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RECENT ADVANCES IN MEDICALLY ASSISTED CONCEPTION

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1. Introduction

A WHO Scientific Group on Recent Advances in Medically Assisted Conception met in Geneva from 2 to 6 April 1990. The meeting was opened on behalf of the Director-General by Dr T. Varagunam, Responsible Officer for Resources for Research, WHO Special Programme of Research, Development and Research Training in Human Reproduction.

Two previous Scientific Groups have been convened by WHO to advise the Director-General on the subject of infertility, of which the first, in 1973, was concerned with agents stimulating gonadal function (1) and the second, in 1975, reviewed the epidemiology of infertility (2). There have been dramatic improvements in the investigation and treatment of infertility in the 17 years since the first of these meetings; public and scientific attention has focused on techniques for the induction of ovulation, extracorporeal fertilization and the subsequent transfer of the fertilized ovum to the mother.

More than a decade has passed since the birth of Louise Brown in the United Kingdom as the result of the first successful *in vitro* fertilization. Since then, there have been many thousands of such births in clinics throughout both the developed and the developing countries. The techniques of ovarian stimulation, oocyte recovery and storage, *in vitro* fertilization, gamete replacement and monitoring of subsequent outcome have been considerably refined, but the ultimate success rate as judged by the live-birth rate remains stubbornly low, in most clinics not exceeding 25%. Given that the conventional treatments of ovulatory disorders result in pregnancy rates of 60-80%, much remains to be learned about the factors influencing the success of what may be called medically assisted conception. This term has been used for the purposes of the present report to cover *in vitro* fertilization, gamete intrafallopian transfer (GIFT) and similar procedures, as well as the longer-established techniques of artificial insemination either by the husband or by a sperm donor.

The considerable scientific and public interest in this area of reproductive medicine has provoked intense debate on the associated ethical, moral and legal issues, a debate that continues in many countries. Medically assisted conception has ramifications beyond the treatment of, for example, women with blocked fallopian tubes or couples in whom the male partner has oligospermia, and covers matters such as the very early detection of genetic disease and the genetic manipulation of ova or spermatozoa.

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1. WHO Technical Report Series, No. 514, 1973 (*Agents stimulating gonadal function in the human:* report of a WHO Scientific Group).

2. WHO Technical Report Series, No. 582, 1975 (*The epidemiology of infertility:* report of a WHO Scientific Group).

2. Background

2.1 Magnitude of the problem of infertility

Infertility affects both men and women of reproductive age in all areas of the world, causing considerable personal suffering and disruption of family life. Although estimates of its prevalence are not very accurate and vary from region to region, approximately 8-10% of couples experience some form of infertility problem during their reproductive lives. When extrapolated to the global population, this means that 50-80 million people may be suffering from infertility (*1*); an accurate assessment of its prevalence is not easy to make, although relevant information can be obtained from a variety of sources.

2.2 Demographic and epidemiological sources

Comprehensive surveys derived from a variety of sources include the World Fertility Survey (*2*) and WHO epidemiological studies (*3-5*). In the WHO studies, infertility was defined as "two years of exposure to the risk of pregnancy without conceiving" (*4*). It should be noted that this is different from the usual clinical definition of infertility, namely inability to become pregnant after one year of regular unprotected intercourse (*6*).

Table 1 shows the primary and secondary infertility rates in selected developing countries, and gives some indication of the regional and interregional variations in these rates.

2.3 Clinical studies

The etiology of infertility is of major importance if any therapeutic or preventive measures are to be implemented, but assessment of the cause of infertility at the level of the individual couple is often difficult and time-consuming. While various factors that may contribute to a couple's infertility can be identified following a full and comprehensive investigation of both partners, it is often difficult to attribute the infertility to any one factor and thus clearly identify a "cause of infertility".

From 1980 to 1986, the Task Force on the Diagnosis and Treatment of Infertility of WHO's Special Programme of Research, Development and Research Training in Human Reproduction conducted a study involving over 8500 couples in 33 centres in 25 countries throughout the world, using a standardized protocol for the investigation and diagnosis of the infertile couple (*6, 8-10*). The characteristics of these couples, analysed on a regional basis, have been described elsewhere (*9, 10*). The principal regional differences were between the four sub-Saharan centres (in Cameroon, Kenya, Nigeria and Zambia) and the 16 in developed countries (including 11 European centres), while the results obtained in the other centres in developing countries (in Asia, Latin America and the Eastern Mediterranean) were in general intermediate between these two extremes.

Table 1
Primary and secondary infertility rates in selected developing countries[a]

Country	Primary (%)	Secondary (%)	Country	Primary (%)	Secondary (%)
Africa			Fiji	4	—
Benin*	3	10	India*	3	8
Cameroon*	12	33	Indonesia	7	15
Kenya	4	7	Korea, Republic of	2	6
Lesotho	7	17	Malaysia	4	9
Senegal	6	13	Nepal	6	12
Sudan	7	10	Pakistan	5	10
United Republic			Pakistan*	4	24
of Tanzania*	5	25	Philippines	2	5
			Sri Lanka	4	11
Americas			Thailand	2	11
Brazil*	2	30	Thailand*	2	13
Colombia	4	4	Viet Nam*	2	15
Costa Rica	2	6			
Guyana	9	9	*Caribbean*		
Mexico	3	6	Dominican Republic	6	8
Panama	3	8	Haiti	7	5
Paraguay	3	8	Jamaica	7	6
Peru	3	4	Trinidad and Tobago	5	6
Venezuela	2	7			
			Eastern Mediterranean		
Asia and Oceania			Jordan	3	2
Bangladesh	4	15	Syrian Arab Republic	3	3

[a] Data from the World Fertility Survey (2) or, for asterisked entries, from WHO epidemiological studies on the prevalence of infertility (3–5).

Primary and secondary infertility are defined as follows:

World Fertility Survey
Primary infertility: the percentage of women aged 40–44 years married for at least five years who are childless (2).
Secondary infertility: the average percentage of women aged 30–40 years exposed to the risk of pregnancy for at least five years without conceiving, minus the primary infertility rate.

WHO
Primary infertility: the percentage of never pregnant women exposed to the risk of pregnancy for at least two years without conceiving (7).
Secondary infertility: the percentage of women aged 30–39 years who, having had at least one pregnancy, have been exposed to the risk of pregnancy for at least two years without conceiving.

The distribution of the most common specific causes of infertility in women in sub-Saharan Africa and developed countries derived from these studies is shown in Figs 1 and 2 respectively, while Figs 3 and 4 show the causes by partner, again for sub-Saharan Africa and developed countries respectively. In women, the percentage of diagnoses that could be attributed to infectious causes varied between 28% and 65% in different centres. The rate of tubal occlusion in sub-Saharan Africa was over three

Figure 1
Distribution of most common specific causes of infertility in women in sub-Saharan Africa

Endometriosis 0.9%
Hyperprolactinaemia 4.3%
Acquired tubal abnormalities 10.3%
Pelvic adhesions 11.1%
Bilateral tubal occlusion 41.9%
No demonstrable cause 13.7%
Ovulatory disorders 17.9%
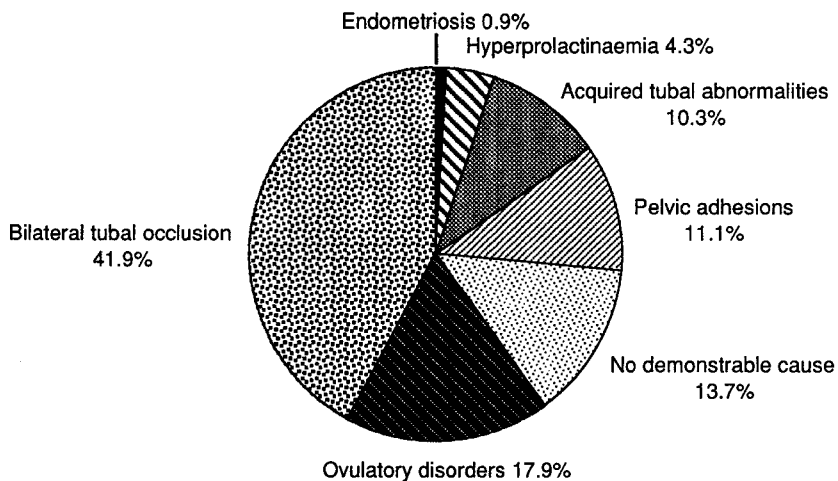
WHO 91755

Figure 2
Distribution of most common specific causes of infertility in women in developed countries

Pelvic adhesions 12.4%
Endometriosis 5.7%
Bilateral tubal occlusion 10.6%
Ovulatory disorders 24.8%
No demonstrable cause 28.6%
Acquired tubal abnormalities 11.2%
Hyperprolactinaemia 6.7%
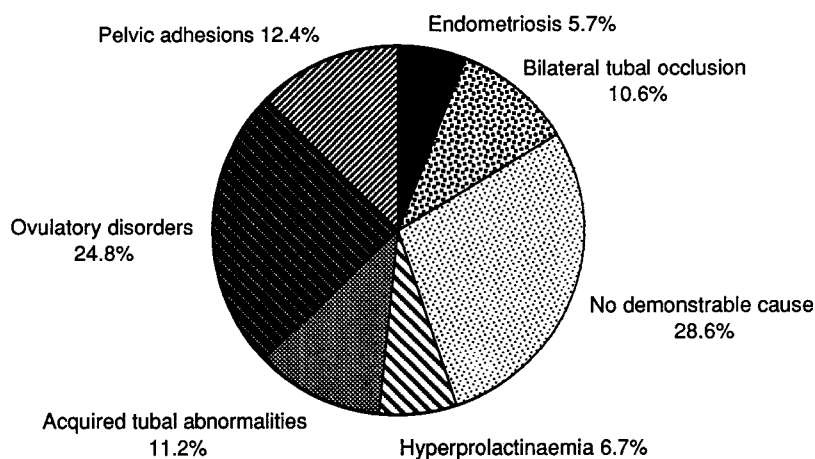
WHO 91756

Figure 3
Distribution of causes of infertility by partner in sub-Saharan Africa

Male cause only
22%

Became pregnant during investigation
12%

No cause found 14%

Cause found in both 21%

Female cause only
31%
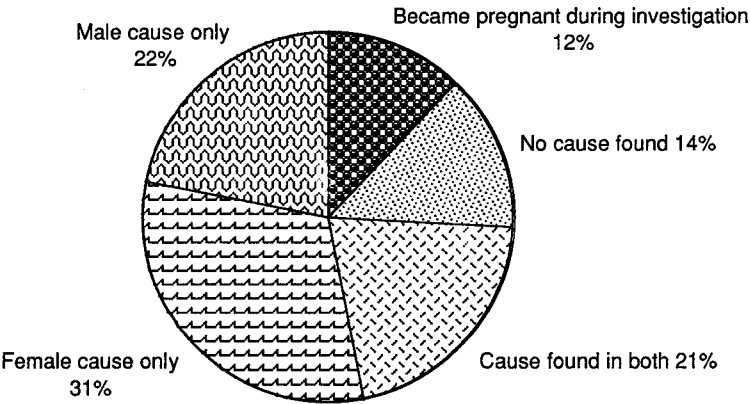
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Figure 4
Distribution of causes of infertility by partner in developed countries

No cause found
5%

Male cause only
8%

Cause found in both
35%

Became pregnant
during investigation
15%

Female cause only 37%
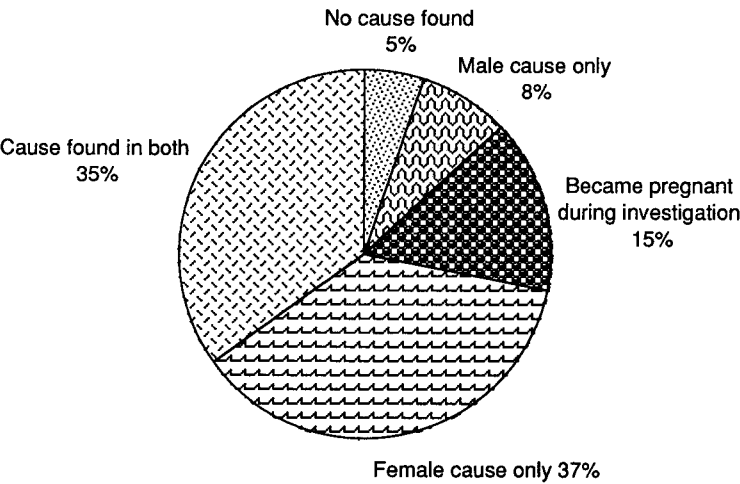
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times that seen in other regions, with the exception of the Eastern Mediterranean. All the developing countries had rates of tubal infertility higher than those in the developed ones. The patterns of male infertility are less clear, but regional variation is seen in the rates of varicocele and accessory gland infection.

Marked differences between the regions are seen in the rates of infection-related diagnoses – bilateral tubal occlusion and pelvic adhesions in the female partner and accessory gland infection in the male. The percentages of women reporting a history of sexually transmitted disease or a postpartum or postabortal infection-related complication were 9% and 8%, respectively, in the sub-Saharan African countries, over three times the rates found in the developed countries. Other developing countries more closely resembled the developed countries than the sub-Saharan African ones.

While the role of sexually transmitted disease (mainly gonococcal and chlamydial infections) and pelvic inflammatory disease in the genesis of female infertility has been recognized for some time, the male partner has largely been neglected. If the data from all subjects in the WHO study in whom a diagnosis was reached in the female partner are taken and women with infection-related diagnoses but with no history of infection in either partner are used as the reference group, there was a 56% higher relative risk of an infection-related cause being found where a positive history of infection was obtained from the male partner alone, 82% in the cases of infertility with a female-only history of infection, and 124% where both partners had a positive history (6).

Other etiological factors in tubal obstruction or pelvic adhesions include postpartum and postabortal infection, tuberculosis, acute appendicitis, filariasis, schistosomiasis, iatrogenic causes, and traditional practices involving the female genital tract such as female circumcision; further studies are needed to determine the importance of these factors, especially in developing countries.

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3. Historical overview

3.1 Origins of medically assisted conception

The aim of medically assisted conception is to overcome the barriers preventing spermatozoa from encountering oocytes in infertile couples. The origins of such technologies can be traced to attempts to fertilize human oocytes outside the body. Although mammalian oocytes were fertilized extracorporeally at the end of the last century (*1*), *in vitro* fertilization of human oocytes was accomplished only in 1944 by Rock & Menkin (*2*). Since then, the procedures for handling gametes have been refined and have led to various techniques for the transfer of either gametes or embryos to the woman (Table 2).

3.2 Artificial insemination with the husband's semen

This form of artificial insemination has been practised for many years and various procedures have been developed, including intravaginal, intra-cervical and intrauterine insemination. The first successful vaginal arti-ficial insemination with the husband's semen (occasioned by infertility due to hypospadias) was achieved by Hunter at the end of the 18th century. In 1886, Marion Sims reported on six women in whom artificial insemination with the husband's semen was attempted, resulting in one pregnancy which terminated in a spontaneous abortion (*3*). Variations on this basic technique have been developed during recent years and include stimulated intrauterine insemination, where insemination with the husband's sperm is

Table 2
Major events in the history of medically assisted conception

Year	Technique	Reference
1878	*In vitro* fertilization of rabbit and guinea-pig oocytes	Schenk (*1*)
1890	First transfer of mammalian embryo from one female to another	Heape (*4*)
1909	Human pregnancy with donor semen	Hard (*5*)
1944	*In vitro* fertilization of human oocyte	Rock & Menkin (*2*)
1970	Laparoscopic ovum pick-up	Steptoe & Edwards (*6*)
1973	Transient rise in human chorionic gonadotrophin (hCG) after transfer of eight-cell embryo fertilized *in vitro*	de Kretser et al. (*7*)
1978	Successful live birth of human baby by *in vitro* fertilization and embryo transfer (IVF-ET) in a natural cycle	Steptoe & Edwards (*8*)
1980	IVF-ET used for treating unexplained infertility	Lopata et al. (*9*)
1981	IVF-ET pregnancy in a clomifene-citrate-stimulated cycle	Trounson et al. (*10*)
1981	Laparoscopic collection of human oocytes for IVF-ET under ultrasound guidance	Lenz et al. (*11*)
1982	Birth following oocyte and sperm transfer into the uterus	Craft et al. (*12*)
1983	Established pregnancy after transfer of donated embryos fertilized *in vitro*	Trounson et al. (*3*)
1983	Successful cryopreservation of human embryos	Trounson & Mohr (*14*)
1983	Transvaginal aspiration of oocytes by culdocentesis	Gleicher et al. (*15*)
1983	IVF-ET used for treatment of endometriosis and for infertility of unknown cause	Mahadevan et al. (*16*)
1984	Intrauterine insemination with washed semen (IUI)	Kerin et al. (*17*)
1984	IVF-ET used for the treatment of immunological infertility	Ackerman et al. (*18*)
1984	Pregnancy following oocyte donation and IVF-ET in a woman with premature ovarian failure	Lutjen et al. (*19*)
1984	IVF-ET used for the treatment of male infertility	Yovich et. al. (*20*)
1984	Pregnancy after gamete intrafallopian transfer (GIFT) by laparotomy	Asch et al. (*21*)
1984	GIFT by laparoscopy	Asch et al. (*21*)
1985	IVF-ET used for the treatment of hostile cervical factor	Hewitt et al. (*22*)
1985	Vaginal oocyte collection under ultrasound guidance	Wikland et al. (*23*)
1986	Pregnancy after human oocyte cryopreservation	Chen (*24*)
1986	Direct intraperitoneal insemination (DIPI)	Forrler et al. (*25*)
1986	Pregnancy after translaparoscopic zygote intrafallopian transfer (ZIFT)	Devroey et al. (*26*)
1987	Pregnancy following pronuclear-stage tubal transfer (PROST)	Yovich et al. (*27*)
1987	Ultrasound-guided peritoneal oocyte and sperm transfer	Mason et al. (*28*)
1987	Oocyte donation and GIFT as treatment for premature ovarian failure	Asch et al. (*29*)
1987	Catheterization of the fallopian tubes from the vagina	Jansen & Anderson (*30*)
1988	Pregnancy after transfer of multiple sperm under the zona pellucida	Ng et al. (*31*)
1989	Fallopian replacement of eggs with delayed intrauterine insemination (FREDI)	Leung et al. (*32*)

carried out following gonadotrophin therapy (*17*), and vaginal intratubal insemination, where spermatozoa are deposited in the fallopian tube under ultrasound guidance (*30*). It has also been shown that direct intraperitoneal insemination can lead to pregnancies (*25*). The advent of embryo micromanipulation techniques has made possible, *inter alia*, the injection of sperm into the periovular space, resulting in fertilization and live birth (*31*).

3.3 *In vitro* fertilization and embryo transfer

The IVF-ET technique was perfected by Edwards & Steptoe and resulted, in 1978, in the birth of Louise Brown, the world's first baby to be born after the use of this technique (*8*). IVF-ET was originally advocated for treating cases of female infertility due to tubal obstruction but has also been used subsequently to treat successfully various other forms of infertility, e.g., endometriosis (*16*), idiopathic infertility (*9*), cervical hostility (*22*), immunological infertility (*18*) and oligospermia (*20*).

3.4 Ovulation induction

Clomifene citrate was first used for ovulation induction in 1961 by Greenblatt et al. and led to a pregnancy (*33*); in 1967, its use combined with human menopausal gonadotrophin (hMG) in the induction of ovulation was reported (*34*).

In 1957, Gemzell et al. reported the induction of ovulation with human gonadotrophins (*35*) and, in 1962, Crooke et al. reported a pregnancy after treatment with human follicle-stimulating hormone (FSH) and chorionic gonadotrophin (*36*). Treatment with either pituitary FSH or hMG is now widely practised in medically assisted conception (*37*).

In 1971, Kastin et al. reported the use of gonadotrophin-releasing hormone (GnRH) to stimulate the secretion of luteinizing hormone (LH) and ovulation (*38*) and, in 1985, GnRH was successfully used in combination with hMG (*39*). The use of GnRH agonists in ovarian-stimulation regimens was first reported in 1984–1985 (*40, 41*).

