

Opinion on the evolution of practices concerning medically assisted procreation. Report.

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Opinion

Scientific progress and its medical applications in the field of human reproduction have created important ethical problems.

For the past ten years, the National Consultative Ethics Committee (CCNE) has been thinking about these problems, and has published the following opinions concerning them :

October 23 1984 : Opinion on the ethical problems created by techniques of artificial reproduction.

December 15 1986 : Opinion on research concerning, and the use of, human embryos in vitro for medical and scientific purposes.

December 15 1989 : State of the art as regards the Committee's studies concerning gamete and embryo donation.

July 18 1990 : - Opinion concerning the research on the embryo which has been subject to a moratorium since 1986 and whose aim is to allow a genetic diagnosis to be performed before transfer.

- Opinion on the present organization of gamete donations and its consequences.

June 24 1991 : Opinion on foetal and embryonic reduction.

In addition, these questions have been publicly discussed on several occasions during the days of annual deliberation organized by the Committee. In the conclusions of a report adopted by the CCNE in February 1993 on Dissociated Motherhood (see enclosed text) a working group recalled the importance of rigorous impartial epidemiological evaluation of the techniques for medically assisted procreation.

Introduction

Medically assisted procreation is proposed as one of the possible solutions to the human and social problem of sterility. In the face of this problem, certain couples prefer to resort to adoption, or give up the idea of having children. For the others, a doctor unable to remove the cause of their sterility may suggest palliative technical intervention.

The practices involved in medically assisted procreation may be perceived as being preferable to adoption, because they give a sterile couple the possibility of procreating. However, this situation, which lays special stress on the advantage for the couple of what is termed biological or genetic parenthood, should not mask the difficulties and uncertainties inherent in this medicalized procreation.

For our society, many issues are at stake in this new medical strategy for the treatment of sterility. These issues concern the following domains :

scientific knowledge, inasmuch as the practices concerned extend the possibilities of research on the biology of reproduction and embryonic development ;

medical institutions, because of the broadening of the physician's field of intervention, which in turn raises unprecedented questions concerning the extension and limits of his or her professional responsibility ;

economic factors and public health policy, insofar as these practices constitute a source of income for the medical profession and for pharmaceuticals manufacturers, and compete with other types of health care for financial coverage by the Social Security Service, and lastly,

social factors inherent in the unprecedented procedures and situations arising from the dissociation of sexual intercourse and fertilization.

The work of critical reflection which these issues require cannot be the exclusive prerogative of the CCNE and calls for efforts to organize coordinated discussion and consultation between the practitioners, specialists and those outside the medical world who are closest to the spheres in which the dilemmas of daily medical practice are encountered. The CCNE, which is anxious to promote these efforts, is prepared to help in their execution.

On the other hand, the CCNE wishes to recall that as in the case of any other medical practice which develops as a function of the research designed to improve it, elementary rules of caution must be observed, so that the health and safety of women, and of the children to be born thanks to these techniques, are not sacrificed to considerations of effectiveness and performance.

The CCNE also recalls that all new technical procedures to be applied to human beings must be submitted to rigorous evaluation, and for this purpose must be the object of a research protocol. This protocol must observe the general rules for the protection of persons stated in

The Nuremberg Code of 1947

The Helsinki Declaration of 1964 as revised in 1975

The Manilla Declaration of 1981, and

The French Code of Medical Deontology (Decree of June 28 1979).

The protocol must also conform to the Law of December 20 1988 on the protection of persons who agree to participate in biomedical research.

The requirement of freely given informed consent assumes that couples have been given clear and pertinent prior information on the advantages, drawbacks and uncertainties of the various solutions proposed.

The aim of the present report is also to draw attention to the possible consequences of certain methods currently undergoing experimentation, because these methods might contribute to the development of practices which must be the subject of thorough ethical and scientific discussion, such as genetic diagnosis based on the embryo in vitro or the transfer of genetic material.

A CCNE working group is at present examining and discussing the evolution of the indications for medically assisted procreation. Such procreation is being used increasingly often to avoid the transmission of hereditary diseases, and its use will be the subject of another report.

Evaluation of practices for the purpose of in vitro fertilization

Medically assisted procreation is undergoing extensive development : new techniques are evolving and the indications are broadening, in a general climate that favours a certain degree of precipitation in treating cases of sterility whose duration and preliminary characteristics do not always justify medical intervention. This climate means that rigorous epidemiological data are indispensable. Despite the efforts made by practitioners' associations and the studies by several teams, evaluation is still insufficient. Nevertheless, the Social Security Service agrees to cover the cost of assisted procreation techniques.

Studies on in vitro fertilization practices as a whole should be conducted by independent teams, and the criterion of success should not be pregnancy, but delivery, taking into account the child's state of health at birth. The conditions and possible mishaps attending attempts at fertilization, pregnancy and delivery are also essential criteria of this evaluation.

These studies should include the following points :

The indications, number of cases per indication and their evolution with time ;

The success rate for each indication, evaluated by relating the number of births to the number of stimulations and transfers performed, including indications of the number of embryos transferred per birth, and any use of frozen embryos ;

Negative events during pregnancy : extrauterine pregnancy, spontaneous abortion, embryo reduction, etc. ;

Condition of the infants, including the incidence of malformations, whether the embryos were transferred just after fertilization, after a few days of ex vivo culture, or after thawing, and lastly,

Information regarding

- the number of frozen embryos and their fate
- donation of oocytes, the indications and their results
- the sequence of events for each woman : repetition of fertilization attempts, births resulting from in vitro fertilization, and spontaneous pregnancies during or after attempts at in vitro fertilization.

