

# Genetic aspects of female reproduction

## The ESHRE Capri Workshop Group

Correspondence address. P.G. Crosignani, II Department of Obstetrics and Gynecology, University of Milano, Via Commenda 12, 20122 Milano, Italy. E-mail: piergiorgio.crosignani@unimi.it

**BACKGROUND:** Sexual reproduction provides the means for preserving genetic identity and in turn, genetic variability may affect the ability to reproduce. This review aims to summarize current research on genetic diagnosis and genetic causes of reproductive disorders. **METHODS:** Searches were done by subject in Medline and other databases, and each subject summary was presented to the Workshop Group and omissions or disagreements were resolved by discussion. **RESULTS:** Single-gene defects are most likely to be found among patients with hypogonadotropic hypogonadism, which may be due to defects in the KAL genes or the gonadotrophin-releasing hormone receptor genes. With premature ovarian failure there is an increased risk of having a premutation of the Fragile X syndrome gene. Complex genetic inheritance may explain the variable familial links in polycystic ovary syndrome and endometriosis, but no definitive genetic pathways are as yet known. With recurrent miscarriage, genetic defects causing thrombophilias are 2-fold more likely. Chromosome abnormalities account for ~60% of all spontaneous abortions, and the most common type, trisomy, is closely associated with advanced maternal age. Three percent of couples have a balanced chromosome abnormality, but live birth rates are better with natural conception than with preimplantation genetic diagnosis. **CONCLUSIONS:** Understanding of the methods used for genetic diagnosis and research is becoming a standard requirement for the clinical practice of reproductive medicine.

*Keywords:* hypogonadotropic hypogonadism; premature ovarian failure; polycystic ovary syndrome; Rokitansky syndrome; pregnancy loss

## Introduction

Genetic variability and epigenetic factors affect reproduction and fertility from gametogenesis to birth. The human genome is contained within 23 pairs of chromosomes, each containing hundreds or thousands of genes. The estimated 20 000 genes each make an average of three proteins. The genome involves three billion base pairs which are subject to countless variations that may affect health and disease (Fig. 1). Single-gene defects may cause female infertility, although this is rare (Fauser and Hsueh, 1995) more commonly complex genetic inheritance contributes to variations in the frequency of diagnoses such as endometriosis.

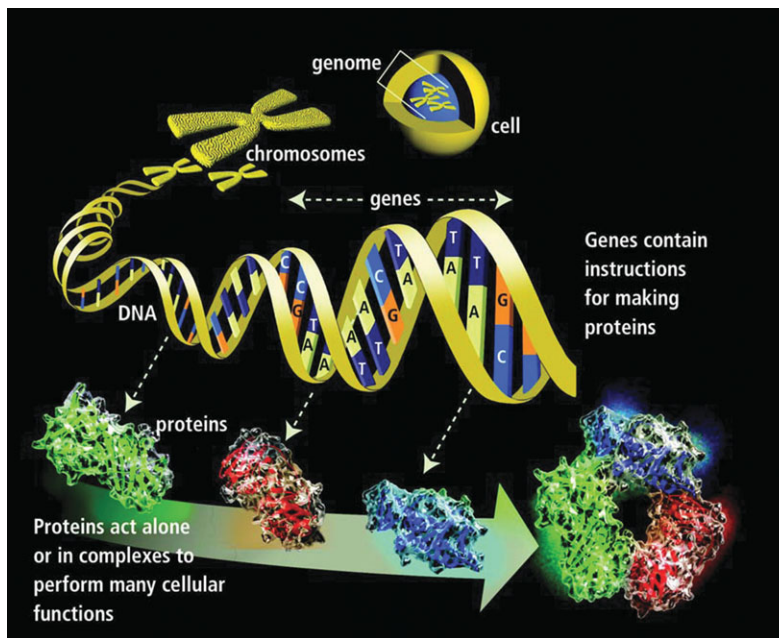
The four mechanisms for inheritance of disease are chromosomal, Mendelian, mitochondrial and complex.

Chromosomal disorders may be numerical, such as polysomy and polyploidy, or structural. Translocation is the most clinically important structural disorder and it may be reciprocal (exchange of two terminal segments from different chromosomes), or Robertsonian (centric fusion of two acrocentric chromosomes). Balanced translocation occurs in about 1 in 600 newborns (Jackson, 2002).

Mendelian types of heritable disease are single-gene defects which are usually autosomal dominant, autosomal recessive or X linked.

**Mitochondrial inheritance:** Mitochondrial DNA (mtDNA) may be 10–20 times more frequently mutated than nuclear DNA. About one-third of the oocyte DNA is mitochondrial, while the sperm mitochondria do not survive in the oocyte, and therefore mitochondrial abnormalities are transmitted only along the maternal line. Abnormalities in mtDNA are likely to cause fertilization abnormalities and disturbances in early development. In patients with mitochondrial disease, the number of mtDNA molecules that carry a mutation is increased compared with healthy carriers. Most pathogenic mtDNA mutations are heteroplasmic, that is, the mitochondrial genomes are not identical, resulting in heteroduplexes after polymerase chain reaction (PCR) amplification of mtDNA. These heteroduplexes can be detected with a technique of denaturing high performance liquid chromatography (van den Bosch *et al.*, 2000), this is also possible via microarray technology (van Eijsden *et al.*, 2006).

**Complex genetic inheritance:** Many of the differences between individuals reflect features that show continuous quantitative variability. The inheritance of such features does not follow the rules of simple, monogenic heredity. Complex genetic inheritance of disease is characterized by familial clustering and a recurrence risk which is higher than the population risk. The incidence is



**Figure 1:** Each human cell contains 46 chromosomes, 2 meters of DNA, 3 billion base pairs and ~20 000 genes which code for proteins that perform most life functions ([http://www.ncbi.nlm.nih.gov/resource\\_center/genomics\\_and\\_bioinformatics/genomics\\_primer.html](http://www.ncbi.nlm.nih.gov/resource_center/genomics_and_bioinformatics/genomics_primer.html)).

always less than that with Mendelian disorders and can be estimated only by observations among families and population samples. In genetic terms the complexity results from the interaction of endogenous (genetic) and exogenous (environmental) factors. Such a pedigree pattern might also result, however, from the interaction of more than one genetic factor. Family and population data help to explain diseases with complex genetic inheritance, but heritability estimates obtained from twin studies are of special importance.

While understanding of complex genetic mechanisms is evolving slowly, knowledge of single-gene defects is expanding rapidly, in part because of new and powerful genetic investigations. When these methods are applied, a distinction has to be made between the research application (to find genes that may underlie disease) and the diagnostic application (to detect chromosome abnormalities or gene mutations in an individual patient or family).

The research methods may include longitudinal family and twin studies and in some cases ethnic studies. Candidate gene studies of genes known to be related to the disease look for differences in the frequency of genetic variants between disease cases and healthy controls, although increasingly the research involves a search across the whole genome. Genome-wide association studies can compare the frequency of up to one million single-nucleotide polymorphisms (SNPs) in cases and controls. The genetic diagnostic methods include pedigree analysis, chromosome analysis, assessment of single-gene defects and examination of the genome for disorders of gene copy number or mutations that may assist with a diagnosis.

The objectives of this review are to: outline genetic diagnostic methods; summarize the impact of chromosomal abnormalities on miscarriage; consider single-gene mutations that cause infertility; assess whether complex genetic mechanisms may influence the frequency of polycystic ovary syndrome (PCOS),

endometriosis and fibroids; and summarize genetic factors that influence the risk for and the management of recurrent miscarriage (RM). Searches were done in Medline and other databases by subject and each subject summary was presented to the Workshop Group, where omissions and disagreements were resolved by discussion.

### Genetic methods for diagnosis

Clinical practice increasingly depends on knowledge of the principles of genetics and the techniques used by geneticists. These include use of pedigrees, cytogenetic analysis, fluorescence in-situ hybridization (FISH) and methods of molecular genetics diagnosis such as PCR and comparative genomic hybridization. Table I summarizes the methods used to detect genetic mutations.

#### Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) can rule out a specific genetic disease known to exist in one or both parents before pregnancy has been established. While PGD is not used to diagnose genetic causes of female infertility, it can be done only within a cycle of *in vitro* fertilization (IVF). One blastomere is removed from Day 3 embryos which are usually at the 6–10-cell stage of development. The blastomere is tested by PCR if there is a single-gene defect, and by FISH if there is a translocation or to exclude sex-linked disorders. Only embryos free from the known defect will be transferred to the mother (Sermon *et al.*, 2007). The difficulties with PGD include a small diagnostic error rate (1–3%), lower live birth rates with IVF than normal conception and high cost. The effectiveness of PGD is self-evident and many couples prefer this approach to prenatal testing and possible selective termination.

**Table I.** *In vitro* methods for genetic investigations.

Method	Submethod	Mutation detected
Karyotyping (Gersen and Keagle, 2004)		All chromosomal abnormalities
Fluorescence in-situ hybridization (FISH) (Gersen and Keagle, 2004)	Probes for chromosome-specific repetitive sequences	Interphase cytogenetics
	Painting probes	Complex structural abnormalities
	Unique sequence probes	Microdeletion syndromes
	Subtelomere probes	Cryptic deletions
Comparative genomic hybridization (CGH) (Shaffer and Bejjani, 2006)	Metaphases	Small structural abnormalities
	Array CGH (microarrays)	Small structural abnormalities and microdeletion syndromes
Multiplex ligation-dependent probe amplification (MLPA) (Schouten <i>et al.</i> , 2002)		Simultaneous determination of multiple copy numbers
Denaturing high performance liquid chromatography (DHPLC) (Strachan and Read, 2003)		Screening of unknown DNA mutations
Temperature gradient capillary electrophoresis (TGCE) (Strachan and Read, 2003)		Idem
Single-strand confirmation polymorphisms (SSCP) (Strachan and Read, 2003)		Idem
Denaturing gradient gel electrophoresis (DGGE) (Strachan and Read, 2003)		Idem
Sequencing (Strachan and Read, 2003)		Diagnosis of small DNA mutations
PCR (Hundscheid <i>et al.</i> , 2003)		Estimation length short trinucleotide repeats
Southern blot (Hundscheid <i>et al.</i> , 2003)		Estimation length long trinucleotide repeats
Metabolite analysis (Forges <i>et al.</i> , 2006)		Screening of inborn errors of metabolism
Enzyme diagnosis (Forges <i>et al.</i> , 2006)		Diagnosis of enzyme deficiencies

### **Preimplantation genetic screening**

Preimplantation genetic screening (PGS), in contrast to PGD, is done for couples who do not have a known genetic defect but appear to be at high risk of aneuploidy because of advanced maternal age, recurrent pregnancy loss or recurrent implantation failure. It was assumed that screening for aneuploid embryos and transferring only euploid embryos would reduce pregnancy losses and increase live birth rates. Two randomized controlled trials have shown, however, that PGS would appear not to be effective in improving live birth rates in IVF or intracytoplasmic sperm injection for women aged 35–41 years (Staessen *et al.*, 2004; Mastenbroek *et al.*, 2007). Trials are needed to determine whether PGS is effective for recurrent pregnancy loss or recurrent implantation failure.