3.5 Techniques for gamete and zygote transfer

The transfer of oocytes retrieved by laparoscopy and sperm directly into the uterine cavity has resulted in live births (*12*), and gametes have also been placed in the fallopian tube in cases of non-tubal infertility. The first report suggesting consistent success with gamete intrafallopian transfer (GIFT) was by Asch et al. in 1984 (*21*). GIFT is now widely practised as a two-step procedure consisting of follicle aspiration for oocyte collection followed by transfer of oocytes and capacitated spermatozoa deep into the ampulla of the fallopian tube using laparoscopy (*21*), mini-laparotomy (*42*) or ultrasound guidance (*30*).

Zygotes have also been transferred to the fallopian tube. The first report of a pregnancy achieved by zygote intrafallopian transfer (ZIFT) was published by Devroey et al. in 1986 (26). Subsequently, further pregnancies and live births were reported by the same group (43). Oocytes at the pronuclear stage or cleaving embryos have also been placed in the tube; these techniques are referred to by the acronyms PROST (27) and TEST (44), respectively. The tubal placement of ova at the time of laparoscopy, followed by intrauterine insemination 24 hours later, was first advocated by Leung et al. in 1989 as an alternative to GIFT (32).

In contrast to gamete transfer to different sites of the female reproductive tract, in 1987, placement of oocytes and semen in the peritoneal cavity was reported, and resulted in a live birth (28).

3.6 Medically assisted conception involving a third party

3.6.1 Artificial insemination with donor's semen

For couples affected by certain types of male infertility, artificial insemination with donated sperm has been in routine clinical use since the 1960s, although the first recorded mention of this technique dates from around 1900 (5).

3.6.2 Oocyte donation

In this technique, a third party donates oocytes, which are fertilized with either the husband's or a donor's spermatozoa. Successful pregnancies following the transfer of such donated oocytes after *in vitro* fertilization (19) or by GIFT (29) to patients with or without endogenous ovarian function have been reported since 1984.

3.6.3 Embryo transfer

The transfer of donated embryos to women with a functional uterus but unable to produce oocytes has led to live births (14, 45). The embryos are either transferred after cryopreservation, as first reported in 1983 (14), or obtained fresh from the donor (46).

3.7 Semen and oocyte cryopreservation

Spermatozoa have been routinely cryopreserved for future use since the 1950s (47) and this has led to sperm-banking in many countries. Frozen spermatozoa from the husband or a donor can be used for artificial insemination.

Oocytes have been successfully cryopreserved, thawed and fertilized, and subsequent embryo transfer has led to pregnancies and live births (14, 24, 48). Oocyte freezing enables the excess oocytes harvested in a stimulated ovulatory cycle to be preserved and offers the possibility of repeating IVF-ET in subsequent cycles without the need for ovarian stimulation.

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4. Psychosocial aspects

The discovery of infertility can provoke a complex psychosocial crisis in either or both members of an infertile couple that may take several years to resolve. The crisis involves an interaction between the physical conditions predisposing to infertility, the medical interventions addressing it, social assumptions about parenthood, the reactions of others, and individual psychological characteristics. The process of diagnosing and treating infertility has a profound impact on the lives of the couples affected. They may concentrate on what they have failed to accomplish and soon start neglecting other goals and needs (*1*).

The discovery of infertility, combined with the couple's own deeply rooted expectations and those of others about conceiving and raising children, sets the stage for a complex series of reactions (*2*). Infertility represents the loss of a potential, but its intangibility makes it difficult to grieve. There is a considerable degree of consensus among experts in the field about the type of feelings experienced by infertile individuals. Menning (*3*) has proposed a developmental progression consisting of the following series of associated reactions: disbelief and surprise, denial, anger, isolation, guilt, grief and resolution. These descriptions are generalizations, and there are

differences in the way that individuals perceive, react to and resolve the problem of their infertility that are not well understood; for example, men and women may experience infertility at different levels of intensity and have different concerns (4).

Infertile couples may confront sterility with a feeling of impotence and frustration because they see it as an obstacle to fulfilling the social function expected of them. The woman is frequently seen as responsible, regardless of the real cause of the problem. In many societies, having children is still the woman's *raison d'être* and establishes the man's identity. For many women infertility affects the most precious years of life and the problems it causes must be resolved virtually without the recognition, understanding or support of others.

The sources of stress directly associated with infertile couples' physical well-being include the medical procedures designed to diagnose the cause of and treat the infertility. These offer the hope that the cause will be found and successfully treated; ironically, however, they also contribute to the infertility crisis because of the stress to which they can give rise.

Medical interventions rarely produce immediate results; the gradual nature of the process often increases the couple's anxiety about their infertility, and each month and each failed treatment may seem like another opportunity of conception lost.

Much of the current literature supports the need for the education, guidance and counselling of couples in order to reduce the stress involved in new infertility treatments (5-9) and emphasizes the relationship between stress and physiological responses (10-12). Counsellors should help the couple both to explore fully the options open to them for having a family and to distinguish between wanting to have a baby and wanting to be a parent (13). The traditional option of adoption is still attractive to many couples, while others may find that a childless life-style offers opportunities that they have not considered (14).

Probably the most important finding of studies on pretreatment psychological profiles has been that, in general, IVF-ET participants score within normal limits on measurements of pre-existing psychological abnormality, despite differences in methodology and in the measurements made (15-17). In fact, the data suggest that these couples often exhibit considerable psychological determination, and some may have developed ways of managing stress without becoming overtly anxious. In addition, some symptoms normally indicative of psychological abnormality may be normal side-effects of, or reactions to, infertility, so that spurious estimates of morbidity may be obtained (18). Follow-up research to date supports these findings and indicates that participation in medically assisted conception is not associated with psychological sequelae (19). Recent research on the use of donor gametes has stressed the need for psychological support for both partners before and throughout the investigations and treatment (20).

The Scientific Group recommended that research should be redirected away from the investigation of pre-existing psychological abnormalities in couples undergoing treatment for infertility to focus on the investigation and management of psychological abnormalities directly associated with the treatment of their infertility and its outcome.

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5. Social, ethical and legal issues

Any discussion of the social, legal and ethical aspects of medically assisted conception must first place these new techniques within the general context of reproductive health care. Indeed, infertility treatment should not be dissociated from the need for preventive health care with regard to infection-related causes of infertility, from fertility control through contraception and from infertility investigation and counselling. Also important are the acquisition and dissemination of knowledge concerning these new techniques, through the education of both practitioners and the public, and the social consequences of infertility.

The social, legal and ethical issues surrounding medically assisted conception require: (a) respect for the dignity of the human being; (b) the security of human genetic material; (c) the inviolability of the person; (d) the inalienability of the person; and (e) the necessary quality of services. The adoption of, and respect for, these principles at the international level will provide a normative framework that can serve as a guide for national legislation.

5.1 The dignity of the human being

The inherent dignity of the human being is universally acclaimed in international covenants and is seen as the foundation and source of all human rights. Nevertheless, its interpretation in relation to the status of the human embryo has been bitterly debated (*1-4*).

This is essentially an ethical question. The very vocabulary used to describe the various stages of embryo development demonstrates that words are tools. The use of terms such as "embryo destruction", "individuation", "unborn child", "wastage" and "experimentation" is indicative of certain moral, emotional and ethical choices. In addition, the debate has not been able to divorce itself from other debates surrounding reproduction, such as that on abortion.

It is clear, however, that embryonic development is a continuous biological process (5-7). A person acquires legal identity at birth, yet the protection offered by the law to the fetus *in utero* through the restrictions on legal abortion, the protection of maternal health, and the acquisition of certain conditional legal rights at various stages of fetal development recognizes that a biological continuum exists. The legal position, then, is one of graduated respect for the human being, but this must now be taken back to an earlier stage to reflect respect for the potential of the human embryo. *The principle of respect for the dignity of the human person demands a measure of protection for the human embryo that is consonant with national, cultural, religious and social mores.*

5.2 The security of human genetic material

The possibility of banking, selecting, retrieving, freezing, splitting and transferring human gametes and embryos during and after the lifetime of the progenitor(s) raises the issue of the security of human genetic material down to the very DNA of human cells (8). Even before the acquisition of legal identity at birth, the basic components of human life – viable germ cells and embryonic material – can now be manipulated outside the body, raising public concerns over possible eugenic applications, sex pre-selection and the use of human beings as instruments or objects.

The 1966 International Covenant on Economic, Social and Cultural Rights proclaims in Article 15.1b the right of everyone "to enjoy the benefits of scientific progress and its applications". All states party to that Covenant undertook "to respect the freedom indispensable for scientific research and creative activity" (Article 15.3). Yet, while research on the causes of infertility or on the improvement of IVF techniques is generally acceptable, there is a universal consensus on the need to prohibit extreme forms of experimentation, such as cloning, interspecies fertilization, the creation of chimeras and, at present, alteration of the germ-cell genome (9, 10).

The principle that the security of human genetic material must be ensured requires the regulation of the conditions for, and limits of, research on human gametes and embryos.

5.3 The inviolability of the person

The generally accepted principle of inviolability protects the person even from "touching" if consent has not been obtained. The ethical and legal duty to obtain free and informed consent before any medical intervention serves to ensure this right of autonomy and self-determination. Now that the body is no longer indivisible but can be split up into its component parts and cells, including the reproductive cells, how is that autonomy to be exercised? Can those component parts or products be regarded as property, or are they part of the person? Up to the present, no clear legal response has been given to the question of the nature of the rights that may be exercised over such human genetic material (11-13).

In addition to raising the problem of the legal nature of control, the donation of human genetic material and its freezing and subsequent implantation disturb the age-old notions of linear genealogical order and the evanescence of human life, as well as the social and cultural notions of the family.

Currently, there is no consensus on the maximum duration of conservation, potential uses, indications for donation or means of storage and disposal of human genetic material (especially following separation, divorce or death), or on the form of control to be exercised over such activities, whether contractual, regulatory or statutory. *The principle of respect for the inviolability of the person requires a distinction to be made between the conditions governing the disposition and use of human genetic material and those governing other human tissues. In the former case, this involves not only the fully informed and free consent of the person concerned but also specification of the rights of the donor.*

5.4 The inalienability of the person

Freedom from subjugation is an absolute human right and the human body is generally considered outside the realm of commerce. Now that human embryos or gametes are accessible before implantation, there is the possibility that human life in its earliest stages of development will be considered a marketable commodity, that financial inducements to participate as a donor will be offered, and that gestational motherhood will be dissociated from genetic motherhood as a result of commercial surrogacy. This fear of exploitation and of the production of human life for gain rather than on the basis of altruism or human solidarity makes the preservation of the inalienability of the human person a matter of public policy.

Distinctions must be drawn, however, between the payment of legitimate expenses and the offer of financial inducements by commercial advertising agencies or intermediaries in profit-making arrangements (*14-16*). Irrespective of the position taken, the respect for the principle of the inalienability of the human person requires the regulation of any such commercialization so as to guard against possible exploitation.

5.5 Quality of services

The low priority given to the techniques of medically assisted conception within different health systems, the disparities in its accessibility and geographical availability, the differences in the medical, social and economic criteria for its application, and the variations in the standards and experience of the centres or individuals offering it have made the quality of services an urgent concern. Indeed the adequacy of health care has been specifically questioned with regard to training, the establishment of services and their availability and accessibility, the standardization of criteria for the delivery of services, and screening of couples as to their suitability for treatment (*17, 18*).

There is a general consensus on the need to integrate or establish medically assisted conception services, however organized, within the context of a health care system. This should be coupled with the appropriate training and qualification of clinical, laboratory, nursing and counselling personnel; counsellors, for example, should be able to describe the techniques available, the medical and social alternatives, and the psychological, social, economic and legal consequences. Quality assurance programmes should be introduced to cover the clinical and laboratory aspects, which include both research and clinical services, through multidisciplinary ethical review. Accreditation procedures are essential. Practices should be audited and the results processed for transmission to participants in an understandable form. Services should be readily available and accessible in line with established and widely publicized medical and social criteria, and should be of comparable quality within a given region. In particular, the criteria for the delivery of services, including the medical and social criteria, and policies on the banking of human genetic material, the number of children to be born from any one donor, record-keeping and anonymity, the linkage of records or registries, and follow-up should be clear and well known. Both donors and recipients must be screened as to their suitability and such screening standardized. Irrespective of whether these requirements are embodied in legislation, regulations, directives or codes of practice, respect for the principle of quality of services requires:

- the initiation and promotion of professional self-regulation early in the development of new techniques so as to contribute to the development of a national policy;
- the integration of medically assisted conception into the health care system;
- the auditing and accreditation of such services in order to ensure that they are of the necessary quality and that proper records are kept;
- the availability of medically assisted conception to the population requiring such services;
- the education of both health workers and the general population in the techniques and role of medically assisted conception in the treatment of infertility;
- the promotion of the international exchange of data.

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6. # Medical indications for treatment by *in vitro* fertilization

Before treatment by *in vitro* fertilization is provided, the infertile couple must have undergone a comprehensive investigation, such as the WHO standardized investigation of the infertile couple (*1, 2*), in which the resources of a division of reproductive medicine and infertility in a university or its equivalent have been used in order to reach one or more diagnoses. Only in exceptional circumstances, such as laparoscopically confirmed bilateral tubal occlusion not amenable to microsurgery, is IVF

indicated as a primary treatment, and even then only after the procedures, including the cost and inconvenience, have been fully explained to the couple, and they have considered other options such as adoption.

6.1 Female infertility

6.1.1 *Tubal factors*

The choice between microsurgery and IVF depends on the skills of the gynaecologist and the results that he or she has obtained with microsurgery and IVF; however, irreversible tubal damage or absence of the fallopian tubes is an absolute indication for IVF. In the few patients studied, repeat tubal surgery has given poor results and therefore does not appear to be justified (*3*). The ease of accessibility of the ovaries using the transvaginal ultrasound approach makes reparative pelvic surgery before IVF rarely necessary. How soon IVF should be attempted after failed tubal surgery depends on the extent and type of initial damage (e.g., postinflammatory or post-sterilization), the age of the patient and other infertility factors. Other assisted reproduction techniques involving the placement of gametes, zygotes or embryos directly or indirectly into the fallopian tube(s) are inappropriate in the presence of tubal disease.

6.1.2 *Endometriosis*

If pregnancy does not occur after medical and/or surgical treatment of endometriosis, medically assisted conception can be attempted. The oocyte recovery rate is much lower in patients with persistent stage III and IV disease than in those with stage I and II disease or later stages that have responded to treatment (*4*). After resection of ovarian endometrioma, ovarian function may be altered and the ovarian response to stimulation with drugs may be suboptimal. A suggestion that the fertilization rate of preovulatory oocytes is reduced in these patients has not been confirmed (*5, 6*).

6.1.3 *Cervical factors*

The persistent failure of sperm from a normal ejaculate to penetrate cervical mucus obtained in midmenstrual cycle may be an indication for IVF when artificial insemination with the husband's sperm has failed, as may the absence of cervical mucus due to local abnormalities (*7*). Failure of sperm penetration may have an immunological basis.

6.1.4 *Immunological causes*

The presence of systemic or local cervical antisperm agglutinating or immobilizing antibodies in the female is rare but can be overcome by *in vitro* fertilization (*8, 9*).

6.1.5 *Diethylstilbestrol*

The offspring of women treated for recurrent abortion with diethyl-stilbestrol have both a higher incidence of ectopic pregnancy due to tubal abnormalities and a higher frequency of uterine abnormalities (*10*). The outcome of IVF depends on the type of uterine malformation; for example, pregnancy in women with uterine hypoplasia has a better prognosis when the uterine cavity has a wide lower segment (*11*).

6.2 Male infertility

As established by means of a standardized system of semen analysis (*12*), the diagnoses of oligospermia, asthenospermia, teratospermia and antibody-coated spermatozoa may all be indications for IVF. However, patients with these disorders only to a minor degree should be referred for IVF only after failure of other treatments and when infertility is prolonged by other factors in each partner. To assist in decision-making, tests of sperm function are useful. Criteria for acceptance for IVF need to be established by each laboratory for tests such as basic semen analysis and sperm–cervical mucus penetration (*12*), penetration of zona-free hamster eggs (*13*), evaluation of acrosomal status (*14*) and hemi-zona assay (*15*). In addition, "swim-up" of the ejaculated sperm (see section 8.3.2) must optimally yield a minimum of 1.5×10^6 per ml of sperm showing progressive forward motility (*16*). If there is evidence of infection in the ejaculate (*12*), appropriate treatment should be given before IVF is undertaken. A high frequency of sperm-head abnormalities, regardless of other abnormalities in sperm morphology, is likely to impair fertilization (*17*). Human IVF is the final function test for sperm, and failure of fertilization may be a necessary prelude to the consideration of other approaches to the couple's infertility problem, such as adoption or surrogacy.

6.3 Multifactorial infertility

In many infertile couples in whom more than one cause has been diagnosed in the female partner, IVF may be just as effective as if there were only a single diagnosis. However, if male infertility is also diagnosed, it becomes the determining element in the prognosis of the couple's infertility.

6.4 Unexplained infertility

Although earlier reports suggested a lower success rate of IVF in unexplained infertility (*18*), recent results show an outcome at least as good as that in infertility of tubal origin (*19*). Secondary unexplained infertility has a better prognosis than primary (*20*). However, it should be borne in mind that spontaneous pregnancy does occur in these patients (*21*).

6.5 Other indications

IVF can be considered in patients with an ovulatory disorder where pregnancy has not occurred despite satisfactory follicle development after induction of ovulation using gonadotrophins for some months (22). The rare diagnosis of persistent luteinized unruptured follicle syndrome may also be successfully treated by IVF (23). Failed artificial insemination by donor or with the husband's semen, with or without ovarian stimulation, can be treated by IVF (24-26).

6.6 Indications for oocyte donation

Oocyte donation is indicated in cases of ovarian failure, including gonadal dysgenesis, "insensitive ovary", failed steroid therapy of autoimmune ovarian failure, and premature menopause, whether idiopathic or the result of surgery, chemotherapy or radiotherapy. In women with normal ovarian function, oocyte donation may be indicated where the ovaries are anatomically inaccessible, in cases of repeated IVF failure despite apparently normal oocytes and spermatozoa, in patients with significant autosomal dominant or sex-linked disorders or autosomal recessive traits. In some circumstances, surgical harvesting of oocytes may be contraindicated, or there may be recurrent and persistent ovarian cysts or abnormal oocytes (27, 28) that preclude ovarian stimulation.

6.7 Indications for techniques other than IVF

The use of GIFT or other methods involving tubal or peritoneal transfer requires the presence of at least one normal fallopian tube. In the later stages of endometriosis, the transabdominal approach to tubal transfer may be possible (29), but if access to a tubal ostium is impaired, the transcervical/transuterine route may be a feasible alternative (30, 31). If higher pregnancy rates are documented in the future following transcervical/transuterine tubal transfer, a reappraisal of the advantages of direct embryo or gamete placement in the uterine cavity and of transabdominal transfer to the fallopian tube will be required.

Where there is a male factor, it is more rational to obtain evidence of sperm fertilizing ability using IVF, at least in the first instance, than to use GIFT or similar techniques (32, 33). GIFT has been used in couples where the male has sperm autoantibodies (34). The direct transfer to the tube of the pronuclear-stage oocyte (PROST) has been advocated in cases of severe oligospermia and asthenospermia, the presence of circulating antisperm antibodies in the female partner, repeated failure with GIFT, and unexplained infertility (32, 35). ZIFT has also been used successfully in cases of male infertility (36, 37).

6.8 **Selection criteria**

The following criteria are applicable in the selection of patients for IVF treatment:

1. The selection of recipients over 40 years of age must be made in the knowledge that results in such women are poor, as a result largely of an increased abortion rate (*38*). Recently, normal blood levels of follicle-stimulating hormone have been found to be a better predictor of good IVF outcome than age alone (*39*).
2. The recipient's uterine cavity should be normal or any abnormalities should have been corrected.
3. Ovulation should occur spontaneously or be readily induced.
4. The recipient should be in good physical health so as to be able to sustain a pregnancy to term, and both participants should be in good mental health.
5. The final selection of couples for treatment must take place only after they have been given the appropriate technical information with which to make an informed decision. They must also be offered counselling and support because of the stress and anxiety provoked by their persistent infertility, the complexity of the procedures involved in medically assisted conception, and the fact that they are embarking on a treatment of last resort.

