Assisted Procreation

October 11, 2011
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The International Consensus adopted by the IFFS is an up-to-date focus on modern techniques of Assisted Medical Procreation (AMP), with respect to the reasons for which they have been proposed, the conditions for their realization and their possible risks.

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IFFS (International Federation of Fertility Societies) has among its objectives to contribute to the standardisation of terminology and evaluation of diagnostic and therapeutic procedures in the field of reproduction. Assisted Reproduction Techniques (ART) have raised new questions which have been addressed with wide differences depending on culture, religion, or health policy. Many countries have issued laws or regulations and many others are about to do so. In issuing this consensus document on a number of ethical issues regarding ART, the objective of IFFS is to produce an official position paper based on the views of the professionals themselves.

Introduction
The International Consensus adopted by the IFFS is an up-to-date focus on modern techniques of Assisted Medical Procreation (AMP), with respect to the reasons for which they have been proposed, the conditions for their realization and their possible risks.

Doctors and biologists who all over the world offering these new techniques to infertile couples are aware that they are considered an upheaval of traditions and, in the case of gamete donation, radically change the concept of the legitimate family. Each country has reacted to these facts and sometimes even passed laws in accordance with their traditions, beliefs, and public opinions. This consensus will allow each one of you to indicate to his/her own governmental authorities what techniques are considered universally as the most useful and least harmful to infertile couples.

Couples must be fully informed of the advantages and inconveniences of the techniques which are offered to them. This consensus will allow them to be informed in an objective way.

Ovarian Stimulation

Background
The use of natural cycle IVF was abandoned soon after it was demonstrated that the use of clomiphene citrate and/or gonadotrophins gave a lower cycle cancellation rate and a higher pregnancy rate. The introduction of GnRH agonist treatment prior to gonadotrophin stimulation has subsequently been successfully used in various types of protocols (ultrashort, short and long). Agonists with both a short half life and depot injections have been utilized. Recently GnRH antagonists have been successfully utilized. However, the use of natural cycles for the replacement of frozen/thawed embryos could be used in patients without hormonal disturbances.

Medical indications
Treatment with gonadotrophins for the induction of follicular growth and ovulation has been used in anovulatory women (WHO group I) since the beginning of the 1960’s. In association with IVF, controlled ovarian stimulation (COH) by the use of gonadotrophins is also adopted in regularly cycling women. Since many of these women’s endogenous gonadotrophins interfere with exogenous gonadotrophins given to them, there is a risk of premature luteinization and premature follicular rupture. The adverse effects are predominantly associated with the actions of LH.

New urinary gonadotrophins with hight FSH and low or absent LH activity have therefore been developed, as well as recombinant FSH. Another way to avoid the release of endogenous LH is to use GnRH agonists or antagonists. For the controlled induction of oocyte maturation prior to oocyte retrieval, hCG (or recominant LH) is utilized in various doses. The increased purity of the
gonadotrophins that are available has made subcutaneous injection possible without causing adverse local reactions. This has widened the possibilities for the self administration. As the hormonal stimulation regimens used during the follicular phase generally demand support of the luteal phase with exogenous hormones in the form of repeated hCG injections or daily progesterone substitution, this improvement in the options for drug delivery represents a major step forward.

**Results and risks**

The exogenous use of gonadotrophins has resulted in a lower cancellation rate, a higher yield in terms of retrieved mature oocytes, more high quality embryos for replacement and frozen preservation and higher pregnancy rates. The replacement of more than one embryo per cycle has resulted in high multiple pregnancy rates compared to natural conception. This in turn leads to an increased premature delivery rate, and consequent morbidity among the offspring. To reduce these serious complications associated with IVF, only one or two embryos should be replaced, and surplus embryos should be freeze preserved for later replacement. If three or more embryos are seen in the uterus on ultrasound examination, the couple must be counselled regarding the risk of multiple pregnancy and possible embryo reduction. Embryo reduction beyond the 10th gestational week seems to be associated with higher risks for complications than embryo reduction at an earlier stage. The spontaneous abortion rate does not seem to be increased while the ectopic pregnancy rate is increased most likely dependent upon that a majority of the treated women have factors predisposing for ectopic pregnancy.

Most multiple gestations are associated with ovarian stimulation (±) intrauterine insemination. To reduce the risk of multiple pregnancies it is suggested:

1. To use low-dose protocols for ovulation induction
2. To selectively reduce the number of transferred embryos to 3, 2 or even 1 depending on several factors:
   
   (i) embryo quality  
   (ii) woman’s age  
   (iii) oocyte number  
   (iv) semen quality  
   (v) fertilization rate  
   (vi) the overall quality of the embryology laboratory

Ovarian hyperstimulation syndrome (OHSS) is another serious complication which seems to have become more frequent since GnRH agonists started to be used in combination with gonadotrophic stimulation. The desire to obtain as many mature oocytes as possible whilst reducing the degree of cycle monitoring has led to the recognition of predisposing risk factors, hyperandrogenism, PCO “necklace sign” in the ovaries etc...., the utilization of preventive measures (low dose protocols, avoidance of HCG luteal supplementation etc) and the early intervention with albumin and the aspiration of peritoneal fluid have decreased the severe life threatening risks of the syndrome considerably.

**Reservations**

1. “The ovarian stimulation protocol should be individualized depending on the patient and the technique used. Every effort should be made to avoid or decrease the chance of complications from this therapy”.
2. Monitoring with ultrasound and serum estradiol determinations increases the possibility of optimizing stimulated cycles and decreases the risk for the development of OHSS.
3. Luteal support is mandatory only when GnRH analogues are used and can be performed by administration of progesterone or hCG for a minimum of 2 weeks following follicular puncture.
4. As a routine, not more than 2 embryos should be replaced in young women, and surplus embryos of good quality freeze preserved.
5. The establishment of multiple pregnancies should, as far as possible be avoided, and triplet or higher multiples should be considered according to the national laws for embryo reduction after extensive counselling of the couple.

