Review

Definition and causes of infertility

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Abstract

Infertility is a common problem affecting one couple in six. It can be defined as the incapacity to fulfill pregnancy after reasonable time of sexual intercourse with no contraceptive measures taken. The evidence for changes in the prevalence of infertility is difficult to establish. This increase could be due to at least four factors: delayed childbearing, alterations in semen quality due to habits such as cigarette smoking and alcohol, changes in sexual behaviour and eliminations of most taboos. The study of infertile couple has always been focussed on different factors: ovulatory factor (present in about 20% of couples), utero-tubal peritoneal factor (present in ~30% of couples), semen migration factor (10% of cases) and male factor (30% of couples). Around 40% of all infertile couples exhibit a combination of factors and about 15 % of couples may not display any objective alteration leading to a definite diagnosis. During the past two decades there have been three important changes in infertility practice. First, the introduction of assisted reproduction technologies has provided an opportunity to study basic reproductive processes. Second, societal changes have occurred such as the increase in the proportion of women over 35 years old seeking pregnancy. This fact is due to a later age for marriage and postponement of pregnancy. Third, the development of molecular biology and genetics has become very important for the study, diagnosis and assessment of couples, many of them considered until now as "unexplained infertile couples".

Keywords: endocrine, genetic, infertility, mechanisms, miscarriage, pregnancy

Introduction

Infertility is a condition affecting one fifth to one sixth of couples in reproductive age. Within the field of reproductive health, infertility implies a deficiency that does not compromise the physical integrity of the individual, nor is it life-threatening. However, such deficiency may negatively impact the development of the individual, bringing about frustration and weakening the personality, since most couples consider having children as a vital objective. As compared to other species, the human being is highly inefficient in terms of reproduction. The fertility rate per cycle is about 20% and the accumulated pregnancy rate in couples with proven fertility is ~90% after 12 months and 94% after 2 years (**Figure 1**).

In the area of reproductive health, problems tend to be different in each country. Similarly, population studies on this issue vary according to the area studied. Hence, knowledge about the prevalence of infertility to establish the potential needs of the population and adapting health care to each particular population is of considerable interest.

The prevalence of infertility

Definition

Infertility is defined as the incapacity to fulfill pregnancy after a reasonable time of sexual intercourse with no contraceptive measures taken. The terms sterility and infertility are sometimes used interchangeably and at times define different populations. In the Spanish literature, the definition of the word sterility is the difficulty to fulfill pregnancy, whilst the term infertility is used when pregnancy develops but is interrupted at some point; hence, the term is used as a synonym of recurrent
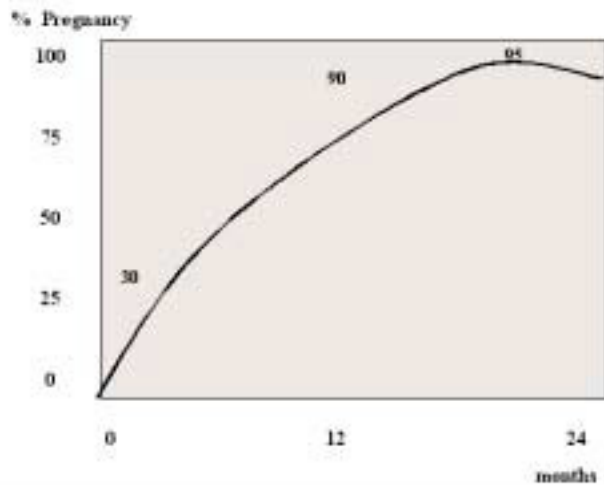
Figure 1. Cumulative pregnancy rate in fertile population after 2 years.

Figure 2. Pregnancy and miscarriage rates per cycle according to the woman's age.

miscarriages. On the contrary, in the English literature the term infertile refers to a couple that fails in achieving pregnancy, either because of the impossibility to become pregnant through natural means (sterility) or whenever the possibilities exist but pregnancy does not occur (subfertility) or if pregnancy does develop but does not lead to a live newborn. In contrast, the fertile population is defined as those who do become pregnant after some reasonable time of regular sexual intercourse.

The concept of "reasonable time" is debatable; the World Health Organization (WHO, 1992a) as well as the European Society of Human Reproduction and Embryology (ESHRE, 1996) in their recommendations mention a 2 year minimum deadline for developing pregnancy. If pregnancy does not occur after that time, the couple is considered to be infertile. From the practical point of view, most physicians initiate study of an infertile couple following 1 year of failed pregnancy attempts. Moreover, due to the impact of age on fertility, when the woman is >39 years, it might be advisable to begin the study after 6 months of unsuccessful attempts. Hence there should not be strict limits on beginning a study of an infertile couple, since the waiting time should be related to the age of the woman, the history of alterations that affect fertility, the desires and wishes of the couple, etc.

Fecundity is the probability of becoming pregnant in one particular menstrual cycle and is ~20%, depending on the age of the woman. This entails that the average time to develop pregnancy is ~4 months. Fertility is the capacity to give birth to one live newborn.

Increased awareness of infertility and assisted reproduction

There is no evidence from population studies for a recent change to a higher incidence of infertile couples; nevertheless, apparently there has been an increase in the number of visits to infertility clinics in the last few years. This increase could be due to at least four factors.

First, the average age at which women wish to become pregnant has increased considerably in recent decades. Education and the parti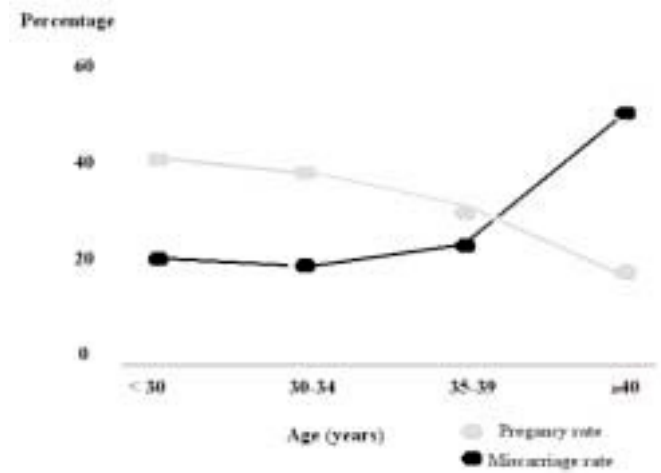cipation of women in professional activities, as well as the need for constant professional advancement, have led to a postponement of the decision about pregnancy. This means that women wish to become pregnant at about 35 years of age, at a time when fertility begins to decline. Similarly, divorce and seeking stability with new partners implies waiting longer before making the decision to have any children.

Second, alterations in semen quality can influence the need for advice on infertility. It has been proven that habits such as cigarette smoking (Sofikitis *et al.*, 1995) and alcohol abuse are harmful for semen quality. Alcohol, for instance, has been related to a reduction in the synthesis and secretion of testosterone and abnormal spermatogenesis. Tobacco abuse leads to spermiogram alterations.

