describe some of the projects which have been undertaken to ensure the quality of cryopreserved sperm.

The first two chapters give an overview of the history and the genetic aspects of cryopreservation. The other chapters describe studies, which have been undertaken in our unit. They also describe in detail the organization of our sperm bank and summarise the result of a survey, which was undertaken amongst Cape Town gynaecologists, to test their attitudes towards artificial insemination by donor sperm (A.I.D.).

The last chapter critically reviews the present South African legislation concerning AID and it's implications on our clinical practice.

A literature review is given at the beginning of each chapter.

In summary, the purpose of this thesis is to give an overview of the methods of cryopreservation of human sperm and the associated problems with specific reference to the situation in South Africa.

## HISTORY OF CRYOPRESERVATION

In 1776 an Italian called Spallanzani first reported observations on the effect of freezing temperatures on human spermatozoa. He exposed spermatozoa to "the freezing cold of winter and its snow" and noted that the spermatozoa became motionless during cooling and regained their motility on rewarming.<sup>1</sup>

Approximately 100 years later Montegazza reported that human spermatozoa resisted freezing to  $-15^{\circ}\text{C}$  and he suggested that "a man dying on a battlefield may beget a legal heir" with his semen frozen and stored at home.<sup>2</sup>

The first report in modern times regarding freezing of spermatozoa came from Jahnel, who was the head of the Institute for the Research into Spirochaetes in Munich, Germany and who studied the resistance of *Treponema Pallida* at very low temperatures.<sup>3</sup> After the freezing of rabbit testicular tissue infected with syphilis in liquid nitrogen he observed that with thawing some of the spermatozoa regained their motility. He repeated these experiments with human sperm and the same effect occurred. Jahnel then suggested experimentation with the freezing and thawing of mammalian spermatozoa but did not pursue this intent any further.

During the early nineteen forties many experiments using deepfreeze techniques for animal sperm were performed. The results were however unsatisfactory.

The great breakthrough came in 1949 when Polge, Smith and Parkes discovered the protective action of glycerol during freezing and thawing of spermatozoa.<sup>4</sup> They reported improved survival rates for spermatozoa after thawing utilising glycerol protection. Polge and Rowson 1952 published a method for the deep freezing of bull sperm which was both practical and promised a reproducible degree of success for the first time.<sup>5</sup>

Preservation of human spermatozoa by freezing was however neglected until the reports of Sherman and associates in 1953 stressed the importance of the rate of freezing and different methods were compared using glycerol in various concentrations.<sup>4</sup> The same investigators also showed that human spermatozoa whose motility was preserved after thawing were capable of fertilization and the production of normal pregnancies.

From 1964 onwards research concentrated on the effect of temperature shock on motility and other deleterious consequences of rapid cooling.<sup>7</sup> The preferred technique of most researchers is the nitrogen vapour technique using plastic straws as semen containers.

The practicality and the many indications for the freezing of human spermatozoa led to the proliferation of sperm banks all over the world and to date most countries in the western world have a network of spermbanks catering for the requirements in respect of cryopreservation.

In a study published by David and Lanzac in 1979 it was estimated, that for the average population, with good access to

medical facilities, there is a need for one sperm bank per 2-4 million inhabitants.<sup>6</sup>

Artificial insemination by fresh donor sperm has been practised in South Africa for many years and articles have appeared in the South African Medical Journal describing this procedure.<sup>7</sup> Consequently the demand for this procedure has increased and as it has become more acceptable and morally permissible it is not surprising that now with a population of approximately 4-5 million requiring this service the need for a sperm bank has become obvious. The sperm bank at Groote Schuur Hospital (G.S.H.) established in late 1984 as the first and until now, the only one operating successfully in South Africa, it has catered for the ever increasing demand for cryopreserved human sperm throughout the country.

### CHAPTER 3

#### GENETIC ASPECTS OF CRYOPRESERVATION

There is a growing appreciation that the clinical use of frozen stored human semen is a practical, successful, safe and valuable method for both infertility management and population control. One of the concerns about cryopreservation has always been, whether there could be any modification of the genetic potential of spermatozoa by freezing or longterm storage, both in terms of embryonic death or later foetal abnormalities.

It would therefore be appropriate to examine the available evidence from previous studies aimed at measuring the genetic effects of cold storage on spermatozoa as well as bacterial and animal cells.

Sherman in 1973 published a study reviewing 564 births which resulted from the use of frozen semen.<sup>10</sup> He reported 7 abnormalities and 50 spontaneous abortions in his group of patients which gives an abnormality rate of about 1.2%. This is below the 1.6% which is accepted as the abnormality rate in the general population. Also in his study the figure of 50 abortions out of 564 pregnancies is lower than the conservative estimate of 15% which occurs naturally in the general population. One should of course not see these figures as proof of a reduced incidence of birth defects after use of frozen stored human semen, though it certainly suggests that there is no increase in

the number of chromosomal abnormalities and abortions associated with the use of cryopreserved spermatozoa.

The thesis that there is minimal or no genetic damage after cryopreservation procedures is supported by Ashwood-Smith in a study published in 1985 where he investigated the influence of cryopreservation on bacterial cells, their mutation rates during longterm cryopreservation and similar influences on the ovarian cells of the Chinese hamster.<sup>11</sup> He showed that the spontaneous mutation rates of E.coli, WP 2, over a period of eleven years of frozen storage at  $-196^{\circ}\text{C}$  showed no basic change in spontaneous mutation rates during these lengthy periods of storage using 10% DMSO as a cryopreservative. This concept is supported by Sperling and Zeindl who studied the effect of deep freezing on the rate of chromosomal aberrations in human lymphocytes, the number of gene mutations at the HGPRT locus, and the infrequency of sister chromatid exchanges in Chinese hamster ova. In all the three test systems no evidence of induced genetic damage after cryopreservation was observed.<sup>12</sup>

Other researchers have demonstrated that the quantity of DNA in glycerolated cryopreserved semen specimens will remain constant when measured after frozen storage for periods up to 75 weeks.<sup>13</sup> Very long periods of storage running into hundreds of years will lead to detectable genetic effects as the background radiation level of approximately 0.1 rad/year would slowly accumulate in the absence of metabolically driven repair processes, which are inoperable at low temperatures in the frozen state. At the moment of thawing the accumulated radiation damage would be made

manifest. Calculation concerning these factors and the interplay of the radioprotective effect of DMSO and to a lesser degree of glycerol were investigated by Whittingham in 1977 and he showed that the amount of background radiation does not play any role for the usual length of storage.<sup>14</sup>

Taking these facts into account recently the Warnock Report<sup>15</sup> recommended that frozen storage of gametes or embryos should not exceed 10 years. The clinical and experimental evidence up to now does not support any arguments that cryopreservation or the use of cryopreserved sperm will increase the chance of genetic mutations in any way or increase the percentage of abnormal children born.