**Risk of ovarian cancer and the use of infertile agents**

It is well established that the risk of ovarian cancer is altered by a number of identifiable factors relevant to fertility and reproductive health. Factors such as parity and contraceptive pill use have well documented protective effects, while the effect of infertility and related treatment is far less certain.

In the recent years some publications concluded to an increase of risk of ovarian cancer while others denied. From the data presented in the studies, it is not possible to identify if the observed increase in the risk of ovarian cancer is due to infertility per se, to a sub group of infertility, to the use of infertility drugs (different types have different modes of action) to diagnostic bias (more investigations in infertile patients) or even to chance.

There is a need for more accurate and precise studies, only appropriate if adequate informations on patients characteristics, nature of infertility, details of the drug treatment and histology, stage and grade of the ovarian cancer can be provided.

In the meantime, it is important that patients who received drug treatment for their infertility should be reassured. Doctors must control the ovarian conditions before prescribing fertility drugs. They must inform the patients and keep detailed files for further retrospective studies.

**Assisted Reproduction Techniques**

The blossoming of new techniques of assisted reproduction has been accompanied by a considerable burden of responsibility being placed on the scientific community which must not only settle the indications for each one of the techniques, weighing the balance between the benefits and the risks derived from its application, but must also ensure that all these techniques are ethically irreproachable and have as their sole purpose the treatment of the human infertility.

**Artificial insemination**

Artificial insemination is one of the most common therapies in infertility clinics. Depending on the origin of the semen, artificial insemination can be of two types : AIP and AID.

The technique consists in placing in the interior of the vagina, the uterine cervix, or the uterine cavity, a sample of total semen or of semen prepared in the laboratory by means of swim-up techniques or of filtration in Percoll gradients.

**a) Indications**

The most common indications for performing an AIP cycle are:

- The impossibility of vaginal ejaculation (psychogenic or organic impotence, severe hypospadias, retrograde ejaculation and vaginal dysfunction)
- The use of sperm cryopreserved prior to cancer treatment or vasectomy.
- Unexplained infertility.
- Infertility due to male factor in which the semen sample shows deficiencies in the number (oligospermia), motility (asthenospermia), or morphology (teratospermia) of spermatozoa. A threshold of at least 1 million motile sperms seems a reasonable requirement.
- Unfavourable cervical factor which cannot be overcome by medical treatment.

**b) Advantages**

The majority of artificial inseminations with partner’s sperm are intra-uterine (IUI), they are simple to perform and for this reason are easily repeatable. The efficacy of intra-uterine artificial inseminations is variable but thanks to the possibility of repetition the accumulated rate of pregnancy after three IUI cycles is usually greater than the natural expectancy of pregnancy which these couples have during the same period of time.

**c) Disadvantages**

One of the drawbacks of this technique lies in the great variability of its efficacy depending on the
different indications. We should not overlook the risks which stem from repeated cycles of ovulation stimulation that are required to achieve a satisfactory pregnancy rate. The treatment needs careful monitoring in order to prevent the risk of ovarian hyperstimulation and/or multiple pregnancy.

d) Ethical evaluation
If there is a medical indication for its practice and the individual evaluation of the case shows a greater expectation of pregnancy with insemination than with programmed coitus, then the technique is ethically acceptable.

**In vitro fertilization and embryo transfer (IVF/ET)**
The technique of in vitro fertilization (IVF) consists in bringing about the fusion of the egg and the spermatozoa in the laboratory instead of in the women’s Fallopian tubes. Thanks to this technique there are now tens of thousands of children who have been born throughout the world. IVF technology involves ovulation in order to obtain multiple oocytes, thus making available more embryos, with which higher pregnancy rates can be achieved. The response to stimulation is controlled by a series of determinations of plasmatic oestradiol and by ultrasound measurements of follicular growth. At the appropriate moment the ovaries are punctured in order to aspirate the contents of the follicles and obtain the oocytes. These oocytes will be incubated together with appropriately capacitated spermatozoa from the husband and if fertilization occurs within 48 hours the embryos obtained will be transferred to the interior of the uterine cavity between day 2 and day 6 after puncture in order that implantation may take place in the following days.

The efficiency of IVF is high and approximately one in every 4-5 women who undergo the attempt achieve pregnancy. IVF is the therapeutic option of reproductive medicine with the highest yield per attempt, close on many occasions to that achieved by fertile couples with natural conception.

**Indications**
The original indication of IVF is the irreversible pathology of the fallopian tubes, resulting from inflammatory processes or previous surgery. However, in recent years the indications of IVF from an abnormal male factor have become more and more common, and owing to assisted fertilization techniques they are becoming the principal indication of many IVF programmes. There are other indications such as unexplained infertility, residual endometriosis, and infertility of immunological origin which can also benefit from the application of IVF technology.

**Techniques derived from IVF**
For patients with normal tubes there has been increasing support for the recommendation to transfer into the tube gametes (GIFT), zygotes (ZIFT) or embryos (TET), although GIFT has the disadvantage of being unable to demonstrate the fertilizing capacity of the gametes. ZIFT and TET are usually applied in cases of infertility that is unexplained, or due to male factor or immunological factor provided that at least one tube is normal. Access to the tube may be gained by laparoscopy or by retrograde catheterization through the uterine cervix. Although the reported pregnancy rates with transfer of zygotes or embryos into the tube are higher than that achieved by conventional IVF with uterine transfer, there are no randomized studies demonstrating this superiority.

