Guidelines on Assisted Reproduction

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Introduction:

Infertility is a disease. The duration of the failure to conceive should be twelve or more months before an investigation is undertaken unless medical history findings dictate earlier evaluation and treatment.

The FOGSI has among its objectives to contribute to the standardization 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 these guidelines on a number of ethical issues about ART, FOGSI wants to formalize the opinion of the professionals themselves.

World wide it has also been variously estimated that between 6-10% of all couples are infertile. In India, since a stable family structure and desire for children is the norm there is often a social stigma associated with infertility. There is hence an ever increasing demand for diagnostic and therapeutic intervention in infertile couples. However many of these technologies require enormous technical expertise and infrastructure, carry a limited success rate even in the best of hands, are expensive, and tax the couple's endurance physically, emotionally and economically.

The guidelines adopted by the FOGSI aim to be an up-to-date focus on modern techniques of ART, on the reasons for which they have been proposed, on the conditions for their realization and their possible risks.

Infertility specialists and biologists, the world over offer who offer these new techniques to infertile couples are aware that they are considered as an upheaval of traditions and that in the case of gamete donation they change radically the concept of the legitimate family. Each country has reacted to these facts and sometimes even passed laws in accordance to their traditions, beliefs, or public opinion. This consensus must allow each one of us to indicate to our patients what is 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 consenses will allow them to be informed in an objective way. Progress never stops. So these guidelines will have to be updated frequently.

Ovarian Stimulation

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 for patients without hormonal disturbances.

Protocols in General Use

In Vitro Fertilization and Embryo Transfr (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 plasma estradiol 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 are incubated together with appropriately capacitated spermatozoa from the husband and if fertilization occurs within 48 hours the embryos obtained are 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 was the irreversible pathology of the fallopian tubes, resulting from inflammatory processes or previous surgery.

1. **Tubal Disease**
   I.V.F./E.T. can be offerd where microsurgical techniques for tubal and peritoneal disease have failed or are unlikely to benefit the patient. The choice between I.V.F. and Microsurgery would be dictated by the presence of peritubal adhesions, condition of the tubal wall, condition of the ciliary epithelium and degree of fimbrial damage. Patients who have already undergone tuboplasty and those with inaccessible ovaries would be more suitable for I.V.F. In cases of history of ectopic pregnancy, I.V.F. could be a better option.

2. **Endometriosis**
   I.V.F. may be a suitable option for women with moderate to severe endometriosis and in those where medical and surgical therapy have failed. It may even be offered for mild to moderate endometriosis in the presence of other factors contributing to infertility.

3. **Unexplained infertility**
   Couples who have prolonged unexplained infertility would benefit from I.V.F. as many factors such as subtle ovulation defects, defects in ovum pick-up, gamete transport, tubal environment, sperm abnormality, oocyte abnormality may come to light when I.V.F. is used.

4. **Immunological factor**
   I.V.F. can be considered when there are antisperm antibodies in the male or the female and when other techniques such as immunosuppression, use of condoms, intrauterine insemination and other therapeutic measures have failed.

5. **Cervical factor**
   IVF should be offered for cervical factor if repeated attempts (6 to 8 cycles) of intrauterine insemination have failed and other therapies have not resulted in pregnancy.

6. **Male factor**
   I.V.F.-E.T. is logical therapy in the presence of low concentrations of sperm (less than 10 million - ml), low motility -less than 30%) and in the presence of abnormal (greater than 60% of abnormal forms as per Yovich). For severe male factor, assisted fertilization by means of micromanipulation can be offered. No universally accepted minimal sperm concentration for IVF success exists.

Techniques Derived From IVF

For patients with undamaged tubes there has been increasing recommendation of the use of transfer to the tube of gametes (GIFT), of zygotes (ZIFT) or of embryos (TET). GIFT is associated with higher levels of pregnancy than IVF but it has the drawback that it is unable to demonstrate the fertilizing capacity of the gametes.