It would be desirable to conduct studies on the physical and mental development of children born after medically assisted procreation, on condition such studies are not a cause of discrimination and do not impinge on family privacy or violate the rules of professional secrecy.

Ovarian hyperstimulation

The CCNE already gave its views on ovarian hyperstimulation in its opinion of June 24 1991 on embryonic and foetal reduction. Multiple pregnancies frequently result from this type of treatment. In view of the large increase in the number of multiple births during the past decade, it has now become indispensable to broaden ethical reflection to include ovarian stimulation as a whole, whether or not it is practised as part of an in vitro fertilization technique.

Ovarian stimulation treatments can have harmful consequences in the short or long term for the health of the mother and of the child to be born. The immediate complications of these treatments for the mother are well known, but for lack of adequate epidemiological studies, the possible long-term consequences, including the risk of ovarian cancer, are still hypothetical. As for multiple births, they constitute an increased risk of pregnancy complications for the mother, and a high risk of prematurity for the child, with all the short and long-term consequences this implies. In addition, multiple births involve serious social and economic problems for the family.

It is necessary to stress once again certain points in the opinion of June 24 1991 :

" Treatments capable of leading to multiple births such as ovarian stimulation of embryo transfer must not be instituted without transmitting complete information that prompts the patient - who, with the help of the medical team, is the one who must take the decision - to think seriously about the possible after effects of these treatments.

The doctors who use these techniques must be thoroughly trained and fully conscious of their possible repercussions, both for the couple and the children to be born, and should try to prevent multiple births caused by medical assistance from being more frequent than those that occur naturally" .

The instructions for the use of the necessary medical drugs should give fuller information about the possible consequences of these treatments.

Rigorous epidemiological studies should be undertaken right now to evaluate the short and long-term consequences of ovarian hyperstimulation for mothers and children.

These studies should not only deal with stimulation as part of the protocol of in vitro fertilization, but also as part of medical practice outside such fertilization.

It would be timely for these measures and their favourable and unfavourable consequences to be evaluated by an independent body, the more so as treatments that may lead to multiple births are financially covered in the framework of a public health campaign.

The methods of spermatozoid injection into an oocyte

Indications

The medical world has encountered many obstacles in its search for treatments of male sterility. One of the greatest was cultural - i.e. the refusal to admit that a man could be sterile. Because of this, artificial insemination with donor sperm has for long been considered the only medical procedure open to a couple wishing to have a child despite the husband's infertility. There are other solutions that do not require medical assistance, such as adoption, but the latter involves attitudes and social arrangements that differ from those involved in medically assisted procreation.

During the past few years, techniques of what is termed assisted fertilization have been developed within the framework of in vitro fertilization. These techniques enable the indications to be extended to cases of male sterility. Thus, failure of fertilization can be due to qualitative or quantitative anomalies of the spermatozoids, or to difficulty in penetrating the pellucid zone of the oocyte. In vitro fertilization by microinjection techniques enables the resort to donor sperm to be avoided in such indications.

In addition, the use of intraoocyte microinjection techniques in these cases constitutes an unusual therapeutic approach for the physician, because he is dealing with a man's infertility by treating his wife, who is fertile. These techniques are invasive and involve risks for the mother and child, all of which are not yet clear. The "advantages" expected from having descendants genetically linked to the couple can thus only be sought at the price of increased medicalization of fertilization, for as long as certain forms of male sterility cannot be cured by an etiological treatment.

Fertilization protocols

Up till now, in vitro fertilization protocols have only consisted of promoting favourable conditions for the gametes to meet. The choice of the egg to be fertilized for transfer to the mother's uterus or for freezing is confined to eliminating fertilized eggs with a clearly abnormal morphology known to be incompatible with embryonic development.

On the other hand, the protocol for Intra-Cytoplasmic Sperm Injection (ICSI) consists of direct invasive intervention, as the embryo is obtained after two procedures :

- choosing of the spermatozoid, for which there are not yet any well-defined criteria but which might in the near future be guided by biological data, and
- piercing the oocyte's protective membrane.

Contrarily to the recognized rules of medical research, the first human trials were conducted at a time when experimentation on non-human mammals was still very limited. The practice of ICSI in man is developing very fast, and more than two hundred children have been born in various parts of the world after fertilization by the intraoocyte injection of a spermatozoid.

Despite these initial results in humans, the CCNE wishes to stress the following :

- efforts must be made to find an animal model and develop experimental protocols that would provide solutions to the problems raised by spermatozoid selection and the piercing of the oocyte.
- couples must be informed of the experimental nature of the method, of its constraints and also of the other possible solutions to the problem of male sterility. They must be granted a period of reflection before making any decision ;

- the technique of intraoocyte injection must not be combined with other techniques of assisted procreation, especially cocultures on cell layers, because the piercing of the oocyte's protective membrane might facilitate the penetration of foreign material ;

- the trial protocol must be such as to permit rigorous evaluation. It is indispensable for the evolution of pregnancy and the state and development of the child to be assessed in a prospective study, which must deal with all trials without exception. Its results will have to be communicated to the CCPPRB (Consultative Committee for the Protection of Subjects in Biomedical Research) responsible for examining the trial protocol, and to the CCNE.

Lastly, it is desirable that only a few highly specialized centres be expressly approved for, and take part in, this evaluation.