**Advantages of IVF**
The greatest advantage of IVF techniques is that, thanks to their efficacy, the advantages which can be reaped from their application are much superior to the theoretical risks which both the couple and the future offspring might incur. Another important advantage of IVF is its diagnostic ability, which means that many IVF cycles serve a dual diagnostic/therapeutic purpose. Finally, a great advantage of IVF is its reproducibility which allows different teams throughout the world to achieve similar results.

**Disadvantages**
There are two main areas of concern regarding IVF techniques. First, there are conceptual reasons such as the separation between procreation and sexual union or the fact that there is no life-threatening risk which justifies the application of IVF or any of the other techniques of assisted reproduction. Likewise, the high cost of these therapies should not be overlooked, bearing in mind that they only bypass the problem and do not solve it definitively.
Second, there are drawbacks specific to the technique itself such as the risk of ovarian hyperstimulation, the necessity of limiting the number of embryos which may be implanted to avoid the possibility of multiple pregnancies and the accumulation of frozen embryos with the responsibilities which this situation places on both physicians and patients.

**Ethical evaluation**

We believe that as long as IVF is applied in order to optimise the possibilities of pregnancy for an infertile couple, the technique is ethically acceptable. If medical indications are respected, the number of embryos normally implanted is limited to three and there is an efficient cryopreservation programme available which can accommodate extra embryos, we can validate the technique.

Although the techniques of assisted reproduction may alter the couple’s sexual intimacy, if we consider them as a therapeutic procedure designed to fight against the disease of human infertility we shall recognise their value, avoid deviations from the technique, and we shall always respect the dignity of the human being.

Intracytoplasmic sperm injection (ICSI) with ejaculated, epididymal and testicular spermatozoa

**Background**

In the last two decades, in-vitro fertilization (IVF) has been successful in the treatment of long-standing infertility due to tubal disease, idiopathic and male-factor infertility. It is a well-documented fact that the results of IVF in male infertility are not as good as those in patients with normal semen parameters. In andrological infertility only 20-30% of the inseminated cumulus-oocyte complexes are normally fertilized, which is much lower than the 60-70% fertilization rate in patients with tubal infertility. Absence of fertilization may occur in about one third of the cycles. It has been the experience of all centers for reproductive medicine that a certain number of patients with andrological infertility cannot be helped by standard IVF treatment. Furthermore, a sizeable number of couples cannot be accepted for IVF if the number of progressively motile spermatozoa with normal morphology available for insemination is below a certain threshold number such as 100,000.

In the past six years, assisted fertilization procedures have been developed to circumvent the barriers that prevent sperm access to the ooplasm, namely the zona pellucida and the ooplasmic membrane. Successful fertilization, embryo development, pregnancies and births have been reported after partial zona dissection (PZD) and subzonal insemination (SUZI). In 1992 the first pregnancies and births obtained by a novel procedure of assisted fertilization i.e. intracytoplasmic sperm injection (ICSI) were reported. The results of several hundreds of cycles of assisted fertilization by SUZI and ICSI and a controlled comparison of ICSI and SUZI on sibling oocytes indicated that the normal fertilization rate after ICSI is substantially higher than after SUZI, while the further in-vitro development to transferable or freezeable embryos is quite similar for the two procedures. The higher fertilization rate and similar cleavage rate resulted in more embryos for replacement after ICSI and high implantation rates have been obtained. By now most groups have adopted ICSI as the sole procedure of assisted fertilization.

**Medical indications**

ICSI can be carried out with fresh or frozen-thawed ejaculated spermatozoa, with fresh and frozen-thawed epididymal spermatozoa and with spermatozoa isolated from a shredded testicular biopsy.

1 - **Indications for ICSI with ejaculated spermatozoa**

   - Severe male-factor infertility and fertilization failure after standard in-vitro fertilization treatment.
   - Too low number of spermatozoa in the ejaculate for standard in-vitro fertilization treatment.

2 - **Indications of ICSI with epididymal spermatozoa**

   - Epididymal spermatozoa can be obtained by microsurgical epididymal sperm aspiration (MESA), or by needle
   - Aspiration, in the following conditions:
Congenital bilateral absence of the vas deferens (CBAVD)
Failed vasoepididymostomy
Failed vasovasostomy
Young’s syndrome
Azoospermia because of bilateral herniorraphy
Obstructions at the level of both ejaculatory ducts
Anejaculation because of spinal cord injury, diabetes, lymphadenectomy,
Retrograde ejaculation
Sexual dysfunction
Masturbation problems at IVF

3 - Indications for ICSI with testicular spermatozoa

- Testicular sperm can be obtained from biopsy specimens or by needle aspiration.
  - All indications for MESA
  - Extensive scarring rendering MESA impossible
  - General anesthesia contraindicated
  - Germ-cell hypoplasia (“hypospermatogenesis”)
  - Germ-cell aplasia with focal spermatogenesis (“Sertoli cell-only syndrome with focal spermatogenesis”)
  - Necrozoospermia

Results
The results of ICSI with ejaculated, epididymal and testicular spermatozoa in terms of fertilization, embryo cleavage and implantation rate after transfer can be considered to be similar to the results of standard in-vitro fertilization treatment in infertile couples with non andrological infertility. The table summarizes the results of four years of ICSI (1991-1994) as collected by the ESHRE Task Force on ICSI. The results of this first survey which included data submitted by 31 December 1993