### **Chromosomal abnormalities and pregnancy loss**

The modern study of chromosomes in reproduction began with the discovery of the histologic distinction between the cells of males and females (Barr and Bertram, 1949). Knowledge of the Barr body gave rise to the Lyon hypothesis that in normal female cells one X chromosome is inactivated during embryogenesis (Lyon, 1961). The first report of how chromosome abnormalities contribute to fetal wastage was based on cytogenetic analysis of 200 miscarriages, of which 44 (22%) had a chromosome abnormality (Carr, 1963, 1965). The three main classes of abnormality were 45X, trisomy and polyploidy. Carr demonstrated that (i) the maternal age of miscarriages with trisomy but not the other categories was significantly increased and (ii) mean gestational age at the time of miscarriage was

significantly lower for a chromosomally abnormal fetus than one that is chromosomally normal. He also suggested that the proportion of chromosome abnormalities detected was likely to be a serious underestimate.

### **Frequency and types of chromosome abnormality in miscarriage**

Many series of karyotyped miscarriages have confirmed that ~50% of all clinically recognized spontaneous miscarriages have a chromosome abnormality. Of these, ~9% have a 45,X constitution, 30% have trisomy, 10% are either triploid or tetraploid and 2% have a structural rearrangement (Table II).

Trisomy is the most common class of abnormality and trisomy frequency in miscarriages is associated with increased maternal age, rising from very low at age 20–24 up to 35% at age 40–44 years. As a result, trisomy rates are rising with the increase in mean age at childbirth. In the last 20 years in the eastern United States, the trisomy rate increased in spontaneous miscarriages from 22 to 42% as the average maternal age rose from 29 to 34 years (Table III) (Warburton, 2007).

Theoretically for each trisomy there should be a corresponding monosomy but the only monosomy that is detected among clinically recognizable pregnancies is that for the sex chromosomes. The autosomal monosomies are likely to be incompatible with survival and a major cause of pregnancy wastage in early pregnancy.

The frequency and outcome of trisomy varies greatly for different chromosomes. As can be seen from Table IV, trisomy for chromosome 16 is by far the most frequent but never survives to birth, while the next most frequent are trisomies for chromosomes 21 and 22. Only three autosomal trisomies (13, 18 and 21) and the three sex chromosome trisomies are compatible with live birth.

**Table II.** Frequency of chromosomal abnormalities in miscarriage.

Population	Abnormality (%)					Total abnormal
	45,X	Trisomy	Polyploid	Structural	Other	
Spontaneous miscarriages	8.60	26.8	9.8	2.0	0.7	47.9
Stillbirths	0.25	3.8	0.6	0.4	0.6	5.65
Live births	<0.01	0.3	–	0.6	0.02	0.93
All clinically recognized pregnancies*	1.30	4.3	1.5	0.8	0.15	8.05

\*assuming 15% spontaneous miscarriages and 1% stillbirths (Jacobs and Hassold, 1995).

**Table III.** Spontaneous miscarriages—effect of increasing maternal age.

Time of observation	Mean maternal age (years)	Normal, %	45,X, %	Trisomies, %	Double trisomies, %	Polyploid, %	Structural, %	Other, %
1975–1986	28.6	60	6.5	22	0.72	8.3	1.6	0.6
2001–2005	34.2	32	7	42	3	12	3	1

Adapted from Warburton (2007).

**Table IV.** Monosomy and trisomy: frequency and probability of survival to birth.

Chromosome	Population			Probability of survival to birth (%) <sup>a</sup>
	Spontaneous miscarriage	Stillbirths	Live births	
1–12	0.1–1.1	0	0	0
13	1.1	0.3	0.005	2.8
14	1	–	–	0
15	1.7	–	–	0
16	7.5	–	–	0
17	0.1	–	–	0
18	1.1	1.2	0.01	5.4
19	–	–	–	–
20	0.6	–	–	0
21	2.3	1.1	0.12	22.1
22	2.7	0.1	–	0
Mosaic trisomy	1.1	0.5	0.02	9
Double trisomy	0.8	–	–	0
XXY	0.2	0.4	0.05	55.3
XXX	0.1	0.3	0.05	70
XYY	–	–	0.05	100
XO	8.6	0.25	<0.01	0.3
Total	34.7	4.25	0.3	4.6

<sup>a</sup>assuming 15% spontaneous miscarriage and 1% stillbirth.

While the single monosomy for the sex chromosomes (XO) can be born alive the great majority are fetal deaths.

Trisomy is usually the result of non-disjunction at the first or second meiotic division, most often in the female. However, the mechanism underlying the error is, surprisingly, very different for different chromosomes. Table V shows the mechanism of non-disjunction for five different chromosomes. The X chromosome is the only one in which non-disjunction in the male makes an important contribution and this is the result of failure of recombination between the X and Y chromosomes at the first meiotic division in the male. This error gives rise to 50% of all XXY males. Non-disjunction of the X in oogenesis occurs at both the meiosis I and II, and may be nulli-chiasmate (failure of recombination) or chiasmate. All mechanisms except nulli-chiasmate are associated with increased maternal age. In contrast all trisomy 16s result

from non-disjunction at the first maternal meiotic division, and are associated with increased maternal age. Trisomy for chromosome 13 is due to non-disjunction at meiosis I or II and both mechanisms are associated with increased maternal age, while trisomy for chromosome 18 is almost entirely due to non-disjunction at meiosis II, again associated with increased maternal age. Trisomy for chromosome 21 is mainly due to non-disjunction associated with abnormal recombination in meiosis I and also in meiosis II, both mechanisms being associated with advanced maternal age.

A quite different increased risk for trisomy is shown by rare individuals who have, even at a young maternal age, two or more trisomy miscarriages involving trisomy for the same chromosome. This can be the result of the mother being a gonadal mosaic for the trisomy which increases her risk of

**Table V.** Chromosomal abnormalities and pregnancy loss: mechanisms of non-disjunction (chromosomes X, 16, 13, 18 and 21).

Chromosomes	Paternal			Maternal			
	Meiosis		Post-zygotic mitotic	Meiosis		Post-zygotic mitotic	
	I	II		I	II	Nulli-chiasmate	Chiasmate
X	A***	*	*	A**	**	**	*
Maternal age	N	N	N	N	⊙	⊙	N
16	—	—	—	*A	***A	—	—
Maternal age				⊙	⊙		
13	*	*	*	A**	A**	**	*
Maternal age	N	N	N	⊙	⊙	⊙	N
18	—	—	*	*A	*	***	*
Maternal age			N	⊙	⊙	⊙	N
21	*	*	*	A*	A***	A**	*
Maternal age	N	N	N	⊙	⊙	⊙	N

\*observed—infrequent maternal age; \*\*observed—frequent maternal age; \*\*\*observed—very frequent maternal age. N, normal; ⊙, raised; A, abnormal recombination.

producing oocytes carrying an additional copy of the trisomic chromosome (James *et al.*, 1998).

### Structural chromosome abnormalities

Approximately 2% of spontaneous miscarriages are associated with structural, as opposed to numerical, chromosome abnormalities. Structural abnormalities are re-arrangements of a portion of a chromosome, and include translocations, inversions, deletions and duplications (see Glossary). Translocations are the most common clinically important structural abnormality. In their balanced form they have no phenotypic effect. However, unbalanced translocations are likely to have an effect on the phenotype and the viability of the pregnancy.

An unbalanced structural abnormality ascertained in a miscarriage may well have caused the miscarriage, and could result from chromosomal segregation errors during gametogenesis in a parent with a balanced rearrangement. When products of conception contain an unbalanced structural rearrangement, it is customary to examine the parental chromosomes and determine whether a parent carries a balanced rearrangement which may put them at risk of having a further conceptus with additional and/or missing chromosome material (Jacobs *et al.*, 1975).

The frequency of unbalanced gametes segregating during gametogenesis in an individual with a balanced translocation depends on the exact positioning of the translocation breakpoints. The great majority of balanced structural rearrangements appear to segregate in families and to be unrelated to any adverse reproductive outcomes (Jacobs *et al.*, 1975; Morton *et al.*, 1975). Hence a structural rearrangement in a couple presenting with repeated miscarriages may be co-incidental and not the cause of the miscarriage.

### Single-gene defects that cause infertility

#### Hypogonadotropic hypogonadism

Normal secretion of pituitary gonadotropins depends on migration of gonadotropin-releasing hormone (GnRH) producing neurons

from the olfactory placode to the forebrain and the development at puberty of pulsatile GnRH secretion from the hypothalamus into the portal circulation. Deficiency of this hypothalamic–pituitary system causes hypogonadotropic hypogonadism (HH), which occurs in approximately 1:8000 newborns in a 4:1 male:female ratio (Simoni and Nieschlag, 2007).

HH is the most likely type of female infertility to be associated with a specific genetic defect, usually affecting the hypothalamus rather than the pituitary. Kallmann syndrome is one genetic cause of primary amenorrhoea which can be suspected on clinical grounds (anosmia) and for which there is a specific treatment (pulsatile GnRH administration). A specific genetic lesion can be identified in 50% of familial cases with isolated HH and in 30% of familial cases with Kallmann syndrome.

Of 315 patients with isolated HH, 6% had one or more affected relatives. Autosomal recessive inheritance was most likely but autosomal-dominant and X-linked recessive inheritance patterns also were observed (Bhagavath *et al.*, 2006). All of the females had normal karyotypes and 30% had anosmia. Approximately 10% of the patients tested had mutations in the *KAL1* gene or the gene for the GnRH receptor (*GNRHR*).

GnRH deficiency due to developmental defects of GnRH secreting neurons is usually associated with anosmia, constituting Kallmann syndrome. Several genes regulating GnRH synthesis, secretion or action have been identified, and systematic studies in humans and mice have confirmed their relevance for human reproduction (Table VI).