Though experimental results are very encouraging they should be viewed with some caution as autosomal recessive mutations will only become evident when mating occurs with an equally affected partner and thus the defect will only become apparent after one or two generations.

To prove or disprove that recessive mutations take place is virtually impossible as an enormous collection of patients is needed for statistical significance, because there is a spontaneous new mutation rate which is estimated to be about 7 per 1000 births.

In summary, with the existing evidence it appears that cryopreservation does not induce a significantly increased genetic risk to the offspring of our patients.

## CHAPTER 4

### QUALITY ASSESSMENT OF CRYOPRESERVED SPERMATOZOA

The success rate of artificial insemination by donor semen depends on many factors, of which the quality of the cryopreserved and thawed semen is only one. Most authors relate the classical sperm features, such as sperm density, morphology and motility to the relative success rate of artificial insemination. The most commonly used parameter is sperm motility.

The subjective assessment by phase contrast light microscopy is practised by most laboratories, but due to its subjective nature is difficult to reproduce and varies between the different laboratories. A number of objective methods which include Laser Dopler Velocimetrie<sup>14</sup>, stroboscopic photography<sup>17</sup> and videographic methods have been developed.<sup>18</sup>

In order to assess the qualities of fresh human sperm by subjective visual scoring with frame lapse videography and to compare the influence of cryopreservation on fresh human sperm by the same method, we performed a study which is now described.

"THE INFLUENCE OF CRYOPRESERVATION ON HUMAN SPERM MOTILITY ASSESSED BY FRAME LAPSE VIDEOGRAPHY"<sup>19</sup>

### Materials and Methods

16 semen specimens were collected from regular healthy donors at the Groote Schuur Hospital fertility clinic. The fresh specimens were assessed subjectively by phase contrast light microscopy on a 37°C heating stage by the same experienced observer for percentage of motile sperm and speed of forward progression which was classified as follows:

- 0 appears to be dead
- 1 vibrating on the spot
- 1+ vibrating with some forward movement but extremely sluggish
- 2 slow forward movement, still sluggish
- 2+ definite forward progression
- 3 fast forward progression with an apparent goal in sight
- 3+ very fast, straight movements
- 4 extremely fast, "only a blur"

The above assessment was made about 30 minutes after liquefaction and immediately thereafter a videotape recording of the sperm preparation was taken for report assessment by frame lapse videography. After the above procedures were completed the specimens were mixed slowly with the cryoprotectant and frozen by a slow stepwise method which will be described later. The specimens were stored in liquid nitrogen for a period of 50 days, which corresponds to the average time period semen specimens are stored in our sperm bank before release.

### Frame lapse videography:

A colour video camera (JVC) was connected to a colour video monitor via a video tape recorder (JVC). A small droplet of well mixed sperm was placed on a microscope slide, covered with a cover glass (22 x 22mm), and kept at a constant temperature of 37 degrees celsius.

Ten different fields were chosen at random and then recorded. The tape was then replayed and by means of a remote control unit could be advanced frame by frame. A transparency was layered over the videoseen and motile sperm were followed from frame to frame by marking their path with a koki pen. In order to draw the path of a sperm the respective points 10 frames apart were joined to each other. The average time per video frame is 0.0206 seconds.

Each trace was then followed by means of a "light pen" on a graphic tablet coupled to an Apple II computer to determine the distance travelled by each sperm. The computer calculated the trace velocity (swimming speed) in micrometer per second, the chord velocity (vector from starting point to endpoint) and the angle of the curvature. The computer also allowed for statistical analysis of the data.

Percentage motility was estimated by random marking on a frame 50 spermatozoa, which were followed for 50 frames. Those spermatozoa, which did not move in this time interval (1.03 seconds) were labelled as nonmotile and their percentage of the

total was calculated. This procedure was repeated twice per specimen.

Another parameter which was calculated was the progressiveness ratio, (Progratio) defined as chord speed divided by swimming speed, which seemed to be a good indicator of the "straightness" of swimming comparable to the subjective assessment of sperm forward progression (S.F.P.). The closer the "progratio" is to 1 the straighter the sperm swims.

### Results

Comparison between subjective light microscopy and frame lapse videography:

	<u>Light microscopy</u>	<u>Frame lapse videography</u>
% Motility	Med. 55%	Med. 73.5%
	Range 55-70	Range 59-90
SFP	Med. 2+	Swimming speed
	Range 1-3	Med. 54.57
Chord Speed		Range 34.74-90.30
		Med. 46.39
		Range 27.18-74.50
		Progratio
		Med. 0.80
		Range 0.69-0.95

Comparison of fresh and postfreeze/thaw human sperm

	<u>Fresh</u>	<u>SD</u>	<u>Fresh/thaw</u>	<u>SD</u>
% Motility	71.5	8.24	38.0	15.34
Swimming speed	57.09	15.04	36.24	6.03
Chord Speed	46.50	14.51	32.02	5.59
Progratio	0.80	0.07	0.87	0.04

After Freezing and thawing

% Motility	47% decrease	(p < 10 <sup>-4</sup> )
Swimming speed	37% decrease	(p = 1.53 x 10 <sup>-5</sup> )
Chord speed	31% decrease	(p = 8.93 x 10 <sup>-4</sup> )
Progratio.	9% increase	(p = 8.50 x 10 <sup>-4</sup> )

The specimens from 2 donors were assessed fresh, after the addition of the cryopreservative and at the postfreeze/thaw.