<table>
<thead>
<tr>
<th></th>
<th>Ejaculated</th>
<th>Epididymal</th>
<th>Testicular</th>
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<tr>
<td>Number of cycles</td>
<td>13.178</td>
<td>539</td>
<td>193</td>
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<tr>
<td>Oocytes injected</td>
<td>111.291</td>
<td>5.744</td>
<td>2.057</td>
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<td>% intact after ICSI</td>
<td>90,3</td>
<td>91,9</td>
<td>89,6</td>
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<td>% of intact oocytes</td>
<td>58,5</td>
<td>50,5</td>
<td>50,9</td>
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<tr>
<td>with 2PN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% transferred or frozen</td>
<td>69,2</td>
<td>61,4</td>
<td>71,9</td>
</tr>
<tr>
<td>embryos</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>% embryo transfers</td>
<td>91,1</td>
<td>93,3</td>
<td>87,6</td>
</tr>
<tr>
<td>% positive HCG/cycle</td>
<td>28,7</td>
<td>34,9</td>
<td>33,2</td>
</tr>
</tbody>
</table>

Recommendations
Patient counselling is an essential component of all types of assisted reproductive techniques. If ICSI has to be applied to alleviate the problem of infertility, the couple should be informed about the novelty of the procedure and about its many unknown aspects.

A thorough andrological and genetic screening will be important prior to perform ICSI in cases of very severe sperm defects. It may be recommended that the couples undergo prenatal diagnosis and participate in a prospective follow-up study of children born after ICSI. Although the results of about 500 prenatal karyotypes and the prospective follow-up of 750 children does not indicate an increase in abnormal foetal kayotypes and in major congenital malformations, it is important to continue this careful follow-up of the children in different centres practicing ICSI. This is one of the goals of the “Task Force on ICSI” established by the European Society of Human Reproduction and Embryology.

Remarks on laboratory safety in assisted reproduction technology
The presence of human gametes and embryos in the laboratory requires the adoption of Good Laboratory Practice, that has been defined internationally. Apart from instituting standardized optimal culture conditions in order to obtain optimal results the following two risks always have to be
considered in this field:

1 - Transmission of disease
Sources of infection in this system are: patient serum, semen and possibility hyaluronidase (ICSI). The diseases most feared are AIDS, hepatitis B and C, and lues. Screening of both partners followed by appropriate measures to deal with positive cases will prevent the possible transmission of these diseases through the Laboratory. The use of patient serum is not necessary in culture medium. Yet, potentially infectious material enters the laboratory. Workers should ideally be protected by immunization against hepatitis B and work under biohazard conditions. Cross-contamination from one patient to another can still occur during the cryopreservation procedure when straws with semen or embryos are filled by dipping the straw in patient medium with semen or embryos, sealing it and passing it into liquid nitrogen without external disinfection. Semen for future IVF use should only be stored after patient screening and kept separate from embryos.

Recommendations
Patient/partner screening, laminar flow working conditions and immunization of workers, external desinfection of straws prior to cryopreservation and the use of gammasterilized hyaluronidase for ICSI. Since heat sterilization can no longer be recommended by hospital authorities, gamma irradiation should be used for pipettes in IVF and ICSI.

2 - Identification errors
All material obtained from the operation room, culture dishes and sperm tubes should bear the name of the patient (also on the lid) and in the incubator eggs and sperm undergoing swim-up should be kept together on the same tray and be double checked. Pipettes used for insemination should be completely disposable. During embryo transfer the doctor should clearly announce to the laboratory staff the name of the patient.

Sperm freezing

History
History of sperm freezing starts after the second world war. When clinicians became aware that some forms of male azoospermia or very severe oligospermia could not be improved by medical treatment, the idea was to create sperm banks. This coincided with general ethical views of the great many couples suffering from infertility, realizing that the cause was mainly on the male side but sharing a sufficient richdom of emotional values into the education of a child. So the first created sperm banks were almost if not totally devoted to the storing of donor sperm.

Donor sperm
The technique of sperm freezing took only few years before receiving general consensus. Several cold chock preventing media were tried before concluding that the optimal medium was one which diluted the sperm by no more than 30% and contained variable amounts of glycerol, egg yolk and other components. The sperm were placed in straws of either 0.5 or 1 ml, and stored in liquid nitrogen. The storage procedures for freezing and thawing have been satisfactorily developed. Despite this, the efficiency of frozen sperm is lower and the pregnancy rate per cycle is about half the normal rate. But this drawback is encompassed by the safety of the procedure. A minimum of 2 millions motile sperms in the inseminate seems a valuable prerequisite.

The safety of using frozen sperm has been abundantly proven both by both experimental and veterinary work and ultimately by the large number of healthy children born after donor insemination with frozen sperm. Whatever risks were considered, they were eliminated by utilizing the large veterinary experience and the actual results in humans.

Despite this, recommendations for optimal management are mandatory. They concern the donor’s health and the necessity to exclude all donors carrying either venerial disease, hepatitis or HIV.

Husband sperm
Clinicians using donor sperm banks soon realized that the same sperm banking could be used for husband sperm. It was mainly in cases of impotence that this approach was used. Indeed, some males do suffer from impotentia coendi, but are perfectly able to produce good sperm samples by
masturbation. Because, for psychological or other reasons, it is not always possible to produce a sperm sample at the appropriate time in the cycle, sperm banking was introduced to help males who may have such difficulty. The procedure has further been extended to those couples in which the male partner may be away for long periods of time for professional reasons so that during his absence his sperm could be used to inseminate the female partner at the time of ovulation. Husband sperm storage and insemination is also used for preservation of fertility before testicular surgery, radiotherapy and/or chemotherapy, and before sterilization (vasectomy).

There is an extensive amount of literature that provides the results of using sperm bank insemination in either natural or induced cycles.