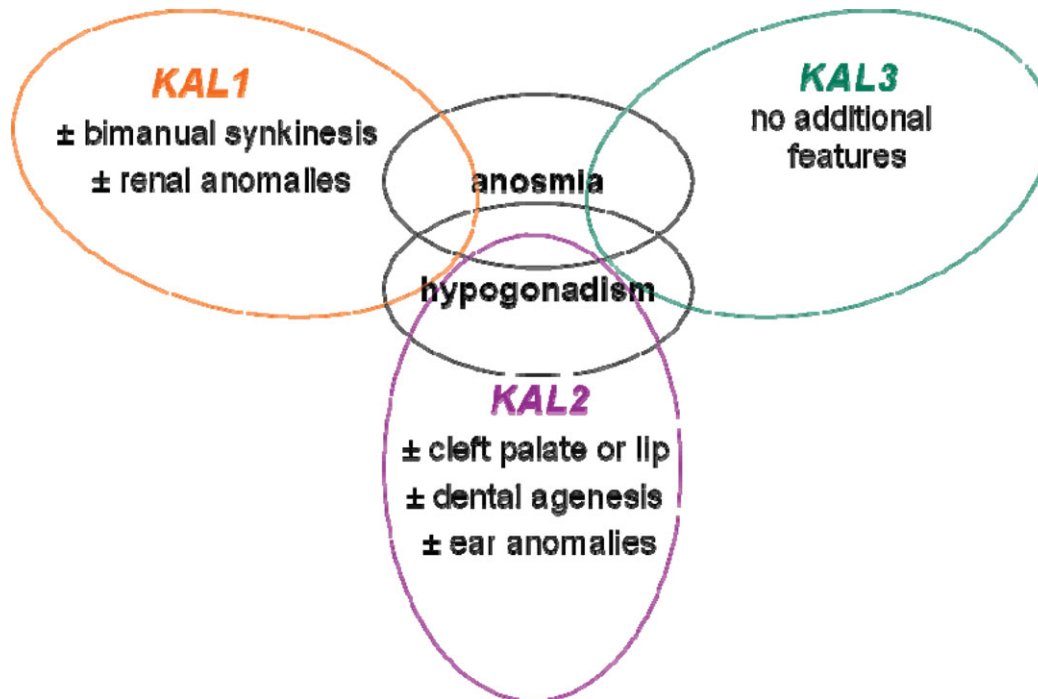
Inactivating mutations of the *KAL1* gene, encoding anosmin-1, which is an adhesion protein involved in synaptogenesis, have been identified in X-linked Kallmann syndrome. Patients with *KAL1* gene mutations may also present with bimanual synkinesia and renal anomalies. The *KAL2* gene encodes the fibroblast growth factor receptor 1 (*FGFR1*), a tyrosine kinase receptor involved in olfactory bulb development which interacts with anosmin-1. Inactivating *KAL2* gene mutations are found in autosomal-dominant Kallmann syndrome with hypogonadism, with or without anosmia (Dode *et al.*, 2003; Pitteloud *et al.*, 2006a). The phenotype of hypogonadism may vary considerably in these patients



**Table VI.** Genetic causes of Kallman syndrome.

Inheritance	Gene	Protein	Function
X-linked dominant	<i>KAL1</i> , <i>KAL2</i>	anosmin-1, FGFR1, FGF8	cell adhesion tyrosine kinase receptor ligand for FGFR1
Recessive or dominant?	<i>KAL3</i>	PROKR2, PROK2	G protein-coupled receptor ligand for PROKR2
Dominant	<i>NELF</i> , <i>CHD7</i>		Neuronal migration, DNA binding protein

*NELF*, nasal embryonic GnRH factor; *CHD7*, chromodomain helicase DNA modelling protein 7 gene.

**Figure 2:** Phenotype of Kallmann syndrome.

including complete hypogonadism without any pubertal development, partial pubertal development and delayed puberty with normal reproductive function (Pitteloud *et al.*, 2006b). Additionally associations with cleft palate or lip, tooth agenesis, ear anomalies and deafness have been described (Fig. 2). The *KAL3* gene, which encodes the prokineticin receptor 2 (PKR2), a transmembrane heptahelical G-protein coupled receptor (GPCR), or its ligand prokineticin 2 (PK2), is involved in olfactory bulb development (Matsumoto *et al.*, 2006). Inactivating mutations have been identified in patients with autosomal-recessive Kallmann syndrome (Dode *et al.*, 2006). Among familial Kallmann syndrome cases, mutations in the *KAL1*, *FGFR1* or *PKR2/PK2* genes were detected with similar frequency. A further candidate relevant for Kallmann syndrome is the nasal embryonic GnRH factor (*NELF*) involved in GnRH and olfactory neuron outgrowth, for which a heterozygous deletion has been found in one patient with Kallmann syndrome so far (Pitteloud *et al.*, 2007). Mutations within the chromodomain helicase DNA binding protein 7 (*CHD7*) gene have been identified in 58% of individuals with CHARGE association, a syndrome in which HH is associated with ocular coloboma, cardiovascular malformations, choanal atresia, growth retardation, anosmia and ear anomalies (Lalani *et al.*, 2005).

Altered GnRH secretion may also be due to defective signalling through the kisspeptin GPCR54 (GPR54) system. Expressed at the surface of GnRH neurons, GPR54 induces GnRH secretion, while patients carrying inactive GPR54 variants exhibit abnormalities in gonadotropin axis activation (de Roux *et al.*, 2003; Seminara *et al.*, 2003). In contrast to secretion defects, resistance to GnRH is caused by inactivating mutations of the GNRHR, another GPCR expressed in membranes of pituitary gonadotropes. Inactivating GNRHR mutations have been found in ~40% of patients with isolated autosomal-recessive HH, while GPR54 mutations are less frequent (15%) (Simoni and Nieschlag, 2007). Individuals bearing inactivating GNRHR substitutions may present a wide phenotypic spectrum from partial to complete hypogonadism depending on the specific functional consequences of each mutation. Because the GNRHR is inactive in these GnRH resistant defects, the necessary treatment is gonadotrophins (Karges *et al.*, 2003a, b).

The difficulty in predicting phenotype from genotype in individuals with HH suggests that phenotypic heterogeneity is due to digenic rather than monogenic inheritance (Pitteloud *et al.*, 2007). In other words, the clinical variability in features associated with GnRH deficiency may reflect mutations of two distinct genes in an individual, which may generate a more severe phenotype than a single-gene alteration.

### Premature ovarian failure

Premature ovarian failure (POF, OMIM 311360–300511) is a heterogeneous group of disorders with cessation of ovarian function before the age of 40 years and elevated gonadotrophins. POF occurs in one per 1000 women by age 30 years and in one per 100 women by age 40 years accounting for up to 10% of ovulatory female sterility (Coulam *et al.*, 1986).

#### Mechanisms

POF may result from (i) a decreased pool of primordial follicles, (ii) an accelerated rate of follicular atresia or (iii) dysfunction in follicular recruitment or maturation. In most cases the aetiology is unknown, but POF can be associated with autoimmune disorders and systemic diseases like galactosemia, or it can be secondary to chemotherapy or radiotherapy. POF can also arise with X chromosome abnormalities, which is the indication to carry out a karyotype analysis in these patients.

#### Familial POF

Familial occurrence of POF and the high frequency of associated chromosomal abnormalities suggest a genetic component (Vegetti *et al.*, 2000). The incidence of familial POF varies from 4 to 31% depending on the inclusion criteria adopted and whether the assessment of family history is detailed (Davis *et al.*, 2000; Vegetti *et al.*, 2000). Pedigree studies of affected families suggest that the inheritance of POF may be autosomal dominant sex-limited or X-linked with incomplete penetrance (Vegetti *et al.*, 2000).

POF is associated with mutations in a small number of genes including INHA, which encodes inhibin alpha (Marozzi *et al.*, 2002) and the gene for the FSH receptor (Fauser and Hsueh, 1995). Recent evidence in experimental and natural models documents the essential role of the paracrine effect of growth/differentiation factors of transforming growth factor (TGF $\beta$ ) superfamily (BMP15, GDF9) produced by the oocytes or granulosa cells in ovary development and progression of folliculogenesis (Fabre *et al.*, 2006). In this regard, variants of these genes have been identified in women affected by POF, presenting variable phenotypes (Di Pasquale *et al.*, 2006; Laissue *et al.*, 2006; Kovanci *et al.*, 2007). Furthermore, the observation that GDF9 gene variants are significantly more prevalent among mothers of dizygotic (DZ) twins than controls suggests that these gene are of particular relevance in the pathway regulating the ovarian follicle growth and maturation (Palmer *et al.*, 2006).

Also, the forkhead box L2 (FOXL2) gene is responsible for the autosomal dominant, blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES, OMIM 110100, which may occur with POF (BPES type I) or without POF (BPES type II) but is rarely involved in idiopathic POF (Bodega *et al.*, 2004). Also rarely involved are mutations in the eukaryotic translation initiation factor 2B genes (EIF2B) found in patients with ovarioleukodystrophy.

#### Fragile X premutation

The fragile X syndrome arising from an X-linked mutation of the fragile X mental retardation 1 gene (FMR1 gene) causes the most common form of familial mental retardation in men (Hundscheid *et al.*, 2003). The FMR1 mutation involves expansion of a CGG

triplet to more than 200 copies in the 5' prime untranslated region (5' UTR) of the FMR1 gene (full mutation). The premutation involves 50 to 200 copies of the CGG triplet and is associated with tremor/ataxia in males (FXTAS) and POF in females (Hagerman and Hagerman, 2004). Production of FMR1 mRNA is decreased in the full mutation, but increased in the premutation (mRNA gain-of-function toxicity), which may underlie the altered ovarian function.

The incidence of POF among female premutation carriers is ~15–20% compared with 1% in the female population. Inversely, the frequency of the premutation allele in POF females is ~5–10% compared with <1% in the general population. The POF risk is 2-fold higher in women with the FMR1 premutation (Bodega *et al.*, 2006; Ennis *et al.*, 2006). Because women with POF are at increased risk of having an FMR1 premutation, they should be informed of the availability of fragile X testing and counselled about the implications for the family if the result is positive (Wittenberger *et al.*, 2007).

#### X monosomy

Turner syndrome with X monosomy causes POF with primary amenorrhoea; mosaic forms may cause POF with secondary amenorrhoea. POF also presents with structural anomalies of the X chromosome such as terminal and interstitial deletion (Toniolo, 2006). Two loci (from Xq22 to Xq26 and from Xq27 to 28) appear to be critical for the POF phenotype. The mapping of different POF translocation breakpoints led to the identification of four interrupted genes, (DIAPH2 gene in proximal Xq22, XPNPEP2 gene in Xq25, DACH2 gene in Xq21.3 and POF1B gene in Xq21.3). It remains unknown, however, whether these genes contribute to the aetiology of POF (Toniolo, 2006).

