human
reproduction
update

# Contemporary genetic technologies and female reproduction

## The Evian Annual Reproduction (EVAR) Workshop Group 2010*

Correspondence address. B.C.J.M. Fauser. Tel: +31-88-755-7524; E-mail: b.c.fauser@umcutrecht.nl

**BACKGROUND:** The Fifth Evian Annual Reproduction (EVAR) Workshop Meeting discussed knowledge regarding contemporary genetics in female reproduction.

**METHODS:** Specialist reproductive medicine clinicians and geneticists delivered presentations based on published literature and current research. The content of this report is based on the expert presentations and subsequent group discussions that took place during this Workshop.

**RESULTS:** Numerous ovarian genes with a role in infertility have been identified. Future challenges for genetic screening of patients, such as those with polycystic ovary syndrome, primary ovarian insufficiency or endometriosis, include the identification of high-throughput strategies and how to apply these findings to infertile patients. The identification of high-quality embryos in IVF using objective technologies remains a high priority in order to facilitate single-embryo transfer. Gene expression profiling of cumulus cells surrounding the oocyte, and proteomic and metabolomic approaches in embryo culture media may significantly improve non-invasive embryo quality assessment.

**CONCLUSIONS:** The way forward in advancing the knowledge of genes involved in reproduction was considered to be through genome-wide association studies involving large numbers of patients. Establishing international collaboration is required to enable the application of such technologies in sufficient numbers of patients.

**Key words:** genes and female reproductive pathologies / embryo assessment / endometriosis / metabolomics / transcriptomics

# Introduction

The study of genetics is a rapidly expanding field in all areas of medicine, including fertility. This review focuses on the genetic aspects of a number of topics of importance in fertility medicine: ovarian function, primary ovarian insufficiency (POI) and poor response to ovarian stimulation, polycystic ovary syndrome (PCOS) and endometriosis. In addition, new technologies for the assessment of oocyte and embryo quality are reviewed, including gene expression profiling of cumulus cells, proteomics and metabolomics.

The number of genes known to be expressed in the ovary and involved in reproductive function has increased dramatically in recent years. Increasing our understanding of the genes involved in ovarian ageing and poor response to ovarian stimulation may lead to improved clinical diagnosis and early detection, along with the development of new agents or technologies for use in fertility treatment. Although familial clustering of PCOS cases has been described, the mode of inheritance remains uncertain. Candidate genes for PCOS are discussed, although few studies have satisfied the rigorous criteria of population selection and size, repeatability and correction for multiple comparisons. Genetics also appear to have a role in complex reproductive diseases, such as endometriosis. A number of candidate gene families that may be differentially regulated in endometriosis have been identified. New developments in this field include the identification of a candidate population of endometrial stem cells, the study of which may provide new insights into this condition.

Improving our knowledge of the genetics of the ovary and the genetic causes of infertility should lead to future improvements in fertility treatment. For instance, it is now recognized that the oocyte is not passive in the ovarian follicle but is a fundamental regulator of somatic cell differentiation and function. The cumulus–oocyte complex plays a central role in the regulation of folliculogenesis and is important for the maturation, reprogramming and fertilization of oocytes. Consequently, cumulus cell gene expression profiling is being explored as a new method for the assessment of oocyte competence and embryo developmental competence, which shows promise for the near future.

Currently, IVF represents the only hope for many couples with fertility problems. Embryo assessment has an enormous impact on the success of IVF and, with moves towards single-embryo transfer (SET), assessment of embryo quality is of the utmost importance. Despite its evident importance, until recently, assessment of embryo quality has depended on subjective morphological assessment (Assou *et al.*, 2011). Any objective method of embryo assessment that could determine its potential for implantation, as well as its health status, will increase success rates.

With the recent expansion of the current knowledge and developments in this area, a thorough review of contemporary genetics and 'omics' technologies in human reproduction is timely.