<u>Donor A</u>	<u>Fresh</u>	<u>Cryopreservative</u>	<u>Freeze/thaw</u>
% Motility	78	38	17.5
Swimming Speed	43.97	34.43	30.83
Chord Speed	35.01	26.60	27.06
Progratio	0.79	0.77	0.87

Donor B

% Motility	42	42	14
Swimming Speed	34.83	32.19	30.78
Chord Speed	26.44	27.17	26.58
Progratio	0.75	0.84	0.86

Discussion

The aim of the first part of the study was to define a correlation between the subjective light microscopic and the more objective frame lapse videographic method of assessment of sperm motility. The subjective assessment was done by the same experienced observer who has been doing routine assessments for the last 15 years. For our clinical practise the results are reliable and reproducible. However the comparison with frame lapse videography showed that our observer underestimated motility to a significant degree, by an average of 20%. This for clinical practise is safe and acceptable, but is not transferrable in quantitative terms and one cannot compare motility results with other units or use the assessment for comparative research work.

SFP (sperm forward progression) is difficult to compare to any of the parameters assessed by videography, though the progratio, which is a measure of straightness of swimming movements seems to be the most likely comparison. No correlation in statistical terms was noted. When assessing human sperm before and after freezing by frame lapse

videography a very significant decrease in the motility is noted, an average of 47%. Rather than use a decrease in motility rates it is more accurate to calculate the cryosurvival rate i.e. is post thaw motility of spermatozoa x 100 divided by the initial motility. The average cryosurvival in our specimens was 53%. This rate seems low but most figures in the literature are derived from subjective light microscopic assessment. All parameters assessed percentage motility, swimming speed and chord speed showed a significant decrease apart from the progratio which showed a 9% increase. This is probably explained by the fact that freezing and thawing is a very stressful process for spermatozoa and only the fittest will survive. One therefore assesses a selected population of spermatozoa which have excellent properties of movement.

It is very difficult to compare these results with other studies in the literature because of the variable freezing methods, different cryoprotective media and mainly the type of motility assessment.

Harrison and Sheppard in 1980 did a comparative study in methods of cryoprotection for human semen and their "complex cryoprotective medium" is very similar to our medium, but their type of assessment is by phase contrast microscopy. The mean cryosurvival rate was 73%<sup>20</sup>

Similar studies, like the one by Pilikian, Czyba and Guerin, who used Doppler Velocimetry as an objective method for motility assessment, showed that cryosurvival rates drop to an average of 38% in a similar cryoprotective medium.<sup>21</sup>

Our subjective assessments show very similar results to those in the literature, but when using an objective method our cryosurvival rates seem to be superior.

It is interesting that in most comparative studies the subjective pre-freeze assessment always underestimates, whilst the post thaw motility always overestimates both by about 20% when compared with objective methods.

In conclusion studies reported in the literature have to be carefully assessed in regard to methods of motility studies. Light microscopic assessment due to its subjectivity is of no value in research work. Frame lapse videography, though laborious, is a practical reproducible method for assessment of sperm motility, where accuracy is essential.

#### OTHER METHODS OF QUALITY ASSESSMENT

It has not always been accepted that motility correlates well with fertilising capacity, though the property of movement is of extreme importance for cervical mucous penetration and the rapid ascent to the ampullary part of the fallopian tube.

The ability to capacitate, to penetrate through the granulosa cells, which needs adequate acrosomal enzymes, to attach to receptor sites on the zona pellucida and finally to fertilise, are all properties of sperm which cannot be adequately assessed with presently available methods.

Goodpasture et al determined the acrosin content of sperm heads before and after cryconservation and found a distinct reduction after thawing.<sup>22</sup>

Several authors describe that substantial damage can be observed to the plasma membrane and the acrosome integrity of the sperm head after the freeze/thawing process. The percentage of spermatozoa with intact sperm heads and preserved ultrastructural detail was found to be significantly reduced after freeze/thawing and this may partly explain the reduced fertilising potential of cryopreserved spermatozoa.<sup>23,24,25,26</sup> It also seems biochemical changes, which indicate cellular destruction, cause substantial damage to sperm heads during the freeze/thaw process.<sup>27</sup> Most recently Jeyendran et al showed that the ability of spermatozoa to penetrate zona free hamster oocytes is significantly reduced after cryopreservation when compared with fresh sperm.<sup>28</sup> Van der Ven et al used this functional test to assess different cryoprotective media and had the poorest results with glycerin only, whilst more complex media showed a much higher penetration rate.<sup>29</sup> Van der Ven et al used this functional test to assess different cryoprotective media and had the poorest results with glycerin only, whilst more complex media showed a much higher penetration rate.<sup>29</sup> Laufer suggested that this functional test should be routinely used to assess the post-thaw fertilising potential of donated semen specimen.<sup>30</sup> The ultimate test is to evaluate the cryopreserved spermatozoa in an in vitro fertilization program, where the ability is tested to penetrate cumulus oophorus complexes. The success thereof can be seen 48 hours later with regular cleavage of the embryo into four cells.<sup>31</sup> Cohen and co-workers studied the incidence of pregnancies in 38 couples after

in vitro fertilization, where both partners were infertile. They found no significant differences between the study group and their controls in regard to fertilization, pregnancy per cycle and abortion rates. In general cryopreserved donor and husbands sperm produce excellent results in an I.V.F. system. 32,33,34

## APPLICATIONS OF CRYOPRESERVED SPERMATOZOA IN CLINICAL PRACTICE:

### 1. Sperm Banking in Oncology

The treatment of Hodgkin's disease, testicular cancer, lymphoblastic leukaemia and other malignancies, which mainly affect young patients has improved dramatically and offers good long term survival rates.