Third, there have been changes in sexual behaviour. An increase in the frequency of sexual intercourse, and in the number of sex partners, has been observed. In addition to the decision to delay pregnancy, the use of contraception methods (not necessarily condoms) expose couples to a higher incidence of sexually transmitted diseases (ST) to tubal–peritoneal infections that have consequences for fertility.

Finally, the elimination of most taboos (not all) about fertility and broader dissemination of the existing studies and treatments available, results in a higher frequency of visits by couples to the doctor.

Age and fertility

One of the most important factors in assessing a couple with fertility problems is the age of the woman. A desire to become pregnant at ~40 years of age does not only entail a low possibility of success, but also an increased risk of developing maternal pregnancy ailments such as pre-eclampsia, hypertension and diabetes, as well as fetal chromosomal abnormalities and miscarriages. The decline in female fertility starts at 30 years of age and becomes more pronounced at 40. The possibility for pregnancy at age 40 is half that of younger women, whilst the incidence of spontaneous abortion doubles or trebles (Cruz & Gindoff, 1999) (**Figure 2**). As shown by the excellent results obtained through ovum donation, the main

effect age has on the reproductive capacity of women is almost exclusively determined by the age of the ovum, since the possibility of pregnancy depends on the age of the donor rather than on the age of the receptor (Sauer *et al.*, 1990).

The negative effect age has on the ovum is mainly its inefficacy to complete a normal first meiotic division and perhaps adequately to start the second meiosis. Accordingly, the number of chromosomes left in the female pronucleus after completion of the second meiosis after fertilization is defective. This gives rise to embryos with such serious chromosomal imbalance as to prevent the evolution of pregnancy. The high incidence of disorders during chromosomal disjunction of the ovum could be the result of an intrinsic failure of the meiotic system or of cytoplasmic ageing such as that resulting from a decrease in the energy-synthezing activity of mitochondria. In addition to ovum quality, age also affects the number of follicles available for ovulation. Follicular depletion from the pool established in the fetal ovary begins in the seventh month of uterine life. Thus, the number of follicles available in week 20 of gestation is $\sim 6-7 \times 10^6$ while at birth it is $\sim 1-2 \times 10^6$. By puberty, this figure declines to 300,000. The number of oocytes available to a woman at a particular age is subject to the balance between the oocytes in month 5 of intrauterine life and the proportion of oocytes lost throughout the life span due to apoptosis or as a consequence of external factors that may decrease the ovarian reserve. This means that in addition to age, other factors also affect follicular availability, such as genetic factors, chromosomal abnormalities, autoimmune diseases, smoking, ovarian surgery, endometriomas, chemotherapy, radiation therapy, pelvic adhesions, chlamydia exposure, and others.

Many men are also sub-fertile. WHO (1992a) has suggested several schemes for the diagnostic classification of male sterility, based upon diverse diagnostic standards for clinical and seminal diagnosis. Some of these classifications are controversial at present, and many of them are really descriptive rather than aetiological. The contribution of environmental, occupational and especially genetic factors is becoming widely acknowledged. While sterility is quite common, it is quite difficult to determine the relative contribution of the male factor to this problem. Many studies attempting to establish the aetiology of the male factor are based upon the semen studies put forward by WHO (1992b). Though of considerable importance, this criterion is limited in its diagnostic value since many men with normal seminal parameters are infertile due to defects in the spermatic function, while others with theoretically normal semen have a normal spermatic function. Few epidemiological studies are based on functional diagnostic criteria. However, despite the use of the currently available diagnostic techniques, the current consensus is that male factor is more frequently present than previously suspected in couples suffering from infertility.

As previously suggested, the greatest obstacle to a meaningful epidemiological study of male factor is the difficulty in making a precise diagnosis about the presence or absence of the problem. Traditionally, the diagnosis of male sterility is based upon the conventional semen assay. This includes information about volume, sperm concentration, motility and morphology. Unfortunately, there are a considerable number of defects that limit the diagnostic value of this study. The greatest obstacle is the marked inter-ejaculate variability that exists in the study of

semen specimens. Moreover, many of the semen studies are done subjectively and show important differences amongst various laboratories and even among technicians in the same laboratory, which leads to high inter and intra-laboratory consistency. Although WHO has established a normal range of values, these are not based on evidence in terms of its diagnostic value of its relationship with the fertile population. Consequently, many couples with an original diagnosis of unexplained sterility may later be found to have a specific cause after applying the appropriate analyses. It seems therefore more logical to have each laboratory define its normal values according to their respective populations.

Identifying the causes of infertility

A methodical study of all probable factors in the failure to achieve pregnancy must be performed on all couples seeking assistance for infertility. The ovulatory factor, which summarizes the adequate development, maturation and rupture of the follicle, is present in about 20% of couples. The utero–tubal–peritoneal factor includes the study of tubal integrity, the uterine cavity and the presence of pelvic adhesions that compromise the anatomy of the female genital tract. It is present in ~30% of couples (**Figure 3**).
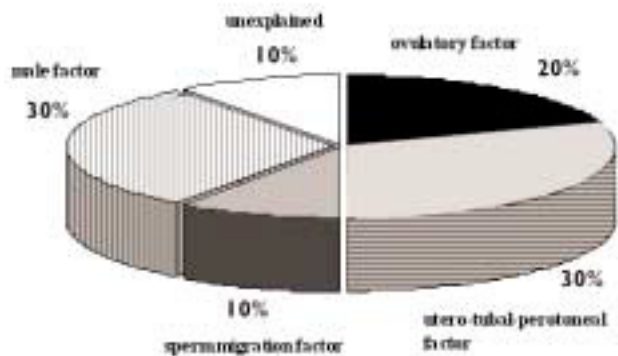

Figure 3. Distribution of infertility causes.

The sperm migration factor includes the study of the relationship between cervical mucus and spermatozoa. Alterations in these variables include a reduction in the number and motility of spermatozoa and their displacement inside the cervical mucus, which are prerequisites in reaching the tubes and fertilizing the ovum. This situation occurs in ~10% of the cases with normal semen (Cohen, 1991).

Male factor also corresponds to the semen study. Several conditions are known to invoke alterations in the amount and quality of the sperm sample. These include varicocele, genital infections, trauma, surgeries, gene dysfunctions, toxic substances, etc. This situation occurs in about 30% of couples. Endometriosis is a disease that may or may not co-exist with infertility. If it does, the quality of ovulation may be affected, together with the structure and patency of the oviducts due to adhesions and implants. According to some authors, endometriosis may even be the cause of miscarriages (Metzger *et al.*, 1986).

Around 40% of all couples who seek help for infertility usually exhibit a combination of factors; i.e. a female factor combined with a male disorder. Hence the study of infertility must always be done considering the couple together: man and woman and interrelationships between them.

Finally, descriptions have been given of immunological infertility and infertility due to genetic factors. Whilst these two categories do not correspond to any particular type of infertility, the presence of some alterations of this nature may cause reproductive failure.