As far as ZIFT and TET technologies are concerned, they are usually applied in cases of infertility due to a male factor, an immunological factor, or unknown cause,

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 it has been postulated that the transfer of zygotes or embryos to the tube surpassed the pregnancy rates achieved by means of conventional IVF with uterine transfer, there are no multi-centre randomized prospective studies, which prove 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

The Reservations with regard to IVF techniques have been of two types. On the one hand there are conceptual reasons such as the separation which they produce between procreation and sexual union, the non-existence of a life-threatening risk which justifies the application not only of IVF but also of any of the techniques of assisted reproduction. Likewise it would be wrong to overlook the allusions to the high cost which these therapies involve, bearing in mind too that they only bypass the problem and do not solve it definitively.

On the other hand there are drawbacks specific to IVF such as the risk from ovarian hyperstimulation and the necessary limitation to the number of embryos which may be implanted in view of avoiding the increasing risk of multiple pregnancies and the possible accumulation of frozen embryos with the responsibilities which this 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 500,000.

In the past six years, assisted fertilization procedures have been developed to circumvent the barriers that prevent sperm access to the ooplasma, 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 freezable 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 spermatozoa isolated from a shredded testicular biopsy.

I. **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) 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
- Retrograde ejaculation
- Sexual dysfunction

3. **Indications for ICSI with testiculor spermatozoa**

- All indications of 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) Masturbation problems at IVF
- Necrozoospermia

Recommendations

Patient counselling is an essential part of all forms of assisted reproductive technology. If ICSI has to be applied to alleviate the couple's infertility, the patients should be informed about the novelty of the ICSI procedure and about its many unknown aspects.

It may be recommended to ask the couples to have prenatal diagnosis and to participate in a prospective follow-up study of children born after ICSI. Although the results of about 1950 prenatal karyotypes and the prospective follow-up of 750 children does not indicate an increase in abnormal foetal karyotypes and in major congenital malformations, it is important to continue this careful follow-up of the children in different centers practicing ICSI.

Embryo Transfer Policy and Multiple Pregnancy in IVF

One of the remaining problems in a successful IVF program is the occurrence of high order multiple pregnancies following transfer of three or more embryos. 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. 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 adaptions 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 implantion 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 day 5 is the same as or higher than that obtained after ET on day 2 or day 3.

Serious consideration must be given 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.

Sperm Freezing

Donor Sperm

The technique of sperm freezing took only few years to come to a general consensus. Several cold chock preventing media had been tried before deciding on one diluting the sperm by no more than 30% and containing variable amounts of glycerol, egg yolk and other components. The sperm is kept in straws of either 0.5 or 1 ml, content and stored in liquid nitrogen. The storage procedure both for freezing and for thawing has been satisfactory mastered to ensure regularly that every donor insemination offered per cycle a fertilization chance that turned out to be close to nature in the human.

The safety of using frozen sperm has been abundantly proven both by experimental and veterinary work and ultimately by the considerable amount of healthy children born after donor insemination with frozen sperm. Whatever risks were considered were totally eliminated by the preceding large veterinary experience and the actual results in the human.

It is necessary to eliminate all donors carrying either venereal disease, hepatitis or, the HIV virus

Husband sperm

Clinicians using donor sperm banks soon realized that the same sperm banking could be appropriately used for husband sperm. It was mainly in cases of impotence that this approch was used. Indeed, some males do suffer from impotentia coendi, but are perfectly able to produce good sperm samples by masturbation. Since, for psychological reasons, this was not easy to do at the appropriate time of the cycle, sperm banking has been introduced to help couples suffering from such conditions. The procedure has further been extended in particular cases, such as husband absent for long times for professional reasons so that during their absence their sperm could be used at the appropriate timing of ovulation. An extensive literature deals with results of the use of sperm bank insemination in either natural cycles or cycles where ovulation had to be induced.

One of the drawbacks of sperm freezing was a +/ 20% loss of motility after thawing of the sperm, so that ejaculates where the seminological parameters are low cannot be considered as appropriate for freezing.

Recent progress in sperm freezing

During the very last years new techniques have been developed mainly in the field of in vitro fertilization. These techniques do offer 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 surgical approach is of course much lower