The toxic effects of cytotoxic drugs and radiotherapy especially on those organ systems which have a rapid cell turnover are well-known.

The primary testicular lesion caused by most agents used in tumor therapy has been depletion of the germinal epithelium lining of the seminiferous tubules. Testicular biopsy in most patients shows complete aplasia of germinal cells lining the tubular lumen. Clinically there is a marked decrease of testicular volume, severe oligozoospermia or azospermia with resultant infertility. Since these malignancies occur mainly in young patients of reproductive age, it is very important to counsel regarding the impact of the treatment on their reproductive potential. For those young patients who have a favourable prognosis and where the planned treatment will most probably result in sterility the possibility of storing cryopreserved sperm would be an important consideration.

Of all the cytotoxics, the alkylating agents have been most extensively studied.

Richter et al in 1970 showed that the effect of chlorambucil on spermatogenesis is dose dependant:<sup>33</sup>

<u>Total dose</u>	<u>Effect</u>
> 400mg	progressive but reversible oligozoospermia
< 400mg	azospermia and aplasia of germinal epithelium

Azospermia after Chlorambucil treatment is usually at least partially reversible after about 3-5 years of treatment.

In 1975 Buchanan studied the effect of cyclophosphamide on spermatogenesis and found that out of his 26 patients studied all became severe oligozoo- or azospermic and in 14 of them no recovery of spermatogenesis was observed.<sup>36</sup>

More critical is the situation when combination cytotoxic therapy is employed as shown in the following table:

Author	No. of patients	Combination regimen	Sperm count		
			azoo	oligozoo	normal
De Vita 1973 <sup>37</sup>		C alone			
Roser 1978 <sup>38</sup>	15	MOPP, CVP	15	-	-
Chapman 1981 <sup>39</sup>	33	MVPP	33	-	-
Whitehead 1982 <sup>40</sup>	44	MVPP	44	8	1
Waxman 1982 <sup>41</sup>	41	MVPP	36	-	5
Santoro 1983 <sup>42</sup>	17	ABVD	6	3	8

A = adriamycin

B = bleomycin

C = cyclophosphamide

D = dacarbazine

M = mechlorethamine                      O = oncovin (vincristine)  
P = procarbazine                            PP = procarbazine/prednisone  
V = vinblastin

It can be seen from the above studies, that when combination chemotherapy is employed the recovery rate of spermatogenesis is very poor and infrequent. Only a few data are available about newer drugs like cisplatinum. Drasga in 1983 studied the effect of cisplatinum, vinblastin and bleomycin on testicular function and showed that all his 24 patients became azospermic during the treatment but after 3 years 20 had recovered and reported 12 pregnancies in this group.<sup>43</sup>

More data are needed but his results are encouraging.

It is important to test patients with Hodgkin's disease and testicular cancer before cryopreservation of sperm, because a significant percentage will have some degree of testicular dysfunction even prior to the start of therapy which will make them poor candidates for sperm preservation.

The effect of radiotherapy is shown in the following table (Greiner 1982).<sup>44</sup>

Total radiation dose (rad)	effect on spermatogenesis
< 30	normal number of spermatozoa
30-100	temporary azospermia, but recovery likely
100-150	incomplete recovery of sperm number
>150	recovery from azospermia unlikely.

Greiner also found that if the total dose of gonadal radiation did not exceed 90 rad, recovery of normal number of spermatozoa was reached after 22 months.

During the first 3-5 months of radiotherapy it is often seen that the patient is still fertile. The reason for this is that spermatozoocytes and spermatids are relatively radioresistant and therefore sperm production continues, whilst spermatogonia are radiosensitive and stop dividing soon.

The possibility of radiation induced chromosomal damage should be considered and contraception is advisable.

### Conclusions

In the circumstances outlined above sperm banking should be considered and should be offered to patients with a favourable prognosis.

All patients who undergo cytotoxic or radiotherapy therapy and are of reproductive age should be counselled about this option as

many pregnancies have been reported from patients, who have had malignant disease treated and whose fertility potential was preserved by semen freezing at an early stage of their disease.<sup>45.46.47.48.49.50</sup>

In the near future the option of cryopreservation of gametes will also be available to female patients who face sterility due to medical treatment and the first reports are available detailing the successful freezing of oocytes.<sup>51</sup>

## 2. ARTIFICIAL INSEMINATION BY DONOR SPERM AND ORGANISATION OF A CENTRAL HUMAN SEMEN BANK

In about 38% of all couples visiting our infertility clinic the male has a major problem.<sup>52</sup> Unfortunately the treatment options are rather limited and the results disappointing.

There has therefore been a considerable demand for and an increase of use of artificial insemination over the past decade in this country. This demand was mainly met by the use of fresh donor sperm, which is logistically difficult to arrange because timing of insemination is critical. More important however, the donor is seldom adequately screened for diseases transmittable by semen. Particularly the threat of AIDS transmission of the virus (HTLV III) which has been reported in South Africa, and Cape Town<sup>53,54</sup> makes semen storage essential to await negative results before distribution of the semen specimen.

There is also a demand for a semen storage facility for patients undergoing vasectomy as a type of "fatherhood insurance".

## THE ORGANIZATION OF THE BANK<sup>53</sup>

### The Recipient Couple

If at the infertility clinic it is found out that the husband is oligo- or azospermic, he is referred to a urologist attached to the infertility clinic. Should the urologist not be able to offer any treatment for the condition, the couple is then carefully counselled about the remaining alternatives, which are artificial insemination by donor (AID) sperm or adoption. As there are many children available for adoption, this step is encouraged. If the couple decides on AID the medical, ethical and legal implications are carefully discussed. For these discussions the help of a social worker and a clinical psychologist is essential. The couple is encouraged not to make an immediate decision but are asked to come back when they have definitely decided, or if they are in need of further counselling.

The woman then undergoes an observation cycle with a temperature chart and a day 21 progesterone level is taken to confirm ovulation. In the same cycle the patient also has a hysterosalpingogram performed to confirm anatomical normality. A detailed phenotypical description is taken from the husband.