One of the drawbacks of sperm freezing is a loss of motility of approximately 20% after thawing. Therefore, ejaculates with low semen parameters cannot be considered appropriate for freezing. However, recent progress in assisted fertilization (ICSI) allowing the use of very low quality samples have deeply modified this, giving the possibility to offer such cryopreservation procedures more frequently.

**Recent progress in sperm freezing**

During the very last years new techniques have developed mainly in the field of in vitro fertilization. These techniques do after the possibility of fertilizing oocytes first with extremely low numbers of spermatozoa, second with spermatozoa collected either from the epididymis or the testis itself. The rapidly spreading interest to use epididymal and testicular sperm included that at the time of collection of these cells some could be used directly and others stored, in order to avoid, in cases of failure, the need for renewed surgical collection of spermatozoa.

The quantity of epididymal or testicular spermatozoa retrieved by the surgical approach is much lower than that of fresh ejaculates. Therefore, the routine procedure of mixing the sperm with culture medium had to be adapted for the use of spermatozoa for microinjection of oocytes. While routine freezing has the drawback of a loss on motility of 20%, it has rapidly been shown that after thawing epididymal spermatozoa, motility was not necessary, although vitality remains an important parameter. Indeed, the fact that the spermatozoa were frozen in a living state is sufficient to use them for microinjection after thawing. A vitality of 20% or more seems to be a reasonable requirement. The storage of such spermatozoa is usually performed in much smaller straws than for ejaculated sperm. Cryopreservation of testicular biopsy specimens for future ICSI cycles is a technique under development.

The use of such sperm is mandatorily linked to micro-insemination of oocytes collected in in-vitro fertilization programs. The first results obtained with frozen and thawed epididymal and testicular sperm, with regard to quality of the obtained pregnancies and born children, are very reassuring with regard to eventual genetic damage.

**Conclusion**

Sperm freezing, both for ejaculated spermatozoa and spermatozoa collected from the male genital tract, is a safe procedure that has proven to offer a valuable help for otherwise unsolvable problems. Genetic damage is unknown and risks are totally eliminated. The medical indications for freezing spermatozoa collected from the genital tract are increasingly recognized and practically applied. The basic recommendation concerning the health of the male partner are of course routine.

**Human Embryo Cryopreservation**

**Background**

Mammalian embryos have been successfully frozen and stored since 1972 when live mice where obtained after the transfer of freeze-thawed morulae. In humans, the first birth from a frozen embryo occurred in 1984 in Australia and this cryotechnology, derived from rodent and cattles routines, became a necessary part of in vitro fertilization programs in order to avoid the risks of multiple pregnancies following the transfer of large numbers of embryos, as well as the wastage of supernumerary embryo arising from the wide oocyte cohorts primed by ovarian stimulation. At present, embryo freezing is a widespread routine procedure which provides possibilities to increase the cumulative pregnancy rates from every cycle of successful ovarian recovery and transfer.
**Medical indications**
To preserve supernumerary embryos in the course of conventional IVF as well as ICSI procedures, after the transfer of limited number of embryos. To assist embryo donation for 2 purposes:

- avoid the synchronization between donors and recipients
- maintain embryos in quarantine while awaiting HIV antibody testing of the donor at a safe interval after donation

To preserve all embryos obtained after IVF, in case of serious risks of severe ovarian hyperstimulation syndrom or in case of a therapy definitely suppressing ovarian function in married women with a desire of children in the future.

**Usual procedure**
Human embryos can be successfully cryopreserved by protocols using 1,2 propanediol (PROH), dimethylsulfoxide (DMSO) or glycerol. Each of these cryoprotectants gives an optimal efficacy when used at a particular embryo developmental stage: PROH or DMSO for zygotes or cleaved embryos, glycerol for blastocysts.

The efficiency of ice-seeding is of major importance and may be impaired in freezers with automatic seeding, thus leading to a failure of the whole freezing program.

Slow cooling in the most widely employed method despite human embryos may survive a simple ultrarapid procedure by fast cooling; the latter method is still scarcely used throughout the world.

No definite conclusions can be made whether embryonic stage is the best to freeze from zygote to blastocyst. On the contrary, although embryos of less quality can be successfully frozen, the best survival rates are obtained with embryos displaying the best morphology.

The transfer cycle should be performed as is best suitable for the patient: natural cycle for normal ovulatory women, stimulation with hMG-hCG or steroid substitution with or without GnRH analogs in case of cycle disturbances. The synchrony embryo-endometrium is accepted as giving the best results.

**Results and risks**
Embryo freezing has resulted in _65%_ embryos keeping at least half of their blastomeres intact after thawing and _15% to 20%_ clinical pregnancies after the transfer of 2 to 2.5 embryos per patients. After an IVF cycle, comprising embryo freezing, the chances to obtain a live birth is enhanced by 8 to 10% on average after the transfer(s) of the frozen-thawed embryos. The outcome of pregnancies arising from frozen-thawed embryos do not seem to differ from fresh embryos transfers. Till now, no difference where found when comparing the congenital malformation rate between the two groups.

A recent report analyzed the long-term effects of embryo freezing in MICE. Although reassuring, as this work confirmed that embryo cryopreservation does not induce major anomalies, the study pointed out delayed effects at senescence such as a body weight increase in males but the data were rather disconcerning because the differences from the controls were of moderate amplitude and depended on genotyp, sex, or age. Such a long-term follow-up is impossible to perform in humans and more animal date (data ???) should be collected.

These results, nevertheless, stress the importance to carefully analyze the outcome of pregnancies and the state of babies born after such procedures.