Thus, POF is a heterogeneous disorder that may have several genetic causes. Some genetic causes may be inherited as Mendelian factors; others seem to carry susceptibility variants that increase the risk of developing ovarian dysfunction.

Karyotype and FMR1 testing are indicated in most cases. Genetic counselling is essential for women who have POF or belong to families with POF.

#### Congenital absence of the uterus and vagina

Congenital absence of the uterus and vagina (Mayer–Rokitansky–Küster–Hauser) syndrome involves a failure of development of the Müllerian derivative of the vagina and the portions of the Müllerian ducts that are destined to become the uterus. The incidence is one per 4000 to 10 000 female births. Type I has complete aplasia of the vagina and uterus with two rudimentary horns and normal Fallopian tubes. Type II has vaginal aplasia with either symmetric uterine hypoplasia or aplasia of one horn, tubal malformation and other associated malformations.

A tendency to familial distribution suggests the potential for a genetic link, although few genes have been identified as causal. One candidate is mutation of the WNT4 gene, a developmental gene which regulates growth and differentiation during embryogenesis.

Anomalies of the Müllerian duct also have been described in association with maturity onset diabetes of the young (MODY) due to mutations of the TCF2/HNF1 $\beta$  gene (MODY5, OMIM 189907) (Fajans *et al.*, 2001; Karges *et al.*, 2007). Since discovery

of the Müllerian duct malformation may precede signs of diabetes, an oral glucose tolerance test should be performed in these individuals to detect this chronic metabolic disorder.

### Folate metabolism genes and fertility

High homocysteine concentrations are associated with pregnancy loss and high concentrations of folic acid have been associated with an increase in twinning (Forges *et al.*, 2007). The folate-methylation cycle demethylates homocysteine to produce methionine and then S-adenylmethionine (SAM), which is a prime source of methyl groups for DNA methylation and for the synthesis of nucleic acids, proteins and lipids. Deficiencies in the enzyme methylenetetrahydrofolate reductase (MTHFR), which is central to the methylation pathway, cause DNA hypomethylation and could be related to imprinting disorders (Dobson *et al.*, 2007). The gene for MTHFR has two common polymorphisms (CC to TT at base pair 677 and AA to CC at base pair 1298) which have been studied in association with embryonic development and live birth after IVF. Among 602 women undergoing IVF, live birth was less likely (odds ratio, OR 0.24, 95% CI 0.08–0.71) with the homozygous 1298 CC variant of the MTHFR gene, than the AA variant; the C677T polymorphism had no effect (Haggarty *et al.*, 2006). In a study of 197 couples, however, neither the C677T nor the A1298C variants were associated with embryo quality or pregnancy rate (Dobson *et al.*, 2007). The conflicting results may reflect gene–gene as well as gene–environment interactions, which may be a source of bias in observational studies (Forges *et al.*, 2007). Alternatively, the effects of the polymorphisms may be too small to have consistent effects in folate-replete populations or in women <35 years of age (Forges *et al.*, 2007).

### Complex genetic mechanisms associated with female infertility

#### The genetic basis of PCOS

PCOS affects 5–10% of the female population and accounts for >75% of anovulatory infertility. It is also characterized by excess androgen secretion and an increased long term risk of type 2 diabetes. The high prevalence of PCOS in families suggests a genetic aetiology. The mode of inheritance is difficult to define because the clinical profile is heterogeneous, the diagnosis is difficult to make in post-menopausal women and there is no obvious male phenotype (Franks *et al.*, 1997; Franks and McCarthy, 2004).

#### Fetal origin

Ovarian dysfunction in PCOS is associated with an increased population of primordial follicles, suggesting that its origins may be in the fetal ovary (Webber *et al.*, 2003). The affected ovary may be genetically predisposed to secrete excess androgens, possibly *in utero*, probably in infancy and certainly at the onset of puberty (Abbott *et al.*, 2002; Franks *et al.*, 2006). Exposure to excess androgen in experimental animals, at any stage from fetal development of the ovary to the onset of puberty leads to many of the characteristic features of PCOS, including insulin resistance. In post-natal life the natural history of PCOS can be modified by factors affecting insulin secretion or function, most importantly nutrition.

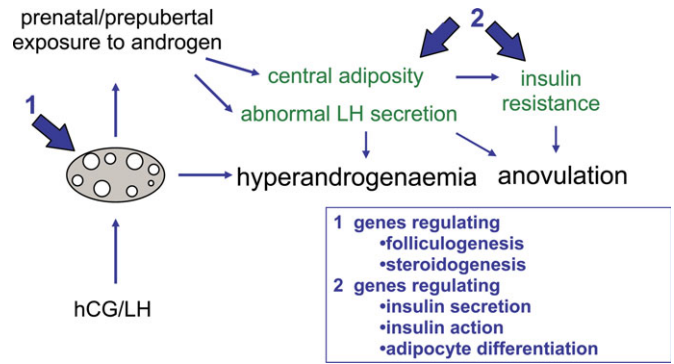


Figure 3: Developmental origin of PCOS (adapted from Abbott *et al.*, 2002).

#### Family and twin studies

Familial clustering of PCOS cases has often been reported, suggesting a genetic origin of the disorder. In a Dutch twin study, concordance of PCOS was greater in monozygotic than in DZ twins (Vink *et al.*, 2006). Hyperandrogenaemia, with or without menstrual cycle abnormalities, is common among sisters of PCOS patients. Familial hyperinsulinaemia is also common; elevated insulin levels were associated with hyperandrogenemia, rather than with menstrual irregularity (Legro *et al.*, 2002).

#### Candidate genes

A large number of case–control and family-based association studies have been performed to search for candidate genes in PCOS. The most obvious candidate genes to consider include (i) genes implicated in folliculogenesis (about which little is known), (ii) genes affecting insulin secretion or action or (iii) genes involved in the androgen biosynthetic pathway (Fig. 3). A plausible candidate gene in the androgen synthesis pathway is *CYP11A*, encoding the cholesterol side chain cleavage enzyme P450<sub>scc</sub>, because the increase in androgen production in PCOS appears to reflect a ‘global’ increase in steroid production by theca cells. A large case–control study found no association, however, between polymorphisms in *CYP11A* and either polycystic ovary morphology or testosterone levels (Gaasenbeek *et al.*, 2004). Among nearly 50 other candidate genes studied, only one, so far, has been clearly associated with PCOS status (Nam Menke and Strauss, 2007; Urbanek, 2007). The one potential exception is a locus on chromosome 19p13.2, which is now thought to be in the region of the fibrillin 3 (FBNN3) gene (Stewart *et al.*, 2006). Fibrillins are modulators of TGFβ proteins, but their potential role in PCOS remains to be determined. The main limitation of many of the published studies of potential candidate genes in PCOS is the lack of appropriate power (i.e. the populations studied are too small to either confirm or exclude candidate loci). This is important because, among the many potential candidate genes, the effect of any one gene is likely to be small.

While the search for candidate genes continues, genome-wide association studies of the kind that have been successfully applied in type 2 diabetes may be the most likely means to uncover the genes contributing to PCOS, even though these studies are expensive and require large populations (thousands) of cases and appropriate controls (Urbanek, 2007).



### *The genetics of endometriosis*

A genetic aetiology of endometriosis was suggested many years ago with a report that first-degree relatives of women with severe endometriosis had a 6-fold higher risk than relatives of unaffected women (Simpson *et al.*, 1980). Endometriosis appears to be inherited not as a Mendelian condition, but as a complex genetic trait influenced by environmental factors (Kennedy *et al.*, 1995).

#### *Family and twin studies*

Clinical evidence of a genetic basis for endometriosis includes familial clustering in humans (Kennedy *et al.*, 1995) and rhesus monkeys (Hadfield *et al.*, 1997a); a founder effect in the Icelandic population (Stefansson *et al.*, 2002); concordance in monozygotic twins (Moen, 1994; Hadfield *et al.*, 1997b; Treloar *et al.*, 1999a); a similar age at onset of symptoms in affected, non-twin sisters (Kennedy *et al.*, 1996) and up to 15-fold higher prevalence in first-degree relatives of cases over the general population (Coxhead and Thomas, 1993; Moen and Magnus, 1993; Kennedy *et al.*, 1998). Genetic traits may create a susceptibility to endometriosis although the disease emerges only after exposure to environmental risk factors (Giudice and Kao, 2004).

As in other diseases with complex genetic inheritance, there are difficulties in the interpretation of the clinical studies. The problems include a heterogeneous clinical profile (not all women with endometriosis have pain, infertility or a laparoscopy); ascertainment bias (relatives of women with endometriosis are more likely to ask for testing) and referral bias (only severe cases attend tertiary clinics where studies are done). Thus, the relative influence of genetic versus environmental factors remains uncertain.

#### *Linkage and association studies*

Plausible candidate genes for the aetiology of endometriosis, including detoxification and tumour suppressor genes, have emerged from linkage analysis and association studies (Giudice and Kao, 2004).

A definitive linkage analysis study is The International Endogene Study which analysed genetic variants in sister pairs and families (Kennedy *et al.*, 1995, 2001; Treloar *et al.*, 1999a, b, 2002). Among women that could be staged 2957 (54%) have minimal to mild disease and 2568 (46%) have more severe disease. A genome-wide scan of over 400 microsatellite markers identified one region of significant linkage (Maximum LOD score = 3.09) on chromosome 10q26 and one region of suggestive linkage (Maximum LOD score = 2.09) on chromosome 20p13 (Treloar *et al.*, 2005). There is also suggestive evidence of one or more high-penetrance susceptibility loci with near-Mendelian inheritance on chromosome 7 in 248 of the families with  $\geq 3$  affected members (Zondervan *et al.*, 2006). The parametric analysis produced a MOD score (equal to maximum LOD maximized over numerous possible inheritance models) of 3.30 at D7S484 for a recessive model with high penetrance. Critical recombinant analysis has narrowed the probable region of linkage down to overlapping 6.4 and 11 Mb intervals containing 48 and 96 genes, respectively.

The identification of endometriosis susceptibility genes should lead to a clearer understanding of the molecular and cellular

mechanisms involved in the aetiology and pathogenesis of the disease and possibly lead to a categorization of the disease into logical sub-types based upon genetic information.