Although the consequences of the use of new techniques of medically assisted procreation have still to be evaluated, the obvious efficacy of certain techniques, including fertilization assisted by intra-cytoplasmic spermatozoid injection, might be an incitement to broaden their indications or even turn them into usual methods. In addition, these techniques may open the way to the development of additional procedures whose scientific value, efficacy, innocuousness and ethical legality must be carefully examined, as regards, for instance, the choice of spermatozoid on the basis of biological criteria such as the choice of sex, the transfer of genetic material into the embryo, and prolonged culture of such material before its transfer for the purpose of conducting genetic studies on a larger number of cells in connection, for example, with the choice of sex, the detection of genes susceptible to certain diseases, or other genetic characters.

These examples show that extremely great vigilance is necessary in developing the new methods of medically assisted procreation.

Report

General data on the practice of artificial procreation in France

For several years now, great efforts have been made to collect data on artificial procreation activities in France, on the following points :

- in the field of artificial insemination with a donor (AID), thanks to the assistance of the Centres d'Etudes et Conservation du Sperme - CECOS (Centres for the Study and Preservation of Sperm) ;

- in the field of in vitro fertilization (IVF) thanks to the Groupe d'Etudes de la Fécondation in vitro en France - GEFF (Group for the Study of in vitro Fertilization) and the Association FIVNAT. The data now centralized by FIVNAT are obtained on a voluntary basis from the reproductive technology centres (there were such about 100 centres in 1992) and are analysed by Unit 292 of l'Institut National de la Santé et de la Recherche Médicale - INSERM (National Institute of Health and Medical Research).

The number of children born after AID is about 1 500 a year, and after IVF, about 4 500, i.e. nearly 1% of births during the past few years. These figures show all the benefit that these methods have brought to infertile couples.

In the field of IVF, more than 100 000 cases of puncture can today be analysed (the point of departure of all analyses is the puncture performed to collect oocytes from the mother). This analysis includes information concerning about 8 000 clinical pregnancies and 6 000 deliveries.

The first question that comes to mind is this : what is the significance of the FIVNAT study in relation to the sum total of IVF activities in France ? During the past few years, there has been a difference between the figures recorded for this activity by the GEF and FIVNAT respectively. Thus, for 1987, the GEF reported 24 000 punctures, and FIVNAT, 15 000, and in 1991, GEF reported 27 000, and FIVNAT, 19 500. This difference should diminish in the coming years thanks to improved data collection. At present, this collection is estimated to cover 90% of the centres in operation.

Evolution with time of IVF activities (report by FIVNAT)

This evolution has been characterized by a steady rise in the annual number of punctures recorded, which increased from 15 000 in 1987 to 19 500 in 1991.

With regard to techniques , the traditional technique of in vitro fertilization and embryo transfer was found to predominate. The other techniques, which at one time were proposed as improvements, have virtually been abandoned, i.e. Gamete Intra Fallopian Transfer and Zygote Intra Fallopian Transfer.

With regard to the indications , there has been an evolution.

- Tubal indications, which were the very reason for developing IVF, have remained stable in absolute terms. Isolated tubal indications account for 7 500 to 8 000 punctures a year. In 1988, they constituted 48.3% of the indications, but in 1992, only 38.6%. The figures for 1988 and 1992 are respectively 68 and 59% if one counts the tubal indications combined with other indications, especially among men ; tubal lesions were only permanent in a little more than half the tubal indications.

- The increase in the number of punctures is chiefly the result of rises in the number of two indications : those concerning men, i.e. in vitro fertilization with the husband's sperm (16.4% in 1992) or donor sperm (5.2% in 1992), and the indications for idiopathic sterility (13.7% in 1992) ; this heading covers cases of sterility lasting more than two years without identified causes.

Results

First of all the parameters of evaluation must be specified.

In the FIVNAT reports, there is a precise denominator : puncture for the collection of oocytes.

The numerical parameters can be the number of positive punctures (at present over 98%), the number of embryos transferred (now around 80%), the number of clinical pregnancies (i.e. a state of pregnancy defined by an echographic examination or a biochemical measurement such as a level of human gonadotropin - hCG - above 1 000 IU/L), the number of deliveries, and the number of live children.

Often, the number of clinical pregnancies has been used to evaluate the results, with large variations in the other evaluations, depending on whether the number of pregnancies is related to the number of punctures or the number of transfers. Evaluation of IVF based on the number of pregnancies is useful within the framework of a centre's activities for the following up of these activities. Account should also be taken of the rates of pre-term ending of pregnancies (5% of extra-uterine pregnancies, 25% of spontaneous abortions, and embryo reductions). Now that sufficient time has elapsed to obtain figures permitting statistical analysis, it would be preferable if in scientific communications, especially communications via the media, the success rate were evaluated by the number of deliveries of at least one live normal child. In 1990 and 1991, this rate was 13.7% in relation to the

number of punctures, and may have been a little higher in 1992. These figures are close to those cited in studies abroad : 14% in the United States and 12.5% in the United Kingdom.

As regards the evaluation of the results of reproductive technologies, there is a need to improve the collection of data on deliveries and the condition of the children. Such improvement is possible, as shown by studies conducted by the CECOS. In 1991, out of 1 700 pregnancies following AID, the outcome and condition of the children were only unknown in 1.3% of cases. Equivalent results were obtained for IVF with donor sperm, thanks to its conservation by CECOS.