**Recommendations**

1. Embryo cryopreservation counselling: before the treatment cycle, patients should be adequately and completely informed about the procedure, the results, the risks, the law and particularly what is to be done with their embryos in any situation. They should sign a consent form concerning the agreement for embryo freezing and future disposition of embryos in storage.
2. Embryos can be frozen at any stage from zygote to blastocyst, but embryos that are not
considered to be of sufficiently high quality to be replaced as fresh embryos should not be cryopreserved.

3. The slow freezing method has led, until now to the vast majority of reported pregnancies and childbirths after transfer of frozen-thawed embryos. DMSO, or more often, PROH-Sucrose are used as cryoprotectants for early embryo stage cryopreservation, whereas glycerol is used for blastocysts. Adequate seeding is mandatory for optimum results.

4. Straws or ampoules used for freezing embryos should be carefully and indelibly labelled for identification purposes.

5. When a serum supplement is used in the preparation of freezing and thawing solutions, any risk of viral transmission should be carefully avoided. It may be preferable to use substitutes.

6. After thawing, only embryos containing at least half of the initial number of blastomeres within an intact zona pellucida have optimal viability and should be replaced. When these criteria are not fulfilled, the success rate is drastically reduced.

7. Data from retrospective studies indicate that the length of storage can be extended to at least 5 years without any impairment of embryo viability.

8. Laboratories must take care to separate liquid nitrogen storage tanks containing virus positive embryos in order to reduce the risk of cross-contaminating clean embryos. This applies also to the storage of cryopreserved gametes.

Oocyte Cryopreservation

Introduction

The first birth following human cryopreservation of human oocytes was in 1986 but there have been few births reported since. The intention with egg freezing would be to thaw and fertilize the cryopreserved eggs by in vitro fertilization or ICSI when the women needs them.

Large numbers of immature eggs can be readily retrieved from the unstimulated ovary. Mature eggs are only retrieved after controlled ovarian hyperstimulation. Immature eggs, ovarian tissue fragments or mature eggs can be frozen.

Indications and advantages

To preserve fertility if ovarian function is about to be lost as a side effect of the treatment of life threatening diseases in young women (or children) with no male partner. Typically this would involve cytotoxic therapy, radiotherapy or surgical removal of the ovary for malignancy.

If surgery is urgent, eggs can be retrieved from unstimulated ovaries.

To assist oocyte donation. The oocyte can remain in quarantine while awaiting HIV antibody testing of the donor at a safe interval after donation. Occasional problems with synchronization of the cycles of donor and recipient may also be overcome.

For some couples, the relative ethical (and legal) simplicity of oocyte cryopreservation makes it preferable to embryo freezing after IVF or discarding excess eggs after GIFT. Most people have fewer concerns at the prospect of discarding or donating unwanted eggs, when compared with the problems created by embryos which become excess to the couple’s needs.

Problems and reservations

There remain significant technical problems with the use of human oocyte cryopreservation in assisted reproduction.

With current techniques it appears that mature eggs frozen, thawed and fertilized by IVF or ICSI have about half the pregnancy potential of eggs fertilized and frozen as embryos. There are still some concerns that fully mature (metaphase II) eggs are at a delicate stage where they may be vulnerable to chromosomal damage by freezing.

On the other hand, the more numerous and robust immature eggs can generally not be matured to metaphase II after thawing.

Recommendations
Oocyte freezing is recommended for young women unmarried who are about to lose ovarian function. Oocyte freezing is not reliable enough to recommend for preservation of excess eggs in routine IVF or GIFT.

Embryo transfer policy and multiple pregnancy in IVF

One of the remaining problems in a successful IVF programme is the occurrence of triplet pregnancies following transfer of three or more embryo. Decisions to transfer three or more embryos are taken on the basis of the following considerations:

1. The belief that the pregnancy rate is correlated with the number of embryos transferred.
2. The triplet risk would be lower above 35 years of age.
3. The ethical necessity of not wasting apparently good embryos.
4. A strong desire of the patient to become pregnant and her willingness to take the triplet risk as a personal responsibility.

Medical statistics clearly show that triplet pregnancies are associated with considerable medical and psychological risks. The cost of intensive care of triplet children and associated aftercare and adaptations in life-style probably outweigh the cost of refunding one extra IVF attempt by the insurance system.

A logical approach to solve this problem is to transfer a maximum of two selected embryos. Embryo selection should theoretically be performed when the embryonic genome is activated (after the 8-cell stage) and contributes to the development of the embryo in vitro. Several studies have now shown that individual late blastocysts have an implantation rate of about 30%. Transfer of two expanded blastocysts leads to a pregnancy rate of over 40%. Taking all embryos transferred into account the pregnancy rate following transfer on the day 5 is the same as or higher than that obtained after ET on day 2 or day 3. In two large unpublished series comprising about 600 day 5 transfers early or late blastocysts could be transferred in 72% of the patients. In order to obtain these results culture conditions, without the use of feeder layers, could be improved according to a standardized protocol by daily checking the incubator CO2 content with capnograph and by the introduction on of the CO2 controller, a device which rapidly restores the after door opening the incubator needs about 20 minutes to equilibrate again.

On day 5 successful embryos can be selected at at time when the embryonic genome has manifested its developmental potency. This policy will prevent the occurrence of triplet pregnancies and lead to a predictable high pregnancy rate in about 70% of the patients with fertilized eggs. The remaining patients with lesser developed embryos receive morulas and non-compacted stages. Only two pregnancies were seen in 190 cases receiving non-compacted stages only day 5. In mixed embryo cultures blastocysts and non-compacted stages grow side by side in the same culture drop indicating that embryonic differences are responsible for the differences in embryo morphology.