Approximately 15% of couples, even when all the above factors have been considered, may not display any objective alterations leading to a definite diagnosis. They are then classified as patients with unexplained infertility, at least at the time of the diagnosis.

Causes of female infertility

Anovulatory Infertility

Anovulation is defined as the condition in which follicular development and rupture is impaired and hence the oocyte is not released from its follicle. Several causes for anovulation have been identified (Franks, 1991). These include intrinsic ovarian failure including genetic, autoimmune and other factors such as chemotherapy. Ovarian dysfunction secondary to gonadotrophic regulation is another cause. It can be subdivided into specific causes such as hyperprolactinaemia and Kallmann's syndrome, and functional causes including low body weight, excess exercise, the use of drugs and idiopathic infertility. Gonadotrophin deficiency arises in case of pituitary tumour, necrosis of the hypophysis and thrombosis. Alterations can occur in the action of gonadotrophins as in polycystic ovary syndrome.

In women with a suspicion of ovulatory failure, the most frequent causes for anovulation may derive from one of the following conditions.

Hyperprolactinaemia
Variations in the dosage of prolactin can be expected, depending on the oestrogen concentrations in the patient; hence, under hypo-oestrogen conditions values ranging from 20–25 ng/ml are considered normal, whilst if the oestrogen concentration is higher, the usual concentrations are 30–40 ng/ml (Lenton, 1982). Prolactin is a hormone with considerable secretion sensitivity because elevated concentrations of prolactin may result from substances such as digestive medications, anti-depressants, neuroleptics, hypotensive drugs, as well as from stress conditions, excessive exercise, high protein intake, chest trauma, surgeries, sexual intercourse and other factors. Hyperprolactinaemia alters the secretion pulses of gonadotrophin-releasing hormone (GnRH) and depending on the circulating prolactin concentrations, clinical manifestations may occur such as inadequate luteal phase, anovulation and amenorrhoea). The study of thyroid function is obligatory in every woman who presents with hyperprolactinaemia, since hypothyroidism usually occurs with elevated concentrations of prolactin (Blackwell, 1992).

Hypogonadotrophic hypogonadism
This condition is expressed with oestradiol concentrations of <40 pg/ml and reduced follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentrations. It is seen in weight disorders and excessive exercise. It can be idiopathic or caused by a hypophyseal or hypothalamic dysfunction.

Hypergonadotrophic hypogonadism
FSH plasma concentrations may be >20 mUI/ml in repeated determinations. This is a usual situation in patients <40 years of age with premature ovarian failure, patients with resistant ovaries or patients with genetic disorders.

Polycystic ovaries
This is the most prevalent endocrine pathology in women and the most frequent cause of anovulation. Women with polycystic ovaries may exhibit a wide range of clinical symptoms and signs; however, anovulation and hyperandrogenism are considered prerequisites in this pathology. In 1844, Chereau (1844) described sclerotic changes in the human ovary. In 1935, Stein and Leventhal (1935) described the classical picture. The elevation in LH was reported for the first time in 1958 (McArthur *et al*., 1958) but in 1976 (Rebar *et al*., 1976) the condition was described with a normal LH. Later on, the presence of this syndrome was associated with insulin resistance and during the 1980s ultrasound findings were described in women with polycystic ovaries. This chronology illustrates the wide range of clinical presentations, the evolution of diagnostic criteria and a rather obscure pathophysiology.

Clinical and scientific background to anovulatory infertility

When assessing ovulatory infertility a careful clinical record becomes of the utmost importance. It must include the age of menarche, menstrual cycles, systemic diseases (e.g. thyroid), drug intake such as cytotoxic agents, neuroleptics, anti-depressants and hypotensive drugs, physical activity, stressful situations, habits including smoking and alcohol intake. The physical examination must be thorough and it should take into consideration body mass index, acne, hirsutism, height, gynaecological examination including breast development, external and internal genitalia.

Ovulation detection tests may be predictive or confirmatory. Predictive tests include the direct ultrasound visualization of the dominant follicle, indirect methods such as serum oestradiol and LH concentrations and evaluation of the cervical mucus. Confirmatory tests include direct tests such as ultrasound or laparoscopic visualization of follicular rupture, and indirect tests such as progesterone dosage, basal temperature curve and endometrial biopsy.

In some situations, ovulation takes place but the quality of ovulation is insufficient to achieve and/or maintain pregnancy. Such is the case for the inadequate luteal phase defined by Jones (1949). An abnormal transformation in the endometrium is due to inadequate or insufficient progesterone secretion by the corpus luteum or to endometrial pathology. This condition usually occurs with infertility or recurrent miscarriages. According to different authors its incidence varies from 3–10% in infertile women and up to 35% in recurrent miscarriages.

The corpus luteum produces over 80% of the circulating progesterone during the luteal phase. Granulosa cells and theca cells present in the ruptured follicle form the constituents of this gland. These cells take up lipids from the circulation to synthesize progesterone that should impinge on one of its target organs, the endometrium, to achieve the necessary secretory transformation to materialize, develop and normally maintain pregnancy (**Figure 4**). Any alteration during the follicular phase will result in alterations of the luteal phase. A change in the pulsatile LH secretion from a high frequency and low amplitude in the follicular phase to a low frequency and high amplitude in the luteal phase for the maintenance of the corpus luteum is essential for an appropriate genesis of the luteal phase. Exposure to LH is increasingly required for luteinization of the granulosa cells and re-initiation of the second meiotic division and follicular rupture, luteinization and appropriate maintenance of the corpus luteum. Thus, the pathologies affecting the pulsatile secretion of LH will generate defects to
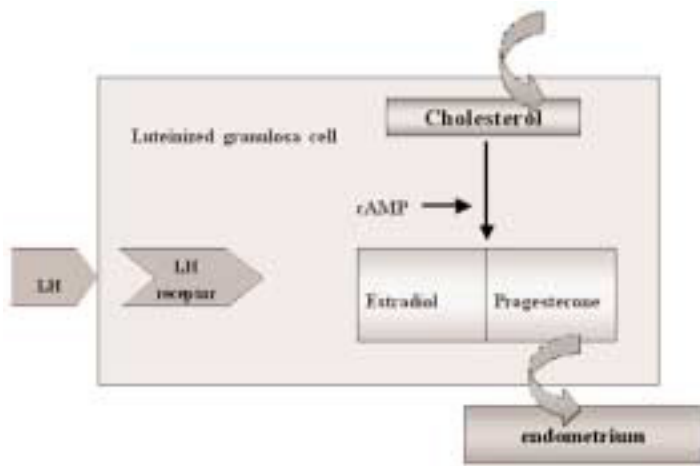

Figure 4. Diagrammatic representation of the role of the luteinized granulosa cell in endocrine regulation and implantation.

the appropriate luteinization level of granulosa cells and genesis of the corpus luteum. This is the case in hyperprolactinaemia, stress, malnutrition, excessive depression, drugs, etc.