On the other hand, of the total amount of data collected by FIVNAT, over 40% of the cases of pregnancy following IVF and their outcome are not included in the collection. It may be that the length of the form to be filled up had a dissuasive effect on reproductive technology centres. It should be recalled that the objective of reproductive technologies is the birth of a healthy child, and the best criterion of success is the number of children who go home with their mother after the delivery (the take home baby rate).

Analysis of the results of IVF shows differences in the success rate, which depends first and foremost on the age of the mother. A recent British study by the group of R. Edwards shows that under the age of 35, the success rate after one cycle of treatment, based on the number of deliveries, is 12 to 14% ; after five cycles of treatment, a rate of 45% can be expected. From 35 to 39 years, the success rate for one cycle is 7.7% and 29% for five cycles ; and from 40 to 45 years, the rate drops to only 2.8% for one cycle and 14% for five cycles. However, the calculations for five cycles were biased by the numbers of pregnancies abandoned, hence the selection of the best indications.

There are as yet no prospective studies in which the sequence of attempts, abandonments, spontaneous pregnancies and births is analysed for the same woman.

The variations in the success rate are fairly small as a function of the different indications, except for those in which fertilization was carried out with donor sperm, for which the results are better.

One factor that greatly affects the success rate is the number of embryos transferred . This is the subject of the second part of the present report.

Analysis of the data collected by the CECOS provides other items of information :

- *on frozen embryos* : in the CECOS centres, more than 22 000 embryos were frozen for 5 500 couples between 1985 and 1991, and 15 000 embryos were thawed. In 1991, 6 500 embryos were frozen for 1 400 couples and 3 800 were thawed. About 9 000 embryos remained in storage.

There are no other data in this connection except those collected by the CECOS. The total number of embryos frozen annually is believed to be around 30 000, and the number in storage is larger.

There is no reliable estimation of the success rate for the transfer of embryos after thawing. It seems that different teams have found very different rates, depending on freezing methods, which would give a very small mean success rate.

- *regarding oocyte donations* : a few centres have offered couples requesting it the possibility of receiving a donation of oocytes. After such donations, 250 couples were followed up in 1990-1991. The donations led to 5 births in 1990, 7 in 1991 and 3 in 1992.

Ovarian stimulation and its consequences

Under physiological conditions, a single mature oocyte is discharged in each menstrual cycle.

For more than thirty years, medical drug protocols that act on ovulation have been developed. At first, they were used to improve natural procreation by inducing previously non-existent ovulation, or to improve the quality of ovulation.

As IVF developed, the aim of these protocols was to place ovulation on a supra-physiological level in order to collect several oocytes and thus increase the chances of success.

Apart from the mother's age, the factor that greatly affects the IVF success rate is the number of embryos transferred. The results in terms of clinical pregnancy rates per transfer (unfortunately, the number of deliveries does not appear in the FIVNAT data) rise from 9% for a single embryo transfer to 18% for the transfer of two embryos and 26% for that of three, but this percentage hardly increases at all for transfers of more than three.

The medical drug protocols comprise two phases : one phase of stimulation whose aim is to obtain the maturation of several oocytes, and one phase of ovulation triggering. The second phase also makes it possible to programme the time of ovulation and therefore of in vitro fertilization within the framework of hospital management. The range of medicinal substances used and the number of protocols for their administration are growing steadily and their development has corresponded to that of IVF. The medicinal substances used in protocols designed to stimulate ovulation act on the hypothalamus (e.g. clomiphene citrate) or on the hypophysis (e.g. Luteinizing Hormone Releasing Hormone agonists) or on the ovary itself, through gonadotropins such as human menopausal gonadotropin (hMG). Ovulation is triggered by hCG.

To evaluate the results and secondary effects of ovarian stimulation methods, the only data of any importance concern IVF, thanks in particular to the FIVNAT surveys.

On the other hand, no data at all are available regarding the medical practice of ovarian stimulation, apart from IVF. Thus, nothing is known about the number of cases, the results or the complications. The elaboration of therapeutic protocols within the framework of IVF has increased the number of prescriptions by practitioners who are under pressure from anxious women after a few months' lack of success of their projects for conception, and from firms that produce the medical drugs required and circulate practitioners with IVF protocols. Thus, the sales of hMG rose from 500 000 ampules in 1985 to about 3 million in 1992. About 2 million of these ampules are thought to have been prescribed for other purposes than IVF, which would represent treatment for 30 to 50 000 women.

There is a risk that under these conditions, medical surveillance by hormonal measurements and echography will be less rigorous, and in the case of in vivo fertilization it is impossible to limit the number of oocytes that will be fertilized and implanted.

The complications resulting from treatments to stimulate ovulation may concern the following :

- the woman herself, with immediate consequences that are now well known, and long-term consequences that are still hard to appreciate ;
- the " products" of the conception ; here, the risks are connected with multiple pregnancies and their consequences for the evolution of pregnancy, and after birth, for the state of the child.

Complications in women

The ovarian hyperstimulation syndrome is a pathological iatrogenic ovarian manifestation caused by the inducers, and results in an increase in the size of the ovary and numerous cysts. There are a few severe forms that induce haemoconcentration and ascites, and the prognosis may be life threatening. This syndrome is moderate in about 6% of cases and severe in about 1 to 2%. Severe cases require hospitalization and intensive care or even resuscitation.

This exacerbated ovarian response is chiefly observed in young women and for this reason is more frequent when stimulation is prescribed outside the framework of IVF.