### *Genetic aspects of fibroids as a cause of female infertility*

Uterine leiomyomas (fibroids) can be identified by ultrasound in 80% of African-American women and almost 70% of white American women by the time they reach menopause (Baird *et al.*, 2003). Although fibroids theoretically may distort uterine anatomy, obstruct the tubal ostia or impede implantation, there is little evidence that fibroids cause infertility (Kolankaya and Arici, 2006). One exception is the observation among IVF patients that submucous fibroids impair success rates (Pritts, 2001). A genetic basis for fibroid presence is suggested by family and ethnic studies.

#### *Family and ethnic studies*

Family studies show that there is a 2.5-fold increased risk for fibroids among first-degree relatives of women with fibroids compared with relatives of unaffected women (Dixon *et al.*, 2006). In ethnic studies, fibroids are three times more frequent in black compared with white women in America (Baird *et al.*, 2003).

Hereditary leiomyomatosis renal cell carcinoma (HLRCC) is a familial cancer syndrome with an autosomal dominant hereditary pattern in which many patients develop uterine and cutaneous leiomyomas as well as renal cell carcinoma. The FH gene, which codes for the fumarate hydratase enzyme, contains mutations in ~90% of patients with HLRCC. This FH mutation may predict a predisposition in white but not black American women (Dixon *et al.*, 2006).

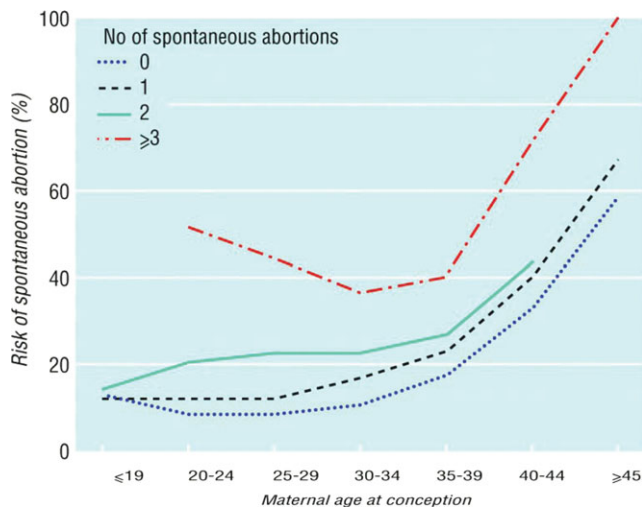
#### *Candidate gene studies*

Analysis of candidate genes has not been successful. Fibroid growth appears to be estrogen-dependent, but microarray and candidate gene studies have not uniformly identified significant changes in genes for estrogen receptors, progesterone receptors or their nuclear receptor cofactors (Arslan *et al.*, 2005).

#### *Gene expression studies*

Eight genes (ADH1, ATF3, CRABP2, CYR61, DPT, GRIA2, IGF2, MEST) were identified as over or under expressed by at least five of nine gene expression studies (Arslan *et al.*, 2005). Genes regulating retinoid synthesis, IGF metabolism, TGF- $\beta$  signalling and extracellular matrix formation have biological plausibility in the aetiology of fibroid development. ADH1 and CRABP2, for example, are involved in retinoid metabolism and also estrogen-dependent. It is also possible, however, that the differences in expression between fibroid and normal myometrial tissue were the result of the alpha error arising from the thousands of significance tests involved in these discovery studies.

Thus, family studies suggest that the development of fibroids may be genetically determined and candidate gene expression studies have identified over or under expression of some genes in fibroid tissue compared with normal myometrium. There remains, however, no conclusively proven genetic pathway for the development of fibroids and the disorder is so common that genetic studies are difficult to interpret.



**Figure 4:** Spontaneous miscarriage rates in nulliparous women by maternal age at conception and number of previous spontaneous miscarriages (Nybo Andersen *et al.*, 2000).

### Genetic aspects of RM

RM is the loss of three or more consecutive pregnancies, affecting 1% of couples trying to conceive (Rai and Regan, 2006). Among women with three or more previous spontaneous miscarriages, the risk of another one rises from 40% at age 35–39 years to 60% at age 40–44 years (Nybo Andersen *et al.*, 2000) (Fig. 4). RM remains unexplained in up to 80% of cases after investigation, suggesting the possibility of genetic causes. Genetic counselling is needed by the 3% of partners that have an abnormal karyotype and in cases involving decisions about karyotyping the products of conception.

### Genetic factors contributing to RM

#### Single-gene defects

Couples with RM are twice as likely as controls to have hereditary thrombophilias due to Factor V Leiden defect and prothrombin gene (G20210A) mutations (Kovalevsky *et al.*, 2004). Since the population prevalence of each mutation is close to 5%, the prevalence in RM couples may be as high as 10%, and screening is indicated.

Levels of plasminogen activator inhibitor-1 (PAI-1) have been reported in some studies to be increased in RM couples. In a systematic review involving 5 studies and 973 women, the prevalence of the homozygous mutant 4G/4G genotype was 30% in the RM cases and 25% in the controls. The OR for RM was not significant (1.65, 95% CI 0.92–2.95) in the presence of 4G/4G genotype (Sotiriadis *et al.*, 2007).

Deficiency of mannan-binding lectin (MBL) was more common in 397 couples with RM than blood donor controls (Kilpatrick *et al.*, 1995). Deficiency in MBL, which is a key activator for humoral innate immunity, usually is caused by mutations in the MBL2 gene. In a 2003 study, however, the frequencies of polymorphisms in the MBL genes were similar in 76 RM couples and 69 control couples without a history of miscarriage and at least one live birth (Baxter *et al.*, 2001).

The C677T polymorphism in the MTHFR gene which controls DNA methylation was the subject of 26 case–control studies involving 2120 couples with unexplained RM and 2949 controls. In a meta-analysis there was a modest effect of the TT genotype on risk for unexplained RM (OR 1.49, 95% CI 1.12–2.00) compared with the CC genotype (Ren and Wang, 2006). The risk was higher in the five studies from China (OR 2.96, 95% CI 1.88–4.66), where the frequency of the MTHFR 677TT polymorphism was 25% in cases and 15% in controls, compared with 13 and 11% in cases and controls from other countries.

### Recurrence risk

A miscarriage with a normal karyotype is more likely to recur than one with an abnormal karyotype, and the repeat miscarriage is more likely to have a normal karyotype (Lauritsen, 1976). Women with trisomy abortion, however, are 2.5-fold more likely to have another miscarriage with the same trisomy (homo-trisomy) (Warburton *et al.*, 2004). When the chromosome involved in the two trisomy conceptions is not the same (hetero-trisomy), there may be an increased risk for non-specific non-disjunction, which is likely to be genetic in origin.

### Recurrent pregnancy loss and structural chromosomal abnormality

One partner in ~3% of couples with RM has a balanced structural chromosomal abnormality (De Braekeleer and Dao, 1990; Clifford *et al.*, 1994; Franssen *et al.*, 2006; Stephenson and Sierra, 2006). While carriers of balanced translocations are normal, more than half of their gametes and embryos are unbalanced because chromosomal segregation is disrupted during meiosis. Thus it is prudent to arrange for peripheral blood karyotyping for couples with RM. If an abnormality is found, the couple should be referred to a clinical geneticist to decide on further family studies and to discuss the prognosis for a future pregnancy.

The prognosis in a future pregnancy is not desolate, however. In 73 pregnancies among 99 couples with a structural chromosomal abnormality, 33 (45%) ended in live birth (Carp *et al.*, 2004). In 58 pregnancies among 51 similarly affected couples, 41 (71%) ended in live birth (Stephenson and Sierra, 2006). In 239 pregnancies among 278 couples with a structural chromosomal abnormality, 205 (83%) ended in live birth; the live birth rate was similar for reciprocal translocations, Robertsonian translocations and inversions (Franssen *et al.*, 2006).

The couple has a risk of unbalanced translocation in a pregnancy ranging from 1 to 5%, indicating the need for prenatal diagnosis (Franssen *et al.*, 2006). PGD is another option for translocation carriers. This requires the couple to undergo IVF to produce embryos for testing. In 469 PGD cycles among patients with translocations there were 123 clinical pregnancies (26% per PGD cycle, 35% per embryo transfer) (Verlinsky *et al.*, 2004). In 282 PGD cycles for abnormal karyotype in which embryos were transferred, the clinical pregnancy rate was 25% per transfer (Sermon *et al.*, 2007). These PGD outcomes are from collected series in which RM was not necessarily the only clinical diagnosis for couples with a translocation, but they are lower than the live birth rates from 45 to 83% to be expected with natural conception (Carp *et al.*, 2004; Franssen *et al.*, 2006; Stephenson and Sierra, 2006).

**Karyotype testing on products of conception**

The pattern of abnormalities found in products of conception from RM couples is not substantially different from the pattern in spontaneous losses shown in Table II, with the exception of fewer Turner syndromes in RM. Trisomy is the leading anomaly (31%, most often chromosomes 15, 16, 22 and 21), followed by polyploidy (9%), Turner syndrome (4%) and unbalanced translocations (2%).

Euploid products of conception would imply that there is a non-cytogenetic cause of RM, leading some authors to advocate karyotyping of all pregnancy losses in couples with RM (Stephenson *et al.*, 2002). On the other hand, chromosome abnormalities are so common that a euploid report is far from re-assuring about a future pregnancy. Given the overall favourable prognosis for idiopathic RM and the cost of karyotyping products of conception, it seems reasonable to limit the procedure to couples undergoing a specific treatment for RM: a normal karyotype in a failed pregnancy would suggest the loss was due to treatment failure rather than aneuploidy.

**PGS for unexplained RM**

Couples with unexplained RM may be at higher than average risk for embryo aneuploidy compared with age-matched controls. In one series of 241 PGS cycles in RM couples with FISH testing for chromosomes 13, 16, 18, 21, 22, X and Y, the pregnancy rate was 37% per transfer (Rubio *et al.*, 2005). In another series of 69 cycles there were 10 pregnancies (15%) (Platteau *et al.*, 2005). The ESHRE PGD Consortium guidelines state that similar couples have a high chance of conceiving naturally (Thornhill *et al.*, 2005). Two studies support that statement: of 325 couples with idiopathic RM, 70% ( $n = 226$ ) conceived, with a 75% success rate (Brigham *et al.*, 1999); and among 201 women with three or more consecutive first trimester miscarriages and normal investigations, the live birth rate in the next pregnancy was 69% (Clifford *et al.*, 1997). In couples with unexplained RM, similar to those with a known translocation, the live birth rate with natural conception is higher than any success rate reported after preimplantation diagnostic procedures.