In the absence of sufficient substrate for steroideogenesis, such as in the case of hypobetalipoproteinaemia, progesterone synthesis may be insufficient. Alterations at the ovarian level have been described in the gonadotropin receptors, to result in anovulation or insufficient luteal phase. Finally, endometrial alterations, such as in hormone receptors, chronic infections etc., may result in an inadequate pre-gestational transformation of the endometrium.

Just as any event that reduces the amplitude of the LH pulses is detrimental, tonic elevations in amplitude during the follicular phase can also be harmful, either because of premature luteinization of the granulosa cells or because of oocyte-related alterations in the early establishment of meiosis II. Cellular luteinization will probably give rise to an elevated circulating progesterone concentration at the time of the cycle, resulting in asynchrony in terms of oocyte maturation, fertilization, embryo cleavage and endometrial maturation. On the other hand,

Table 1. Detrimental effects of LH pulse alterations.

Reduction in LH pulse amplitude	Tonic elevation of LH pulses
• Inadequate luteinization of granulosa cells	• Premature luteinization of granulosa cells (elevated circulating proegesterone, asyncrony in oocyte maturation, fertilization, embryo cleavage and endometrial maturation
• Absence of follicular rupture	
• Inappropiate luteinization and maintenance of the corpus luteum	• Oocyte alterations in the establishment of meiosis II

stimulating maturation to the second meiotic division in advance of follicular rupture may generate an "old ovum" incapable of undergoing fertilization (**Table 1**).

There are other circumstances in which mechanical or biomechanical factors including endometriosis or insufficient LH peak respectively complete the follicular and luteinization stages. Nevertheless, the dominant follicle still does not rupture, resulting in luteinization of the intact follicle and thus infertility.

Tubal–peritoneal infertility

Tubal–peritoneal factors account for ~30% of the causes of infertility. The functions of the Fallopian tubes are closely linked to the integrity of ciliated epithelium responsible for oocyte uptake. Fertilization takes place in the outer end or ampullar section. The tubes are also involved in early embryo development and in the transport of the embryo into the uterine cavity. Consequently, any anatomical or functional alterations of the tubes are associated with infertility. In contemporary society, cultural changes including the use of contraceptives have anticipated the onset of sexual activity several years before partner stability or fertility is even considered. Hence,
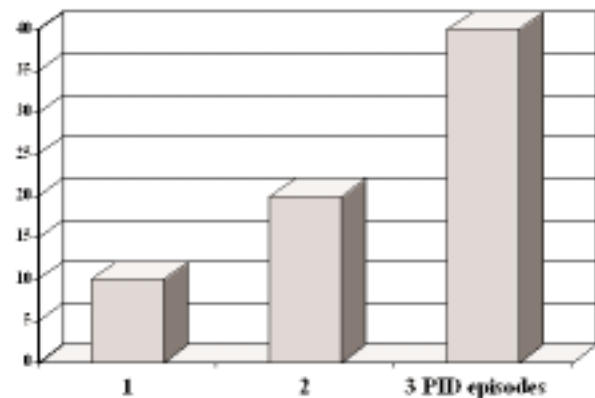

Figure 5. Number of pelvic inflammatory disease episodes related to percentage incidence of tubero–peritoneal infertility.

there is an increased risk of developing certain conditions involved in the genesis of the tubal–peritoneal factor (Westrom, 1994). These include pelvic adhesions secondary to infections, pelvic inflammatory disease (PID), prior surgeries or endometriosis (**Figure 5**).

Genital infections are among the main culprits of tubal–peritoneal damage. Many sexually transmitted diseases (STD) can be indirectly associated with infertility, but only two micro-organisms have proven to have any direct effects on fertility post-infection. These are *Neisseria gonorrhoea* and *Chlamydia trachomatis* (World Health Organization, 1995). Genital infections caused by *Chlamydia* are currently the most important cause of sexually transmitted bacterial infections. This organism is responsible for ~60% of acute salpingitis in young women. It has been suggested that the probabilities of tubal factor infertility, as well as ectopic pregnancy, are considerably increased with each infectious episode (Westrom, 1994); the occurrence of tubal–peritoneal infertility is also associated with the severity of the infection.

In terms of prevention of STD associated with infertility, two lines of action should be considered. First, primary prevention is aimed at avoiding the occurrence of infections, and advice should be given as to the use of barrier contraceptive methods. Secondary prevention requires early evaluation and treatment in cases in which salpingitis is suspected, with partner treatment and subsequent control in order to avoid re-infection.

Endometriosis

The association between endometriosis and infertility has long been established. A higher incidence has been shown in infertile patients (48%) as compared with fertile patients (5%). Several situations have been suggested to explain the presence of infertility in patients with endometriosis, among them anatomical alterations, anovulation and luteal phase alterations. Nevertheless, it has not been possible to describe one single mechanism totally responsible for the clinical manifestations of the disease. There is no doubt that both the presence of endometriosis and adhesions produce anatomical distortions, limit the mobilization of fimbriae, and obstruct the tubes or cause phimosis. Distal tubal obstruction is generally associated to adhesions, whilst proximal occlusions are usually associated to intramural endometriosis foci or with invasive growth of peritoneal lesions (**Table 2**)

The main options for evaluating the tubal–peritoneal integrity are hysterosalpingography and laparoscopy. The former is an ambulatory procedure which assesses the tubal patency and the uterine cavity with minimal complications of infections and bleeding, and is highly useful when assessing tubal patency, the diameter of the tubes and their mucosa. It cannot be used, however, as the only tool for the study of tubal condition, since its sensitivity is low for evaluating peritubal adhesions. Laparoscopy performed in the operating room under general anaesthesia allows for a complete pelvic evaluation and an

Table 2. Proposed mechanisms of infertility in patients with endometriosis

Dysfunction	*Defective changes*
Ovarian function	Prolonged follicular phase Reduced rate of follicular growth Reduced pre-ovulatory follicle size Reduced pre-ovulatory serum oestradiol concentration Disordered and impaired LH surge Disordered early luteal phase patterns of oestradiol and progesterone Luteinized unruptured follicle
Tubal function	Alterations in normal tubo-ovarian relationships Hydrosalpinges Alterations in tubal motility by prostaglandins with accelerated tubal motility
Sperm function	Phagocitosis by macrophages
Fertilization - embryo defects	Impaired fertilization Embryo toxicity, impairs early embryo development
Endometrial defects	Luteal phase defects Endometrial antibodies Implantation defects
Early pregnancy failure	Abnormal embryos Immune reaction Auto-santibodies Cytokines (interleukin I)

examination of the extraluminal and peritubal conditions, as well as detection of the presence of other conditions, for instance endometriosis. No information is gained about the tubal lumen or the condition of the mucosa.