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7. Induction of multiple follicular development

The first successful results with IVF were obtained in spontaneous or natural cycles of ovulatory women (*1*). In 1980, Edwards & Steptoe (*2, 3*) reported the first series of patients from whom preovulatory oocytes had been recovered after the onset of the LH surge in a natural cycle. The subsequent use of ovarian hormonal stimulation was shown both to provide greater numbers of oocytes for fertilization and to maximize the yield of embryos that could be transferred, with a consequent increased pregnancy rate (*4-9*). However, further work on the natural or spontaneous menstrual cycle suggests that it may still be useful. Although various drugs have been used to induce multiple follicular development (Table 3), disagreements exist as to which regimen produces the best results. It should be noted that batch-to-batch variation has been reported

Table 3
Agents used to induce multiple follicular development

Clomifene citrate with human chorionic gonadotrophin (hCG)
Human menopausal gonadotrophin (hMG) alone or combined with clomifene citrate,
    followed by hCG
Pure follicle-stimulating hormone (FSH) alone or combined with hMG, followed by hCG
Gonadotrophin-releasing hormone agonist analogues (GnRH-a) plus hMG, followed by
    hCG

in the gonadotrophin preparations used to induce follicular development, and this may account for variations in the patient's response from cycle to cycle.

7.1 Clomifene citrate with hCG

Clomifene citrate, although capable of inducing multiple follicular development (8), has been found to have the following three major disadvantages:

— its use results in a high incidence of spontaneous LH surges, leading to cancelled cycles;
— it may inhibit endometrial development as a result of its antiestrogenic activity, so that implantation is impaired;
— the yield of oocytes is low.

The side-effects of clomifene citrate include hot flushes in about 10% of patients, nausea, vomiting and breast discomfort in 2%, mild visual disturbances in 1.6%, dermatitis or urticaria in 0.6% and reversible hair loss in 0.4%. Severe ovarian hyperstimulation syndrome is rare with clomifene citrate/hCG therapy (10).

7.2 Human menopausal gonadotrophin

7.2.1 Combined with hCG

The hMG stimulation proposed by Edwards (2), and further developed by workers in Norfolk, Virginia, USA (11-13), is associated with a higher yield of oocytes than when clomifene citrate is used. Various low- and high-dose protocols have been used in which daily doses of 75-225 IU of hMG are administered, starting on day 2-3 of the menstrual cycle, and continuing until 5000-10 000 IU of hCG are injected; this is usually timed for the sixth day of continuously rising levels of plasma estradiol and/or when estradiol levels in plasma reach 200-300 pg/ml per follicle, provided that follicle diameter is greater than 17-18 mm on ultrasound imaging.

7.2.2 Combined with clomifene citrate and hCG

A combination of clomifene citrate and hMG at various doses is the most commonly used regimen (7, 14-16); the constituents can be administered

simultaneously or sequentially. The majority of clinicians (17-19) prefer a protocol in which the hMG dosage is adjusted in the range 75-225 IU daily, depending on the ovarian response; this is monitored in terms of levels of plasma estradiol and ultrasound measurements of follicular growth. Increasing the dose of hMG to more than 150 IU daily does not increase the number of ova retrieved (20).

A "fixed" schedule of hMG administration has also been used. This regimen entails administration of combined oral contraceptives starting on days 1-5 of the pretreatment cycle for a mean of 25 days, or of a progestogen starting from days 15-18 of the pretreatment cycle for a mean of 19 days (21). Clomifene citrate (100 mg/day) is then given from days 2 to 6 and hMG (150 IU) on days 2, 4, 6, 8 and 10 of the treatment cycle, while 5000 IU of hCG are administered on day 11 at 22h00 and oocyte retrieval is scheduled 35 ± 1 hours later. Follicular growth is not monitored either hormonally or by means of ultrasound.

7.3 Pure follicle-stimulating hormone with hCG alone or in combination with hMG

Pure FSH with hCG alone or in combination with hMG may be used for multiple follicular recruitment (22).

Some clinicians have not found that ovarian stimulation with the addition of pure FSH to the hMG stimulation protocol or with FSH alone results in a larger number of mature high-quality oocytes or fewer spontaneous LH surges (23, 24). The optimal dose of pure FSH remains to be determined (25).

7.4 GnRH analogue with hCG and hMG

The use of a GnRH analogue (GnRH-a) in combination with other ovulation-induction regimens such as hMG and pure FSH decreases the spontaneous LH surge (26, 27), results in lower rates of cancelled cycles and improves oocyte recovery rates, thus increasing the number of embryos available for transfer; there is also a higher pregnancy rate than with cycles stimulated with hMG alone or hMG combined with clomifene citrate (28).

Several analogues of GnRH are available, having different forms and routes of administration; these include nasal sprays and subcutaneous and intramuscular injection. Side-effects resulting from the use of GnRH-a are uncommon but include menopausal symptoms, headaches and, rarely, neurological symptoms, such as numbness and paraesthesia, weakness of the limbs and face, and sensory ataxia (29).

The two principal protocols used are a short or "flare up" protocol and a long or "blocking" protocol.

In the short protocol (28, 30-32), exogenous stimulation (hMG, hMG/clomifene citrate, FSH, etc.) begins at the same time as, or shortly after, the GnRH-a is administered. Administration of the analogue is

started on the first or second day of the menstrual cycle, and can be followed on the third day by that of hMG or FSH. The GnRH-a and hMG or FSH are then administered together until the day on which the hCG is injected. One comparative study showed greater numbers of follicles and fewer cancelled cycles when GnRH-a administration was started on the third day of the treatment cycle instead of on the first day (*32*).

The goal of the long protocol is to reduce endogenous gonadotrophin production to basal levels. This is generally achieved approximately 21 days after the start of treatment with GnRH-a and is confirmed by measurement of estradiol in the serum. When adequate hypothalamo-pituitary desensitization is obtained (serum estradiol less than 30 pg/ml), GnRH-a administration is continued and ovarian stimulatory treatment started with any of the previously mentioned combinations (*33, 34*).

In one study, patients allocated to the short treatment protocol required less FSH and hMG and had significantly higher pregnancy and lower abortion rates than patients allocated prospectively to a long treatment protocol (*35*).

The use of GnRH-a in ovarian-stimulation regimens is considered to be advantageous because it results in:

- a greatly reduced incidence of spontaneous LH surges and therefore fewer cancelled cycles;
- a better follicular response in the "poor responders" (patients who, on other regimens, develop few follicles and have low estradiol levels); and
- better synchronization of follicular development since, in the long protocol, the hypothalamo-hypophyseal axis is inhibited and the ovulation-stimulation drugs act only on the follicles (*30, 31, 34-42*).

The protocols still need to be improved, and better results will probably be obtained by defining the clinical indications for the various protocols more precisely and by simplifying the mode of administration of drugs by avoiding daily injections.

7.5 Oral contraceptives for gonadotrophin suppression

In an attempt to coordinate personnel better and to increase efficiency, modifications of standard ovulation-induction protocols in medically assisted conception procedures have been employed. In one such modification (see section 7.2.2), oral contraceptives for gonadotrophin suppression have been used before the initiation of ovulation induction (*43-45*). This makes it possible:

- to schedule a preselected number of subjects to undergo ovulation induction as a group;
- for the duration of induction and time of oocyte retrieval to be reasonably predictable; and
- for more follicles of diameter 5 mm or greater to develop and more oocytes to be retrieved (*46*).

It has been reported that follicular fluid estradiol/progesterone ratios and FSH levels were lower in a group of women who received oral contraceptives prior to ovulation induction (47). On the other hand, higher concentrations of FSH have been associated with increased fertilization rates (48). High follicular fluid progesterone and progesterone/estradiol ratios have also been found in women with high pregnancy rates (49).

7.6 Monitoring follicular stimulation

Monitoring multiple follicular development is of the utmost importance in determining the quality of the cycle, including hormonal response and the potential number of oocytes. It is also needed for the precise timing of hCG administration (see below). In such monitoring, ultrasound or hormonal parameters can be used, either alone or in combination (50-53).

7.7 Timing of hCG administration

The doses of hCG administered are in the range 2000–10 000 IU. Oocyte retrieval is scheduled for 33–36 hours after hCG administration to allow for final maturation of the oocytes. The regimen used by many clinics (50-53) calls for the administration of hCG on the sixth day of a sustained increase in serum estradiol levels. Patients who fail to achieve adequate follicular development after 6–8 days of ovarian stimulation do not receive hCG and the treatment cycle is classified as "cancelled". This treatment schedule has proved to be easy to administer and monitor and spontaneous LH surges occur in only 2.5% of cases (51-53). If a spontaneous LH surge occurs in a stimulated cycle, some centres cancel the treatment cycle while others give hCG if there is a satisfactory estradiol response and adequate follicular growth has taken place (54). In these cases, it is necessary to adjust the timing of oocyte recovery.

In other centres, hCG is administered when the serum estradiol level reaches 200–300 pg/ml per follicle greater than 17–18 mm in diameter. Patients with poor follicular development or with only one developing follicle are not given hCG. It is inadvisable to give hCG to patients in whom the serum estradiol level is seen to increase rapidly (i.e., doubling in 24 hours) in order to minimize the risk of the hyperstimulation syndrome.

7.8 Complications of ovarian stimulation

The most serious complication of ovarian stimulation is the formation of multiple ovarian cysts, both follicular and luteal, associated with excess steroid production and ovarian enlargement in mild cases, abdominal distension, nausea, diarrhoea and vomiting in more severe cases, and sometimes also ascites, hydrothorax, electrolyte imbalance, haemo-concentration, hypovolaemia, oliguria and thromboembolic phenomena (55, 56). This syndrome has been well recognized from the early days of hMG/hCG therapy (55). The incidence of severe hyperstimulation syndrome reported in a series of papers between 1970 and 1982 varied between 0.2 and 1.8% (10).

The severe form of the syndrome has not been observed when hCG has not been administered, but the mild form can occur with hMG and the endogenous production of LH (*10*). The severe form has also been reported where hCG has been administered during the luteal phase after replacement of the fertilized ova, GnRH-a and hMG having been used to stimulate follicular development (*57*). It has been suggested that GnRH-a-treated cycles may be associated with a higher incidence of severe ovarian hyperstimulation (up to 1.8% in one series (*58*)), whereas in the nine years and more than 4000 treatment cycles before the introduction of GnRH-a, only three cases of such hyperstimulation were recorded. The risk can be reduced by careful monitoring of follicular development when GnRH-a and hMG are used and by withholding hCG when multiple large follicles and very high levels of estradiol are present.

Other complications of ovarian stimulation include torsion of adnexa following the hyperstimulation syndrome (*59*). An attempt to unwind the twisted adnexum should be made before resorting to oophorectomy (*60*).

7.9 *In vitro* fertilization in unstimulated cycles

Although the first successful pregnancy following *in vitro* fertilization occurred during a spontaneous cycle (*1*), the use of controlled ovarian stimulation in medically assisted conception was soon adopted in the majority of centres. This resulted in the development of several preovulatory follicles and increased the number of ova for fertilization and hence the number of embryos available for transfer.

As culture techniques and methods of follicular aspiration have steadily improved over the past decade, medically assisted conception in natural spontaneous cycles has become practicable, and pregnancy rates of 22.5% per cycle have, in fact, been reported (*61*).

This approach offers a number of advantages:

- shorter duration of monitoring of the follicular phase and therefore reduced costs;
- reduction in the cost of drugs as no medication is used except hCG for ovulation induction;
- no risk of ovarian hyperstimulation;
- the simplicity of the technique and its acceptability to patients permit a number of attempts at ovum recovery with a consequent increase in the efficiency of the method.

7.10 Oocyte recovery

Once adequate multiple follicular development has been attained and ovulation has been triggered by the intramuscular administration of hCG, it is necessary to retrieve the oocytes from the follicles some 33–36 hours later. This time interval is selected to permit *in vivo* oocyte maturation on the one hand and to reduce the possibility of premature ovulation and loss of the oocytes on the other (*62-64*). A further 4-6 hours of *in vitro*

maturation is allowed before insemination, to reduce the incidence of polyspermia (65). Initial attempts to harvest oocytes for use in medically assisted conception involved laparotomy (66-68) but this was soon replaced by laparoscopy. The major advantage of the latter is that it provides an equally good or better view of the pelvic organs during aspiration while involving less manipulation and a smaller risk of morbidity than laparotomy. However, it causes the patient discomfort and there is some morbidity. In addition, prolonged exposure of oocytes to anaesthesics (69) and the possible lowering of the intrafollicular pH associated with the carbon dioxide pneumoperitoneum might have a detrimental effect on the capacity of the oocytes to be fertilized (70, 71).

In most centres, laparoscopy is now used only for GIFT and similar procedures and oocyte recovery is usually effected by means of a transvaginal approach. In this approach, an ultrasonically guided probe is used, and thanks to the location of the ovary adjacent to the vaginal vault, a clear view of the ovaries is usually obtained together with easy access to the follicles, while the risk of injury to other pelvic organs is minimized. Complications are rare, the most frequent being bleeding from the site of puncture and pelvic infection (72, 73). The latter can be minimized by the routine administration of an appropriate antibiotic. Other advantages of the ultrasound-guided transvaginal approach are that it is simpler, less expensive and has a lower morbidity than other oocyte-recovery techniques.

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8. # Oocyte fertilization and subsequent embryo culture

Successful IVF and embryo growth require the recovery of a mature oocyte with full developmental potential and the maintenance of its viability during fertilization and growth *in vitro.* Spermatozoa must be collected and prepared and both gametes placed together in an environment in which they are capable of retaining their function.

The methods used for sperm preparation, insemination and the culture of oocytes and embryos are relatively straightforward. However, many variables within the IVF laboratory system are crucial to the overall success of IVF and related procedures. These variables may be biological, such as the quality of the oocytes and spermatozoa, or intrinsic to the laboratory, including water quality and the precision with which equipment is calibrated.

8.1 Quality assurance

Regular but simple quality-control procedures are essential to maintain a high fertilization rate and satisfactory embryo growth. These procedures must be consistent and well regulated and should include the regular checking, maintenance and cleaning of all items in the laboratory to ensure optimum performance.

8.1.1 *Equipment*

Incubators used for oocyte and sperm incubation and for embryo culture should be regularly cleaned and sterilized. Each incubator should be monitored for temperature fluctuations by taking independent tem-perature readings from a calibrated thermometer, and for fluctuations in carbon dioxide levels by means of a recording device used as recommended by the manufacturer of the incubator. Accurate control of the temperature of equipment such as heating blocks and water baths is also important. If temperatures are too low or too high, fertilization and embryo growth may be impaired. A factor often overlooked is the temperature of the laboratory. Electrical equipment such as incubators may not operate properly if the temperature of the room is too high. Controlled air-conditioning of the laboratory is strongly recommended. Filtering of the air into the laboratory will help reduce airborne contamination.

8.1.2 *Glassware and consumables*

All glassware and other items, such as embryo-transfer catheters and ovum-aspiration sets, that come into contact with culture medium, spermatozoa, oocytes or embryos should be washed using purified water and a nontoxic detergent specifically designed for tissue culture, and then sterilized. All new glassware should be soaked in dilute acid solution overnight and then washed extensively and adequately rinsed in purified sterile water before use to remove any oil, dust or other contaminants that may accumulate during manufacturing. Dry heat sterilization is recommended for IVF glassware and ovum-aspiration equipment. Items such as embryo-transfer catheters and ovum-aspiration sets that are autoclaved must be flushed out with culture medium before contact with oocytes or embryos. Items that are sterilized with ethylene oxide gas must be left 10-14 days before use to ensure that all traces of gas are removed.

8.1.3 *Water*

A supply of purified water is necessary for washing glassware and consumables and for the preparation of culture media. The water may be purchased commercially but, if space and economics allow, a water purification system installed in the laboratory is recommended. Water purification systems need to be regularly checked for the presence of organic contaminants, microorganisms and pyrogens, all of which may influence the successful culture of oocytes, spermatozoa and embryos. The manufacturers of these systems are able to recommend the tests that need to be completed to check the integrity of their systems.

8.1.4 *Toxicity testing of culture vessels and media*

A variety of vessels may be used for oocyte incubation and embryo culture. Test-tubes were used in the pioneer IVF programmes, but since then many alternatives have become available, dishes and culture wells now being the most commonly employed. The plastic composition of these vessels varies and all new products should be tested for toxicity before introduction into the IVF laboratory. This also applies to new brands of other items that come into contact with oocytes, spermatozoa and embryos, such as embryo-transfer catheters, ovum-aspiration sets, syringes for collecting and filtering culture media, and filters for sterilizing culture media or serum supplements.

Mouse embryos can be used for toxicity testing of new materials used in IVF and for the quality control of culture media. Two different tests have been employed. The first uses two-cell embryos that have developed *in vivo* and are flushed from the uteri of mice in which superovulation has been induced. These embryos are then grown *in vitro* in the presence of the item being tested, and their growth to blastocyst stage is observed (*1*). The second method involves the *in vitro* fertilization of mouse oocytes and observation of embryo growth to the blastocyst stage (*2*). The latter method is considered to give a more sensitive indication of embryo growth and hence toxicity (*3*).

A bioassay has also been used that determines the survival of human spermatozoa over several days after exposure to various products used in IVF procedures (*4*). Progressive motility is monitored and test samples are compared with controls to give an indication of the possible presence of cytotoxic substances. This assay may not be sensitive enough to use for routine quality-control testing for more subtle effects that reduce embryo viability. Cultures of amniotic-fluid cells have been used to screen for toxic effects attributable to the different types of urethral catheters used in IVF (*5*).

Quality control in IVF has provoked much discussion as no simple test can ensure absolute control over every variable in an IVF laboratory (*6*).

Table 4
Commonly used IVF culture media

Medium	Reference
Ham's F10	Lopata et al. (*7*)
Human tubal fluid (HTF)	Quinn et al. (*8*)
Whittingham's T6	Quinn et al. (*9*)
Earle's solution	Purdy (*10*)
Menezo's BZ	Testart et al. (*11*)

8.2 Culture conditions

The correct environment is essential for successful fertilization and embryo growth. IVF techniques involve the use of an incubator containing carbon dioxide and culture media to mimic conditions *in vivo*. Temperature, pH, the osmolality of the culture media and metabolic factors must all remain stable and be within physiological limits.

8.2.1 *Media*

Many types of culture media have been used for successful human IVF. There is no evidence to suggest that any one medium is superior to another with respect to oocyte fertilization, embryo cleavage and pregnancy outcome. Culture media may be obtained from commercial sources or can be prepared in the laboratory. The choice will depend on the laboratory; for example, smaller laboratories might wish to buy commercially available culture media as their scientific staff and equipment resources may be limited. The preparation of culture media requires the use of highly purified water and analytical-grade chemicals; those most commonly used are listed in Table 4 and contain a combination of basic salts and energy substrates such as glucose and sodium pyruvate, supplemented with antibiotics and, in some commercial media, amino acids and vitamins. Culture media prepared in the laboratory have a limited shelf-life, in particular if they contain pyruvate, and fresh batches should be prepared every 10–14 days.

Culture media containing a phosphate buffer or Hepes[1] organic buffer should be used for procedures that involve exposure of gametes to prolonged normal atmospheric conditions, e.g., the flushing of follicles.

8.2.2 *Serum supplementation*

Addition of serum to the culture medium is a widely accepted procedure. The serum protein ensures that oocytes and embryos do not adhere to the glass surface of the pipette used to manipulate them. At the early cleavage stage, embryos metabolize very little protein, so the protein component of

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[1] 4-(2-Hydroxyethyl)-1-piperazine-ethanesulfonic acid.

Table 5
Protein supplements

Human serum	Leung et al. (*13*)
Human cord serum (HCS)	Leung et al. (*13*); Holst et al. (*14*)
Human serum albumin (HSA)	commercially available
Fetal calf serum (FCS)	commercially available
Bovine serum albumin (BSA)	commercially available
Synthetic serum	commercially available, Saito et al. (*15*)

serum is unlikely to play a significant role in embryo nutrition (*12*). Protein supplements that may be used are shown in Table 5.

The human source of any protein supplement should have no evidence of infection with viruses such as hepatitis B virus, human immunodeficiency virus or cytomegalovirus. Toxicity testing of batches of commercial supplements is highly recommended (see section 8.1.4).

Physiological fluids such as amniotic fluid (*16*) and human follicular fluid (*17*) have been used by some laboratories as culture media, for IVF in the former case and for GIFT in the latter. Evidence of consistently improved pregnancy rates needs to be provided before these fluids can be said to be better than the more commonly used media containing serum supplements.

Experiments involving the supplementation of culture medium with a phospholipid (platelet activating factor) have shown a significant difference in pregnancy rates between treated embryos and controls, which suggests that the phospholipid factor facilitates embryo development (*18*). Multicentre trials are being organized to look at the reproducibility of these results.