# Methods

Prior to the Fifth Evian Annual Reproduction (EVAR) Workshop Meeting, held on 16–17 April 2010, expert speakers prepared presentations based on published literature and current research. Presenters were asked to include comprehensive information using systematic literature search criteria. Combinations of the following keywords were used for PubMed searches: genes and fertility-associated pathologies, primary ovarian insufficiency, premature ovarian failure, genomics, proteomics, metabolomics, endometriosis, polycystic ovary syndrome (and PCOS), floating granulosa cell gene expression, cumulus cell gene expression and protein expression profiling.

Following each presentation, a group discussion to reach joint conclusions on the topics covered was facilitated by the chairmen: Professors Fauser and Diedrich. The content of this report is based on the expert presentations and subsequent group discussions that took place during the workshop meeting.

The discussions relating to each topic were complemented as necessary with key publications that were known to the authors and electronic literature searches. Articles of any type and published in English were permitted and were unlimited by the date of publication.

# Genetics of the ovary

## Key genes involved in ovarian function

Our knowledge of the number of genes known to be involved in ovarian function has increased dramatically in recent years. Approaches to elucidate the role of specific genes involved in human reproduction include the use of PCR, real-time PCR, fluorescence *in situ* hybridization (FISH), single-nucleotide polymorphisms (SNPs), array SNPs, comparative genomic hybridization (CGH), array CGH (aCGH), genome-wide linkage analysis, candidate gene-association studies, genome-wide association studies (GWAS) and transgenic animal models. PCR and FISH have been available for some time, but the more recent development of microarray technologies has increased substantially the volume of information available on the genetics of reproduction. Indeed, the online Ovarian Kaleidoscope Database now contains information on more than 3100 ovarian-expressed genes and is a useful resource of knowledge on ovarian genetics (http://ovary.stanford.edu).

Figure 1 shows a model of recognized genes involved in the hypothalamo–pituitary–ovarian axis (Matzuk and Lamb, 2008). Gene mutations shown to affect gonadotrophin synthesis or actions and to cause infertility in humans have been summarized in a recent review and are shown in Table I (Edson *et al.*, 2009). Pre-antral follicular growth appears to be independent of gonadotrophins, but further functional characterization of thecal cells (through antral, pre-ovulatory, ovulation and corpus luteum phases) requires gonadotrophin stimulation (Edson *et al.*, 2009).

An important basic helix–loop–helix (HLH) transcription factor that is essential for fertility, factor in the germline alpha (FIGLA), was identified in 1997 (Liang *et al.*, 1997). FIGLA is involved in formation of the primordial follicle and knockout mice are infertile. In FIGLA knockout mice ovaries, OCT4, a protein required for primordial germ cell survival (Kehler *et al.*, 2004), is absent (Joshi *et al.*, 2007). FIGLA is also involved in coordinating expression of the zona pellucida (ZP) genes. Analysis of newborn ovaries has identified many oocyte-specific genes that are down-regulated in FIGLA knockout mice (Joshi *et al.*, 2007). Thus, FIGLA acts as a central regulator of oocyte-specific genes that play roles in folliculogenesis, fertilization and early development.