Hysterosonography and sonosalpingograghy can be effective especially with the use of saline solutions or contrast materials (Holz *et al*., 1997). However, the first morphological evaluation of the uterus and the tubes must be done with hysterosalpingography. Laparoscopy will then follow, depending on each particular case. Hysterosonography and salpingosonography are not yet substitutes for the first two methods, but provide an excellent applicability and their use may be quite promising.

Uterine infertility

A wide spectrum of uterine abnormalities, either congenital or acquired, has been associated with the presence of infertility or recurrent abortions. Examples include congenital abnormalities, intrauterine exposure to drugs, submucosal myomas, polyps and synechiae. Whilst such an association is certainly true, these conditions may also occur simultaneously with the evolution of pregnancy, hence leading to difficulty in establishing a cause and effect relationship. This is possibly due to the lack of data on the frequency of occurrence of these findings in infertile patients. These types of alterations are seldom detected through interrogation and physical examination. The first line method of evaluation is hysterosalpingography.

Laparoscopy usually complements the information in case of congenital alterations. Hysteroscopy permits the evaluation and correction of uterine cavity defects in the same surgical procedure. Echography, hysterosonography and magnetic resonance imaging (MRI) contribute to the diagnosis of uterine alterations and provide information about the urinary system, which is an important target for investigation in cases of congenital abnormalities in the development of the genital tract.

Altered sperm migration

In 1888, Marion Sims described for the first time the interaction between the cervical pre-ovulatory mucus and sperm motility. The study of sperm migration leads to the determination of whether sexual intercourse is appropriate, the quality of the cervical mucus and the semen and any interactions between them. The constituents of mucus are water, electrolytes and proteins that show qualitative changes throughout the cycle. Oestrogens play an important role in the receptivity and migration of spermatozoa since pre-ovulation mucus promotes this activity to a maximum. Once the spermatozoa are deposited in the vaginal sac, they meet the cervical mucus in 180 s and the cervix becomes a reservoir of spermatozoa that keep moving upwards (first rapidly and then slowly) into the remaining section of the genital tract.

The way to assess this relationship between mucus and semen in the first instance is with a post-coital test or the Simms-Hubner test. The test is simple and allows for the detection of sperm migration alterations, which occur in about 10% of couples that visit the clinic because of infertility. The normal result is determined by the observation of 10 motile

spermatozoa in one microscope field of peri-ovulatory mucus under large magnification. If the result is poor in the presence of adequate cervical mucus and a normal spermiogram, an in-vitro test for the mucus–semen relationship may follow (Kremer test) or cross-testing can be used to determine the presence of either male or female causes.

The presence of antisperm antibodies in the cervical mucus, as well as of certain pathogenic agents results in a reduced in-vivo sperm motility. These are possible causes of infertility.

Causes of male infertility

Despite all the diagnostic difficulties, the WHO (1992b) has suggested a diagnostic classification protocol for the male factor in infertile couples (Rowe *et al*., 1993). By using WHO data, Comhaire *et al*. (1987) collated reports from 33 centres in 25 different countries to show that the incidence of varicocele is considerable, as is idiopathic oligozoospermia (**Figure 6**). In this study, the incidence of endocrine and genetic disorders was rare. It is important to keep in mind that the infertile couple displays a large number of general epidemiological factors. For example, the age of the woman is certainly the most important parameter in determining infertility. Lower sperm concentrations have been detected in smokers than in non-smokers. The recent advances in assisted reproduction have without doubt caused a revolution in the treatment of these couples with a male factor. They have also contributed to a better knowledge about the aetiology of the male factor, emphasizing the importance of genetic factors in this problem. Paradoxically, this has reduced interest in the clinical study of the patient, due to the meagre existing therapeutic possibilities. The risks of adopting such an approach include concerns about the safety and costs of assisted reproduction techniques.

Ejaculatory dysfunction

There are several different types of alterations in ejaculation. Anejaculation means absence of ejaculation resulting from trauma such as in the case of patients with complete or
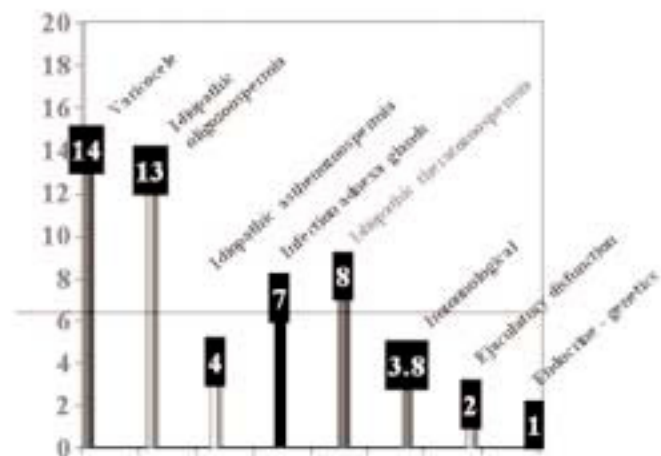

Figure 6. Percentage distribution of male infertility causes.

incomplete marrow section, iatrogenic as following a retro-peritoneal lymph node resection, pharmacological due to the intake if antihypertensives, antidepressants, antipsychotics, etc., metabolic such as diabetes and psychological. Retrograde ejaculation can also be of traumatic, iatrogenic, pharmacological, metabolic or psychological origin.

Premature ejaculation which does not allow for adequate vaginal insemination may occur in cases of multiple sclerosis as a systemic cause. It also arises due to inflammations such as those involved in prostatitis. The most frequent cause is psychological.

Varicocele

The issue of varicocele has generated controversy since the first publication about the apparent benefits of treating the condition. Experience certainly suggests that varicocele is a frequent pathology, particularly in men with lower sperm concentrations. The frequency observed in young and healthy men is between 10 and 25%. According to WHO (1992), amongst men who visit physicians because of fertility problems, varicocele was identified in 11% of those having normal semen and in 25% of those with abnormal semen. The greatest difficulty lies in determining whether the varicocele really affects testicular function and hence semen. On the other hand, questions arise as to whether its cure really improves fertility and if that is the case, in which groups of patients.

It is apparent that varicocele does affect spermatogenesis and shows a clear relationship to abnormal semen (WHO, 1992a); however, the mechanisms have not yet been established. It has certainly been possible to correlate a failure in temperature regulation and the subsequent temperature rise with deterioration in sperm quality in the presence of varicocele. Whichever the physiopathology of varicocele, there is enough evidence to show that it causes progressive testicular damage, and possible mechanisms include reflux of catabolites to suprarenal glands, low oxygen concentration, and increased concentrations of CO_2, lactic acid and norepinephrin. Nevertheless, there is an important controversy concerning whether treatment really improves fertility, with evidence in favour and against. There is even one publication (Sofikitis *et al*., 1996) that establishes a reduced capacity of spermatozoa to develop embryos with assisted fertilization in men with varicocele.