**Conclusions**

The clinical practice of reproductive medicine is increasingly dependent on methods for genetic diagnosis to identify the correct management.

Chromosomal defects explain a high proportion of human pregnancy failures. Nevertheless, karyotyping tests only at the most superficial level of genetic abnormality and testing in the future should be developed to detect other potential genetic defects which may be clinically important.

Single-gene defects are rare causes of infertility, but in clinical presentations such as HH single-gene defects may be found in a high proportion of the cases.

Karyotype and FMR1 testing are indicated in most POF cases and genetic counselling is essential for women who have POF or belong to POF families.

Complex genetic inheritance patterns may explain the small tendency to a familial distribution found in common disorders such as PCOS and endometriosis. Genetic research methods are

likely to improve the understanding of what are now thought to be cases of unexplained infertility and unexplained RM. Couples with infertility or RM who have a single-gene defect or a translocation should have professional genetic counselling.

**Acknowledgements**

The secretarial assistance of Mrs Simonetta Vassallo is gratefully acknowledged.

**Funding**

The meeting was organized with an unrestricted educational grant from Institut Biochimique SA. University of Milano provided the secretarial support for the manuscript preparation.

**Glossary**

(Definitions not referenced are from Stedmans Online Medical Dictionary).

**Genetics terms**

**Acrocentric:** a chromosome which has the centromere close to one end as in chromosomes 13–15 and 21–22.

**Aneuploidy:** the occurrence of one or more extra or missing chromosomes leading to an unbalanced chromosome complement, or, any chromosome number that is not an exact multiple of the haploid number. <http://ghr.nlm.nih.gov/ghr/glossary/aneuploidy>.

**Genome:** an organism's complete set of deoxyribonucleic acid. <http://www.genome.gov/11006943>.

**Mosaic:** the occurrence of two or more cell lines with different genetic or chromosomal constitutions within a single individual or tissue. The result is an organism or one of its parts that is composed of cells of more than one genotype. <http://ghr.nlm.nih.gov/ghr/glossary/mosaic>.

**Non-disjunction:** failure of one or more pairs of chromosomes to separate at the meiotic stage of karyokinesis, with the result that both chromosomes are carried to one daughter cell and none to the other.

**Polyplloidy:** the cell nucleus contains three or more haploid sets. Cells containing three or four multiples are referred to as triploid or tetraploid.

**Polysomy:** a specific chromosome is represented more than twice. Cells containing three or four homologous chromosomes display trisomy or tetrasomy.

**Single-nucleotide polymorphism:** or SNP (pronounced 'snip'), is a small genetic change, or variation, that can occur within the DNA sequence. The genetic code is specified by the four nucleotide 'letters' A (adenine), C (cytosine), T (thymine) and G (guanine). SNP variation occurs when a single nucleotide, such as an A, replaces one of the other three nucleotide letters—C, G, or T. <http://www.ncbi.nlm.nih.gov/About/primer/snps.html>.

**Types of chromosome abnormalities**

**Numerical chromosome abnormalities:** a number of chromosomes in a somatic cell other than two, such as monosomy or trisomy, or more than a duplicate set of chromosomes (triploidy, tetraploidy).

**Structural chromosome abnormalities** <http://ghr.nlm.nih.gov/handbook/mutationsanddisorders/structuralchanges>.



**Inversion:** an inversion involves the breakage of a chromosome in two places; the resulting piece of DNA is reversed and re-inserted into the chromosome. Genetic material may or may not be lost as a result of the chromosome breaks. An inversion that involves the chromosome's constriction point (centromere) is called a pericentric inversion. An inversion that occurs in the long (q) arm or short (p) arm and does not involve the centromere is called a paracentric inversion.

**Deletion:** deletions occur when a chromosome breaks and some genetic material is lost. Deletions can be large or small, and can occur anywhere along a chromosome.

**Duplication:** duplications occur when part of a chromosome is copied (duplicated) too many times. This type of chromosomal change results in extra copies of genetic material from the duplicated segment.

**Translocation:** a translocation occurs when a piece of one chromosome breaks off and attaches to another chromosome. This type of rearrangement is described as balanced if no genetic material is gained or lost in the cell. If there is a gain or loss of genetic material, the translocation is described as unbalanced. Translocation may be reciprocal or Robertsonian.

Reciprocal translocation involves exchange of segments from two different chromosomes.

Robertsonian translocation involves the acrocentric chromosomes 13, 14, 15, 21 and 22, where the entire long arm attaches to another chromosome at the centromere and the short arms of both are lost.

#### Investigation methods

**Comparative genomic hybridization (CGH):** a molecular cytogenetic technique that allows detection of DNA sequence copy number changes throughout the genome in a single hybridization. CGH is based on co-hybridization of differentially labeled tumor and normal DNA to human metaphase chromosomes. Also called chromosomal microarray analysis (CMA). <http://cgap-mf.nih.gov/Protocols/DNARNAProteomicAnalysis/DNA/CGH.html>.

**Cytogenetics:** the study of chromosomes, their structure and their inheritance.

**Fluorescence in-situ hybridization (FISH):** a process which paints chromosomes or portions of chromosomes with fluorescent molecules. This technique is useful for identifying chromosomal abnormalities and gene mapping. [http://www.genome.gov/glossary.cfm?key=fluorescence%20in%20situ%20hybridization%20\(FISH\)](http://www.genome.gov/glossary.cfm?key=fluorescence%20in%20situ%20hybridization%20(FISH)).

**Genome-wide association study:** a study design that involves genotyping cases and controls at a large number (up to 500,000) of SNP markers spread (in some unspecified way) throughout the genome to look for associations between the genotypes at each locus and disease status. [www.stats.ox.ac.uk](http://www.stats.ox.ac.uk).

**LOD score:** a likelihood ratio statistic which estimates whether two loci are likely to lie near each other on a chromosome and are therefore likely to be inherited together. An LOD score of three or more is generally taken to indicate that the two loci are close. <http://www.genome.gov/glossary.cfm?key=LOD%20score>.

**Polymerase chain reaction:** a powerful method for amplifying specific DNA segments which exploits certain features of DNA replication. Replication requires a primer and specificity is determined by the sequence and size of the primer. The method amplifies specific DNA segments by cycles of template denaturation; primer addition; primer annealing and replication using thermostable

DNA polymerase. [http://www.biochem.northwestern.edu/holmgren/Glossary/Definitions/Def-P/polymerase\\_chain\\_reaction.html](http://www.biochem.northwestern.edu/holmgren/Glossary/Definitions/Def-P/polymerase_chain_reaction.html).

#### ESHRE Capri Workshop Group

A meeting was organized by ESHRE to discuss the above subjects. The speakers included: J. Collins (McMaster University, Hamilton, Canada), K. Diedrich (Klinik für Frauenheilkunde und Geburtshilfe, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Germany), S. Franks (Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Hospital, London, UK), J.P.M. Geraedts (Head, Department of Genetics and Cell Biology, University Maastricht, The Netherlands), P.A. Jacobs (Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, UK), B. Karges (University Children's Hospital, Pediatric Endocrinology and Diabetes, University of Ulm, Germany), S. Kennedy (Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford, UK), A. Marozzi (Department of Biology and Genetics for Medical Sciences, University of Milano, Italy), L. Regan (Division Surgery, Oncology, Reproductive Biology, Anaesthetics (SORA), Department of Obstetrics and Gynaecology, Imperial College at St Mary's Hospital Campus, London, UK). The discussants included: D.T. Baird (Centre for Reproductive Biology, University of Edinburgh, UK), P.G. Crosignani (II Department of Obstetrics and Gynecology, University of Milano, Italy), P. Devroey (Centre for Reproductive Medicine, Universitair Ziekenhuis Vrije Universiteit Brussel, Belgium), E. Diczfalussy (Karolinska Institutet, Stockholm, Sweden), J.L.H. Evers (Department of Obstetrics and Gynecology, Academic Hospital Maastricht, The Netherlands), B.C.J.M. Fauser (Department of Reproductive Medicine and Gynecology, University Medical Center, Utrecht, The Netherlands), L. Fraser (Reproduction and Rhythms Group, School of Biomedical and Health Sciences, Kings College London, UK), L. Gianaroli (S.I.S.Me.R., Bologna, Italy), A. Glasier (Family Planning and WW Services, Edinburgh, UK), I. Liebaers (Centre for Medical Genetics, Universitair Ziekenhuis, Vrije Universiteit Brussel), G. Ragni (U.O.C. Sterilità di Coppia ed Andrologia, Fondazione Policlinico, Mangiagalli e Regina Elena, Milano, Italy), A. Sunde (Department of Obstetrics and Gynecology, University of Trondheim, Norway) B. Tarlatzis (Infertility and IVF Center, Thessaloniki, Greece), A. Van Steirteghem (Centre for Reproductive Medicine, Universitair Ziekenhuis Vrije Universiteit Brussel, Belgium). The report was prepared by J. Collins (Hamilton) and P.G. Crosignani (Milano).

#### References

- Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome—a hypothesis. *J Endocrinol* 2002;**174**:1–5.
- Arslan AA, Gold LI, Mittal K, Suen TC, Belitskaya-Levy I, Tang MS, Toniolo P. Gene expression studies provide clues to the pathogenesis of uterine leiomyoma: new evidence and a systematic review. *Hum Reprod* 2005;**20**:852–863.
- Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. *Am J Obstet Gynecol* 2003;**188**:100–107.
- Barr ML, Bertram EG. A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. *Nature* 1949;**163**:676–677.
- Baxter N, Sumiya M, Cheng S, Erlich H, Regan L, Simons A, Summerfield JA. Recurrent miscarriage and variant alleles of mannose binding lectin,