### The Donor

Our donorpool mainly consists of medical students from our medical school. At the initial interview a careful family history is taken with the help of a genetic check list.

The donor is medically examined and blood is taken for blood grouping and rhesus factor, VDRL, Hepatitis B and HTLV III antibodies (AIDS).

A semen analysis is performed and when satisfactory the specimen is frozen and thawed after 24 hours. An individual is only accepted as a donor if his sperm count is above 80 million/ml and the post thaw recovery rate is above 70%, which is considerably higher than the minimum parameters required by other authors.<sup>56</sup> Care is taken that the donor is mentally and physically normal and that he has appealing features.

### The Freezing Technique

The technique will be described in detail in the next chapter.

### Insemination Technique

All inseminations are carried out by a gynaecologist or by the senior registrar attached to the clinic. 3-4 days before the presumed day of ovulation a cervical mucous assessment is performed and repeated until the cervical score is at least 9 out

of 12 and a rich cervical mucous cascade is present. The specimen is thawed and percentage motility is checked under the microscope. If the specimen is satisfactory it is drawn up into a Jelco catheter and tuberculin syringe and injected into the cervical canal under speculum vision.

Two inseminations per cycle 24 hours apart are usually performed.

### Results

During the past 6 months ten couples from the infertility clinic were treated. Four women became pregnant, one with a twin pregnancy. All the pregnancies are ongoing and no abortions have occurred.

Reports in the world literature indicate that pregnancy rates of below 10% per treatment cycle can be expected in comparison to just under 20% with fresh donor sperm<sup>57,58</sup> though some centres report similar pregnancy rates after insemination with either fresh or frozen sperm.<sup>59,40,41</sup> Unfortunately no accurate results are available from specimens sent to centres all over Southern Africa but we have reports of several pregnancies in East London, Johannesburg and as far afield as Harare. Specimens to these centres are sent on dry ice via air freight.

### The problem of AIDS

At the end of July 1985 the sperm bank was closed for three months because of the report from Australia where three women had

been contaminated by the AIDS Virus via artificial insemination by donor sperm.<sup>42</sup>

We therefore introduced a rotation programme where a semen specimen can only be released when the donor has been screened for a second time three months after sperm donation and has been found negative for HTLV III antibodies. This will diagnose the unlikely false negative test, and more importantly a second screen will detect if a donor was in the incubation period for AIDS, at the time of initial semen donation, as the antibody titre only becomes positive after about 6-8 weeks whilst the patient is already viraemic and infective. Until the present no positive results from either our old or our new donors have been recorded. Donors are also excluded who belong to any of the groups at high risk for AIDS. Thus homosexuals, drug addicts or the recipients of any blood products over the 5 years preceding semen donation are excluded.

### Discussion

An efficient service has been established to provide for couples in need of artificial insemination. Extreme care is taken to provide a sample which is safe and matched to the husbands phenotype. Special consideration is given to the special needs of the couple in terms of counselling facilities.

This service is available to private gynaecologists in this country, who can on demand be provided with properly matched and screened specimens. With the presence of AIDS in this country

insemination with fresh sperm may become a gamble unless adequate screening is introduced.

With the establishment of a sperm bank the facility exists to freeze sperm samples of patients undergoing cytotoxic or radiation therapy, which can give the patient peace of mind about future progeny in case his testicular function should be irreparably damaged during treatment. Oncologists should counsel their patients in this respect.

A further aspect is the storage of sperms for patients undergoing vasectomy. Some patients may prefer this type of "fatherhood insurance" in case something should happen to their children.

In summary, a valuable service has been established which caters for a growing need while at the same time considering the special ethical and moral problems of many desperate childless couples.

## CHAPTER 6

### FREEZING TECHNIQUE

A variety of techniques are described, using different cryopreservation media, different types of biological freezers, differing cooling rates and different methods for storage ampoules or straws in pallets.<sup>43,44,45,46,47,48</sup> The pre-requisites for a good method are: reproducible good sperm recovery, inexpensive equipment, a simple method of freezing and storing and preservation of the fertilizing capacity of sperm after thawing. A simple method of cryopreservation of human sperm was developed which is employed in the sperm bank at Groote Schuur Hospital.<sup>49</sup>

#### Materials and Methods

Fifteen samples of semen were collected randomly from patients who were having routine semen analysis performed by the andrology laboratory and were obtained by masturbation after 3 days of abstinence. The samples were assessed for: 1) sperm count per millilitre, ii) percentage motility, iii) forward progression, iv) and morphology.

After liquification had occurred the semen samples were frozen an hour after ejaculation. In the interim they were kept at ambient temperature in capped glass tubes.

The cryopreservative consisted of 41% sodium citrate (3%), 41% purified egg yolk, 16% glycerol and 2% glucose.

The sperm samples were mixed with the medium in a ratio of 1:1 and equilibrated at room temperature for 20 minutes before freezing. Specimens were thawed after 2 hours, 1 week and 4 weeks in liquid nitrogen storage and motility and forward progression was assessed. All assessments were done by one observer.

#### Freezing technique

The freezing apparatus is shown in Figure 1. It consists of a single wall unsilvered Dewar flask immersed in liquid nitrogen. This flask contains 500ml of absolute alcohol which is stirred at high speed to avoid a temperature gradient. The samples are kept in plastic tubes (0.5ml) and are immersed in the alcohol bath using simple holders. The temperature is controlled with a Crison-637 thermocouple. The freezing of the samples is achieved by slowly lowering the alcohol container with the specimen into the container with liquid nitrogen. The freezing rate is 10 degrees per minute from +20 to -80 degrees and samples are thereafter plunged into liquid nitrogen. They are then stored in specially designed containers (Union Carbide 35 VHC). Thawing is performed at room temperature and lasts 5-7 minutes. Assessments are done 20 minutes after removal of the specimen from liquid nitrogen bath when it has equilibrated to room temperature.