The long-term consequences are harder to appreciate, as the follow up period presently available since IVF practices became general is short. There is no epidemiological study of women who have had ovarian stimulation apart from IVF. Ovarian hyperstimulation is not very physiological, and has been suggested to cause ovarian scarring that might lead to ovarian dystrophy. Hyperstimulation has also been suggested to have a role in the development of endometriosis. In addition, it might possibly involve a risk of early menopause, but no epidemiological studies have been conducted in this connection either.

A recent event in this domain was the announcement of a rise in the frequency of ovarian cancer and granulosa tumours among women who had had ovarian hyperstimulation. According to some studies, this risk might have increased five or sixfold.

Note, however, that among the general population, these types of cancer are relatively rare. If ovarian treatments were to double their incidence, this would amount to 600 cancer cases a year, but with a bad prognosis. A link between the cumulative number of ovulations and the risk of this cancer has been reported.

Many practitioners have noticed the frequent occurrence of breast cancer among young women, sometimes in serious forms. Present knowledge of the genesis of breast cancer and its hormonal dependence indicates that ovarian stimulation might play a role in causing this disease.

Rigorous epidemiological studies are absolutely indispensable, but they will be long and difficult. Right now, the potential risks inherent in the indications for ovarian hyperstimulation should be taken into account, especially outside IVF, among young women who are only hypofertile, but are impatient to conceive a child. The transmission of adequate information by practitioners should avoid excesses in connection with stimulation treatments.

Is there any need to recall that in the recent past, the abusive prescription of diethylstilbestrol (Distilbene) had dramatic far-reaching effects on girls exposed to it in utero ?

The problem of multiple pregnancies

This is the most obvious visible problem, which is serious for the mother and the children.

Information in this respect is only available for live births.

The FIVNAT survey shows that after IVF, there are 27.4 of multiple births for every hundred deliveries, including 23% of twins and 4% of triplets, i.e. 133 children per hundred deliveries.

Demographic surveys of the population as a whole have shown that the frequency of multiple births has been rising steadily since 1972, and more especially since 1984. For the overall population, the incidence of twin births increased from 8.8 per thousand in 1972 to

4.4 in 1990, i.e. by 37%. The frequency of triplets among all births rose even more spectacularly, from 0.9 per 10 000 births in 1972 to 4.4 in 1989, which corresponds to a rise of more than 450%. A similar evolution was observed in the United Kingdom, the United States and Australia.

According to these figures and the results of the FIVNAT survey, IVF is responsible for about 25% of these multiple births, and medical indications for ovarian stimulation account for over 50%.

Note that the rising curve for triplet births, which became steeper from 1984 to 1989, is parallel to the rising curve for the sales of hMG hormone, whose role in the occurrence of multiple births has been demonstrated.

The medical problem is the seriousness of multiple pregnancies for the expectant mother, her children and subsequently the family.

A multiple pregnancy involves risks, and a marked increase in all the complications of pregnancy (toxaemia, possible premature delivery, placing of a ring around the uterine cervix, premature rupture of the membranes and caesarian section).

The risk for the child rises with the number of births.

After IVF, the premature birth rate is 9% for single births (which is higher than the corresponding figure of about 5% for the general population) to 90% for triplets. Thus, 11% of single birth children, 42% of twins and 89% of triplets are transferred to neonatal or intensive care departments. Infantile mortality is three times higher among twins and thirteen times higher for triplets than for single birth babies.

It would also be desirable to evaluate the long-term after-effects of multiple births, especially for premature children. It is known that among those born before 31 weeks of gestation, 10 to 25% will be subject to after-effects, half of which will involve a severe handicap. In the results for IVF, these highly premature births account for 5% of twins and 24% of triplets. A recent Australian study showed that at the age of one year, cerebral palsy is eight times more frequent among twins and 40 times more frequent among triplets than among single birth children.

These results show that of every hundred children born after IVF, 21 will be sent to neonatal care departments, and 8, to intensive care units ; 3 will die and 4 to 5 will be highly premature. These figures are three times those observed for births without IVF.

It is also appropriate to recall the psychological, social and economic problems of mothers who have to bring up twins and more especially triplets.

It should be stressed that the results available today only concern multiple pregnancies ending in birth. There is no evaluation of the frequency of multiple pregnancies at their inception and their subsequent outcome, especially in cases of ovarian stimulation without IVF. The problems arising from embryonic reduction performed as a palliative for multiple births are already known (see the CCNE opinion of June 24 1991). According to the FIVNAT survey, this reduction was performed in 2.4% of the pregnancies that led to a birth. The number of reductions causing the termination of pregnancy is not known.

In view of the frequency of multiple pregnancies and the seriousness of their consequences, IVF centres have tried to reduce their number. Already, during the past three years, there has been a moderate decrease in the number of embryos transferred : thus, in 1988 and 1989, 4 or more were transferred in 38% of cases ; in 1990, 32%, in 1991, 25% and in 1992, 23%. A FIVNAT analysis has shown that the most important factors affecting the frequency of multiple births were the number of embryos transferred, and the age of the mother. On the basis of these findings, FIVNAT proposes a strategy that would limit multiple

births of triplets or more to 3%. This is not sufficient, and the aim should be to limit triplet births to 1%.

In the United Kingdom, the Good Practices Code of the Human Fertilization and Embryology Authority restricts the number of embryos transferred to three. Certain centres, including the centres in Hammersmith (Pr Wilson) and in Belgium (Pr Schoysman) and a few French centres are cutting this down to two and have reduced triplet births to 1%.