8.2.3 *Temperature*

The human oocyte is temperature-sensitive (*19*) and a humidified incubator with a temperature setting of 37.0-37.5 °C must be used for oocyte fertilization and embryo culture.

8.2.4 *pH and osmolality*

All media should be equilibrated with carbon dioxide before use in order to obtain the correct pH (*20*), which must be in the range 7.4-7.6. The osmolality of the culture medium must be in the range 275-290 mosmol/kg (*7*).

8.2.5 *Culture vessels*

Important factors to consider when choosing which type of culture vessel to use are the maintenance of pH and osmolality, humidity control, space,

and ease of visualization of the ova or embryos in the vessel. Culture can be conducted under paraffin oil, which gives good protection from humidity, pH and temperature fluctuations; however, the oil can contaminate the laboratory and other solutions and be transferred to the patient at the time of embryo transfer. Paraffin oil can also be very toxic to IVF material and batches of oil must be screened before use in culture.

8.3 Methods for oocyte fertilization and embryo culture

8.3.1 Evaluation of oocytes

There is no universal standard of classification of oocytes but each laboratory must adopt a consistent grading system. The characteristic morphological features that are observed at oocyte collection relate to the oocyte–cumulus/corona cell complex (OCCC) and to the ooplasm when it is visible through these cells (21). The cumulus cells form a cloud-like mass that nurtures the ovum during its growth in the follicle; the corona radiata consists of the cells immediately surrounding the oocyte that provide cell-to-cell communication between the oocyte and the follicle. During follicular maturation the cumulus expands and disperses and the corona cells withdraw from contact with the oocyte, which gives them a radiating appearance.

An example of oocyte classification is shown in Table 6. In this system, a combination of distinctive features is used to classify the OCCC as immature, intermediate or mature (22-28). Another method of classification depends on examination of oocytes for polar-body extrusion; a high-resolution inverted microscope is used to grade each oocyte as being in (a) metaphase II – the first polar body is present; (b) metaphase I – the first polar body has not been extruded and there is no germinal vesicle; or (c) prophase I – the germinal vesicle is present. Oocytes in metaphase II have higher fertilization rates than less mature oocytes (21). For observations made by this method, the oocyte must be spread on the surface of a culture dish so that the cumulus and corona radiata cells do not obscure it; proficiency in this procedure is needed if the oocyte is not to be

Table 6
Classification of OCCC maturity[a]

Grade of maturity	Cumulus	Corona radiata
Very immature	Absent	Compact, adherent to zona pellucida
Immature	Dense	Tight
Intermediate	Expanded cell mass	Tight
Mature	Dispersed cells (zona pellucida is often visible)	Radiant layer
Atretic	Absent, cell clumps surround oocyte	Generally absent

[a] Source: references 22–28.

damaged. Time and available skills and equipment will determine what classification procedure each laboratory chooses.

8.3.2 Insemination of oocytes

Oocytes are incubated for several hours before insemination with the spermatozoa; for oocytes inseminated between 3 and 20 hours after recovery there are no differences in fertilization and pregnancy rates (*29, 30*). There are various methods of preparing the spermatozoa for IVF insemination. The normal "swim-up" technique involves mixing a portion of the ejaculate with culture medium, centrifuging it, discarding the supernatant containing the seminal plasma, and overlaying fresh culture medium on to the pellet containing the spermatozoa. During subsequent incubation of the pellet for 30–60 minutes the motile sperm migrate into the overlay medium. The medium containing these spermatozoa is then collected by aspiration with a pipette and used for insemination (*31*). The purpose of this procedure is to:

– remove the seminal plasma, which inhibits fertilization (*32*);
– concentrate the motile sperm; and
– remove contaminating cells and debris.

Usually, motile sperm are added to oocytes at a concentration of 100 000 sperm per ml.

Provided that sperm counts are greater than 20×10^6/ml, semen containing sperm of which a moderate proportion (e.g., 40%) show abnormalities in motility or up to 90% are morphologically abnormal can be treated by various techniques using density gradients to separate out the sperm with progressive motility (*33, 34*). The couple in which the man has severe oligospermia or asthenospermia has an uncertain prognosis, but various insemination techniques are being developed that involve micromanipulation of the gametes (*35*), including partial zona dissection of the oocyte to assist penetration of the zona pellucida by sperm. In this method, mechanical force is used to open the zona pellucida and the oocyte is then exposed to spermatozoa (*36*). Alternatively, acidic Tyrode's medium is used to digest a hole in the zona pellucida ("zona pellucida drilling") (*37*). Another approach involves the microinjection of a number of spermatozoa into the perivitelline space (the space between the oocyte membrane and the zona pellucida) (*38, 39*).

8.3.3 Evidence of fertilization

Oocytes should be observed for fertilization 12-20 hours after insemination. The cumulus of the oocyte is dissipated by spermatozoal enzymes, especially hyaluronidase, but fine needles or finely drawn pipettes must be used to remove the remaining cells around the oocyte. The cytoplasm of the oocyte should be examined for the presence of pronuclei, two pronuclei being a positive sign of fertilization. Occasionally, there is only one pronucleus or three or more pronuclei are seen. Such

fertilizations are classified as abnormal (*40-42*) and the resulting embryos should not be transferred; they may either be kept in culture and their growth patterns observed or be discarded. Delayed pronucleus formation (after 20 hours) is associated with an abnormal chromosomal arrangement in 87% of embryos, compared with 29% when pronucleus formation has occurred earlier than 20 hours after insemination (*42*). This stresses the importance of checking for fertilization with respect to timing after insemination.

8.3.4 *Embryo culture and classification*

The requirements for media and vessels and their environment are the same for embryo culture as for oocyte incubation and fertilization. The cell divisions in the preimplantation embryo are associated with a progressive reduction in cell size and are referred to as cleavage. The first cleavage normally occurs in the human embryo 20-30 hours after *in vitro* insemination (*43*). Subsequent cleavages progress in a regular pattern and typically result in embryos of four cells 48 hours after insemination. It is generally agreed that embryos that divide evenly, regularly and rapidly are those most often associated with successful pregnancy, but retarded and unevenly cleaved embryos can still develop to term pregnancies.

As is the case with oocyte assessment, embryo classification is based on a subjective assessment of the morphological appearance of the embryos. An example of a grading system is shown in Table 7 (*44*). Other methods of embryo classification relate to the symmetry of blastomeres and the percentage of the mass of the embryo that is fragmented (*45*):

A – symmetrical, no fragmentation
B – < 10% fragmentation
C – 10-25% fragmentation
D – > 25% fragmentation.

8.4 Embryo cryopreservation

The introduction of drug regimens that induce multiple follicular development and permit the harvesting of numerous oocytes has resulted

Table 7
Embryo grading system based on morphological characteristics

Good:	embryo with blastomeres of equal size; no cytoplasmic fragments; clear cytoplasm
Fair:	embryo may have slightly irregular blastomeres; some cytoplasmic fragments; cytoplasm may be granular
Poor:	blastomeres indistinct, irregular, abnormally shaped, shrunken or lysed; many cytoplasmic fragments; granular cytoplasm with retracted organelles

in a dilemma. The maternal and perinatal risks of high multiple pregnancy following the transfer of more than four embryos are well documented, but the transfer of only one embryo results in low pregnancy rates. The alternatives are to use the excess embryos for research purposes (which is legally or ethically unacceptable in many countries), to discard the excess embryos, or to use cryopreservation to save them for future transfer.

Between 1971 and 1979, the successful cryopreservation of embryos from mice (*46*), rabbits (*47*), sheep (*48*), goats (*49*) and cattle (*50*) was reported, and in 1981, the first report appeared on the cryopreservation of four- to eight-cell human embryos (*51*). The clinical outcome of the transfer of thawed human embryos was reported in 1983 (*52*).

Embryo cryopreservation has now become a routine adjunct to IVF procedures, and various methods of freezing have been employed. The method that has yielded the best results in terms of simplicity, efficiency and reproducibility is one that involves freezing of one- to three-day-old embryos (one to eight cells) in a controlled biological chamber that cools the embryos to sub-zero temperatures in the presence of the cryo-protectant 1,2-propanediol (*53*). Other cryoprotectants that are used are dimethyl sulfoxide (*52, 54*) and glycerol (*55*). For these so-called "slow-cooling" techniques, specific training and equipment are needed. Poor results are frequently reported by untrained staff and, even in experienced IVF clinics, difficulties have been encountered in successfully freezing human embryos.

Thawing of the embryos may be carried out in a specially adapted biological freezer. Embryos frozen in the presence of dimethyl sulfoxide, which have been cooled to below $-60\,°C$, require a slow controlled thaw of $10\,°C$/minute, to prevent damaging osmotic effects on the dehydrated cells. However, if cooling was terminated between $-30\,°C$ and $-40\,°C$, the embryos should be thawed rapidly to avoid the growth of small ice crystals because of incomplete dehydration. For example, embryos frozen in the presence of glycerol or 1,2-propanediol are thawed at a rate of $300\,°C$/minute, usually by submerging the freezing vessel in a water bath at 30–$37\,°C$ for one minute.

The thawed embryos are assessed for survival (*53, 57*) in terms of the number of cells that are intact after cryopreservation. Generally embryos having at least half of their blastomeres intact are as capable as complete embryos of establishing pregnancy. As the blastomeres of early embryos retain totipotency and the ability to produce a complete fetoplacental unit, the loss of some blastomeres appears inconsequential. The subsequent embryo-transfer cycle may be a natural cycle or one in which steroid replacement or ovarian stimulation has been used (*58*).

The expense of equipment and the time required for the slow-cooling techniques have stimulated research on rapid-cooling methods for cryopreservation, which do not involve the use of a biological freezing chamber. Vitrification, for example, has been used to preserve mouse

embryos. The embryos are placed in a concentrated solution of cryoprotectants, which, during the rapid cooling process, solidify to form a glass instead of crystals. This process may avoid the damage caused by intracellular ice formation and the osmotic effects caused by extracellular ice formation (*59*). Vitrification has not, however, been proved to be successful in the cryopreservation of human embryos and needs further research.

An ultrarapid cooling method using dimethyl sulfoxide in high concentrations has proved very successful for snap-freezing mouse embryos (*60*). High survival rates have been observed for human embryos frozen by this method, but rates of blastocyst formation are low (*61*). More-detailed studies are needed before this technique can replace the more traditional slow-cooling cryopreservation methods (*62*).

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9. Intrauterine and intrafallopian transfer techniques

9.1 Embryo transfer after *in vitro* fertilization

In the now-traditional method, a stiff polytetrafluoroethylene catheter with an internal diameter of 0.8-1.0 mm is attached to a tuberculin syringe loaded with culture medium and embryos to a total volume of up to 75 μl. Several kinds of media have been employed for embryo growth and transfer, the most common being the patient's own serum, heat-inactivated and filtered through a 0.2-μm microfiltration system, and used either undiluted or at a concentration of 50% or above in culture medium (*1*). In cases of unexplained infertility, another patient's serum is used instead. Fetal cord serum from fresh placentas has also been used (*2*). It has been claimed that serum is not necessary for embryo culture and transfer (*3*) and serum substitutes have been tested (*4*).

With the patient in the lithotomy or knee-chest position, the catheter is introduced through the cervix so that its tip is at the fundus of the uterus (*5-8*). In order to reduce the incidence of multiple pregnancy, it is usual not to transfer more than three or four embryos.

The effect of the interval between insemination and embryo transfer has been studied (*9, 10*). No differences in pregnancy rates as between transfer on day 3 and day 4 after insemination have been seen when the same number of embryos have been transferred (*9*).

Many factors affect the success of IVF-ET. Thus it has been reported that higher pregnancy rates are achieved by transferring embryos with high cleavage rates, but live births have resulted from the transfer both of 1-8-cell embryos (*11*) and of 8-16-cell embryos (*12*).

It is well recognized that embryo transfer must be performed as gently as possible. Leeton et al. (*6*) classify transfers as easy, difficult and very difficult, depending on whether the catheter passes easily or whether mucus and/or blood enter it, the highest pregnancy rates being achieved in the easy-transfer group. Performing the transfer under ultrasound guidance (*13*) has not led to a higher success rate (*15*).

9.2 Gamete intrafallopian transfer

The GIFT technique (see also section 10.9.3) involves the recovery of ova by laparoscopy and the transfer of gametes into the fallopian tube(s) during the same operation (*15, 16*). In general, up to three mature oocytes are loaded into a transfer catheter with 100 000-200 000 washed sperm in 25-50 μl of culture medium. The loaded catheter is inserted through the fimbrial orifice, usually to a depth of 2-4 cm, and its contents are gently discharged by means of an attached tuberculin syringe; a higher pregnancy rate has been reported when the gametes have been injected at a depth greater than 4 cm (*17*). This procedure is repeated in the opposite tube, if

possible. Follicular fluid has been advocated as an additive or substitute for culture medium, as it has been shown to promote the acrosome reaction and improve the *in vitro* fertilization of both human and zona-free hamster oocytes (*18-20*). In one study, no significant advantage resulted from the use of follicular fluid instead of culture medium (*21*), whereas in others an improvement in pregnancy rates was found when follicular fluid was used as a sperm capacitation and gamete transport medium (*22, 23*), in contrast to conventional Ham's F-10 or Earle's medium (*24*).

9.3 Zygote intrafallopian transfer and pronuclear stage transfer

In 1986, Devroey et al. reported the occurrence of pregnancy after translaparoscopic ZIFT in a patient with sperm antibodies (*25, 26*). The development of a safe and efficient procedure for transvaginal oocyte recovery offered the possibility of obtaining oocytes at an outpatient clinic, *in vitro* insemination of the oocytes, and transfer of the cleaving embryos into the fallopian tubes, using a technique similar to that used in GIFT (*27, 28*).

Transferring the embryos at the pronuclear stage (PROST) has the advantage that their exposure to laboratory conditions is minimized (*29*). It is also possible to perform a transcervical/transuterine transfer of either gametes or zygotes into the fallopian tube, as long as there is no underlying tubal disease (*30*).

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10. Results of *in vitro* fertilization, embryo transfer and related procedures

Precise data on the clinical outcome of all the methods used in assisted conception are not available as not all countries have national registries; even in countries that have such registries, information is provided voluntarily and is therefore probably not complete. Reporting deficiencies may affect the overall picture since the missing data may relate to centres that have obtained poor results. Moreover, the format for data collection varies from one centre to another and from one country to another, which makes it difficult to compare data.

The results of medically assisted reproduction (excluding artificial insemination) have been expressed in the following ways:

- *Pregnancy rate per treatment cycle,* calculated from the number of clinical pregnancies occurring and the number of treatment cycles.
- *Pregnancy rate per oocyte-retrieval cycle,* derived from the number of cycles in which oocyte retrieval was attempted.
- *Pregnancy rate per transfer cycle,* calculated from the number of cycles in which embryo transfer was carried out.
- *Live births per treatment cycle.*
- *Live births per 100 attempted oocyte-retrieval cycles (1-3).*
- *Live births per transfer cycle,* i.e. the ratio of the number of live births to the number of cycles in which transfer was performed.

There is an urgent need to use standardized terminology in expressing the results, so that: (1) data from different centres can be compared; and (2) accurate information is made available, and results are not distorted by the use of inappropriate definitions.

Although it has been the practice in some centres to include both biochemical and clinical pregnancies in estimating the number of pregnancies achieved, data from clinical pregnancies should alone be used as these are of much greater relevance to the patient. Pregnancy rates per oocyte-retrieval cycle should not be used to express results, since cancelled

cycles are thereby excluded. The same applies to pregnancy rates per transfer cycle, since treatment cycles where gamete collections were unsuccessful or fertilization did not occur are not then taken into account. It is therefore recommended that the following two definitions be used:

- *Pregnancy rate per treatment cycle,* which should include all treatment cycles irrespective of gamete collection or fertilization.
- *Live births per treatment cycle,* the so-called "take-home baby" rate.

10.1 Clinical results

As of 1989, there were 708 IVF units in 53 countries (see Fig. 5). However, the most recent published information on clinical results dates from 1987 (*4*). Published data from different geographical areas accounting for 286 IVF centres (40% of the total), including centres in Africa, America, Australasia and Europe, in which more than 30 000 women underwent 51 362 treatment cycles, are analysed below and in Figs 6–8. The pregnancy rate per treatment cycle was 11.6% and the live-birth rate per treatment cycle 7.6% (Fig. 7). The pregnancy rate per treatment cycle varied from one geographical area to another, from 8.1% in Africa and Asia to 17.5% in the United Kingdom (*5-9*).

Figure 5
Number of IVF units by geographical region, 1989[a]
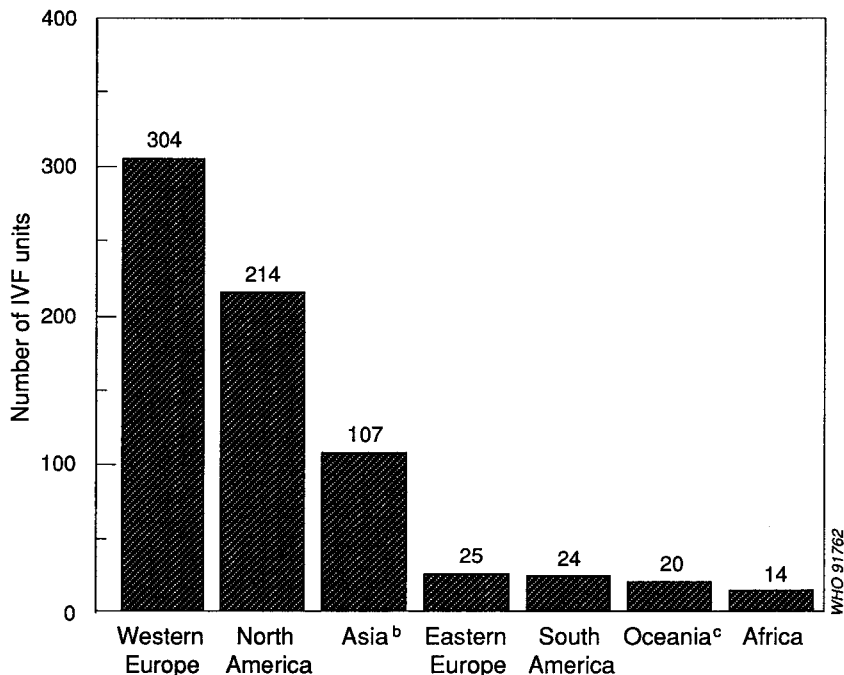
[a] Source: references *4–9*.
[b] China (Province of Taiwan), Hong Kong, Indonesia, Japan, Republic of Korea and Singapore.
[c] Australia and New Zealand.

Figure 6
Results of IVF-ET in selected countries (40% of units), 1987[a]
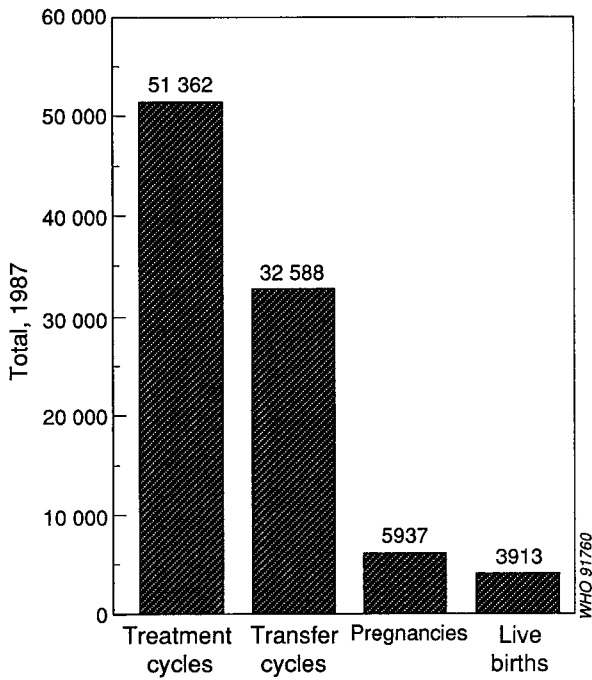
[a] Source:
references *4–9*.