## Results

The mean percentage motility before freezing was 62%. The mean percentage motility after 2 hours in liquid nitrogen was 48%. The mean recovery index expressed as Post thaw motility x 100 divided by the pre-freeze motility was 77.4% as assessed by phase contrast microscopy. The mean percentage motility after 1-4 weeks in liquid nitrogen was 48.5% which was not significantly different from that after 2 hours of storage. It should be noted that 2 samples were oligospermic and had good recovery rates. SFP did not change and remained as before freezing. Morphology was assessed by light microscopy before and after storage and no significant changes could be detected.

## Discussion

As mentioned before the most striking effect of cryopreservation on sperm is the decrease in percentage motility after freezing and thawing. The results, in this study done only by light microscopy, compare very well with any in the literature and even after the observer error is quantified, as in a previous chapter the recovery index is excellent. There was no further decrease in the recovery rate after 1 - 4 weeks of storage in liquid nitrogen, which confirms the finding by others that freezing damage is only minimally increased after long storage. The method described is highly reproducible and simple to perform without the use of expensive equipment or special training.

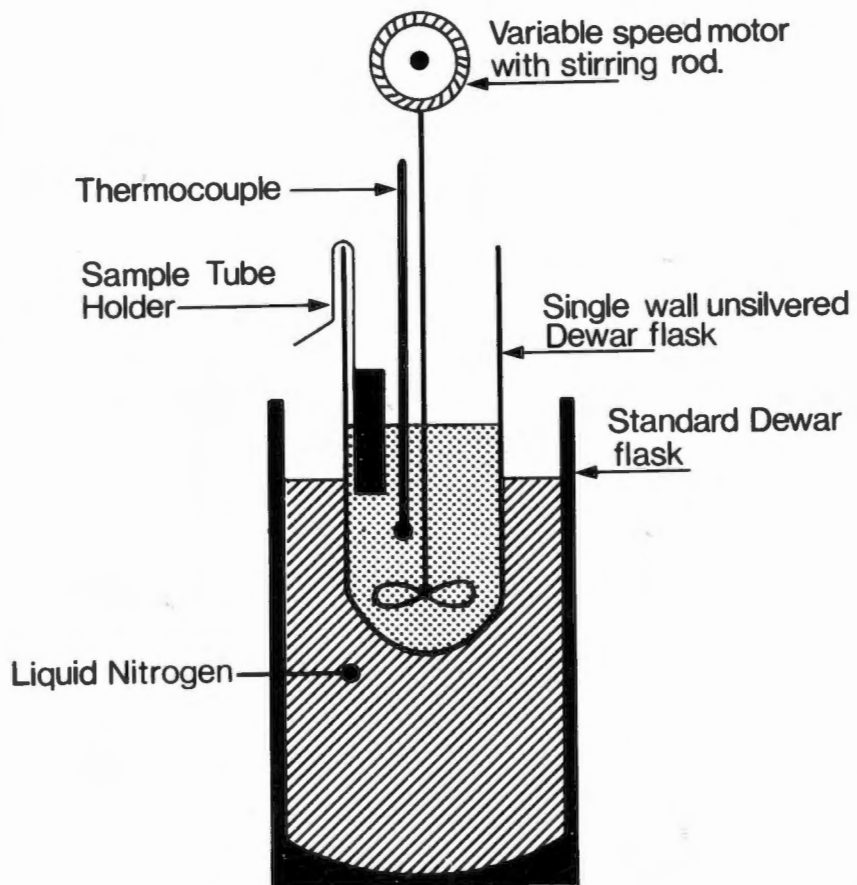
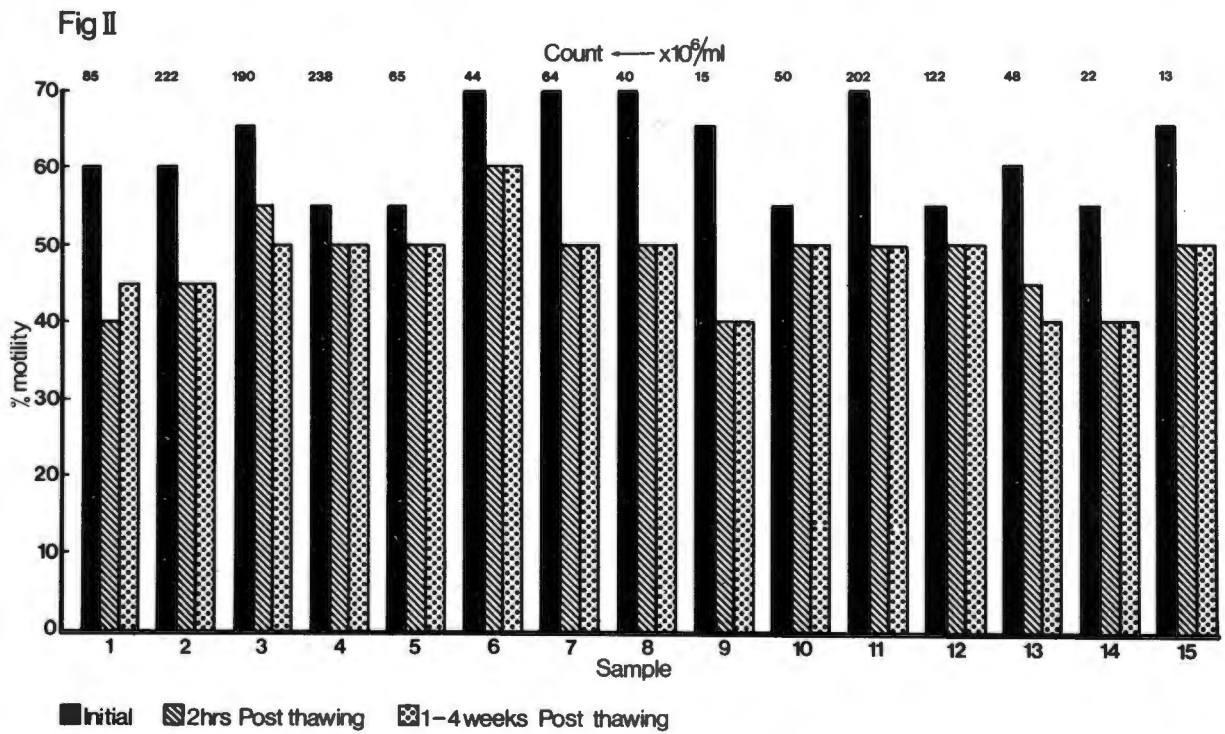


Fig.1.