In this domain, there is a very real debate, and an ethical choice, between the success rate for IVF and the attempts to reach a record level on the one hand, and the serious consequences of multiple births with a record-breaking frequency that it would be better to avoid on the other.

In 1991, the commercial transactions connected with IVF activities in France totalled about a thousand million francs, for a number of children which was probably around 4 000. About 900 of them were hospitalized at birth in neonatal care or intensive care departments. Financial evaluations of the cost of this practice should take account of the extra cost of looking after these children. Is it necessary to recall that a day of hospitalization in a neonatal intensive care department costs about 8 000 FF and that for a child weighing less than 1 500 g, the mean total cost is estimated at 450 000 FF ?

Current research in reproductive technologies

This research aims at improving the results of IVF.

Methods designed to improve the success rate of fertilization : assisted fertilization

In the fertilization process, the spermatozoid has to cross three layers to transmit its genetic material to the oocyte : the cumulus oophorus cell layer, the pellucid zone and the oocyte's plasma membrane. The pellucid zone constitutes a specific obstacle as it cannot be penetrated by a spermatozoid of another species.

The indications for IVF have been extended to cases of infertility in which a male factor is demonstrated or suspected. This can happen in the following circumstances :

- repeated failure of IVF with sperm that was apparently normal but in which no oocyte was fertilized, suggesting that the cause was the non penetration of the pellucid zone by the spermatozoid ;
- failure of IVF with abnormal sperm ;
- abnormal sperm, agenesis and obstruction of the deferent duct.

In these last cases, IVF by conventional methods was not formerly envisaged, and it is the advent of new methods of assisted fertilization that might permit it to be extended to these indications.

Several such methods have been proposed and are under study, including

- perforation of the pellucid zone, by mechanical or chemical means, or simply by a " scrape" . These techniques are designed to ease the passage of the mobile spermatozoids towards the oocyte ;
- insertion by microinjection of a few spermatozoids under the pellucid zone in contact with the oocyte membrane (SUZI, Subzonal Insemination) ; and lastly,

- intracytoplasmic injection of a spermatozoid (ICSI, Intra Cytoplasmic Sperm Injection).

The largest number of attempts has been made with the SUZI method of microinjection. However, in relation to the number of couples for whom this was tried after IVF had failed, the results seem poor : thus, the fertilization rate was 17%, so that few embryos resulted, and only 5 to 8% of clinical pregnancies.

Some results are available concerning the condition at birth of the children born after the use of the SUZI technique, especially the results obtained by the team of J. Cohen in New York. According to the State of New York's malformation criteria, major and minor malformations occur in 6.4% of births among the general population, 8.2% of births after "conventional" IVF, and 9.5% after SUZI.

The Belgian team of Van Steirteghem recently reported very favourable results with the ICSI technique. Their experience covered 800 punctures that permitted the harvesting of 7 900 oocytes. After microinjection, 88% of the oocytes remained intact, and two pronuclei (diploid state) were present in 65% of the oocytes injected, a result that was better than that for conventional IVF. The success rates for clinical pregnancies were very satisfactory after the transfer of two embryos (24% of pregnancies) or of 3 embryos (35% of pregnancies). In view of these rates, the Belgian team is now limiting transfers to two embryos, to avoid triple pregnancies.

As these different investigations are recent, it has not yet been possible to analyse the state of the children.

With the ICSI technique, three important steps have been taken in the practice of reproductive technologies :

Not a single successful experimental study has been conducted with this method on non human mammals . Experiments with the ICSI technique on mice, hamsters and rabbits failed (in particular, those done by the Belgian team using the human protocol).

Selection of the fertilizing spermatozoid

The spermatozoid population always constitutes a mosaic. On the cytogenetic level, there is a non negligible proportion (about 7%) of anomalies of chromosomal structure (deletions, translocations, etc.), in addition to the difference in the sexual chromosome that determines the sex of the embryo. On the molecular level, certain germinal mosaics are known to be capable of causing neomutations giving rise to monogenic diseases, particularly in sex-related diseases like Duchenne's muscular dystrophy and haemophilia. In diseases resulting from one form of molecular instability (i.e. lengthening of a triplet) great variety was recently observed as regards the characteristics of triplet lengthening in the sperm, especially in Huntington's disease.

With respect to spermatozoid selection, many questions have arisen and still await answers.

- What should one think of the fact that a spermatozoid is collected for fertilization randomly, thus eliminating the usual "competition" that occurs in natural fertilization processes or in conventional IVF ?

- No animal model of hypofertility due to defective spermatogenesis is available. Selection by animal breeders has eliminated bad breeding stock.

- One might think that genetic anomalies, the cause of male sterility, could be transmitted to descendants.

- One wonders about the consequences of the different manipulations to which spermatozooids are submitted, manipulations whose harmlessness has not been

demonstrated.

Piercing the plasma membrane of the oocyte .

This is an important stage in the evaluation of the techniques practised on the oocyte, and no preliminary experimentation has allowed its possible consequences to be evaluated.

In animals, transgenesis techniques consist of introducing genetic material into the fertilized egg, either at the single cell stage, when this is preferably done by injection into the paternal pronucleus, or at the two-cell stage. The aim of these experiments is to obtain a few transgenic animals, but no overall evaluation exists concerning the other consequences of the technique for the animals born after this manipulation.

Recent animal experiments in which nuclei were transferred into the cytoplasm of another zygote suggest that the cytoplasmic environment of the nucleus might have long-term effects on development. In this case, experimental conditions were a little different from those prevailing for the intraoocyte injections.