Figure 7
Results of IVF-ET per transfer/treatment cycle in selected countries (40% of units), 1987[a]
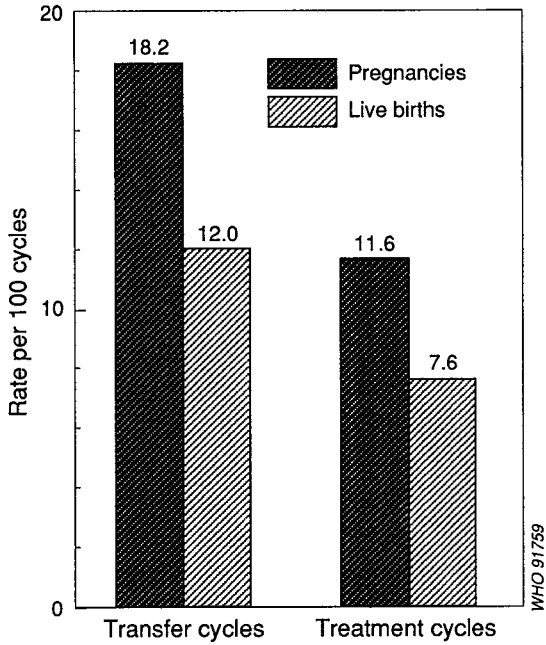
[a] Source:
references *4–9*.

Figure 8
Outcome of pregnancies after IVF-ET, 1987[a]
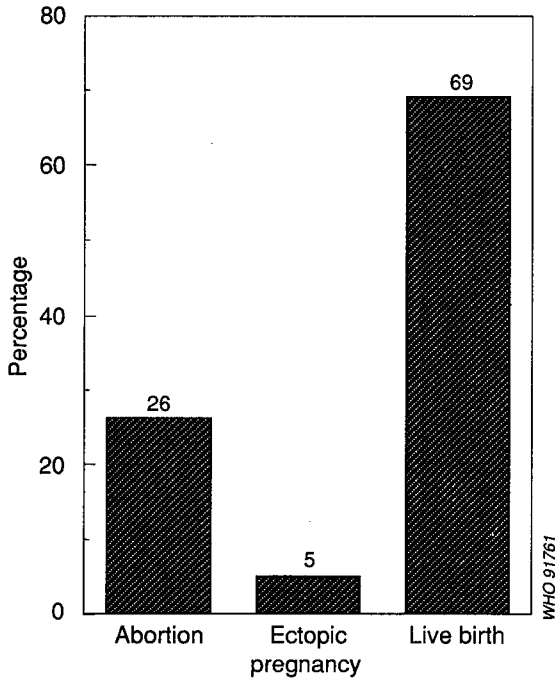
[a] Source: references 4-9.

10.2 Pregnancy outcome

Only about 65% of all pregnancies achieved by IVF have resulted in live births. Spontaneous abortions, ectopic pregnancies and multiple pregnancies have contributed to early and late pregnancy losses and increased perinatal mortality rates. It has been estimated that at least 73% of natural single conceptions do not survive beyond six weeks of gestation and that, of the remainder, about 90% will survive to full term. IVF conceptions do nearly as well as natural pregnancies once they have been diagnosed clinically but have a much higher rate of loss before the clinical diagnosis is made (*10, 11*).

10.2.1 Spontaneous abortions

The incidence of spontaneous abortions following natural fertilization has commonly been quoted as 10% of all pregnancies (*12*). A higher abortion rate has been found in pregnancies in women aged over 40 years, when the couple has been treated for infertility (*13, 14*). The spontaneous abortion rate among pregnancies following induction of ovulation was found to be 20% in patients treated with clomifene citrate (*15*); following induction of ovulation with gonadotrophin preparations, it was in the range 17-31%

(*16*). In these selected groups of infertile patients treated by different procedures, the abortion rate is influenced by the fact that pregnancy is diagnosed during the first few days following conception, whereas abortion rates in normal populations are usually calculated on the basis of data from the eighth week of gestation onwards. The 1987 data for the USA show that, of 1307 clinical pregnancies achieved after IVF, 344 (26%) ended with a spontaneous abortion (*7*). The data from 20 centres in Australia and New Zealand during the period 1979–1987 showed 24% abortions following pregnancies achieved by IVF-ET (641 out of 2624 pregnancies) (*8*), and the global data for 1987 show an abortion rate of 26% after IVF-ET (Fig. 8). The higher rate of spontaneous abortion among pregnancies following IVF-ET may be due to the following factors:

- the women may be older than those who conceive naturally;
- the patients may have a bad reproductive history;
- pregnancy has occurred following induction of ovulation;
- there is a higher rate of multiple pregnancies;
- conception is usually diagnosed very early.

In one study, a comparison was made between the outcome of pregnancy in a group of patients with a history of "preclinical abortion" (biochemical pregnancy that does not progress to clinical pregnancy) after IVF and two sets of matched controls (patients with a history of normal-term pregnancy and patients selected irrespective of previous pregnancy outcome) (*17*). The abortion rates per embryo transfer (preclinical abortion, total miscarriage and total pregnancy wastage rates) in the study group were significantly higher and the ongoing pregnancy rate was significantly lower than in the two sets of matched controls. No correlation was found between the incidence of preclinical abortion and maternal age, type of ovarian stimulation used or the number of embryos transferred. The overall incidence of preclinical abortion in this series was 11.6%, but in another series, it was 3.8% (*18*). Variations in the quality of the culture medium used have been suggested as contributing to an increased incidence of early abortion (*19*).

The rate of spontaneous abortion among 430 women undergoing medically assisted conception (excluding artificial insemination) has been compared with data from other studies (*20*). The indices of pregnancy used were serum hCG concentration of ≥ 25 IU/litre 16 days after follicular rupture, a gestational sac on ultrasound examination at 28 days, and a fetal heart detected 35 days after follicular rupture. Of the total number of pregnancies, 6% were found to be ectopic by 28 days of gestation. Of the remaining 404 pregnancies, 23% ended in spontaneous abortion. At 28 days, 270 women had a gestational sac visible on ultrasound, and 13% of these pregnancies ended in spontaneous abortion. Of the 330 women in whom a fetal heart was demonstrated, 3.6% ended in spontaneous abortion, which is similar to the rate of 3.7% reported after natural conception. When considered together with 4604 natural, 1700 IVF and 406 GIFT conceptions reported elsewhere in the literature, no difference

was found between the spontaneous abortion rates in IVF pregnancies and natural conceptions and between GIFT pregnancies and natural conceptions, but the rate in GIFT pregnancies was higher than that following IVF. The 95% confidence limits for spontaneous abortion rates 5 weeks after follicular rupture or 7 weeks after the onset of the last menses were: natural conception, 7.4–26.1%; IVF, 6.2–20.0%; and GIFT 10.2–43.3%. However, the results of a study with matched, internal controls have yet to be published.

10.2.2 *Ectopic and heterotopic pregnancy*

Ectopic or extrauterine pregnancy is one of the major complications of IVF-ET. The first reported pregnancy achieved by IVF-ET in 1976 was an ectopic pregnancy (*21*). The ectopic pregnancy rate, as reported at the third World Congress of IVF and ET in Helsinki in 1984, was 1.8% of 1084 pregnancies (*22*). A collaborative study on 2342 pregnancies during the period 1979–1984 showed an incidence of 5.2% (*23*). The global data for 1987 show that the incidence was around 5%, i.e., far greater than the 1% reported for spontaneous conceptions (*5-9*).

Heterotopic pregnancy (concomitant ectopic and intrauterine pregnancy) has been recognized as a potentially serious hazard of IVF-ET and other procedures. It has been reported after ovarian stimulation with clomifene citrate and after gonadotrophin treatment (*24*), as well as after IVF-ET (*25-27*) and GIFT (*28*). In one study, the incidence of heterotopic pregnancy was 2.9% of clinical pregnancies (*29*). Other reports have given incidences of 1/4112 (0.02%) to 1/2600 (0.04%) (*29*). The incidence depends on the type of patient attending the clinic for medically assisted conception, being much higher in centres specializing in patients with tubal damage (*29*).

The risk of ectopic pregnancy must be discussed with patients during counselling, before any of the techniques of medically assisted conception are used. After assisted conception, patients should be urged to maintain close contact in early pregnancy with a well-equipped medical facility and skilled staff in view of the morbidity and mortality associated with ectopic and heterotopic pregnancy.

10.2.3 *Multiple pregnancies*

Multiple pregnancy must be regarded as a complication of IVF-ET and similar procedures in view of its frequent association with maternal morbidity and a higher rate of pregnancy wastage. The latter includes early and late abortion, stillbirth, and increased perinatal mortality and morbidity rates primarily due to prematurity. In many IVF units, up to four embryos are transferred at one time, whereas in others the maximum is three (*5-9*). The world survey concluded in 1987 showed a multiple pregnancy rate of 24.2% after IVF-ET or GIFT (*5-9*). The Australian and French registries have reported an increased perinatal death rate in multiple pregnancies. In Australia, the perinatal death rate was 30.4 per

1000 singleton births as compared with 65 per 1000 multiple births after IVF-ET or GIFT (8). In France in 1987, the perinatal death rate was 16.9 per 1000 for singleton pregnancies after IVF-ET or GIFT, 20 per 1000 for twins and 70.7 per 1000 for triplets (6). Multiple pregnancy rates are higher after GIFT than after IVF (38% versus 22%) (30). In the USA, the twin pregnancy rates after IVF-ET or GIFT were 19.9% and 21.5% for 1987 and 1988, respectively, and overall multiple pregnancy rates were 23.7% and 25.4%, respectively (2, 7, 31).

Some centres practise selective fetal reduction in cases of high multiple pregnancy (32-34), although this practice can lead to requests from patients to reduce twin pregnancies to a singleton as well as to select a fetus of a specific sex, with all the attendant moral, legal and ethical difficulties (35).

10.3 Perinatal mortality and premature delivery

The perinatal mortality rates vary from one IVF registry to another; this may be due to the use of different definitions. The IVF data from Australia show a perinatal mortality rate of 42 per thousand as compared with 23 per 1000 in France (6, 8). Even when corrections are made for multiple pregnancy and other factors, IVF-ET and other procedures are associated with a rate of premature delivery of about twice the national average (30).

10.4 Mode of delivery

The caesarean section rate after IVF-ET and GIFT is higher than in normal populations, being affected mainly by the occurrence of multiple pregnancy. The rate of caesarean section in the UK between 1978 and 1987 was 42% (30) and the data from Australia show that this rate has not significantly changed during the last decade (8).

10.5 Birth weight

There has been some concern that the birth weights of infants delivered after IVF are lower than those in a normal population. The Australian registry reported that 30% of such infants weighed 2500 g or less (36). In the United Kingdom, 32% of infants delivered following IVF weighed less than 2500 g as compared with 7% for all births in England and Wales (30). When the birth weights of singletons and twins were considered separately, the singletons' birth weights were significantly lower than those of all singletons born in England and Wales over the same ten-year period. There were no differences between IVF and GIFT with respect to birth weights. Reports from smaller centres show much lower rates of low birth weight (37).

10.6 Sex ratio

The sex ratio in the series of 1581 babies born after IVF or GIFT in the United Kingdom between 1978 and 1987 was 1.07:1 male to female, i.e., not different from the national average (30).

10.7 Chromosomal abnormalities and congenital malformations

Chromosomal anomalies represent the major causes of embryonic loss during the pre- and peri-implantation period. Of all preimplantation embryos resulting from IVF, 25-30% have a chromosomal abnormality (*38*). Such abnormalities have been reported to be present in 62% of embryos/fetuses lost by spontaneous abortion after IVF (*39*), which is the same as the figure reported for abortions following natural conception (60%) (*40*). A higher prevalence of chromosome abnormalities has been reported for abortions from women in whom ovulation has been induced (*41*), but no differences have been observed between groups treated with clomifene and hMG or GnRH and hMG. It would seem that the increase in the incidence in spontaneous abortions following IVF is probably related more to maternal factors than to meiotic or mitotic disorders (*39*). The rate of congenital malformations in children born after IVF-ET does not exceed that observed after natural conception (*5-9*).

The data from the Australian and New Zealand registry for the years 1979-1987 show that the prevalence of major congenital malformations in single and multiple IVF births after at least 20 weeks of gestation was 2.2% (56 out of 2543 births). The rate was higher in singleton than in multiple births (2.6% and 1.6%, respectively). There were more infants than expected with two specific malformations: spina bifida and transposition of the great vessels (*8*). Data from the United States registry for the years 1985-1987 showed a congenital-malformation rate of 2.3% (*9*), while those from Asia and Africa showed a rate of 1.5% (*9*). The French registry data for 1986 showed malformations in 3.4% of 580 infants; the corresponding figure for 1987 was 2.4% of 1079 infants. Three infants with congenital malformations were reported in 329 born following the use of cryopreservation procedures (*42*). There have been some reports of an increased incidence in neural-tube defects in live births following ovulation induction with clomifene, IVF and GIFT, but it is not clear whether the association is real or not (*43-45*). In the United Kingdom, one or more major malformations were detected during the first week of life in 2.2% of 1581 babies born after IVF (2.0% of singleton and 2.5% of multiple births). The total number of malformations reported was within the range of values expected for normal conception (*30*).

10.8 Factors affecting success rates

10.8.1 Indications for IVF

The best results are obtained with IVF-ET when tubal disease or peritubal adhesions are the sole cause of infertility. In cases of severe abnormalities of the tubes, the presence of hydrosalpinges or the absence of fimbriae, the pregnancy rate after microsurgery is only about 15-20%. In such cases, therefore, the primary approach should be IVF-ET (*46*). IVF is less successful when infertility is due to oligospermia and/or asthenospermia and/or teratospermia (*47*). In cases of mild or moderate endometriosis, IVF or GIFT should be considered when conception cannot be achieved

after conventional therapy. In cases of infertility associated with immunological factors, the fertilization and pregnancy rates are lower than when infertility is of non-immunological origin (48).

In a randomized controlled study in which the effectiveness of GIFT and IVF-ET in the treatment of unexplained infertility and oligospermia (less than 20×10^6 spermatozoa per ml) was compared, no significant differences in pregnancy rates were found in either diagnostic group as between the two techniques (49). In cases of unexplained infertility treated by IVF, the fertilization rates are lower than in patients with tubal disease but the implantation and pregnancy rates are similar (50, 51). In a series of 76 women in which the couple's diagnosis was unexplained infertility, one of the following three treatment regimens was used: (a) pure FSH with hMG and hCG; (b) GnRH-a followed by treatment (a); and (c) treatment (a) followed by one cycle of treatment (b). The use of GnRH-a increased the pregnancy rate to 26.7%, which compares favourably with the pregnancy rates achieved by IVF-ET for other indications (52).

10.8.2 Age of the female

Pregnancy rates, especially live-birth rates, are lower in women over 40 years of age (7). The lower birth rates following clinical pregnancies in women of age 30 or more at the time of fertilization have been attributed to the higher incidence of spontaneous abortion among older women (53, 54). One group advocates transferring 11 or more oocytes by GIFT to women aged 40 years or more in order to obtain a 19.2% pregnancy rate, as compared with 12.5% when one to four oocytes are transferred (55). In a series of 613 couples in one IVF programme, the most important prognostic factors were found to be female age, the pregnancy rate showing a linear decline after the age of 25, and previous failed fertilization due to abnormal sperm (56).

10.8.3 Type of ovarian stimulation

The most common protocols used for ovarian stimulation up to 1985–1986 were hMG–hCG and clomifene citrate–hMG–hCG, whereas more recently the combinations FSH–hMG–hCG, GnRH-a–hMG–hCG and GnRH-a–FSH–hCG have gained in popularity. The comparison of the pregnancy rates obtained with different ovarian-stimulation protocols is difficult, therefore, and perhaps inappropriate, not only because centres may differ in the protocols they favour, but also because different protocols may have been used at different times. Patients vary in their responses, as measured by serum estradiol levels and the oocytes retrieved, so that no programme of stimulation can be said to be the best. The results obtained in France in 1988 (56) demonstrated that the use of GnRH-a in long protocols was associated with a higher pregnancy rate than their use in short protocols. Addition of growth hormone to gonadotrophins may offer a new approach to ovarian stimulation, especially for women with a poor response to conventional protocols (57, 58).

Patients with a poor response to ovarian stimulation have a higher incidence of cancelled cycles in subsequent treatment cycles, and fewer follicles are aspirated and oocytes retrieved (*54, 59*). Improved pregnancy rates in this group of patients have been obtained by ovarian stimulation using protocols that include GnRH-a (*60, 61*).

10.8.4 *Embryo quality*

Embryonic development affects the pregnancy rates achieved with IVF-ET. A number of factors can affect the quality of an embryo: the quality of the gametes as judged by morphological appearance (*62-64*), the timing of insemination (*65*), the culture conditions (*66, 67*) and the timing of fertilization (*68*). Pregnancies have been achieved when embryos were transferred at two, three, four and even higher numbers of cell divisions. The optimal stage of development at which to transfer a human embryo to the uterus is at the 2-4-cell stage, as practised in some centres (*69*), or at the 4-8-cell stage, as advocated by others (*70*). It should be noted that thawed embryos, when transferred, give lower pregnancy rates.

10.8.5 *Number of embryos transferred*

The relationship between the number of embryos transferred (see Fig. 9) and the pregnancy rate and frequency of multiple pregnancies has been a

Figure 9
Number of embryos transferred, all pregnancies (1979–1987)

Number of embryos transferred
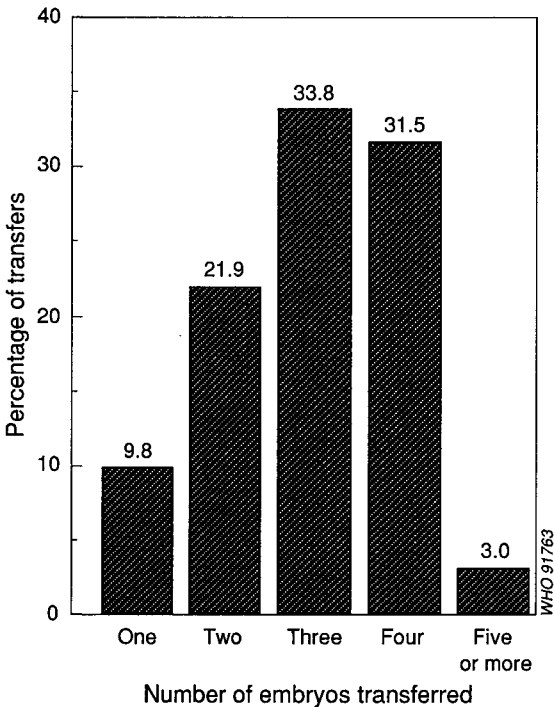
controversial issue in recent years. Some centres have found a significant overall linear increase in pregnancy rates with increasing numbers of embryos. In view of the increased possibility of multiple pregnancies as the number of embryos replaced is increased, together with the increased maternal and perinatal morbidity and mortality, many centres have limited the number of embryos transferred to three. The Australian registry shows that, for clinical pregnancies during the period 1979-1987, the live-birth rate was 64.5% after transfer of one embryo, 69.5% after two, 68.7% after three, 71.2% after four and 56.5% after five (8). In a series of 613 couples treated in 716 IVF-ET cycles, the pregnancy rates per transfer were 9% for one embryo, 14% for two, 18% for three and 17.5% for four (55).

10.8.6 Number of IVF attempts

The clinical pregnancy rate per attempt remains the same up to the seventh attempt, provided that embryo transfer is successful, but decreases thereafter (13, 71).

10.8.7 Implantation

A considerable difference exists between the usual fertilization rates of 70-90% and subsequent pregnancy rates of approximately 20%; this has been attributed to a suboptimal peri-implantation hormonal environment (11, 72, 73). The chance of implantation occurring in IVF-ET is about 20%. Implantation is influenced by both the uterine environment and the quality of the embryo. The uterine contribution to implantation is difficult to assess, but the factors believed to influence it include:

*   Pathological changes in the uterine cavity: congenital malformations, myomas, adenomyosis, endometrial tuberculosis and other intra-uterine infections, and uterine adhesions;
*   Endocrinological factors:
    *   synchrony of the development of the embryo with the preparation of the uterine endometrium for implantation: the "window of implantation" (74-78);
    *   adequate endocrine function in the luteal phase (79);
    *   the secretion of the uterine proteins required for embryo growth.

It is not possible at present to evaluate and measure the contribution of uterine factors both to the establishment of pregnancy and to the normal development of the embryo and the fetus.