In the future about 15% of the patients with fertilization will not undergo ET on day 5 in view of these results thus saving the patient the stress of expecting a pregnancy. These considerations should lead to a universal adoption of proven and standardized optimal culture conditions and to a renewed look at the restrictions to induce the formation of more human embryos in vitro than are required for transfer.

Preimplantation Genetic Diagnosis (PGD) of Chromosomal and Single Gene Defects

Background

For an increasing number of inherited diseases for which the molecular basis is known, couples who are at risk of having affected children can be offered prenatal diagnosis following amniocentesis or chorion villus sampling in the second or first trimester of pregnancy, respectively. Until safe and effective methods for gene therapy or other therapeutic approaches are available, however, in the event of an affected diagnosis, the couple has to face the decision of whether or not to terminate the pregnancy. Over the last eight years, methods have been developed for biopsying cleavage stage human embryos and analysing chromosomal and single gene defects in single cells following in vitro fertilization (IVF). This very early preimplantation genetic diagnosis (PGD) allows only embryos
unaffected by specific defects to be selected for transfer and any pregnancy should be normal.

For the detection of chromosomal abnormalities, fluorescent in situ hybridization (FISH) techniques are being developed which allow rapid and sensitive analysis and interphase cytogenetics. For specific detection of mutations, DNA amplification using the polymerase chain reaction (usually two rounds of nested amplification) are being developed to provide sufficient DNA for mutation detection.

**Medical indication**

Couples known to be at risk of having children with an inherited disease because of their family history.

1. Single gene defects

(a) X-linked recessive disease: this group of disease are caused by defects in genes on the X chromosome, typically inherited from unaffected carrier mothers, and only affect homozygous boys. Identification of the sex of embryos and transfer of females is possible either by amplification of X (or other control) and Y chromosome sequences or by FISH with X and Y-specific probes preferably combined with at least one other autosomal probe. FISH is currently the method of choice since it also allows identification of aneuploidy.

(b) Specific diagnosis of single gene defects: the specific mutation in the affected gene in one or both partners must be known. If not preliminary genetic analysis or linkage analysis is necessary. To date PGD has been applied clinically and unaffected pregnancies confirmed in cystic fibrosis (CF) (DF508), Lesch-Nyhan syndrome (private mutation), Tay-Sachs disease (common insertion mutation) and Duchenne muscular dystrophy (exon deletion). Methods are currently being developed for several others including minor CF mutations, beta-thalassaemia, fragile-X (FRAXA) and Huntington’s. Potentially any gene defect characterised at the DNA level or associated with a closely linked marker could be diagnosed.

2. Chromosomal abnormalities

(a) Advanced maternal age: women of advanced maternal age are at increased risk of aneuploidies. Multicolour FISH methods are being used to detect frequent trisomies either in polar bodies and single cleavage stage cells. Clinical experience is too preliminary to assess the benefit if any.

(b) Translocation carriers and gonadal mosaics: multiple probe and comparative genome hybridization methods are being developed for these couples often at high risk of aneuploid pregnancy.

**Results**

As of February, 1995, 12 centres have attempted at least one cycle of PGD. The outcome as reported to the ESHRE Special Interest Group in Preimplantation Genetics (excluding polar body biopsies and screening for trisomies in older women) is as follows:

<table>
<thead>
<tr>
<th></th>
<th>No of patients</th>
<th>No of IVF cycles</th>
<th>No of embryo transfers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75</td>
<td>113</td>
<td>95</td>
</tr>
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<td></td>
<td>78</td>
<td>70</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>123</td>
<td>183</td>
<td>159</td>
</tr>
</tbody>
</table>
No of pregnancies 24 19 43
No of deliveries 15 11 26
No of babies born 21 11 32

Pregnancy rate per patient : 35%
Pregnancy rate per cycle : 23.5%
Pregnancy rate per embryo transfer : 27%

Misdiagnosis
There have been three misdiagnoses reported so far:

1. Misidentification of sex following amplification of Y-specific repeat sequence alone.
2. Two misdiagnoses in couples at risk of having compound heterozygous CF affected children.

The reason for the first misdiagnosis was single cell amplification failure. The reason(s) for the CF misdiagnoses have not been established. Possible contributing factors include sperm contamination, allele dropout and chromosomal mosaicism. Intracytoplasmic sperm injection (ICSI) may therefore be preferable for PDG. Allele dropout can be minimised by using high denaturing temperatures in the first round of PCR and should be assessed on single carrier for compound heterozygotes unless extensive testing on cells from affected family members demonstrates acceptable levels of diagnostic accuracy. Finally, independent analysis of two cells from each embryo is desirable.

Recommendations
Centres offering PGD should have acceptably high pregnancy rates following IVF and ICSI and need close contact with clinical genetics. The number of genetic risk couples is only a fraction of those with infertility and a small number of regionally based national specialist centres may be the best strategy.

Couples need to be advised of primarily the low (30% per embryo transfer) pregnancy rate and the possibility for misdiagnosis. A follow-up prenatal diagnosis is desirable not only for the couple themselves but for monitoring accuracy in relation to later cases.

DONOR GAMETES

Artificial insemination with donor sperm (AID)
The use of semen an anonymous donor, which has been stored frozen in a sperm bank allows the resolution of certain cases of infertility which are considered as insoluble.

a) Indications
The majority of AID cycles are carried out because of serious semen anomalies which cannot be treated with other techniques (for example the total absence of spermatozoa or azoospermia). AID is also indicated in cases in which the husband has a hereditary genetic alteration or an infectious illness or the couples have a problem or Rh incompatibility.