It is known, from the first human trials, that about one out of ten oocytes does not survive injection.

However, for the oocytes that remain intact, can there be other risks or adverse consequences ?

- the opening of the plasma membrane might favour the penetration of foreign genetic or other material ;

- the trauma due to the micropipette might harm the cytoplasm's components (membranes, organelles, etc.) and lead to epigenetic changes affecting embryogenesis and development. These changes could in turn lead to anomalies in the child's development, or to other types of disorder.

Methods designed to improve the successrate of transfers

Cocultures

The techniques presently used for human embryo culture in artificial mediums do not, in practice, permit evolution to the blastocyst stage. Embryo transfer must therefore be done very early, at the 4 to 8-cell stage.

Development up to the blastocyst stage should allow more detailed morphological examination, such as would lead to the selection of the embryos with the best chance of developing normally in utero after transfer.

This preliminary culture does indeed allow the elimination of the embryos that are incapable of reaching the blastocyst stage normally. The chief aim of this selection is to raise the percentage of successes while reducing the number of embryos transferred to the mother, since each one has a better chance of leading to a normal pregnancy.

Some of the teams performing transfers of the blastocysts obtained by culture on cell layers restrict the number of embryos transferred to two, thus reducing the risk of multiple pregnancies.

In addition, the preselection of embryos capable of developing ex vivo up to the blastocyst stage might, according to some authors, improve resistance to freezing, which is presently extremely weak, thus explaining the very poor results obtained today after the transfer of frozen embryos.

Another objective of certain teams is the availability of multicellular embryos, from which a few cells could be sampled for preimplantation diagnosis.

Technical aspects

The extension of human embryo culture until the fifth day requires nutritional or growth factors which are supplied by the presence of an under-layer of epithelial cells. Two sources are envisaged for these cells :

- human cells from the cumulus oophorus follicular cells surrounding the oocyte. Culture of these cells starts when the oocytes are collected, but its success is not always certain, and in practice, the use of the cells so cultured is limited.

- cells from an established cell line. Embryonic human cell lines are all fibroblastic and are not suitable for embryo culture. As there are no non-transformed human epithelial cell lines, the cells used - called Vero cells - come from a monkey epithelial cell line.

The Vero cell line was established in 1962 in Japan by Y. Yasumura, from the kidney cells of an African green monkey (*Cercopithecus aethiops*). Dr. Yasumura brought these cells to the NIH in 1964, at their 93rd passage. In France, the cells used by the Institut Mérieux were received from the United States in 1979 at their 129th passage. The cells used to prepare viral vaccines come from a stock of cells frozen at their 137th passage. It is on this stock of cells that all the verifications for infectious or oncogenic risks were made, and the negative results of this screening led to the licensing of the stock for the preparation of vaccines.

Although the firm of Mérieux refuses to accept any responsibility for the use of these cells for the culture of human embryos, they are already widely used for this purpose by many specialists in medically assisted procreation, particularly those responsible for elaborating it. However, the cells marketed under the name Vero include lines that have undergone different numbers of passages in different laboratories. To a certain extent, genetic markers make it possible to verify the phenotype of these different cell populations.

Scientific evaluation of cocultures

The absence of adverse effects of embryo coculture on subsequent embryonic and foetal development seems to have been established by a certain number of animal experiments. In man, there are at least twelve IVF centres in France that practise this technique, which has resulted in evolutive pregnancies.

The first known or published results for humans do not make it possible to state that the percentage of successes is today larger when the embryo is transferred early. Strictly controlled studies are necessary to estimate the real benefit of this technique.

In the light of present knowledge, it would be hazardous to prolong cocultures beyond the fifth day. In addition, the embryo matures at that date and leaves the pellucid membrane that protects it. Prolonging its culture would involve the risk of depriving the maternal organism of biological information which is transmitted by the embryo as from the third day and might have a role in the implantation process.

Theoretically, there might be a problem because of the great fragility of the genome of embryos cultured in vitro. Mosaic mutations have been known to result from embryonic events, and in certain cases the aggravation of genetic anomalies is known to be possible at the time of the first mitoses of the embryo. This explains the occurrence during embryonic development of an increase in the number of nucleotide triplet repeats which is at the root of the manifestations of the fragile X syndrome or of myotonic dystrophy (Steinert's

disease). Although there is so far no experimental evidence in favour of such genetic fragility in cocultures, the problem nevertheless deserves to be identified and considered.

A more critical factor in the evaluation of cocultures is their biological safety for the embryo and the mother. The issue here is the risk of contamination of the cell underlayer by infectious agents such as mycoplasmas, viruses or possibly prions.

In the case of recent stocks of Vero cells, duly checked and validated for use in the production of human vaccines, the risk seems very small. On the other hand, it could be far from negligible when many different subcultures of Vero cells are made in the user's laboratory, or when cells whose source and phenotype are uncertain are involved.

In the case of follicular cells, there is no intrinsic a priori risk of contamination. However, the manipulations necessary to establish a culture could, in practice, cause such contamination. This is why none of the culture methods envisaged seems to have any obvious advantage over the others from the point of view of biological safety. The evidence shows that the use of completely synthetic culture mediums containing the necessary growth factors would, from this point of view, be greatly preferable.

In this connection it should be noted that the biological products currently used in the IVF technique are products designed for diagnostic use in biological laboratories, and not for human use. Certain forms of these products on sale are labelled " for human use" without any definition of the criteria of quality applied to them. The only clearly changed feature is the large rise in their price. No quality control seems to have been established.