Infection of the adnexa glands

A second group of frequent etiologies is, according to WHO (1995), also controversial. There is no doubt that STD may result in pathological semen; however, there is still some question about subclinical infections. The transmission of sexual diseases is highly dependent on races and cultures. In Asia for instance there is just a 3% incidence, whilst the corresponding value is 12.2% in Africa (Rowe, 1988). Gonorrhoea for example may bring about an obstruction of the seminal ducts. Chlamydia in males may cause a tubal obstruction in the female partner.

One of the consequences of seminal infection is the higher production of leukocytes which may be associated with an increase in oxygen reactive substances. The increase in these substances is associated with difficulties for spontaneous conception and also for in-vitro fertilization (IVF). It is important not to forget that leukocytes may give rise to an excessive amount of oxygen reactive substances although such increases can also be caused by abnormal spermatozoa. Hence, the diagnostic and therapeutic implications still need to be appropriately elucidated.

Systemic and iatrogenic causes

Exposure to high temperatures has also been postulated as a cause for testicular failure, both in workers exposed to high temperatures, as in those who frequently take steam baths. Claims have also been put forward about the effect of radiation in its various modalities on fertility, including ionizing and high frequency electromagnetic radiation absorbed by electric welders, by radiologists or by people working with telecommunications equipment (Weyandt *et al*., 1991; Larsen *et al*., 1991). People who work with, or are exposed to inorganic lead, cadmium, mercury, manganese, hexavalent chromium, pesticides, organic solvents, anaesthetic gases and plastic monomers also run a risk of developing more or less serious alterations in their testicular function.

In any case, it is clear that in ~60% of the cases, regardless of how comprehensive the study of the patient, the aetiological diagnosis in andrology is still to be determined.

In recent years, advances in molecular biology and in molecular genetics have helped to identify different forms of male infertility that were formerly classified as idiopathic. These findings are extremely important because genetic or gene disorders that result in sterility may also affect other non-reproductive organs. A case in point is bilateral agenesis of the spermatic ducts, which is a mild variant of pancreatic cystic fibrosis. Another example is dysplasia of the fibrous sheath that can co-exist with other pathologies of the flagellum of respiratory cilia (Brugo *et al*., 1997) (**Table 3**). It must also be kept in mind that, thanks to IVF and to intracytoplasmic sperm injection (ICSI), those men who were considered irreversibly sterile are now capable of having their own children. If their sterility is due to genetic disorders, they could pass on the anomaly to future generations.

Table 3. Severe failure in sperm motility due to sperm flagella defects.

Systemic alterations	Acrosome hypoplasia Kartagener's syndrome Dysplasia of the fibrous sheath Acephalic spermatozoa
Non-systemic alterations	Absence of microtubulae

Special categories of infertility

Immune infertility

Immune responses among some tissues in the female and male reproductive systems can be a cause of infertility. It is well established that both men and women may develop antibodies that react against spermatozoa and interfere with fertility. This is believed to occur if agglutination or immobilization arises

due to the presence of those antibodies whenever sperm transport or fertilization are affected (**Table 4**). In men, antisperm antibodies may exist and adhere to the spermatozoon in seminal plasma and in blood. In women, antisperm antibodies may occur in the cervical mucus, in genital fluids and in blood. The incidence of antisperm antibodies is ~9% in the infertile man and 13–15% in infertile women. During evaluation of an infertile couple, the presence of antisperm antibodies is shown by anomalies in the post-coital test or in the evaluation spermiogram. With regards to the prognosis of pregnancy, pregnancy depends on the titre of antibodies and the duration of the quest for pregnancy.

Table 4. Sites of action of antisperm antibodies

Sperm transport
- Clumping and agglutination in seminal plasma
- Inhibition of migration through cervical canal
- Slowed forward progression of sperm in uterus
- Sperm destruction by complement activation in uterus

Gamete interaction
- Inhibition of sperm capacitation and acrosome reaction
- Inhibition of sperm attachment, binding, and penetration of zona pellucida
- Inhibition of sperm fusion with oocyte vitelline membrane
- Inhibition of sperm activation and pronuclear formation

Embryo development
- Inhibition of fertilized oocyte cleavage
- Direct action against developing embryo to reduce viability

Pregnancy itself could stimulate immune diseases in the woman. The fetal–placental unit is a semi-graft due to the fact that the male genetic contribution has an antigenic expression partially unknown to the mother. The normal evolution of pregnancy is contradictory to the rules of immunology in a transplant. For each protection mechanism, an alteration arises which needs to be strong enough to trigger miscarriage. The immune response generated against genetic differences in different individuals of the same species is called allo-immunity. In order to ascertain that miscarriage resulted from an allo-immune cause, other causes should be excluded including genetic, infectious and endocrine factors. Histocompatibility leukocyte antigen (HLA) typing and the mixed culture of lymphocytes are two of the different laboratory tests requested to investigate such causes.

The concept of auto-immunity associated with infertility is controversial. In women, auto-immunity is usually related to the presence of antibodies against components of the ovarian tissue, whilst in men auto-immunity refers to antibodies mainly directed towards the spermatozoa. The presence of non-organ specific antibodies is also accepted; however, there is some controversy as to whether these non-organ specific antibodies should be blamed for disease or should be considered as an epiphenomenon of an immune defect that causes infertility.

In women, the most commonly accepted cause of auto-immunity associated with infertility is premature ovarian failure. The definition for this condition is the cessation of menses before 40 years of age, with the subsequent increase in circulating gonadotrophins. The incidence ranges from 2–10% in amenorrhoeic women and from 1–3% in the general population, with autoimmune abnormalities detected in 66% of cases. In this manner, premature ovarian failure has been associated with rheumatoid arthritis, myasthenia gravis, pseudo-hypoparathyroidism, Addison's disease and thyroid alterations. Premature ovarian failure can also be part of a multiple autoimmune endocrine syndrome. No specific antigens have yet been detected in humans that trigger a selective autoimmune response in the ovary. Circulating antibodies have been reported but detection methods vary considerably. The investigation of auto-antibodies can be severely obstructed because premature ovarian failure can be the final stage in the process. By the time the diagnosis of the follicular defect, which is the target of the autoimmune system, is achieved, the follicular system is exhausted. Autoimmune oophoresis may very well be one of the initial extremes of premature ovarian failure, but unfortunately its clinical manifestation is quite rare. Unexplained infertility and endometriosis are associated with a significant level of antibodies. The immune profile of these three entities usually indicates T-cell alterations.

Recurrent gestational losses represent a form of infertility that can be associated with a normal autoimmune function. The presence of organ and non-organ specific antibodies can be predictive for the risk of abortion. Anti-phospholipid antibodies have been implied in this process. Autoimmune reproductive failure, either combined with infertility or with miscarriages, represents a T-cell dysfunction. The clinical use of antibodies for diagnostic purposes should always be directly related to the clinic and adapted to each particular case.

Infertility and genetics

Advances in molecular biology have resulted in determinant genetic causes for reproductive disorders in both men and women. However, this represents a very small group of patients. There are some cases with a well-defined pattern of purely gene alterations and others with multiple gene involvement. Abnormal developmental and functional forms have been described up to the present, which may be caused by alterations at different levels of the hypothalamic–pituitary–ovarian axis.