- tumour necrosis factor and lymphotoxin alpha genes. *Clin Exp Immunol* 2001;**126**:529–534.
- Bhagavath B, Podolsky RH, Ozata M, Bolu E, Bick DP, Kulharya A, Sherins RJ, Layman LC. Clinical and molecular characterization of a large sample of patients with hypogonadotropic hypogonadism. *Fertil Steril* 2006;**85**:706–713.
- Bodega B, Porta C, Crosignani PG, Ginelli E, Marozzi A. Mutations in the coding region of the FOXL2 gene are not a major cause of idiopathic premature ovarian failure. *Mol Hum Reprod* 2004;**10**:555–557.
- Bodega B, Bione S, Dalpra L, Toniolo D, Ornaghi F, Vegetti W, Ginelli E, Marozzi A. Influence of intermediate and uninterrupted FMR1 CGG expansions in premature ovarian failure manifestation. *Hum Reprod* 2006;**21**:952–957.
- Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. *Hum Reprod* 1999;**14**:2868–2871.
- Carp H, Feldman B, Oelsner G, Schiff E. Parental karyotype and subsequent live births in recurrent miscarriage. *Fertil Steril* 2004;**81**:1296–1301.
- Carr DH. Chromosome studies in abortuses and stillborn infants. *Lancet* 1963;**2**:603–606.
- Carr DH. Chromosome studies in spontaneous abortions. *Obstet Gynaecol* 1965;**26**:308–326.
- Clifford K, Rai R, Watson H, Regan L. An informative protocol for the investigation of recurrent miscarriage: preliminary experience of 500 consecutive cases. *Hum Reprod* 1994;**9**:1328–1332.
- Clifford K, Rai R, Regan L. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. *Hum Reprod* 1997;**12**:387–389.
- Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. *Obstet Gynecol* 1986;**67**:604–606.
- Coxhead D, Thomas EJ. Familial inheritance of endometriosis in a British population: a case control study. *J Obstet Gynaecol* 1993;**13**:42–44.
- Davis CJ, Davison RM, Payne NN, Rodeck CH, Conway GS. Female sex preponderance for idiopathic familial premature ovarian failure suggests an X chromosome defect: opinion. *Hum Reprod* 2000;**15**:2418–2422.
- De Braekeleer M, Dao TN. Cytogenetic studies in couples experiencing repeated pregnancy losses. *Hum Reprod* 1990;**5**:519–528.
- de Roux, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the kiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci* 2003;**100**:10972–10976.
- Di Pasquale E, Rossetti R, Marozzi A, Bodega B, Borgato S, Cavallo L, Einaudi S, Radetti G, Russo G, Sacco M *et al.* Identification of new variants of human BMP15 gene in a large cohort of women with premature ovarian failure. *J Clin Endocrinol Metab* 2006;**91**:1976–1979.
- Dixon D, Parrott EC, Segars JH, Olden K, Pinn VW. The second National Institutes of Health International Congress on advances in uterine leiomyoma research: conference summary and future recommendations. *Fertil Steril* 2006;**86**:800–806.
- Dobson AT, Davis RM, Rosen MP, Shen S, Rinaudo PF, Chan J, Cedars MI. Methylenetetrahydrofolate reductase C677T and A1298C variants do not affect ongoing pregnancy rates following IVF. *Hum Reprod* 2007;**22**:450–456.
- Dode C, Levilliers J, Dupont JM, de Paep A, Le Du N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F *et al.* Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet* 2003;**33**:463–465.
- Dode C, Teixeira L, Levilliers J, Fouveau C, Bouchard P, Kottler ML, Lespinnas J, Lienhardt-Roussie A, Mathieu M, Moermann A *et al.* Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet* 2006;**2**:1648–1652.
- Ennis S, Murray A, Youings S, Brightwell G, Herrick D, Ring S, Pembrey M, Morton NE, Jacobs PA. An investigation of FRAXA intermediate allele phenotype in a longitudinal sample. *Ann Hum Genet* 2006;**70**:170–180.
- Fabre S, Pierre A, Mulsant P, Bodin L, Di Pasquale E, Persani L, Monget P, Monniaux D. Regulation of ovulation rate in mammals: contribution of sheep genetic models. *Reprod Biol Endocrinol* 2006;**4**:20.
- Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* 2001;**345**:971–980.
- Fausser BCJM, Hsueh AJ. Genetic basis of human reproductive endocrine disorders. *Hum Reprod* 1995;**10**:826–846.
- Forges T, Monnier-Barbarino P, Leheup B, Jouvot P. Pathophysiology of impaired ovarian function in galactosaemia. *Hum Reprod Update* 2006;**12**:573–584.
- Forges T, Monnier-Barbarino P, Alberto JM, Gueant-Rodriguez RM, Daval JL, Gueant JL. Impact of folate and homocysteine metabolism on human reproductive health. *Hum Reprod Update* 2007;**13**:225–238.
- Franks S, McCarthy M. Genetics of ovarian disorders: polycystic ovary syndrome. *Rev Endocr Metab Disord* 2004;**5**:69–76.
- Franks S, Gharani N, Waterworth D, Batty S, White D, Williamson R, McCarthy M. The genetic basis of polycystic ovary syndrome. *Hum Reprod* 1997;**12**:2641–2648.
- Franks S, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. *Int J Androl* 2006;**29**:278–285. discussion 286–290.
- Franssen MT, Korevaar JC, van d V, Leschot NJ, Bossuyt PM, Goddijn M. Reproductive outcome after chromosome analysis in couples with two or more miscarriages: index [corrected]-control study. *BMJ* 2006;**332**:759–763.
- Gaasenbeek M, Powell BL, Sovio U, Haddad L, Gharani N, Bennett A, Groves CJ, Rush K, Goh MJ, Conway GS *et al.* Large-scale analysis of the relationship between CYP11A promoter variation, polycystic ovarian syndrome, and serum testosterone. *J Clin Endocrinol Metab* 2004;**89**:2408–2413.
- Gersen SL, Keagle MB. *The Principles of Clinical Cytogenetics*, 2nd edn. Humana Press, 2004.
- Giudice LC, Kao LC. Endometriosis. *Lancet* 2004;**364**:1789–1799.
- Hadfield RM, Yudkin PL, Coe CL, Scheffler J, Uno H, Barlow DH, Kennitz JW, Kennedy SH. Risk factors for endometriosis in the rhesus monkey (*Macaca mulatta*): a case-control study. *Hum Reprod Update* 1997a;**3**:109–115.
- Hadfield RM, Mardon HJ, Barlow DH, Kennedy SH. Endometriosis in monozygotic twins. *Fertil Steril* 1997b;**68**:941–942.
- Hagerman PJ, Hagerman RJ. The fragile-X premutation: a maturing perspective. *Am J Hum Genet* 2004;**74**:805–816.
- Haggarty P, McCallum H, McBain H, Andrews K, Duthie S, McNeill G, Templeton A, Haites N, Campbell D, Bhattacharya S. Effect of B vitamins and genetics on success of in-vitro fertilisation: prospective cohort study. *Lancet* 2006;**367**:1513–1519.
- Hundscheid RD, Smits AP, Thomas CM, Kiemeneij LA, Braat DD. Female carriers of fragile X premutations have no increased risk for additional diseases other than premature ovarian failure. *Am J Med Genet A* 2003;**117**:6–9.
- Jackson L. Cytogenetics and molecular cytogenetics. *Clin Obstet Gynecol* 2002;**45**:622–639. discussion 730–732.
- Jacobs PA, Hassold TJ. The origin of numerical chromosome abnormalities. *Adv Genet* 1995;**33**:101–133.
- Jacobs PA, Frackiewicz A, Law P, Hilditch CJ, Morton NE. The effect of structural aberrations of the chromosomes on reproductive fitness in man. II. *Results Clin Genet* 1975;**8**:169–178.
- James RS, Ellis K, Pettay D, Jacobs PA. Cytogenetic and molecular study of four couples with multiple trisomy 21 pregnancies. *Eur J Hum Genet* 1998;**6**:207–212.
- Karges B, Karges W, Mine M, Ludwig L, Kuhne R, Milgrom E, de Roux N. Mutation Ala(171)Thr stabilizes the gonadotropin-releasing hormone receptor in its inactive conformation, causing familial hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2003a;**88**:1873–1879.
- Karges B, Karges W, de Roux N. Clinical and molecular genetics of the human gonadotropin releasing-hormone receptor. *Hum Reprod Update* 2003b;**9**:523–530.
- Karges B, Bergmann C, Scholl K, Heinze E, Rasche M, Zerres K, Debatin KM, Wabitsch M, Karges W. Digenic inheritance of hepatocyte nuclear factor-1alpha and -1beta with maturity-onset diabetes of the young, polycystic thyroid, and urogenital malformations. *Diabetes Care* 2007;**30**:1613–1614.
- Kennedy S, Mardon H, Barlow D. Familial endometriosis. *J Assist Reprod Genet* 1995;**12**:32–34.
- Kennedy S, Hadfield R, Mardon H, Barlow D. Age of onset of pain symptoms in non-twin sisters concordant for endometriosis. *Hum Reprod* 1996;**11**:403–405.
- Kennedy S, Hadfield R, Westbrook C, Weeks DE, Barlow D, Golding S. Magnetic resonance imaging to assess familial risk in relatives of women with endometriosis. *Lancet* 1998;**352**:1440–1441.
- Kennedy S, Bennett S, Weeks DE. Affected sib-pair analysis in endometriosis. *Hum Reprod Update* 2001;**7**:411–418.
- Kilpatrick DC, Bevan BH, Liston WA. Association between mannan binding protein deficiency and recurrent miscarriage. *Hum Reprod* 1995;**10**:2501–2505.