Selective assisted maturation

The elaboration of this technique was based on the assumption that in certain cases, the pellucid zone prevents the spontaneous maturation of the embryo necessary for its implantation.

After the oocyte has been fertilized by the spermatozoid, a reaction called the zonal reaction occurs in the pellucid zone. It seems to lead to a hardening of this zone which might prevent other spermatozoids from penetrating it after fertilization. This hardening of the pellucid zone is useful because it blocks polyspermy and also protects the embryo, especially during its transport in the fallopian tubes. Once the embryo is in the uterus, it has reached the blastocyst stage ; the pellucid zone becomes thinner, and the maturation of the blastocyst allows its nidation in the endometrium.

Among the hypothetical reasons agreed on for the failure of implantation, J. Cohen in New York raised the possibility that the pellucid zone might not open, thus preventing embryo maturation and implantation. To avoid this, two techniques for the artificial opening of this zone may be envisaged : one consists of dissolving it by the use of an acidified culture medium (this technique has been criticized), and the other, which is mechanical, consists of making a small split in the pellucid zone by micromanipulation, after which the embryos are returned to the culture medium, observed and transferred. According to recent information, this technique does not seem to constitute any great progress.

Comments by the working group on" Dissociated Motherhood"

Introduction

Although the first definition of motherhood centres on the mother, the one who has given birth, and refers to delivery, it is clear that the notion, like that of fatherhood, is complex.

Besides biological motherhood, there is also legal, social, educative, emotional and foster-motherhood.

Throughout the ages, the continuity of the different stages of motherhood has sometimes been broken. In the 19th century, the development of the custom of entrusting a child to a foster-mother provides an example of a practice widely accepted by society. Another example is that of having recourse to a surrogate mother in cases of female sterility, a practice that prevailed long before the advent of artificial insemination. All such situations can be defined as dissociated motherhood.

It is in fact the recent surge of artificial reproduction practices that has given new meaning to this notion. By breaking the hitherto indissoluble chain of events of conception and gestation, the collection of oocytes and in vitro fertilization have given rise to a new form of motherhood dissociation, into genetic or ovarian motherhood, and gestational or uterine motherhood. This dissociated biological motherhood is the subject of the present report.

The National Consultative Ethics Committee stated its attitude to the practice of surrogate motherhood in its opinion of 23/10/1984, and to the donation of embryos, in that of 15/12/1989. In the first case, it was unfavourable, and in the second, it only agreed to the principle of donation under stringent conditions.

Since then, the Committee has given additional thought to these questions, grouping them together under the general heading of Dissociated Motherhood. A few of its members formed a working group and together examined the different aspects of the problem - medical, biological, social, philosophical, anthropological and legal. They asked for the cooperation of leading figures outside the Committee, who provided valuable assistance. The result was two series of reports drafted by the two categories of experts taking part in the study.

Taken together, these reports did not provide an opinion that could be described as final, but a summary of the questions at issue and the precautionary rules which it is at present important to observe, in the light, also, of the parliamentary work now in progress.

This summary should only be considered as a necessary stage in the drafting of a synthesis that will reflect the CCNE's views in the face of the evolution of the problems concerning procreation.

Conclusions

The collection of oocytes and in vitro fertilization have given rise to a new form of motherhood dissociation - i.e. biological dissociation, by breaking the hitherto indissoluble chain of conception and gestation. The present text deals with this biological dissociation. The introduction into medical practice of techniques for such dissociation is already prompting us to revise our ideas of parenthood and filial relationships, at a time when reflection should find a place for an analysis of the role that is now incumbent on medicine, above and beyond the task of treatment.

It should be stressed that the donation of oocytes and the donation of embryos only constitute palliatives to infertility and do not treat its cause. Anything that tends to facilitate, develop or favour their acceptance must not, for that reason, reduce either overall efforts to search for preventive or curative solutions to sterility, or individual recourse to other remedies.

The donation of oocytes and the donation of embryos imply the use of techniques involving risks which, although infrequent, should not be underestimated.

- For the oocyte donor, these risks are inherent in sampling because of hormonal treatments or even the act of sampling.

- For the recipient, the risks are infectious and are inherent in any transfer of human material ; whatever precautions are taken, they can never be completely eliminated.

- For the parents and children, there may be psychological effects which are not presently known but deserve exploration.

Certain principles that today govern the modalities relating to these practices deserve more thorough investigation, in particular, secrecy with regard to modes of conception, the anonymity of oocyte donors and of couples donating embryos, and the recourse to related donors.

Acceptance of an oocyte or embryo donation for certain genetic indications, and donor screening to avoid the transmission of a serious inherited disease, must be subjects of reflection designed to secure a balance between preventive medicine and the risk of abuse for eugenic purposes.

The availability of embryos outside the initial parental project raises specific problems that go beyond gamete donation and are not confined to the domain of medical competence and responsibility.

The fact that at the present time it is impossible to grasp all the problem raised by the donation of oocytes and the donation of embryos, let alone find permanent solutions for them, implies the need for especially great vigilance with regard to these practices and their indications.

Their application should be confined to a limited number of centres with experience of clinical, biological and psychological research in this field. Research work should not only be subject to authorization by a competent body but also to a follow up of the results.

In addition to assessment of the problems concerning research protocols, the results of these practices as a whole should be analysed statistically and undergo short, medium and long term evaluations of their results.

The objective nature of this analysis and these evaluations would be guaranteed by independent survey of the biomedical organizations responsible for running this type of activity.