10.8.8 Luteal-phase treatment

As mentioned above, implantation after IVF may be affected by the asynchrony between the endometrium and the stage of maturation reached by the embryo when it is placed in the uterine cavity (74-78). The embryos are placed in the uterine cavity 36-48 hours after follicular aspiration whereas oocytes fertilized *in vivo* implant 5-7 days after ovulation (80). Ovarian stimulation regimens may lead to altered luteal function, resulting

in persistently elevated estradiol levels (*81*), and there is evidence that luteal-phase hormonal patterns are different in IVF cycles (*82*). It has therefore been claimed that corpus luteum dysfunction may be responsible in part for early pregnancy loss (*83*). Human chorionic gonadotrophin (10 000 units five days after the ovulatory dose) has been shown in one study (*84*) to correct the midluteal-phase decline in plasma estradiol and progesterone levels and to produce a statistically significant increase in the successful pregnancy rate, but two other studies have failed to confirm this finding (*85, 86*).

Other workers have attempted luteal-phase supplementation using progesterone; while some have claimed that this gives higher pregnancy rates (*87*), others have failed to obtain similar results (*88*). A higher pregnancy rate has also been reported following the administration of progesterone 33-34 hours before follicular aspiration (*89*), but these findings were not confirmed by another group (*90*).

It should be pointed out that the doses, route of administration and pharmaceutical composition of the progesterone preparations used in these studies differed, with consequent differences in bioavailability. Further research is needed to compare the effects of bioequivalent preparations of progesterone given in the peri-ovulatory and luteal phases and those of different dosage regimens of hCG.

10.8.9 *Male factors*

IVF-ET has been attempted in the management of all causes of male infertility except, of course, idiopathic azoospermia (*51, 91-93*).

A low sperm concentration is often associated with an increased incidence of sperm abnormalities and/or decreased sperm motility. IVF with abnormal sperm results in lower fertilization and pregnancy rates than fertilization with sperm of normal motility and morphology (*94, 95*). When more than 14-16% of the spermatozoa used for *in vitro* insemination are normal, the probability of fertilization is greater than 87% (*47, 96*). In one study, when more than 14% of spermatozoa were morphologically normal, the pregnancy rates per cycle were 39-41%; the corresponding figures when less than 14% were normal were 24-25% (*47*). If the percentage of morphologically normal sperm is less than 4%, the fertilization rate can be increased from 64% to 90% by increasing the sperm concentration at *in vitro* insemination 10-fold to 500 000 sperm per ml per oocyte, but the pregnancy rate only increases from 0 to 6.3%. There would appear to be little difference in oocyte fertilization rates whether the proportion of sperm showing progressive motility is 10-20% or 31-40% (*47*). The fertilization rates for these two groups in one series were 69% and 73% respectively — still well below those for normal sperm — and the subsequent pregnancy rates per embryo transfer were also lower (*47*).

Sperm density and total concentration must be evaluated in conjunction with morphology and motility, but it is generally accepted that the fertility

prognosis is poor if the sperm concentration is less than 10×10^6 per ml or when the sperm concentration in the motile sperm fraction is less than 1.5×10^6 per ml (*47, 97-99*). One group found that sperm concentration did not correlate well with fertilization rate; sperm morphology and motility were the factors most predictive of successful fertilization. The fertilization rate fell when the proportion of sperm showing progressive motility with normal morphology fell below 20%. Computerized image analysis did not appear to be more predictive than the conventional semen parameters (*106*). Other studies have shown that sperm morphology is the best predictor of successful IVF (*95, 106-108*). If the percentage of sperm with normal morphology and motility is 14 or greater, the fertilization rate is not seriously impaired (*107, 109*). Patients in whom semen analysis reveals single defects (sperm number, motility or structure) have a better prognosis that patients with two or more defects (*100*).

Immunological factors associated with male infertility include antisperm agglutinating and immobilizing antibodies and sperm-coating antibodies. When fetal cord serum is used for culturing oocytes from women with antisperm antibodies, pregnancy rates after IVF are similar to those in women without such antibodies (*101*). When sperm-coating antibodies have been detected in the male partner, pregnancy rates following IVF have been reported as 44% per transfer cycle (*102*), 6% per treatment cycle (*54*), and 53% per patient, 34% per cycle and 38% per transfer (*47*).

Congenital absence of the vas deferens and consequent obstructive azoospermia has been treated by using alloplastic reservoirs or sperm aspiration to collect epididymal sperm for use in IVF (*103-105*).

10.8.10 *Cryopreservation*

As already pointed out, in many centres, in order to reduce the incidence of multiple and especially high multiple pregnancies, the number of embryos transferred at any one time is limited to three or four. The excess embryos can be cryopreserved for use in subsequent spontaneous or stimulated cycles, or donated. The ovarian-stimulation regimens currently used result in the development and retrieval of numerous oocytes so that there are often oocytes surplus to the patient's immediate requirements. It should be noted that the results obtained following the transfer of thawed embryos are worse than those obtained in routine IVF-ET, both because the embryos have been subjected to deep-freezing and because fewer thawed embryos are usually transferred.

Human embryo freezing is a routine procedure in many IVF units. Data from 106 centres were presented at the 1989 World Congress of *In Vitro* Fertilization and Alternative Assisted Reproduction: 30 850 embryos had been cryopreserved, of which 18 322 had been thawed and 10 290 (56.2%) judged to be suitable for transfer. A total of 6441 embryos had been transferred, 632 clinical pregnancies achieved (9.8%) and 329 children born, while there were still 220 ongoing pregnancies; 19% of pregnancies resulted in clinical abortion (*42*). The maturation stage at which human

embryos should be frozen and the cryoprotectant that should be used remain controversial (see section 8.4), and no reliable, simple and inexpensive method is available (*110*). Some centres cryopreserve embryos at the blastocyst stage, others at the eight-cell stage, and some even preserve pronuclear oocytes (*111*). Lassalle et al. (*112*) found no differences between the standard cryoprotectants in terms of embryo survival. Embryo survival has been reported to be correlated with morphological features, but pregnancies have occurred even when less than half of the blastomeres were intact after thawing (*113*).

The collected data from the USA indicate that, in 1987, 7397 embryos and oocytes from 2085 patients were frozen. The centres where the smallest number of embryos were transferred per pregnancy had transferred more than 150 embryos that had been frozen using 1,2-propanediol as cryoprotectant. A total of 127 oocytes had been thawed but the fertilization rates were low (17.3%), as were the cleavage and transfer rates (36.4%), and there were no pregnancies (*114*).

The duration of storage in liquid nitrogen seems to have no effect on the survival or viability of embryos, and pregnancies have been obtained with embryos stored for up to 21 months (*115, 116*).

10.9 Ovum donation and related procedures

10.9.1 *Ovum donation*

Ovum donation is indicated in several clinical conditions, and especially in cases of ovarian failure. For legal and ethical reasons, ovum donation is practised only in certain countries. The pregnancy rate for ovum donation is higher than that for other assisted reproductive techniques, namely about 20–36% (*117*); this may be because the oocytes are obtained from a woman of proven fertility.

10.9.2 *Embryo donation*

The indications for embryo donation include infertility of both male and female origin, and repeated early pregnancy loss, including repeated failure of assisted conception. Embryo donation is practised in only 10 countries and no global data are available. It is obvious that the legal and ethical problems associated with embryo donation may be greater than those with sperm or oocyte donation.

10.9.3 *Gamete intrafallopian transfer*

The GIFT technique (see also section 9.2) was first reported by Asch et al. in 1984 and has achieved considerable popularity (*118-124*). GIFT is claimed to be more physiological than IVF and, because fertilization occurs *in vivo*, the procedure may be accepted by those who have religious or other objections to IVF. In addition, there is no need for IVF culture techniques and embryo transfer. The disadvantages of GIFT are that it is

limited to women in whom the tubal ostia are readily visible at laparoscopy or mini-laparotomy (*49*), that sperm preparation and laparoscopic oocyte retrieval must be scheduled simultaneously, and that the complete operative procedure takes longer than IVF (*124*). The most common indications for GIFT are unexplained infertility (*49, 125*), endometriosis (*124-126*), immunological causes of infertility (*49, 101, 126, 127*) and oligospermia (*124, 125, 128, 129*). It has been used in combination with oocyte donation in cases of premature ovarian failure (*130*) and following failed artificial insemination by donor (*126, 131*). Candidates for GIFT must have at least one patent fallopian tube that appears to be anatomically and functionally normal.

The technique consists of the following stages: ovarian stimulation, sperm preparation, oocyte retrieval, and transfer of gametes by laparoscopy (see section 9.2). Not more than two oocytes per tube are transferred by most centres (*5, 7*). Although the pregnancy rate may be higher when three or more oocytes are transferred (*132*), the multiple pregnancy rate may also be higher (*133*). Transfer of large numbers of oocytes has been advocated in the treatment of older infertile women; in such cases, pregnancy has been reported to be more than twice as common in those receiving 11 or more oocytes as in those receiving between one and four oocytes (*134*).

The pregnancy rate per treatment cycle in the GIFT procedure is similar in different centres, namely around 25%; the spontaneous abortion rate is approximately 24%; the ectopic pregnancy rate is 4-6%; and the multiple pregnancy rate is similar to that obtained with IVF when up to four oocytes are replaced (*5-8*).

10.9.4 *Other procedures*

Procedures similar to GIFT include zygote intrafallopian transfer (ZIFT) for the treatment of unexplained infertility (where the pregnancy rate has been reported as 48%; as compared with 22% with IVF-ET and 30% with GIFT (*135*)), male infertility (*102*) and infertility due to immunological causes (*136*). Pronuclear-stage transfer (PROST) (*137, 138*), tubal embryo transfer (*139*) and fallopian replacement of eggs with delayed intrauterine insemination (FREDI) (*140*) are more limited in their application, and the published results are insufficient to enable firm conclusions to be drawn as to their relative merits.

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11. Collection, storage and preparation of donor semen for artificial insemination

There has been a considerable increase in the demand for artificial insemination with donated semen in several countries during the past 10 years: in the USA by May 1988, 30 000 births had resulted from artificial insemination by donor, and there have been 3000 requests each year in France since 1980. In view of this increased demand, several countries have considered establishing a nationwide system of semen banks for the treatment of infertility as well as to store semen as "fertility insurance" before the male partner undergoes chemotherapy, radiotherapy or vasectomy (1). In some countries, e.g., the United Kingdom and the USA, there are commercial semen-preservation facilities or hospital- or university-based banks, while in others, such as Denmark and France, a national system exists (2, 3).

11.1 Donor recruitment

Students have been the donors most commonly used, followed by hospital personnel and the husbands of women who have recently been pregnant or given birth. Ideally, semen should be donated on an altruistic basis and compensation should be minimal and not the main motivation for the donation. Proven recent fertility is desirable but not an absolute requirement; if fertility has not been demonstrated, semen quality should satisfy the WHO criteria for a normal semen analysis (Table 8) (4). In both situations, the proportion of sperm showing progressive motility after thawing should be higher than 40% (5). Donors should be younger than 55 years of age. They should be informed of the conditions under which the

Table 8
Normal semen analysis [a]

Volume of ejaculate	≥ 2.0 ml
pH	7.2–7.8
Sperm concentration	$\geq 20 \times 10^6$ spermatozoa/ml
Total sperm count	$\geq 40 \times 10^6$ spermatozoa
Motility	$\geq 50\%$ with forward progression or $\geq 25\%$ with rapid linear progression when examined within 60 minutes of collection
Morphology	$\geq 50\%$ with normal morphology
Viability	$\geq 50\%$ live
White blood cells	$\leq 1 \times 10^6$/ml
Mixed antiglobulin reaction	$< 10\%$ with adherent particles
Immunobead test	$< 10\%$ with adherent beads

[a] Source: reference 4.

donation is to be made and written evidence of informed consent to them retained by the clinic. Use of a consent form of this type should be mandatory; it should include a statement to the effect that the donor agrees not to attempt to find out the identity of the person(s) to whom he is donating the semen.

Donor recruitment can be promoted by: (1) publicizing the semen bank through the mass media; (2) making use of contacts among the medical profession; (3) contacting vasectomy candidates; and (4) encouraging candidate couples for artificial insemination by donor to approach close friends or relatives, who might be incorporated into the pool of donors but would not be used for the couples that recommended them.

The size of the donor pool required for a specific centre can be estimated from the number of requests received for artificial insemination by donor (6).

$$\text{Number of donors required} = \frac{\text{Number of requests}}{6}$$

11.2 Donor screening

Donors who present a risk either to the recipient (presence of sexually transmitted diseases, including infection with human immunodeficiency virus (HIV)) or to the child to be born (sexually transmitted disease or hereditary disease) should be excluded (7). Microorganisms that have been reported as being present in donors' semen include *Neisseria gonorrhoeae, Mycoplasma hominis* (4), *Ureaplasma urealyticum* (8), *Chlamydia trachomatis* (9), *Trichomonas vaginalis* (10), HIV (11) and hepatitis B virus (12). The transmission of sexually transmitted diseases can be minimized by requesting a complete sexual history and excluding individuals with multiple sex partners and/or homosexual or bisexual experience, as well intravenous-drug users, those with a history of a blood transfusion in an HIV-endemic area, and those with a sex partner positive for hepatitis B. The screening tests usually employed (3, 13) are listed below. The potential donor should also have a physical examination. Fresh semen from a donor should not be used for artificial insemination because of the risk of infection and, in particular, of HIV transmission.

11.2.1 *Microbiological screening of donors and semen*

Chlamydial infection
If a potential donor has a recent history of painful micturition or urethral discharge, a urethral swab should be taken for fluorescent antigen staining or testing by the enzyme-linked immunosorbent assay. It should be noted that the sensitivity of these tests in asymptomatic men is only 50–60%. If either test is positive, the man and his sexual partner should be treated before semen donation. If two successive swabs are negative, the man can be allowed to donate semen.

Cytomegalovirus

The American Fertility Society has recommended that all semen donors be screened for cytomegalovirus (CMV) antibodies (*12*); sperm from donors who are antibody-negative should preferably be used for all recipients, whether negative or positive (*2*). CMV complement-fixation or passive latex-agglutination tests can be used for screening (*14, 15*). Potential donors who are positive for CMV antibodies should be rejected unless the recipient is also positive.

Neisseria gonorrhoeae

A urethral swab should be taken for gonococcal culture if the potential donor has a history of urethral discharge or dysuria. If the urethral culture is positive, the man should not be accepted as a donor and both he and his sexual partner should be treated.

Hepatitis B virus

The potential donor should be screened for hepatitis B surface antigen. If the test is positive, the man should not be accepted as a donor. In countries with a low prevalence of hepatitis, donors positive for hepatitis B core antibody may also be excluded.

Human (alpha) herpesvirus [herpes simplex virus]

The man should be rejected as a donor if clinical lesions on the genitalia are present.

Human immunodeficiency virus

The potential donor must be rejected if he is HIV-positive, and appropriate counselling and contact-tracing should be undertaken. If the man is HIV-negative and is accepted as a donor, HIV antibody testing must be repeated after three months and thereafter every six months. All semen samples should be kept in quarantine until the *next* blood sample has been shown to be HIV-negative.

Human papillomavirus

The donor should be rejected if clinical lesions are present.

Opportunistic pathogenic bacteria

If the semen culture is positive for pathogenic bacteria, the man should be treated, and accepted as a donor only after two successive semen cultures are negative.

Treponema pallidum

All potential donors should be screened by the venereal disease research laboratory (VDRL) test. If both the original test and confirmatory test(s) (e.g., a haemagglutination assay or fluorescent antibody absorption test) are positive, the man should be treated for the infection and should not be accepted as a donor.

11.2.2 *Transmission of hereditary disease*

The possibility of transmission of hereditary disease can be greatly reduced by genetic screening and karyotyping: up to 4.2% of potential donors are eliminated by karyotype screening alone (*16, 17*).

Donors with a specific risk factor, such as an allergy or diabetes mellitus, can be accepted as long as their semen is not used for recipients with similar illnesses (*18*). Donors from racial groups with a high prevalence of diseases such as sickle-cell anaemia and thalassaemia should be screened for the corresponding traits and excluded if positive.

11.3 Cryopreservation of semen

The semen is obtained by masturbation after 2–3 days' abstinence, collected, preferably in the laboratory, in a sterile, warm glass container and left to stand for up to 20 minutes at room temperature (20–30 °C). Immediately after liquefaction, a cryoprotecting agent is added to give a dilution of 1:1 or 1:2. The agent employed is usually glycerol (*19*) or a medium such as Ackerman's or that developed by Mahadevan & Trounson (*20*). However, it has been reported that the use of other buffer systems, and in particular Hepes and zwitterion (Tes–Tris) buffers,[1] with egg yolk, citrate and glycerol (*21*), gives a higher post-thaw motility. Rao & David (*22*) have suggested that the use of 1,4-dithiothreitol in the freezing medium may improve the recovery of motile and live spermatozoa in the post-thaw samples.

The glycerol or the medium should be added to the semen drop by drop at room temperature and the temperature of the sample lowered at the rate of 1 °C per minute to 5 °C. This temperature should be maintained for at least 5 minutes but not for longer than 1 hour (*21-24*). The semen sample should then be drawn into 0.25-ml straws, which are identified by colour and bar coding.

The sample can be frozen by immediate immersion in liquid nitrogen (rapid method) or by progressive temperature reduction to that of liquid nitrogen (slow technique). The temperature drop is 16–25 °C per minute in the rapid method and as little as 0.5 °C per minute in the slow method. Neither method has obvious advantages as compared with the other (*25*). The semen is stored in liquid nitrogen at −196 °C until used.

11.4 Thawing

Both rapid and slow thawing techniques have been used with human sperm. In the slow method, the specimen is allowed to warm up to room temperature, firstly to 5 °C and then to 25 °C. When straws are used for

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[1]Hepes: 4(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.
Tes: *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid.
Tris: 2-amino-2-hydroxymethyl-1,3-propanediol.

storage, the best results seem to be obtained by leaving them at room temperature for a few minutes, which allows them to thaw fairly slowly (26). Little difference in sperm motility is seen whether the specimen is thawed rapidly or slowly (27).

For artificial insemination, the straws of donated semen are used directly after thawing, the first drop of semen being used to estimate the post-thaw sperm motility. Semen with a motility greater than 40% is suitable for use. For IVF with donor sperm, the contents of two or three straws are either gently mixed with medium and then washed and processed using a swim-up method (see section 8.3.2), or subjected to a discontinuous density gradient, centrifuged and washed in the medium.

11.5 Effects of cryopreservation on spermatozoa

Post-thaw motility is the best parameter for use in evaluating the quality of the sperm-preservation procedure.

The pregnancy rate per cycle has been reported as 7.1% if the proportion of sperm showing progressive motility is less than 25%, and 18.1% if the proportion is more than 60% (13). Cryopreservation causes a decrease in the consumption of fructose and glucose, modifies the production of lactic acid and reduces oxygen consumption (28).

Deep-freezing does not appear to alter the sperm genome, since the incidence of congenital abnormalities in children born as a result of artificial insemination with thawed donated sperm is not different from that in the normal population (13). The DNA content may be reduced by cryopreservation and subsequent thawing (29), but freezing does not appear to diminish the sperm's fertilizing capacity, as shown by the equally good results obtained with fresh and frozen sperm in terms of the fertilization and pregnancy rates obtained in IVF (8). However, frozen semen has been shown to result in lower pregnancy rates than fresh semen when the recipient has been used as her own control (30); this has been attributed to the reduction in motility seen in post-thaw semen specimens (31). A variety of compounds have been used to improve post-thaw motility, and the use of pentoxifylline-treated sperm in cases of oligoasthenospermia has resulted in pregnancy by both pronuclear-stage transfer (32) and IVF-ET (33).

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12. Artificial insemination — indications and techniques

Although the principle of artificial insemination was recognized in the Talmud (2nd century), the first description of the process of fertilization is attributed to Spallanzani of Modena (*1*). In 1770, Hunter successfully performed the first human insemination, using the semen of a husband with hypospadias (*2*). In 1890, Dickinson first used artificial insemination with donor semen in England (*2*). The first successful freezing of human semen was reported by Bunge & Sherman in 1953 (*3*).

The United States Office of Technology Assessment estimated that, by May 1988, 172 000 women had undergone artificial insemination in the USA, resulting in 35 000 births from insemination with the husband's sperm and 30 000 from insemination by donor (4). In France, with 55 million inhabitants, requests for artificial insemination by donor have been running at about 3000 per year since 1980, 0.50-1% of new couples presenting with infertility (5).