Genetics and female infertility

In women, these can be divided into abnormalities in the sex chromosomes, gene alterations and others (**Table 5**). Abnormalities in the sex chromosomes are involved in Turner syndrome (45X). This entity was first described in 1930 in short women with gonadal dysgenesis, ulnar valgus and short neck. This syndrome corresponds to several X chromosome abnormalities, ranging from the deletion of one of them (45X) to partial deletions associated with irregularities in the menstrual cycle and during pregnancy (Lippe, 1991). The incidence of different types of chromosome X variation is estimated at ~1: 2000 children born alive. Some of the partial X deletions include the deletion of the short arm of chromosome X which results in the typical Turner syndrome

Table 5. Genetics of female infertility

Alterations in the X chromosome	
	Turner syndrome
	Primary amenorrhoea
	Irregularities in the menstrual cycle
Gene mutations affecting female reproduction	
ZFX	Shorter reproductive life
FRAXA	Premature ovarian failure
GnRH gene	Kallman´s syndrome
Gene mutations in sex steroidal hormones	
StAR	Cholesterol transport
CYP 11 A	P450 scc
CYP 17	P450 c17
CYP 19	Aromatase
SDR 5A	α-reductase
Gene mutations in adrenal steroids	
CYP 21	p450 c21
CYP 11 B1	p450 c11
Mutations in nuclear hormone receptors	
SF-1	Development and differentiation of reproductive system
DAX-1	Congenital adrenal hypoplasia and hypogonadotrophic hypogonadism
Oestrogen receptor	
Androgen receptor	

and the deletion of the long arm which results in primary amenorrhoea free of all Turner syndrome stigmata. Partial deletions of the long arm of chromosome X convey various clinical manifestations, depending on the chromosomal region involved.

Various gene alterations could impact on female reproduction. Deletion of the gene *ZFX* is associated with a shorter reproductive life span, mimicking early ovarian failure. Fragile X syndrome, *FMR1* is associated with mental retardation in men, and the presence of *FRAXA* (repetition of the CGG trinucleotide) is associated with premature ovarian failure in women. All the above occur due to genetic changes in chromosome X. Other manifestations may arise at different anatomical and physiological levels. In the *GnRH* gene, the *Kal* gene is responsible for the migration of neurons from the olfactory placode to the brain and genetic modifications lead to defective development of the olfactory bulb (Hardelin, 1993). This is the origin of the so-called Kallmann's syndrome involving mutations in the GnRH receptor in 7% of infertile women patients, mutations in the genes that code for gonadotrophins and their receptors.

Mutations in genes that code for several factors involved in the synthesis of sex steroidal hormones may invoke infertility, e.g. *StAR* which provides the protein responsible for cholesterol transport to the inner mitochondrial membrane, cytochrome P450 scc and the gene *CYP 11 A*, 3-β–hydroxysteroid dehydrogenase, cytochrome P450 cl7 (the gene *CYP 17*); β-steroid dehydrogenase; aromatase (*CYP 19*); α-reductase (*SRD 5A*).

Gene mutations that code for the synthesis of adrenal steroids may also influence fertility. These include cytochrome P450 c21 (gene *CYP 21*) and cytochrome P450 c11 (gene *CYP 11*

B1). The following gene mutations are effective at the level of nuclear hormone receptors: *Sf-1* which is involved in the development and differentiation of the reproductive system, *DAX-1* which is a cause of congenital adrenal hypoplasia and hypogonadotrophic hypogonadism, and oestrogen receptor and androgen receptor genes.

There are gynaecological entities such as the polycystic ovary syndrome and premature ovarian failure in which several gene alterations could be involved. In 1985 Simpson (1985) provided evidence on the genetic nature of several conditions associated with infertility. He included Mullerian abnormalities, leiomyomata, endometriosis and polycystic ovary syndrome.

A genetic study is called for in every couple seeking advice for infertility, whenever the suspicion of an abnormality arises, such as in the case of recurrent abortions or women with premature ovarian failure.

Genetics and male infertility

Genetically speaking, male infertility may be classified as being due to a single gene defect, or to a numerical structural chromosomal defect. If the defect affects hormone production, a pre-testicular defect is involved. If the gene failure affects spermatogenesis, a testicular factor should be involved. On the other hand, when the defect affects the transport of spermatozoa, the cause is related to a post-testicular factor. If the gene or genetic disorder affects the spermatozoon, a spermatic factor should be involved.

Single gene defects that generate male sterility are due to mutations in one allele or in two alleles at the same locus. The inheritance of the defect may be dominant or recessive in

Table 6. Genetic factors in male infertility

Pathology	Frequency	Phenotype	Heredity/genotype
Kallman's syndrome	1 in 30 000	Anosmia Delayed puberty Azoospermia or oligozoospermia	X-linked recessive
Cystic fibrosis	1 in 2500	Respiratory infections Pancreatic insufficiency Poor Wolffian duct development Obstructive azoospermia	Autosomic recessive *CFTR* gene, chromosome 7
Immotile cilia syndrome	1 in 30 000	Blonchiectasias Situs inversus Immotile spermatozoa	Autosomal recessive Chromosome 19
Myotonic dystrophy	1 in 8000	Muscle weakening Testicular atrophy	Autosomal dominant Chromosome 19
Androgenic insensitization	1 in 60 000	Feminizing testicle	X-linked recessive
Renal polycystic disease	1 in 800	Multiple kidney liver, epididymis cysts	Autosomal dominant
Usher syndrome	1 in 30 000	Deafness Retinitis Spermatic axonemal defects	Autosomal recessive

nature. **Table 6** describes the most frequent forms of male sterility arising from a single gene defect. These genes will be considered briefly.

Kallman's syndrome is a type of hypogonadotrophic hypogonadism that evolves with anosmia and may be associated with unilateral renal aplasia. It is an X-chromosome recessive condition. Hypothalamic failure of GnRH agonist can be due to a defect in the gene. It codes for the protein responsible for neuronal migration from the olfactory plate to the diencephalon, so affected patients are either severely azoospermic or oligozoospermic.

Pancreatic cystic fibrosis is an autosomal recessive condition resulting from a defect in a locus with 27 exons on the long arm of chromosome 7. The mutated gene is *CFTR*, which regulates transmembrane conductance. This condition affects calcium channels and patients suffer from chronic broncho-pulmonary infections, pancreatic insufficiency and bilateral agenesis of the deferent ducts. However, this latter defect is sometimes the only symptom of the disease. Keeping in mind that most of these patients are carriers of *CFTR* mutations, it is most important to study the female partner before attempting ICSI.

The immotile cilia syndrome is genetically speaking heterogeneous. The most frequent example is Kartagener's syndrome, characterized by bronchiectasis, sinusitis, recurrent middle ear infections and situs inversus. The absence of dynein arms in sperm flagellae prevents the rupture of the spermatic ATP molecules, so rendering the flagellae immotile. The mutation might occur in the short arm of chromosome 1.