## CHAPTER 7

### PRESENT STATE OF AID IN CAPE TOWN

A survey was undertaken during the period July to September 1986 to investigate aspects of artificial insemination by donor in Cape Town.<sup>70</sup> The differential practise of, and attitudes towards AID amongst Cape Town Gynaecologists, as well as a demographic profile of the recipient population were investigated.

The only previously published survey on AID in South Africa is that being of Van Delft in 1985, who presented the results of a questionnaire mailed to 370 gynaecologists in South Africa, Namibia and the "independent black states".<sup>71</sup>

#### Methods

Apart from private gynaecologists there were at the time of the study only two other medical sources of AID in Cape Town:

1. Groote Schuur Hospital Infertility Clinic has offered AID since 1979 and the sperm bank, to date the only one in this country, was established in late 1984.
2. Tygerberg Hospital Infertility Clinic have provided AID services since June 1986. Semen is obtained from the sperm bank at Groote Schuur Hospital.

Telephonic interviews were conducted with every privately practising gynaecologist listed in the 1986/7 Cape Peninsula telephone directory. Names were crosschecked with the 1985 South African Society of Obstetricians and Gynaecologists membership

list and additionally with a list of Cape Town doctors who had utilised the services of the sperm bank.

Results of the calls revealed that of the total listed population of 64, 3 had left Cape Town and a further 6 were not in private practice. The actual population of privately practising gynaecologists in Cape Town was thus taken as 55. It is noteworthy, that only 2 of these were women.

Doctors were asked whether or not they practised AID. Those answering in the negative were asked why they didn't perform AID, while those who answered affirmatively were asked if they had patients currently on an AID programme and if so whether they would be prepared to furnish details as to the number, ages, occupations, racial classification and residential areas of such recipients. The same information was asked of the two hospital sources.

## RESULTS

### 1. Gynaecologists attitudes towards AID:

Doctors responses fell into four categories, (see table).

- (a) those who practised AID and had at least one patient attempting a pregnancy at the time of the interview.
- (b) those who currently practised AID but had no patients on the programme at present.

(c) those who did not practice AID, yet were not opposed to it: 3 of these doctors had performed AID in the past, but each had given it up due to practical problems such as difficulties in obtaining donors, timing of inseminations, etc. Another 6 had practical reasons for not providing this service felt it should be restricted to specialised clinics. Three explained their non-involvement in terms of lack of demand. Two stated it was not their area of work and interest. The three remaining doctors had nonspecific or no particular reason for not practising AID.

(d) Those who did not practise AID provided the following reasons for their non-involvement:

ethical (1), ethical and religious (2), religious (3, 1 Catholic, 1 Moslem, 1 Reborn Christian), religious and legal (2), legal (3), legal plus inadequate donor selection procedures (2), disruption of marital relationships (1).

	<u>Number</u>	<u>% of Total</u>
AID practitioners with AID patients	12	21.8
without AID patients	9	16.4
subtotal	21	38.2

Non-practitioners not opposed to AID	19	34.5
opposed to AID	15	27.3
subtotal	34	61.8
Grand Total	55	100

Recipient population:

The next table indicates the relative distribution of services and recipients in Cape Town:

<u>Facility</u>	<u>Number of recipients</u> <u>currently on programme</u>	<u>% of total</u>
Groote Schuur Hospital	10	11.1
Tygerberg Hospital	14	15.6
Private Gynaecologists	66	73.3
Total	90	100%

Place of residence of recipients

Regarding residential areas, data on only 53 (58.8%) recipients was obtained. Of the patients whose geographical areas were ascertained, 38 (72.3%) were resident within Cape Town. The remaining 15 came from

a great range of distances, the furthest from Port Elizabeth, Upington, Karasburg and Windhoek.

### Age

The mean age of recipient was  $x = 30.2$  with a range of 22 - 41 years.

The partners mean age was 32.6 years with a range from 24 - 44 years.

### Racial Classification

Data for this was available for 72 of the recipients (80%). None of the couples was "mixed" (interacial marriages) and none of the recipients was single. The results are displayed in the following table and these are compared to the 1985 population census.

<u>Category</u>	<u>No. of couples</u>	<u>% of total</u>	<u>Racial Groups</u> <u>% in Cape Town</u>
African	1	1.4	16.5
Asian	2	2.8	1.0
"Coloured"	19	26.4	54.4
White	50	69.4	28.1

Whites are considerably over-represented, whilst "Coloureds" and more conspicuously Africans are under-represented.

Groups differential usage of AID services.

98% of White recipients consulted private gynaecologists for AID, whilst "Coloureds" tended to utilise private services and the hospital roughly equally (53% versus 47%).

Occupations

Fifty-three (58.8%) of recipients and 39 (43.3%) of their partners occupations were identified and sufficiently specific for them to be classified in terms of Schlemmer and Stopforth's (1979) socio-economic classification by occupation code.<sup>72</sup> The results are displayed in the following table:

<u>Occupational categories</u>	<u>Recipients</u>		<u>Partners</u>	
	<u>No.</u>	<u>% of total</u>	<u>No.</u>	<u>% of total</u>
Professional Managerial	15	28	13	33
White collar	17	32	12	31
Skilled artisans	1	2	8	21
Semi-skilled	2	4	4	10
Unskilled labour	1	2	2	5
Housewife	17	32	-	-
Total	53	100	39	100

DISCUSSION

It is interesting to compare the gynaecologists attitudes towards AID with the results of Van Delft's more general survey in 1985.