12.1 Evaluation of the female partner

When couples seek artificial insemination, the female partner should first be evaluated before any time is invested in the procedure and hopes are raised. History-taking is focused on detailed family screening for genetic abnormalities. Normal ovulatory function is documented by means of the basal body temperature and a midluteal serum progesterone determination or endometrial biopsy. Possible uterine or tubal abnormalities can be excluded by hysterosalpingography and/or laparoscopy.

Tests for sexually transmitted diseases, such as syphilis, and for hepatitis B and HIV must be performed for medicolegal reasons. Laboratory evaluation must include determination of the blood group and rhesus factor, and a rubella and toxoplasmosis titre. Testing of sperm penetration of the cervical mucus is important. In cases of post-coital test failure, the sperm defect must be confirmed by examination of the spermatozoa and possibly by an animal-model IVF test, such as the zona-free hamster oocyte or hemizona assay (6).

12.2 Artificial insemination with the husband's semen

For insemination with the husband's semen, the sperm can be introduced intracervically (7-10) or directly into the uterine cavity (11-17); various sperm preparations, such as split ejaculate, and sperm recovered after passage through a density gradient (swim-up technique) or after centrifugation, can be used. The timing of the insemination will depend on the quality of the cervical mucus, the presence of an LH peak, or ultrasound monitoring of follicular development. Attempts have been made to improve the results by using ovarian stimulation during the cycle of insemination (18-23).

12.1.1 Indications for intracervical insemination

This method can be used in cases of ejaculatory dysfunction such as retrograde ejaculation and anejaculation (24). Pretreatment ejaculates should be obtained where the male partner is to undergo chemotherapy or pelvic radiotherapy. There is no evidence that the method is effective when other factors are responsible for male infertility.

12.2.2 *Indications for intrauterine insemination*

Many indications for intrauterine as opposed to intracervical insemination can be found in the literature. Nevertheless, the effectiveness of the former in terms of pregnancy rate per cycle is generally below 10%, and the method appears to be effective only in the first six cycles of insemination (*8-10, 16*). The most common indications include cervical hostility and cervical immunological factors, but in a randomized study no advantage was found when intrauterine insemination was compared with normal intercourse (*9, 16, 17*). Two prospective randomized studies have given satisfactory results in couples with unexplained infertility when the procedure was used in combination with ovarian stimulation (*18, 20*).

12.2.3 *Use of fresh or frozen semen*

Several studies have shown that fresh semen is more effective than frozen semen (*22, 25*). Other studies have shown, however, that the advantages of fresh semen are limited (*23, 26*). Nevertheless, even if fresh semen seems to be more effective, it will probably be used less and less frequently because frozen semen has the following advantages:

- it is readily available;
- bacteriologically and virologically screened samples can be used (*27*);
- it is easily transported;
- it is possible to perform several inseminations;
- the semen can be stored for several years, thus allowing a couple to conceive several children.

12.3 Artificial insemination by donor

In order to minimize the possibility of HIV transmission, only suitably quarantined semen should be used.

12.3.1 *Indications*

In the great majority of cases, artificial insemination by donor is requested by heterosexual couples; in one national scheme, single women and female homosexuals accounted for only 3% of those requesting this service (*4*).

Artificial insemination by donor is usually suggested when the male partner has an irreversible cause of infertility, such as idiopathic and obstructive azoospermia (50% of requests), idiopathic oligospermia, a genetic disease (*5*), or non-psychogenic ejaculatory dysfunction (traumatic surgery, neurological disease, spinal cord injury). It is also indicated for severely Rh-sensitized women.

12.3.2 *Donor–recipient matching*

In most artificial insemination programmes, phenotypic matching with the husband includes height, hair and eye colour, race and Rh blood group. Some centres obtain photographs of the donors and recipients to aid in

matching facial features and physical characteristics more closely. Donor blood types are selected so that the blood group of the infant will be consistent with those of the recipient couple (*28*).

Blood groups are specifically matched when the recipient is Rh-negative and has been sensitized to the Rh antigen (*23*). When a woman is Rh-negative but not Rh-sensitized, a Rh-negative donor is preferred. If a Rh-negative donor is not available, the recipient is so informed and given the option of proceeding with the insemination and receiving anti-D (anti-Rh_o) immunoglobulin injections during the pregnancy and post-partum period (*28*).

12.3.3 *Risk of consanguinity*

It is possible to calculate the risk of chance consanguinity resulting from the use of artificial insemination by donor in a given population (*29*). This varies with the population size and the number of offspring resulting from artificial insemination per donor. Unless the population constitutes a truly isolated subgroup, it would appear that the risk of consanguinity can be minimized if each donor is limited to five successful pregnancies (*5, 30*).

12.3.4 *Techniques*

The insemination technique is simple but far from being standardized. The site of insemination is usually the cervix and the volume of semen used varies from 0.25 ml to 0.5 ml. A cervical cap may be used. It is essential to perform the insemination(s) on the most appropriate day of the cycle for conception. Examination of the curve of basal body temperature during previous cycle(s) is the simplest way to determine the approximate timing. It has been shown that conception is most likely to occur from day −3 to day −1 (day 0 being the last day of hypothermia taken from the temperature chart) (*31*). Three other factors play a role: (1) dilatation of the cervical canal; (2) the amount of cervical mucus; and (3) its viscosity (*32*). Better prediction of ovulation may be achieved by ultrasonography or by serial measurement of the plasma levels of estradiol and especially of LH (*28*). In the absence of ovulatory dysfunction, exogenous ovarian stimulation has not been found to increase the pregnancy rate (*28*). The success rate increases with the number of inseminations per cycle, the best results being obtained with three; a higher number has not proved to be of additional benefit (*32*).

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13. Results of artificial insemination

13.1 Assessment of results

Assessment of the results of artificial insemination should be based on the mean success (i.e. pregnancy) rate per treatment cycle and the cumulative success rate; a life-table method of analysis should be used (*1, 2*). The use of pregnancy rates expressed as a percentage of patients treated is not recommended.

13.2 Insemination with the husband's semen

The results achieved will depend on the indications for artificial insemination with the husband's semen. Results in cases of sexual dysfunction, such as retrograde ejaculation or anejaculation, depend on semen quality (*3*), as do those with cryopreserved semen obtained before the patient underwent chemotherapy or radiotherapy. The usual pregnancy rate is 8–10% per cycle if the sperm count is more than 2 million per straw (8 million per ml) (*4*) or if at least 10 million motile sperm are inseminated in women treated with ovulation-stimulation drugs (*5*). Semen may be frozen for up to 10 years without loss of fertilizing capacity (*4*).

Pregnancy rates of up to 16% per cycle have been reported in patients with cervical mucus abnormalities, such as dysmucorrhoea, or antisperm antibodies (*1, 6, 7*). In the latter case, the pregnancy rate is approximately 3% per cycle with fresh semen, 6% with washed spermatozoa and 9% with sperm after swim-up treatment (*8*).

Opinions differ as to the benefits to be obtained from the use of the male partner's semen in cases of oligoasthenospermia. Variability in patient populations, techniques of insemination, and method of timing of apparent ovulation make comparison of data difficult.

Intrauterine insemination seems to be more effective than intracervical (*9, 10*), but, except in patients with antisperm antibodies, the method of processing the semen (washing, swim-up, or centrifugation) does not influence the pregnancy rate (*11, 12*).

Certain investigators (*6, 13-15*) but not others (*1*) have reported that ovulation stimulation is effective in improving the pregnancy rate. In some studies, the large majority of conceptions were achieved within 3-6 months of artificial insemination (*1, 6, 11, 12, 16*) but the drop-out rate was high (17-72%) (*17*).

The few randomized studies that have been conducted have suggested that, in cases of oligoasthenospermia, neither intracervical nor intrauterine insemination is of benefit. In a controlled trial, Kerin et al. (*18*) reported that LH-timed intrauterine insemination with washed semen produced higher pregnancy rates than normal intercourse, but their pregnancy rate of 22% per cycle could not be duplicated by others (*10, 19*).

Another study involved 345 couples with non-tubal infertility and covered 702 treatment cycles in which high intrauterine insemination of the husband's washed semen was performed daily for three days in the periovulation period. The pregnancy rates were as follows: couples in whom the only abnormality was a persistently negative postcoital test: 15.8%; antisperm antibodies in the male: 18.5%; antisperm antibodies in the female: 17.1%; poor cervical mucus: 4.7%; asthenospermia: 0%; endometriosis: 4.1-7.7%, depending on the severity of the disease; and unexplained infertility: 8.5%. The authors concluded that insemination with the husband's semen could be considered a first line of treatment in cases of poor semen-cervical mucus interaction or antisperm antibodies before IVF-ET or GIFT procedures were used (20, 21).

In unexplained infertility, two studies (13, 15) showed that intrauterine insemination combined with superovulation is a possible alternative to GIFT or IVF-ET. The pregnancy rate increased from 2.7% per cycle with intrauterine insemination alone to 26.4% with intrauterine insemination and superovulation. The multiple pregnancy rate with this method of treatment has been reported as 30% (13). These results have yet to be confirmed.

Apart from those on its use in cases of sexual dysfunction (3), there have been no large-scale studies showing that artificial insemination with the husband's semen improves fertility in the case of male and unexplained infertility. Standardization of indications, more precise timing of ovulation, standardization of the insemination technique and the use of a control group are all necessary before it can be said that the benefits of intrauterine insemination have been demonstrated.

13.3 Donor insemination

The results obtained in a large national series showed that the mean pregnancy rate per cycle was 10.3% over the first six cycles of treatment with frozen semen, after which it diminished: at 24 cycles the mean rate was 2.3% per cycle (22). The cumulative pregnancy rate with cryopreserved semen is approximately 50% after the first six cycles and approximately 70% at the end of the first year (22, 23); these results are very similar to these obtained with fresh semen (24-26).

The factors influencing the pregnancy rate include the quality of the cervical mucus, ovulatory status, the normality of the women's genital tract and her age (2, 8, 27, 28). In one study, the pregnancy rate was increased from 4% to 20% per cycle by increasing the number of spermatozoa inseminated to 15 million per straw or 60 million per ml (above this figure, the rate remained unchanged) (29). The most important semen parameter has been found to be post-thaw motility. It is therefore recommended that only semen having a good post-thaw motility (greater than 40% of sperm) be used (29).

13.3.1 *Length of treatment*

In women without a history suggestive of pelvic disease who have not become pregnant after six appropriately timed insemination cycles, diagnostic laparoscopy should be undertaken to detect asymptomatic pelvic abnormalities, such as minor degrees of endometriosis or pelvic adhesions. After one year of unsuccessful artificial insemination by donor, couples should be counselled by the clinician and a psychologist on the options available, including living without a child, adoption, and resorting to other medical techniques, such as GIFT or IVF-ET.

Except for women with tubal disease and those above 35 years of age, it is probably simpler and more economical to use artificial insemination by donor as a first line of treatment. After 12 unsuccessful treatment cycles, IVF using donor sperm should be considered, especially if the woman is 35 years or older, if she has ovulatory dysfunction, or if a factor such as endometriosis or the presence of only one normal tube is contributing to infertility (*22*).

13.3.2 *Outcome of pregnancy*

A number of studies have been undertaken on the outcome of pregnancy following artificial insemination by donor (*28, 30, 31*), but often without a control group, and without always distinguishing between insemination with frozen semen and that with fresh semen (*25, 28*).

Spontaneous abortions and ectopic pregnancies
The spontaneous abortion rate after artificial insemination varies between 11 and 22%, with a mean value of 16.1% (*31-33*); this is the same as in spontaneous pregnancy.

Ectopic pregnancies occur in up to 2% of cases (*31, 32*); their occurrence is associated with risk factors such as previous pelvic inflammatory disease.

Multiple pregnancy
The reported incidence of multiple pregnancy following artificial insemination can be up to 4.5% per treatment cycle with concomitant ovarian stimulation (*24*), but the frequency of maternal complications following conception by artificial insemination by donor is no higher than normal (*31, 32*).

Maternal complications
In only one study was a higher incidence of pre-eclampsia (9.3% versus 5% expected) found (*34*). However, maternal age was not taken into consideration in this study and is usually greater in cases of artificial insemination (*22*); this could contribute to an increase both in the incidence of pre-eclampsia and in operative delivery rates (*32*).

Sex ratio, congenital malformations and chromosomal abnormalities
The sex ratio following artificial insemination by donor is not different from that in spontaneous pregnancy (*30, 33, 35, 36*). The overall incidence of congenital malformations is less than 2% and is lower than that reported in the general population (*22*). However, the incidence of chromosomal abnormalities following insemination with cryopreserved semen is at the upper limit of normal (*30*), and a large-scale prospective study should be conducted to examine this question in detail.

13.3.3 Follow-up studies

Very few follow-up studies have been carried out on the development of the several hundred thousand children conceived following artificial insemination by donor (*37-39*). There does not seem to be any adverse effect on their development, but this can only be confirmed by formal follow-up studies.

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14. **Equipment and personnel**

Experience over the past decade has shown that the performance of centres offering medically assisted conception depends a great deal on the quality of the available technology and the skills of the personnel as well as on the volume of the clinical workload. Innovations are constantly being made in this field and the personnel of such centres must be committed to the adoption of new techniques, skills and drug regimens as they become available. An adequate throughput of patients is therefore necessary so as to maintain the skills and motivation of the staff.

It is important to distinguish between centres offering clinical services only and those engaged in research projects as well. The two activities should be quite distinct, the research requiring formal protocols drawn up in accordance with accepted guidelines; it must also be scientifically justified and approved by independent peer review as well as by a formal ethics and human experimentation committee. National guidelines have been published by the competent professional bodies in Australia (*1*) and the USA (*2, 3*), and by the Voluntary Licensing Authority in the United Kingdom (*4*).

All the activities involved in medically assisted conception should be confined to a specific clinical unit. However, local circumstances, such as the existence of an already well established and functioning hormone laboratory, may be grounds for not housing the hormone laboratory within the IVF unit. One of the most important requirements is that the embryology laboratory should be in close proximity to the surgical operating facilities.

Centres for medically assisted conception can provide facilities for *in vitro* fertilization and/or *in vivo* fertilization. In this context, *in vivo* fertilization includes direct intraperitoneal insemination and GIFT, and *in vitro* fertilization includes the conventional IVF-ET procedure, PROST, tubal embryo transfer and ZIFT. The *in vitro* techniques require the skills and equipment necessary for extracorporeal fertilization, including gamete and embryo culture and close supervision of embryo development. The equipment required for such centres and the training needed by their personnel will depend on the services offered.

14.1 **Equipment**

The equipment required as a minimum by centres offering IVF services includes:

- Laboratory facilities for semen analysis at least to the specifications laid down in the *WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction* (*5*), including light microscopes with phase-contrast optics, a haemocytometer, counting chambers, and the necessary reagents for determining sperm viability.

- A completely separate laboratory for handling gametes and embryos; this should be equipped with a stereomicroscope, a laminar air-flow bench, an incubator and disposable plastic vessels and glassware for cell culture.

- Culture media and purified water; these can either be bought ready made or be prepared in the laboratory. The water should be sterile and deionized. It should be noted that sophisticated apparatus is required to produce sterile deionized water, and for most laboratories it is more economical to purchase this item. All culture media currently in use require incubation with 5% carbon dioxide.

- Hormonal assay facilities. These should either be available at the centre or provided on a daily basis by another laboratory closely linked to it. The hormones for which assays are most likely to be required are estradiol, progesterone, FSH, LH and hCG. Assay of these hormones usually requires radioimmunoassay systems and a gamma-radiation counter. Commercially available radioimmunoassay kits with validated internal and external quality control are recommended. Enzyme-linked immunosorbent assays (ELISA) are becoming available for most of these hormones and offer the advantages that radioactive reagents are not required and that large numbers of samples can be processed rapidly.

- Ultrasound equipment. This should be readily available in the centre for monitoring ovarian function and may include a probe attachment to be used for ultrasonically guided retrieval of ova through the vagina.

- An operating theatre equipped for minor surgery. This is required when general anaesthesia is used. The theatre should be equipped for laparoscopy, oocyte collection, vacuum aspiration of the follicles, and embryo transfer. Access to facilities for emergency laparotomy and general resuscitation must be readily available.

For centres providing only *in vivo* fertilization services, the minimal requirement will include all the items listed above except for the specific IVF equipment.

The basic requirement for voltage regulators and standby electricity generators should not be overlooked in centres where a continuous constant supply of electricity cannot be guaranteed. In countries where equipment maintenance is difficult and spare parts not readily available, a mechanism must be established for ensuring both that the necessary equipment and spare parts can be procured and that regular maintenance is carried out.

If cryopreservation is among the activities carried out by the centre, the appropriate equipment, including a controlled biological freezer and liquid nitrogen facilities maintained by automatic filling (6), will be required. In centres undertaking research as well as providing services, additional and more sophisticated equipment will be required.

14.2 Personnel

For centres providing medically assisted conception to be successful, close teamwork among those involved is essential. The team providing such services should include:

- A specialist in gynaecology with training and experience in infertility management, and especially in reproduction endocrinology and gynaecological surgery.

- An ultrasonographer for monitoring follicular development. This task could be performed by a clinician with special training in gynaecological ultrasonography who could also undertake the retrieval of ova via vaginal endosonography.

- A biologist with experience in clinical embryology, tissue culture and the handling of gametes.

- An embryologist or biologist skilled in cryobiology, if cryopreservation is among the activities carried out by the centre.

- Laboratory technicians with basic training in laboratory procedures and previous experience of tissue culture techniques. Hormonal measurement, if performed in the centre, must be carried out by properly trained technicians.

- Experienced and qualified nursing or midwifery staff to provide continuous patient care and support, and to undertake the coordination and detailed planning of all the procedures.

- Secretarial staff for record-keeping, data registration and ensuring the safekeeping and confidentiality of the records.

- Other professional health workers, such as psychologists, andrologists and social workers.

It should be borne in mind that a centre providing medically assisted conception operates round the clock and seven days a week, including public holidays. Adequate numbers of staff must therefore be available to provide cover for staff members who are on leave or sick, and for night duty and weekends.

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15. Current research and needs for the future

Areas relevant to medically assisted conception that are currently the subject of research include oocyte quality, oocyte-sperm interaction, the choice of the best embryo(s), embryo and endometrial interaction, and the process of implantation, as well as functional aspects of the endometrium.

15.1 The oocyte

15.1.1 Ovulation induction

More precise regimens using recombinant FSH and LH in individualized protocols for ovulation induction should be possible, thus reducing the risk of hyperstimulation syndrome and high multiple pregnancy.

The replacement of hCG by recombinant LH in ovulation-induction regimens would have the advantage, thanks to its short half-life (*1*), of better selection of oocytes for ovulation; for example, only those oocytes with an optimal level of maturation could be harvested. It may well be that LH has superior physiological properties, yet to be determined, as compared with hCG for ovulation induction.

With pure recombinant products, the possibility exists of mimicking the natural cycle by keeping LH to minimal levels in the follicular phase, and by simulating the midcycle FSH surge.

Recent work has shown the advantage of using GnRH agonists in combination with already available ovulation-induction regimens (*2*). It is possible that the use of GnRH antagonists will further reduce the incidence of side-effects.

15.1.2 Peripheral markers of oocyte maturation

Current standards for use in the evaluation of oocyte maturation depend on plasma estradiol levels and vaginal ultrasound monitoring of follicular numbers and size. Daily measurement of progesterone and LH (*3*) does not provide a better indication of oocyte maturation, and further research will be necessary to identify more specific markers.

15.1.3 *Local markers of oocyte maturation*

Important work has been carried out on evaluating the paracrine regulation of folliculogenesis. The regulatory mechanisms involved include growth factors (*4*) and their binding proteins, the renin-angiotensin system (*5*) and the local effect of protein factors (*6*).

15.1.4 *Chromosomal analysis*

It has been shown that up to 50% of oocytes resulting from ovulation induction have an abnormal chromosomal pattern (*7*), while frozen-thawed oocytes frequently have a disturbed chromosomal composition. The mechanisms underlying both these important biological phenomena need to be explored. Preliminary work has already been done on aberrations in the spindle tubulin component (*8*).

15.1.5 *In vitro maturation*

A report on research on the maturation of immature oocytes *in vitro* and its implications for fertilization and the establishment of pregnancy capabilities has recently been published (*9*).