Myotonic dystrophy is the result of a gene mutation in the long arm of chromosome 19. Approximately 80% of patients suffer from some type of testicular atrophy. Androgen insensitivity, also known as feminizing testicle, is an alteration in androgen receptors that hinders the action of circulating androgens. Several forms of the disease have been described, such as the

atrophy of the bulbar and spinal muscles. The most severe form is complete androgen resistance, a recessive X-chromosome disorder.

Usher syndrome is the most frequent cause of hereditary deafness and blindness. Some Usher syndrome patients present with a degeneration in the spermatic axoneme that causes total immotility of the spermatozoa.

Two types of chromosomal abnormalities affect male fertility: numerical and structural. Usually they are de-novo chromosomal pathologies, resulting from mutations in the parental germ line. The most frequent chromosomal abnormality is Klinefelter's syndrome, the first chromosomal abnormality reported in the literature, which can be pure or mosaic. It has an incidence of 1:1000 live births and 1:300 fetuses from spontaneous abortions. The frequency in sterile men is ~1–2%, but in the azoospermic population is ~7–13%. The pre-pubertal phenotype is normal but after puberty the most salient characteristics are bilateral testicular atrophy with a firm consistency, deterioration of the secondary sex characteristics, sometimes a certain level of dyslexia and even mental retardation. One half of the cases show gynaecomastia, high FSH and LH concentrations and low doses of testosterone. Obviously, almost all of these patients are sterile, with severe oligozoospermia or azoospermia. The testicles usually present hyalinosis and tubular sclerosis, but there are some cases of tubules with spermatozoa and pregnancies by means of assisted fertilization have been reported (Nodar *et al*., 1999). There are variants to the syndrome such as 48,XXXY, 48,XXYY and 49,XXXY. It is generally accepted that the more X chromosomes present in the patient, the worse the testicular lesion.

Noonan syndrome is the male equivalent of Turner's syndrome. Patients display short stature, lymphoedema, low ears, short neck, cubitus valgus and cardiopathies. The incidence in men is 1:2500. Most patients present with testicular atrophy, and

cryptorchidism is also frequent. The karyotype is 46,XO/XY and inheritance is autosomal dominant. FSH concentrations are elevated but the LH and testosterone concentrations are normal. Usually these patients are severely azoospermic or oligozoospermic.

Men with 47,XYY syndrome are phenotypically healthy. They occur with an incidence of 1: 1000. These men may have a higher risk of personality disorders and even show an anti-social personality. Some of these patients suffer from oligozoospermia, but many of them are normozoospermic. The risk of having abnormal children is minimal, since the extra Y chromosome is lost early in the germ line and hence does not pass into the next generation.

Y chromosome deletions can be associated with male infertility. Approximately 10% of men with oligozoospermia and 15% of patients with genetic azoospermia have a deletion in the azoospermia factor (AZF), which is localized in the long arm of chromosome Y (Reijo *et al.*, 1996). Several gene sequences have been identified to date, localized in the long arm of chromosome Y, which are candidates for the azoospermia gene. The gene most frequently deleted in azoospermia is DAZ, in 70% of cases. However, this gene has also been sequenced in chromosome 3. It codes for a protein with 366 amino acids involved in regulating meiosis. Those patients with this defect who may become parents following ICSI, have a high risk of transmitting it to their offspring.

Translocations can be balanced or Robertsonian in nature. The latter are 10 times more frequent in sterile men or in men with pathological semen. The most frequent translocation is 13;14. It can be accompanied by normal semen or severe oligozoospermia, and even with azoospermia.

Alterations in mitochondrial DNA have recently been related to semen defects. An example identified in several patients with oligozoospermia involves the deletion of the 4977 mtDNA gene, which could be responsible for this seminal abnormality.

Summary of genetic diseases
More and more genetic or gene diseases are being described that account for seminal disorders leading to infertility. This concept must be kept in mind when administering treatments such as ICSI, because of the future consequences for heredity. These consequences need not necessarily be malformations, and the frequencies of malformations in newborn children conceived through ICSI or IVF seem to be equivalent. There may be serious fertility problems for the newborn babies or, even worse, alterations in population genetics jeopardizing future generations.

Preimplantation genetic diagnosis of cleaving embryos can be suggested for women over 35 years of age. The diagnosis of aneuploidy in spermatozoa with in situ hybridization and using fluorochromes (FISH) is indicated for severe oligoasthenoteratozoospermia, severe teratozoospermia, couples with recurrent abortions and those with no apparent cause for infertiltiy. Following treatment, de-novo chromosomal alterations have been described in gonads and embryos.

Genetic counselling before, during and after assisted reproduction treatment is a sound practice for making a correct diagnosis and adapting specific genetic recommendations to couples and their families.

Unexplained infertility

Unexplained infertility or infertility with no apparent cause is a term used for those cases in which infertility studies show normal results. This situation occurs in about 15% of couples and is usually frustrating to both the physician and the couple due to that feeling of missing expectations because no specific diagnosis exists. Couples with unexplained infertility could have a subtle defect in their reproductive ability, which cannot be identified through a standard evaluation. It is also possible that specific causes do exist but are unknown to date or their detection is beyond the realm of the available diagnostic procedures (Crosignani *et al.*, 1993).

From a therapeutic point of view it might be important to consider these couples as individuals with a limited reproductive capacity, since many achieve pregnancy but it takes them much longer than for normal couples. From this perspective, the underlying reason for administering treatment for infertility when no apparent cause has been identified would be to increase the monthly probability of establishing pregnancy. With regard to the prognosis of these couples, the duration of infertility becomes an important piece of information. After 3 years of untreated infertility, the pregnancy rate per year drops by 24% when the woman is over 30 years old. The prognosis is more optimistic in couples with a history of prior pregnancies. Couples with unexplained infertility should be aware of the fact that pregnancy is likely to occur without treatment, but it will take them longer than for other couples.

Conclusions

Infertility is a condition affecting one fifth to one sixth of couples at reproductive age. Investigating the cause of infertility necessitates a study of both partners, since 40% of all infertile couples exhibit a combination of causes. Ovulatory factor needs an exhaustive interrogation , physical examination, ovulation detection tests and hormone profile for a complete evaluation. Tubal– peritoneal factor accounts for ~30% of the causes of infertility. The main options for evaluating this factor are hysterosalpingography and laparoscopy. Endometriosis has a high incidence in infertility patients (48%) and several mechanisms have been proposed in infertility-related endometriosis. Male factor is present in 30% of the couples and several entities are related to male infertility. Varicocele is the most frequent pathology, followed by idiophatic oligozoospermia. The study of both female and male infertility has been influenced extensively by the development of molecular biology and genetics. These two disciplines are now crucial for the study, diagnosis and assesment of infertile couples.

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