	<u>This survey</u>	<u>Van Delft's</u>
AID practitioners	38.2%	32.3%
Non practitioners not opposed	34.5%	26.9%
Non practitioners opposed to AID	27.3%	40.8%

On the assumption that the whole population of privately practising gynaecologists was interviewed, the fact that only 27.3% of this population was opposed to AID, as compared to Van Delft's figure of 40.8% may indicate some or all of the following:

1. Cape Town gynaecologists are on average more liberally disposed towards AID than South African gynaecologists in general,  

or
2. Van Delft's figures are not representative of the population of South African gynaecologists (return rate of questionnaire 25.1%),  

or
3. There is a trend amongst South African gynaecologists to become more liberal towards AID.

Most of the AID in the upper income brackets is mediated by private gynaecologists and most of them are White. This is not surprising and is a pattern found in all medical specialities. The fact that Tygerberg Hospital, which has offered AID only since mid 1986, was found to have more patients undergoing AID than the well established GSH service, might indicate a considerable demand and backlog in the northern suburbs of Cape Town.

From this study it can be concluded that AID has become an integral part of infertility treatment in greater Cape Town and is acceptable to the majority of gynaecologists and to both teaching hospitals.

## CHAPTER 8

### PRESENT LEGISLATION ABOUT AID IN SOUTH AFRICA

While the legal problems surrounding AID have received much attention internationally, local publications on the subject have been scant. In 1980 a "suggested code of practise for artificial insemination by donor" appeared in the South African Medical Journal,<sup>73</sup> and was published the following year by the Department of Health and Welfare as the "AID code of Practise".<sup>74</sup> Reports from gynaecologists place the first practise of AID at least as early as 1952 yet the donation of sperm was only legalised in 1983 with the "Human Tissue Act".<sup>75</sup> An annexure to this act published on 20 June 1986 obliges AID practitioners to disclose, amongst other details, the identity numbers of all their recipients and donors to the Director-General of National Health and Population Development for purpose of central registration.<sup>74</sup>

Other important facets of the act are:

- that the premises of the AID practitioners must be inspected and approved for AID
- the recipient must be married
- extensive files have to be opened for recipient and donor
- not more than 5 children per donor are allowed
- the possibility should not exist, that 2 simultaneous pregnancies of the same donor develop

- the donor must have written permission of his wife, if married, for semen donation.

It has been suggested that a consequence of this new regulation, apart from constituting a breach in doctor-patient confidentiality, and due to its impracticality might effectively prevent AID in South Africa.<sup>77</sup> It is possible there is such a demand for this procedure that it will continue to be practised, but without the knowledge of the authorities. This, in the presence of AIDS (HTLV III) in South Africa, would be a dangerous practise and a severe hazard to the patient.

The new law does not regulate for the legality of a child conceived by A.I.D, which is one of the main shortcomings of the legislation. At the moment the parents of a newborn child conceived by A.I.D., have to register this child as theirs with the respective authorities. This is not true in the strict legal and of course genetic sense. A legislation has to be created which clearly gives a child born through AID the same rights as children born after natural conception.

In respect of legislation concerning AID the Council of Europe's recommendations, have become law.<sup>78</sup> These recommendations are an excellent example of a legislator following the advice of professionals active in the specialised field of infertility.

In these recommendations the anonymity of recipient couple and donor is paramount; the identity of the recipient can only be revealed in a court of law, whilst the identity of the donor is to be protected under all circumstances. No government authority has the right to inspect any files concerning the treatment of couples for AID, which

is seen as a breach in confidentiality. Both partners have to consent to AID and the practise of insemination is governed by a code of ethics supervised by the professional bodies.

When artificial insemination has been administered with the consent of the husband the child is considered the legitimate child of the woman and her husband and nobody may contest the legitimacy on the sole ground of artificial insemination.

In most other countries where AID is practised, there are no specific laws, but the practise of AID is governed by a code of ethics issued by the respective professional organisations.

In no country other than S.A. is there any obligation by the practitioner to reveal the identity of either donor or recipient to a government department.

It is hoped that the present legislation in South Africa will be reviewed to adequately deal with the legal, ethical and moral problems of artificial insemination by donor.

The effective use of semen cryopreservation backed up by a sperm bank, now gives these couples now a chance to conceive their own child, which also society accepts as being theirs.

### CONCLUSION

After careful review of existing literature a method of freezing sperm has been developed which reveals excellent results in terms of sperm survival.

A sperm bank has been organised which delivers a much needed and necessary service to the Grootte Schuur Infertility Clinic and private gynaecologists patients in South Africa and neighbouring countries.

It is also possible for patients undergoing therapy for malignant disease to preserve their fertility potential. Special precautions are taken to deliver a safe specimen, which is free of semen transmissible diseases.

Future problems include the improvement of sperm recovery rates after thawing to approximate pregnancy rates achieved by insemination with fresh spermatozoa. This could be possibly achieved by further decreasing cooling speeds and further refinement of the cryoprotective media. A further aim is to find reliable predictors of fertilising capacity of human sperm and the possibility of using the zona free hamster test has been mentioned.

It would be desirable if the legislature would design a frame work of laws which would make it possible to practise AID with a minimum of administration which at the same time ensures the absolute anonymity of recipient and donor. The legality of the child conceived by AID should also be legally defined.

After decades during which artificial insemination has been practised secretly, general acceptance of this valuable method of infertility treatment is spreading.

Unfortunately no representative longterm results are available about the offspring after AID, as these children are now only entering reproductive age.

Up to the present all the results from sociological and psychological studies<sup>79,80,81</sup> seem to be positive and encourage AID.

For a couple, where the husband is infertile, for whatever reason, the prospects for a fulfilled and satisfied marriage is poor. The decision to proceed to artificial insemination by donor sperm originates from the intense desire of both partners to raise a child of their own a product of their love and respect for each other.

In contrast to adoption, which is the only alternative, the couple experiences pregnancy and delivery of their own child, which is so important for bonding between parents and offspring. Experience has shown that when couples where given the choice between AID or adoption, AID is preferred unless there are strong religious convictions.

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