The application of artificial insemination with a donor’s semen to women without a partner or lesbian couples constitutes a limit indication which should be analysed with care.

b) Advantages
The advantages of AID combine its acceptable yield with an average of 50% pregnancies after six attempts, together with a greater availability of semen which is suitable to perform the insemination on several successive days and a lower risk of transmission of certain infectious diseases. In addition, AID has the advantage over adoption that the mother can carry the child and both partners can share the experience of pregnancy.

c) Disadvantages
The main disadvantages are those derived from the introduction of gametes from a third person into the bosom of couple with the psychological and physical implications that that involves.

The possible transmission of diseases from the donor to the future child and the theoretical risk of consanguinity, constitute other additional drawbacks.

d) Ethical evaluation
We believe that insemination with donor sperm is ethically acceptable if:

- There is a medical indication and the couple has undergone a psychological evaluation.
- The normal conditions of anonymity and screening of the donor are met.
- Frozen sperm samples that are used have undergone the appropriate quarantine period to exclude infectious diseases such as HIV, hepatitis B and C, and syphilis.

Serum and sperm samples should be cryopreserved for any possible future analysis in case of the emergence of new pathologies. This also applies to oocyte donation.

**Oocyte Donation**

**Background**
Oocyte donation (OD) was introduced as treatment for ovarian failure in 1984 and is at present an integral part of management of female infertility and for achieving pregnancy in elderly and post-menopausal women.

Patients for oocyte donation fall into two major categories:

1. Women with gonadal function
2. Women without gonadal function

**Indications**

1. Primary ovarian failure due to ovarian agenesis and/or gonadal dysgenesis.
2. Premature ovarian failure (POF) - hypergonado-trophic amenorrhea prior to age 35, or iatrogenic due to ovarian surgery or radiation, or chemical castration.
3. Resistant ovary syndrome.
4. Women in the late reproductive age - low responders.
5. Carrier of autosomal recessive disorders.

**Donor selection**

Selection of an oocyte donor is of critical importance to the prospective parents and the resulting child. Donors should be:

1. In the fertile age of 18-35, preferably with previous fertility.
2. Healthy, as determined by medical examination and psychological evaluation.
3. Screened for hereditary disorder and sexually transmitted diseases. All donors should be screened for AID antibodies.

**Genetic screening**

Genetic screening for donors is important and should be decided by the institution and the country where ovum donation is practiced.

Oocytes may be obtained for donation mostly by surgical intervention under to following circumstances:

1. From women participating in an IVF program, or other assisted reproductive program.
2. During a sterilization procedure.
3. From laparoscopy performed for the donor’s own infertility.
4. At the time of therapeutic surgery in women of reproductive age.

Donation of oocyte from women not undergoing IVF treatment involves the following medical risks to the ovum donor, that should be considered:

1. Ovarian hyperstimulation (induction of ovulation)
2. Risks of oocyte retrieval (anesthesia, infection, trauma, etc...)

**Methods of retrieval**
1. Ovum pick-up during IVF procedure.
2. During therapeutic surgery when the elective procedure is performed at the suspected pre-ovulatory period from patients who agree to undergo ovarian stimulation before the surgical procedure.

**Selection and screening of recipients**
The only contraindication to ovum donation are medical or psychological conditions of the recipient under which pregnancy is ill-advised. A primary diagnostic work-up is indicted before the infertile couple is treated by means of donation.

The following examinations should be performed:

1. A physical examination of the female partner.
2. Hysterosalpingography for evaluation of the adequacy of the uterine cavity, a patent Fallopian tube for the GIFT method for transfer of pre-embryos.
3. A cardiopulmonary work-up, especially in women with dysgenesis of ovaries and in older candidates.
4. The male partner: sperm evaluation.
5. The couple should undergo psychologic and social evaluation.
6. Serological examination for blood groups, rubella antibodies, VDRL, HIV, hepatitis, CMV, VDRL, gonorrhea, and chlamydia, mycoplasma and trichomonas infections.

**Age of the recipient**
It has been shown that ovum donation can be practiced in menopausal women. The endometrium of menopausal women has the ability to respond to sex hormones and provide a receptive environment for implantation of pregnancy. Pregnancies beyond ordinary child-bearing age raises medical, psychological, familial and social problems. In several countries the recommendation is to limit the age of the recipients to the natural reproductive life-span.

**Preparation of recipients**
ET cycles using of donated oocytes initially involved synchronization between recipient and donor cycles. Presently such synchronization is not required, because various protocols with estrogen and progesterone preparations are used to prepare the recipient’s endometrium for implantation.

Hormones are needed for maintenance of human gestation as provided in the early stages by the corpus luteum. Different regiments of estrogen and progesterone administration are applied in the early stage of gestation until the placenta takes over the function of maintaining the gestation.

**Results**
The overall clinical pregnancy results that have been reported with the use of donor oocyte vary from center to center. Published reports have demonstrated clinical pregnancy rate rating from 25% to 60%. The live birth rate per embryo transfer for IVF-ET in infertile women was 18%. Better results were obtained in menopausal women after OD.

**Payment of donors**
There should be no compensation to OD donors for providing the oocytes. However, this does not exclude the reimbursment for expenses, time and risk which are associated with the donation.

**Legal aspects of oocyte donation**
The donor and the recipient couple should provide an informed consent. According to the legislation and regulations, where they exist, the woman who gave birth is recognized as the legal mother of the offspring.

**Anonymity**
The donors can be either anonymous or known to the couple. Most ethical committees in various countries recommended that the anonymity of the donor be preserved.

**References:**
The contents of this document were discussed during two meetings in Vienna and Brussels (1995) with the participation of:
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It has been approved by the Executive Committee of IFFS in Montpellier (France) on September 22nd, 1995.

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Source URL: http://www.physicianspractice.com/infertility/assisted-procreation

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