- Kolankaya A, Arici A. Myomas and assisted reproductive technologies: when and how to act? *Obstet Gynecol Clin North Am* 2006;**33**:145–152.
- Kovalevsky G, Gracia CR, Berlin JA, Sammel MD, Barnhart KT. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: a meta-analysis. *Arch Intern Med* 2004;**164**:558–563.
- Kovanci E, Rohozinski J, Simpson JL, Heard MJ, Bishop CE, Carson SA. Growth differentiating factor-9 mutations may be associated with premature ovarian failure. *Fertil Steril* 2007;**87**:143–146.
- Laius P, Christin-Maitre S, Touraine P, Kuttann F, Ritvos O, Aittomaki K, Bourcigaux N, Jacquesson L, Bouchard P, Frydman R *et al.* Mutations and sequence variants in GDF9 and BMP15 in patients with premature ovarian failure. *Eur J Endocrinol* 2006;**154**:739–744.
- Lalani SR, Safiullah AM, Fernbach SD, Harutyunyan KG, Thaller C, Peterson LE, McPherson JD, Gibbs RA, White LD, Hefner M *et al.* Spectrum of CHD7 mutations in 110 individuals with CHARGE syndrome and genotype-phenotype correlation. *Am J Hum Genet* 2005;**78**:303–314.
- Lauritsen JG. Aetiology of spontaneous abortion: A cytogenetic and epidemiological study of 288 abortuses and their parents. *Acta Obstet Gynecol Scand Suppl* 1976;**52**:1–29.
- Legro RS, Bentley-Lewis R, Driscoll D, Wang SC, Dunaif A. Insulin resistance in the sisters of women with polycystic ovary syndrome: association with hyperandrogenemia rather than menstrual irregularity. *J Clin Endocrinol Metab* 2002;**87**:2128–2133.
- Lyon MF. Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature* 1961;**190**:372–373.
- Marozzi A, Porta C, Vegetti W, Crosignani PG, Tibiletti MG, Dalpra L, Ginelli E. Mutation analysis of the inhibin alpha gene in a cohort of Italian women affected by ovarian failure. *Hum Reprod* 2002;**17**:1741–1745.
- Masterbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, Vogel NE, Arts EG, de Vries JWA, Bossuyt PM *et al.* In vitro fertilization with preimplantation genetic screening: a randomized controlled trial. *N Engl J Med* 2007;**357**:9–17.
- Matsumoto S, Yamazaki C, Matsumoto K, Nagano M, Naito M, Soga T, Hiyama H, Matsumoto M, Takasaki J, Komohara M *et al.* Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. *Proc Natl Acad Sci* 2006;**103**:4140–4145.
- Moen MH. Endometriosis in monozygotic twins. *Acta Obstet Gynecol Scand* 1994;**73**:59–62.
- Moen MH, Magnus P. The familial risk of endometriosis. *Acta Obstet Gynecol Scand* 1993;**72**:560–564.
- Morton NE, Jacobs PA, Frackiewicz A, Law P, Hilditch CJ. The effect of structural aberrations of the chromosomes on reproductive fitness in man. *I. Methodology. Clin Genet* 1975;**8**:159–168.
- Nam Menke M, Strauss JF, III. Genetics of polycystic ovarian syndrome. *Clin Obstet Gynecol* 2007;**50**:188–204.
- Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. *BMJ* 2000;**320**:1708–1712.
- Palmer JS, Zhao ZZ, Hoekstra C, Hayward NK, Webb PM, Whiteman DC, Martin NG, Boomsma DI, Duffy DL, Montgomery GW. Novel variants in growth differentiation factor 9 in mothers of dizygotic twins. *J Clin Endocrinol Metab*. 2006;**91**:4713–4716.
- Pitteloud N, Acierno J, Meysing A, Elisenskova A, Ma J, Ibrahim O, Metzger D, Dwyer A, Hughes V, Yialamas M *et al.* Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci* 2006a;**103**:6281–6286.
- Pitteloud N, Meysing A, Quinton R, Acierno JS Jr, Dwyer AA, Plummer L, Fliers E, Boepple P, Hayes F, Seminara S *et al.* Mutations in FGFR1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. *Mol Cell Endocrinol* 2006b;**254–255**:60–69.
- Pitteloud N, Quinton R, Pearce S, Raivio T, Acierno J, Dwyer A, Plummer L, Hughes V, Seminara S, Cheng Yu-Zhu *et al.* Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. *J Clin Invest* 2007;**117**:457–463.
- Platteau P, Staessen C, Michiels A, Van Steirteghem A, Liebaers I, Devroey P. Preimplantation genetic diagnosis for aneuploidy screening in patients with unexplained recurrent miscarriages. *Fertil Steril* 2005;**83**:393–397.
- Pritts EA. Fibroids and infertility: a systematic review of the evidence. *Obstet Gynecol Surv* 2001;**56**:483–491.
- Rai R, Regan L. Recurrent miscarriage. *Lancet* 2006;**368**:601–611.
- Ren A, Wang J. Methylene tetrahydrofolate reductase C677T polymorphism and the risk of unexplained recurrent pregnancy loss: a meta-analysis. *Fertil Steril* 2006;**86**:1716–1722.
- Rubio C, Pehlivan T, Rodrigo L, Simon C, Remohi J, Pellicer A. Embryo aneuploidy screening for unexplained recurrent miscarriage: a minireview. *Am J Reprod Immunol* 2005;**53**:159–165.
- Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res* 2002;**30**:e57.
- Seminara S, Messenger S, Chatzidaki E, Thresher R, Acierno J, Shagoury J, Bo-Abbas Y, Kuohung W, Schwino K, Hendrick A *et al.* The GPR54 Gene as a regulator of puberty. *N Engl J Med* 2003;**349**:1614–1627.
- Sermon KD, Michiels A, Harton G, Moutou C, Repping S, Scriven PN, SenGupta S, Traeger-Synodinos J, Vesela K, Viville S *et al.* ESHRE PGD Consortium data collection VI: cycles from January to December 2003 with pregnancy follow-up to October 2004. *Hum Reprod* 2007;**22**:323–336.
- Shaffer LG, Bejjani BA. Medical applications of array CGH and the transformation of clinical genetics. *Cytogenet Genome Res* 2006;**115**:303–309.
- Simoni M, Nieschlag E. Genetics of hypogonadotropic hypogonadism. *Horm Res* 2007;**67**(Suppl 1):149–154.
- Simpson JL, Elias S, Malinak LR, Buttram-VC J. Heritable aspects of endometriosis. I. Genetic studies. *Am J Obstet Gynecol* 1980;**137**:327–331.
- Sotiriadis A, Makrigiannakis A, Stefos T, Paraskevaides E, Kalantaridou SN. Fibrinolytic defects and recurrent miscarriage: a systematic review and meta-analysis. *Obstet Gynecol* 2007;**109**:1146–1155.
- Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, Devroey P, Liebaers I, Van Steirteghem A. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Hum Reprod* 2004;**19**:2849–2858.
- Stefansson H, Geirsson RT, Steinthorsdottir V, Jonsson H, Manolescu A, Kong A, Ingadottir G, Gulcher J, Stefansson K. Genetic factors contribute to the risk of developing endometriosis. *Hum Reprod* 2002;**17**:555–559.
- Stephenson MD, Sierra S. Reproductive outcomes in recurrent pregnancy loss associated with a parental carrier of a structural chromosome rearrangement. *Hum Reprod* 2006;**21**:1076–1082.
- Stephenson MD, Awartani KA, Robinson WP. Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. *Hum Reprod* 2002;**17**:446–451.
- Stewart DR, Dombroski BA, Urbanek M, Ankener W, Ewens KG, Wood JR, Legro RS, Strauss JF, III, Dunaif A, Spielman RS. Fine mapping of genetic susceptibility to polycystic ovary syndrome on chromosome 19p13.2 and tests for regulatory activity. *J Clin Endocrinol Metab* 2006;**91**:4112–4117.
- Strachan T, Read A. *Human Molecular Genetics*, 3rd edn. Garland Science/Taylor and Francis Group, 2003.
- Thornhill AR, deDie-Smulders CE, Geraedts JP, Harper JC, Harton GL, Lavery SA, Moutou C, Robinson MD, Schmutzler AG, Scriven PN *et al.* ESHRE PGD Consortium 'Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)'. *Hum Reprod* 2005;**20**:35–48.
- Toniolo D. X-linked premature ovarian failure: a complex disease. *Curr Opin Genet Dev* 2006;**16**:293–300.
- Treloar SA, O'Connor DT, O'Connor VM, Martin NG. Genetic influences on endometriosis in an Australian twin sample. *Fertil Steril* 1999a;**71**:701–710.
- Treloar SA, Do KA, O'Connor VM, O'Connor DT, Yeo MA, Martin NG. Predictors of hysterectomy: an Australian study. *Am J Obstet Gynecol* 1999b;**180**:945–954.
- Treloar S, Hadfield R, Montgomery G, Lambert A, Wicks J, Barlow DH, O'Connor DT, Kennedy S International Endogene Study Group. The International Endogene Study: a collection of families for genetic research in endometriosis. *Fertil Steril* 2002;**78**:679–685.
- Treloar SA, Wicks J, Nyholt DR, Montgomery GW, Bahlo M, Smith V, Dawson G, Mackay IJ, Weeks DE, Bennett ST *et al.* Genomewide linkage study in 1,176 affected sister pair families identifies a significant susceptibility locus for endometriosis on chromosome 10q26. *Am J Hum Genet* 2005;**77**:365–376.
- Urbanek M. The genetics of the polycystic ovary syndrome. *Nat Clin Pract Endocrinol Metab* 2007;**3**:103–111.
- Van den Bosch BJ, de Co RF, Scholte HR, Nijland JG, van den Bogaard R, de Visser M, de Die-Smulders CE, Smeets HJ. Mutation analysis of the entire

- mitochondrial genome using denaturing high performance liquid chromatography. *Nucleic Acids Res* 2000;**28**:E89.
- van Eijsden RG, Gerards M, Eijssen LM, Hendrickx AT, Jongbloed RJ, Wokke JH, Hintzen RQ, Rubio-Gozalbo ME, De Coo IF, Briem E *et al.* Chip-based mtDNA mutation screening enables fast and reliable genetic diagnosis of OXPHOS patients. *Genet Med* 2006;**8**:620–627.
- Vegetti W, Marozzi A, Manfredini E, Testa G, Alagna F, Nicolosi A, Caliari I, Taborelli M, Tibiletti MG, Dalpra L *et al.* Premature ovarian failure. *Mol Cell Endocrinol* 2000;**161**:53–57.
- Verlinsky Y, Cohen J, Munne S, Gianaroli L, Simpson JL, Ferraretti AP, Kuliev A. Over a decade of experience with preimplantation genetic diagnosis: a multicenter report. *Fertil Steril* 2004;**82**:292–294.
- Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI. Heritability of polycystic ovary syndrome in a Dutch twin-family study. *J Clin Endocrinol Metab* 2006;**91**:2100–2104.
- Warburton D. 2006 William Allan Award Address: Having it all. *Am J Hum Genet* 2007;**81**:648–656.
- Warburton D, Dallaire L, Thangavelu M, Ross L, Levin B, Kline J. Trisomy recurrence: a reconsideration based on North American data. *Am J Hum Genet* 2004;**75**:376–385.
- Webber LJ, Stubbs S, Stark J, Trew GH, Margara R, Hardy K, Franks S. Formation and early development of follicles in the polycystic ovary. *Lancet* 2003;**362**:1017–1021.
- Wittenberger MD, Hagerman RJ, Sherman SL, Conkie-Rosell A, Welt CK, Rebar RW, Corrigan EC, Simpson JL, Nelson LM. The FMR1 premutation and reproduction. *Fertil Steril* 2007;**87**:456–465.
- Zondervan KT, Treloar SA, Lin J, Weeks DE, Nyholt DR, Mangion J MacKay IJ, Cardon LR, Martin NG, Kennedy SH, Montgomery GW. Significant evidence of one or more susceptibility loci for endometriosis with near-Mendelian inheritance on chromosome 7p13-15. *Hum Reprod* 2006;**22**:717–728.

*Submitted on December 4, 2007; resubmitted on February 7, 2008; accepted on March 10, 2008*