15.2 **Sperm**

15.2.1 *Species-specific sperm surface receptors for the zona pellucida*

Investigation of sperm surface receptors, including epitope location, characterization and specific biochemical analysis, is of vital importance in achieving a better understanding of sperm fertilizing capacity (*10*).

15.2.2 *Chromosomal investigation of abnormal sperm*

Most publications seem to indicate a low frequency of around 8% of structural or numerical chromosomal aberrations in the normal sperm population, but no chromosomal studies have been reported on abnormal sperm.

15.2.3 *Biochemical markers*

Various biochemical markers of sperm function have been identified. Most involve energy metabolism, e.g., creatine kinase levels are increased in sperm from oligospermic males, indicating defective energy metabolism (*11*). Further research on the metabolism of sperm with regard to their motility and fertilizing potential needs to be undertaken. The anatomical integrity and functional capabilities of the acrosome need to be accurately evaluated since it plays an essential part in sperm penetration of the zona pellucida. Some methods of evaluating the acrosome have been tested (*12*), but better and more reproducible ones are necessary.

15.2.4 *Sperm–oocyte interaction*

The hemi-zona assay is a crude method of evaluating sperm–zona pellucida affinity and interaction (*13*). Another technique involves removal of spermatozoa that fail to fertilize *in vitro* from the zona pellucida and reinsemination of the oocyte with fertile donor sperm; this method has been suggested for use in assessing the fertilizing ability of spermatozoa and the relative contribution of the oocyte to failure of fertilization (*14*). Further work on defining precise receptors on the sperm and oocyte is necessary. The processes involved in sperm changes, such as de-condensation of the sperm head, occurring between binding to the zona pellucida and the appearance of pronuclei, require scrutiny.

15.2.5 *Micromanipulation*

Most of the current work on the oligospermic male's low fertilizing ability is associated with micromanipulation of the oocyte to facilitate fertilization (*15*). The methods used include zona drilling or dissection, zona puncture, and direct injection of sperm into the perivitelline space of the ovum or into the ooplasm (see section 8.3). Adjunct therapy with corticosteroids has been claimed to improve implantation rates of zona-pellucida-dissected oocytes (*16*), but this treatment is now considered not to be effective.

A preliminary report has appeared on the use of microsurgical techniques involving sperm pronuclear extraction to correct polyspermy (*17*). Chromosomal studies are required to confirm that a supernumerary male pronucleus can be completely removed before such techniques can be applied clinically (*18*). Micromanipulative insemination procedures have been recommended for cases with immotile sperm, severe oligospermia (less than 5×10^6 per ml) and putative inability to penetrate the oocyte's outer vestments (*19*). Polyspermy accounts for approximately 5% of *in vitro* fertilizations (*20, 21*), but is much more common after microsurgical fertilization, which is increasingly being used in cases of oligoastheno-spermia (*22*).

15.3 Endometrium

Little is known about implantation and embryo–endometrial interactions in humans. Work with donated embryos has demonstrated that the "window of receptivity" of the endometrium is wider than was previously suspected; more precise information is needed on its determinants. The regulation of endometrial maturation and differentiation needs further study, especially of the underlying biochemical, genetic, and molecular mechanisms.

15.4 Embryo research and cryopreservation

Universal ethical constraints make embryo research difficult. Many authorities and organizations have developed scientific and ethical

guidelines on research on embryos, one example being the guidelines proposed by the British Voluntary Licensing Authority (23). Indirect methods of embryo evaluation have, of necessity, to be used.

Even though cryopreservation is becoming a common adjunct to medically assisted conception procedures, further research is needed on the effect of the various techniques employed on the outcome of pregnancy and on fetal and neonatal development. Studies on oocytes are also needed to determine whether there is a limit to the time for which they can be cryopreserved and remain viable.

15.4.1 *Embryo biopsy*

Advances in genetic technology, including use of the polymerase chain reaction and restriction-fragment-length polymorphisms, have facilitated the precise diagnosis of genetic disease. A portion of the embryo, i.e., one blastomere or its nucleus, can now be removed and analysed for a specific genetic defect (24), and this has been successfully achieved in two of the more than 200 known recessive X-chromosome-linked diseases, namely adrenoleukodystrophy and X-linked mental retardation (25). The same technique can be used for the purpose of sexing (26). The long-term effects of embryo biopsy are yet to be fully established. Future developments in this field can be expected as knowledge of the human genome increases.

15.4.2 *Co-culture*

Efforts have been made to enhance embryo maturation by means of co-culture techniques. In these experiments, bovine fibroblasts (27) and human tubal epithelium (28) have been used as the matrix, the theory being that implantation will be enhanced if the embryo is cultured in contact with tissue.

15.4.3 *Biochemical markers of embryo quality*

Indirect biochemical markers of embryo quality have already been proposed, including platelet activating factor (29), various growth factors and their binding proteins, enzymes, FSH, LH and prolactin (30). Adding certain substances to the culture media might therefore enhance embryogenesis (29).

As the human embryo appears to be controlled by oocyte-derived genetic factors up to the eight-cell stage of development, when it fully activates its genome, tests using markers of embryonic gene activity released into the culture medium may be of prognostic value in determining embryonic viability. However, advances in this area will depend on the development of improved embryo culture techniques up to the eight-cell stage (31).

15.4.4 *Facilitated hatching*

It has recently been reported that laceration of the zona pellucida prior to embryo placement facilitates implantation. This work was based on the

observation that up to 30% of embryos fail to hatch in the endometrial environment (*32*).

15.4.5 *Improved embryo placement*

New techniques of placing gametes and embryos in the uterus and the fallopian tubes have been developed and offer distinct advantages as compared with older placement methods, as they do not require general anaesthesia or an operative approach. Catheters are placed in the fallopian tube using tactile pressure, imaging or direct observation. One such new technique is tubal embryo transfer, in which the embryo is introduced into the tube through a catheter which has previously been placed in it under ultrasonic guidance by means of the transcervical/transuterine approach (*33*). Data on the efficiency, efficacy and safety of the new procedures have yet to be reported.

15.5 Technological improvements

Since IVF and allied procedures are highly technical, it is reasonable to expect that changes and improvements will be made in the equipment currently employed, including improved imaging through the use of Doppler, colour Doppler, and higher resolution probes. In the laboratory, significant improvements are being made in media composition, and micromanipulation with laser beams is being developed. In addition, as advances in human genetics and molecular medicine are made, their rapid translation into human reproductive technology is to be expected.

15.6 Health service and social science research

The implications for health service research of the new techniques mentioned in previous sections need to be addressed. Assessment of their psychosocial impact, mainly in relation to the culture of the particular population involved, constitutes an important area for research.

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16. National documentation as an aid to quality assurance in medically assisted conception

In the 1980s, when IVF and allied procedures changed from being purely experimental in character to become accepted treatments for certain types of infertility and the number of centres offering them increased rapidly, the need to monitor their use, side-effects and clinical efficacy was generally recognized. In most countries, an informal registration scheme has been established. In the United Kingdom, all centres have to be registered, are inspected at intervals and have to submit their results (*1*). The voluntary scheme in the United States includes 181 of the estimated 200 clinics practising medically assisted conception and operates under the auspices of the national professional body – the American Fertility Society (*2-4*).

The Australasian scheme covers 22 centres in Australia and three in New Zealand and is organized by the National Perinatal Statistics Unit at Sydney University (5).

In most countries, no one clinic treats sufficient numbers of patients to generate enough data to provide evidence of the relative safety and efficacy of the procedures. In addition, many of the techniques depend to a large extent for their success on the skills of the personnel involved. Each centre follows a "learning curve" when it first starts, but improvements can only be made if the data and results are recorded accurately and systematically and can be compared with those of other centres. Established clinics also require access to other centres' results in order to monitor their own performance.

Although a registry serves as an information resource for clinical and public health workers, politicians and the general public, its major objective should be to record all reported data on the assisted reproductive technologies, including IVF, GIFT, frozen embryo and donor oocyte transfer, and related procedures (in many countries, the results of artificial insemination are reported separately). Thus, a first priority is to describe and standardize the use of the various procedures and their relative efficacy. Another major priority is to record the frequency of unfavourable results, such as chromosomal or congenital anomalies in the offspring, failure to achieve clinical pregnancy, and adverse reactions in the women undergoing treatment.

16.1 Methodology

The initial goals of a registry are to identify the necessary basic information required to develop an effective data-collection process, and, most important, in voluntary schemes, to enrol clinics and gain their continuing cooperation and interaction. Once established, the registry's long-term goals centre on developing a data base that can be of use to both research workers and clinicians in investigating clinical and epidemiological hypotheses.

Reports by centres to the registry should include patient-specific data-collection forms. A computer-generated data base should record demographic information, medical and infertility history, spouse and donor information, ovarian-stimulation protocols, retrieval and embryo-transfer information, luteal-phase drug support, the occurrence of pregnancy and its course, and outcome data, including congenital anomalies and chromosomal abnormalities.

A suitable form can also be developed for use in collecting retrospective data from clinics without access to a computer (5), and should record the clinics' summary for each procedure, namely IVF with fresh oocytes, GIFT, ZIFT, PROST, tubal embryo transfer, and donor oocyte and frozen embryo transfer. The information should include the numbers of ovarian-stimulation cycles, cancelled cycles and patients treated, the

procedure used for oocyte retrieval and the number and maturity of the oocytes retrieved, the number of embryos transferred for IVF and of oocytes transferred for GIFT, and the frequency of occurrence of ovarian hyperstimulation, pregnancy (with details of its outcome), congenital anomalies and chromosomal abnormalities.

16.2 International experience

In the USA, the American Fertility Society IVF Registry, which has been in existence since 1986, has allowed the monitoring of the number of cycles of medically assisted conception performed, as well as the success rates, with emphasis on clinical pregnancy, spontaneous abortion, ectopic pregnancy, multiple gestation, and delivery (2-4). As of April 1989, there were 181 member clinics, i.e., an estimated 90% of all clinics performing IVF and related procedures (5). The new reproductive technologies represent a unique aspect of medical care in the USA because they have come under strict public as well as professional scrutiny, both at the regulatory level and in terms of the evaluation of the results (5). The establishment of the Registry has been extremely beneficial in both of these areas. In addition to collaborating in the establishment of the Registry, the American Fertility Society has established revised minimum standards for the performance of IVF, GIFT, and related procedures in order to ensure uniformity both within and among centres (6). The intention is for all embryo research laboratories in the USA to follow the example of the Registry and be monitored by a professional association.

The IVF statistics for Australia and New Zealand are compiled by the National Perinatal Statistics Unit at Sydney University, which was established in 1979 by the Australian Commonwealth Department of Health to collect and analyse national birth data and, in particular, to monitor the incidence of congenital malformations. In 1983, the Unit assumed the responsibility of monitoring IVF and related procedures (Australian IVF Register). In 1983, data were available from eight units in Australia; in 1987, 14 in Australia and one in New Zealand; and in 1988, 22 in Australia and three in New Zealand. The Register is updated annually from information provided by the participating units; data are collected on a standard questionnaire form, batched and sent to the Register, where they are checked, collated and analysed. Cumulative individual printouts are sent to the units, and the combined statistics are published at regular intervals (4, 7, 8). One of the Unit's most important tasks is that of evaluating the outcome of IVF in both the short and the long term.

In the United Kingdom, all centres offering IVF and related services, but not artificial insemination alone, are inspected and certified by the Interim (previously Voluntary) Licensing Authority (1). In April 1989, there were 38 approved centres, of which 17 were engaged in licensed IVF research. At present, participation in the national registry in France is voluntary (9), and in Sweden preliminary steps have been taken to establish a national

registry, possibly to be followed by a Nordic or Scandinavian collaborative scheme (*10*).

The principal benefits of a national registry for centres offering artificial insemination include: standardization of clinical indications for treatment; standardization of facilities for cryopreservation and storage of semen and for quality control; increased availability of donors for individual centres and closer matching of donor and recipient; follow-up of the outcome of the treatment; and multicentre research.

Once established, and provided that it is representative either of all the clinics practising IVF and related procedures in that particular country, or of the vast majority of them, in the case of a voluntary system, the registry can be used to monitor and supervise the introduction of new techniques by means of multicentre trials. Only a few centres in any one country will have sufficient numbers of patients to make scientifically valid judgements on the relative merits and adverse effects of new treatment regimens that may be advocated from time to time.

There are, however, certain drawbacks to the reporting of results on a named centre-specific basis, including pressure to present the results in a more favourable light when economic considerations are important. In addition, in order to improve a clinic's success rate, the selection of patients may be biased; some clinics might select only young women with tubal disease and reject older women or couples where the male is oligospermic. Centres in countries with a compulsory registration scheme must conform to the reporting requirements laid down. In a voluntary scheme, both conformity of reporting and general acceptance of uniform definitions and practices are more difficult to enforce. There are strong arguments both for the identification of individual centres in the results published by the registry (the public's right to know) and against this practice (the need to avoid commercialization).

16.3 Data reported

The national registry should produce regular reports giving details of the pregnancy results, adverse outcomes, the annual total of stimulated cycles and retrievals, pregnancy rates, and pregnancy and delivery rates by age and method used.

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17. **Recommendations**

In addition to the specific recommendations to be found elsewhere in this report, the Scientific Group made the following recommendations for those concerned with the planning and implementation of programmes on medically assisted conception, including artificial insemination. These recommendations relate, in particular, to the investigation and treatment of infertility, basic research, the conduct and analysis of clinical research, and the evaluation of treatment regimens.

17.1 **Investigation and treatment of infertility**

1. All members of a department of reproductive medicine and infertility should be aware of the relative merits of, indications for, and risks associated with the different types of medically assisted conception.

2. In centres providing medically assisted conception services, various types of IVF and related procedures should be available so that a technique appropriate to the couple may be chosen.

3. Information about the various techniques and their relative merits

should be available to referring doctors so as to ensure that unrealistic expectations are not entertained by the couples referred by them.

4. A full range of treatments for infertility should be incorporated in a unified service so that an undue length of time does not elapse before medically assisted conception is considered.

5. Counselling should include the provision of information on alternative options in the event of failure. Psychosocial support should be available both during and following any attempt(s) at medically assisted conception.

6. In contexts in which infertility and its treatment are discussed, terms such as "male factor" and "unexplained infertility" should always be precisely defined.

7. Suspected tubal obstruction should be confirmed laparoscopically before treatment for infertility is undertaken.

8. The basic investigation of the male partner of the infertile couple should include semen analysis in accordance with standards such as those laid down in the *WHO laboratory manual for the examination of human semen and semen–cervical mucus interaction (1)*.

9. Reports on the results achieved by medically assisted conception should include, *inter alia*, the live-birth rate per 100 treatment cycles.

10. Life-table analysis should be used in the computation of pregnancy rates for treatments involving artificial insemination.

11. Prospective double-blind randomized trials are to be preferred to other types of studies of more than one treatment regimen or laboratory procedure.

12. The effect of the sperm count on natural fertility is well established, but further information is needed on the influence of other parameters, such as sperm morphology and motility, on the process of natural fertilization.

13. Further studies on the use and safety of embryo biopsy in the detection of genetic disease should be conducted.

17.2 Research needs

17.2.1 Basic research

1. Basic research should be carried out on:
   - The role of non-fertilizing spermatozoa in embryonic cleavage and implantation (2).
   - The use of timed transfer of donor oocytes to define more precisely the "window of implantation".
   - The place of immunomodulation and pregnancy-related proteins in successful implantation in primates (3–5).

- The maturation of oocytes *in vitro* from the arrested dictyate stage to resumption of meiosis.
- The role of tubal function in early embryo support.
- The role of the endometrium in the "window of implantation".

2. Further studies are required on the thickness of the endometrium on the day immediately before oocyte retrieval as a predictor for successful implantation (*6*).

17.2.2 *Artificial insemination*

1. The rate of shedding of cytomegalovirus into the semen of potential donors who are seropositive for this virus should be investigated (*7*).

2. Research should be carried out on the pathogenicity of the different strains of cytomegalovirus and the effect of cryopreservation on pathogenicity (8).

17.2.3 *Preservation, fertilization and culture techniques*

1. Further research on the use of vitrification and snap-freezing methods in cryopreservation is needed.

2. Techniques should be developed to permit cryopreservation of oocytes without the development of zona pellucida defects.

3. The effect of direct injection or introduction of spermatozoa into the oocyte requires study.

4. Research on the maturation of primary oocytes *in vitro* could lead to the successful treatment of oocyte maturation arrest.

5. There is a need to develop techniques for identifying oocytes that fail to develop into "normal" embryos and for selecting viable embryos.

6. The reported improvement in pregnancy rates after addition of phospholipids such as platelet activating factor to the culture medium requires confirmation.

17.2.4 *Treatment regimens*

1. Further studies are needed on treatment regimens involving natural-cycle IVF.

2. Research is required on co-treatment with human growth hormone in ovarian-stimulation regimens in order to determine whether the requirement for gonadotrophin stimulation is thereby reduced. If this is the case, the incidence of side-effects of gonadotrophin therapy, such as the ovarian hyperstimulation syndrome, may be reduced or eliminated (*9*).

3. Further clinical studies are needed on the beneficial effects of pure FSH in men with very poor sperm morphology ($<14\%$ normal forms) (*10*).

4. Randomized double-blind studies are required on the possible benefits in medically assisted conception of luteal-phase support with progestogens, including progesterone and/or hCG.

5. Prospective randomized trials should be carried out on controlled ovarian stimulation with hMG, with or without concomitant GnRH-a treatment (*11*).

6. Further studies are required on the combined use of GNRH-a and gonadotrophins to stimulate ovulation in cases of unexplained infertility (*12*).

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Annex
Explanations of technical terms used in the report

The definitions of some of the terms given below may vary from those used in the literature. They are not intended to supersede the latter but were adopted by the Scientific Group for the purposes of its report.

Artificial insemination (*in vivo*): the introduction of male gametes into the cervical canal or uterine cavity.

Biochemical pregnancy: evidence of conception based on biochemical data only, i.e., rising titres of hCG in serum or urine before the expected onset of a menstrual period.

Blastocyst: an embryo with a fluid-filled cavity.

Blastomere: the stage of development of the embryo from the first cellular division to the appearance of the blastocyst.

Cancelled cycle: a treatment cycle in which oocyte retrieval is not attempted because of abnormal follicular development, premature ovulation, excessive ovarian stimulation or other side-effects of the ovulation-induction regimen.

Clinical pregnancy: pregnancy indicated by both clinical and ultrasound parameters.

Cryopreservation: the freezing of gametes or embryos in order to store them for subsequent use.

Ectopic pregnancy: a pregnancy in which implantation has taken place outside the uterine cavity.

Embryo: the product of conception from the time of fertilization to the end of the embryonic stage eight weeks after fertilization. (The term "pre-embryo" has been avoided in the Scientific Group's report. It has been used elsewhere in certain contexts to signify a stage in development starting from the completion of fertilization and ending when the primitive streak appears 15-17 days later.)

Embryo donation: the transfer to a woman of an embryo resulting from both sperm and oocyte donation.

Fertilization: the process that begins with the penetration of the secondary oocyte by the spermatozoon and is completed shortly before the first cleavage. It usually takes up to 24 hours to complete fertilization in the human.

Fetus: the product of conception from the end of the embryonic stage (eight weeks after fertilization) until birth.

Hatching: the process whereby an embryo escapes from the zona pellucida.

Implantation: the process that starts with the attachment of the zona-free blastocyst to the uterine wall (5–6 days after fertilization). The blastocyst then penetrates the uterine epithelium and invades the endometrial stroma. The process is complete when the blastocyst develops primary villi and the surface defect on the epithelium is closed (13–14 days after fertilization).

Insemination *in vitro*: the introduction of male gametes into the culture medium containing the oocyte for the purpose of fertilization.

Medically assisted conception: fertilization brought about by non-coital conjunction of the gametes.

Micromanipulation: the use of special magnification technology that allows operative procedures to be performed on the oocyte, sperm or embryo.

Ovarian stimulation: medical treatment to induce the development of multiple ovarian follicles in order to obtain multiple oocytes.

Polyspermic fertilization: fertilization of an ovum by more than one spermatozoon.

Pregnancy rate per treatment cycle: the number of clinical pregnancies per treatment cycle.

Still birth: the death of a viable fetus before birth is completed.

Unexplained infertility: failure to conceive after all available routinely used methods of infertility evaluation have failed to demonstrate a cause of infertility in either partner.

Vitrification: a method of cryopreservation.

Zygote: the diploid cell produced by the union of a spermatozoon and an ovum at fertilization.

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