Regulators of the development of primordial to primary follicles include the following genes: newborn ovary homeobox (*NOBOX*), a
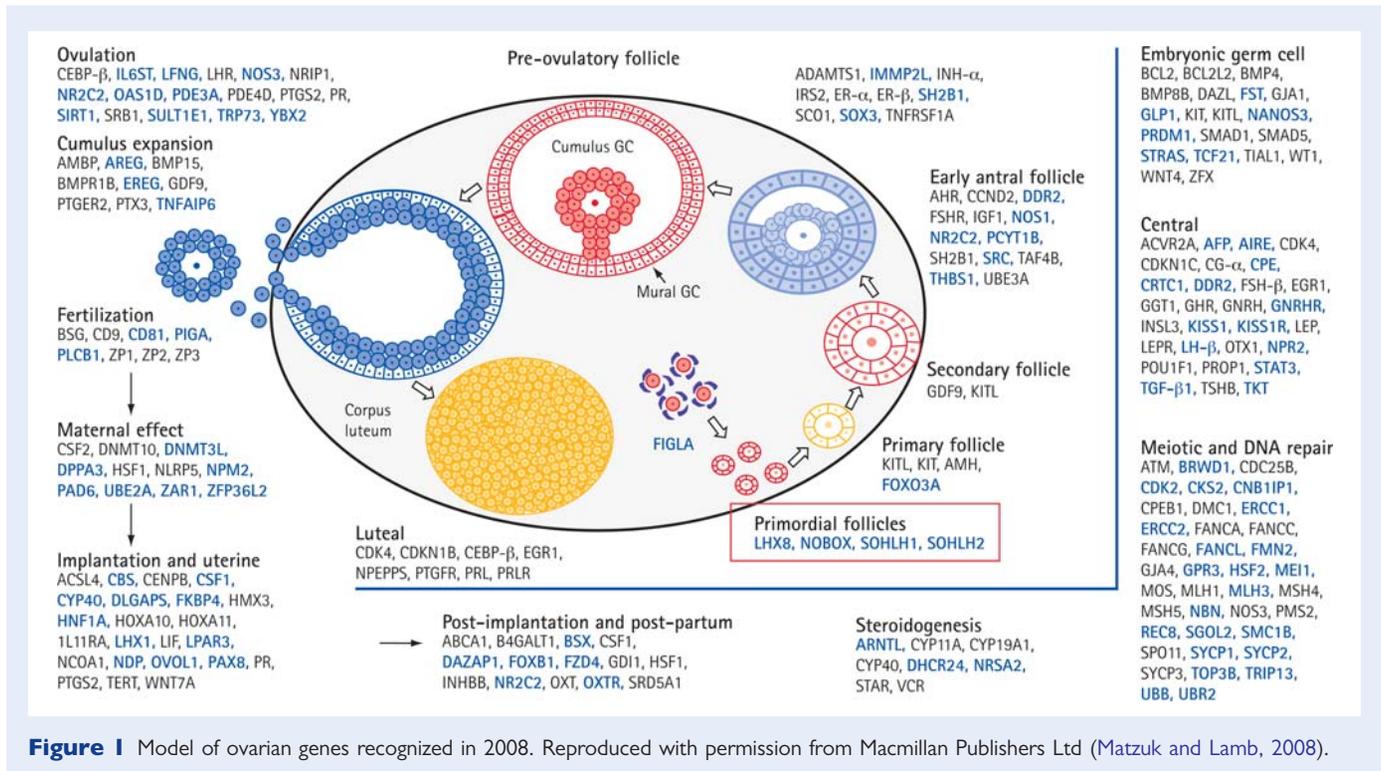
**Figure 1** Model of ovarian genes recognized in 2008. Reproduced with permission from Macmillan Publishers Ltd (Matzuk and Lamb, 2008).

homeobox-encoding gene (Suzumori *et al.*, 2002; Rajkovic *et al.*, 2004), spermatogenesis- and oogenesis-specific basic HLH1 (*SOHLH1*) and 2 (*SOHLH2*) transcription factors (Pangas *et al.*, 2006; Hao *et al.*, 2008; Toyoda *et al.*, 2009) and Lim homeobox protein 8 (*LHX8*) (Choi *et al.*, 2008). All of these are expressed in the late embryonic and newborn ovaries, and knockout models lack primordial follicles and are sterile.

SOHLH1 disruption has been shown to down-regulate the expression of *FIGLA* and *NOBOX* genes and their targets: *ZP1*, *ZP2*, *ZP3* and *OCT4*, growth differentiation factor 9 (*GDF9*) and *LHX8* (Pangas *et al.*, 2006) and up-regulate the expression of stimulated by retinoic acid gene 8 (*STRA8*), which is directly or indirectly repressed by *NOBOX* and *LHX8* (Choi *et al.*, 2008). SOHLH1 and SOHLH2 appear to be master–master regulators of other master transcription factors. The presence of two master–master regulators (*SOHLH1* and *SOHLH2*) supports the concept that it is important to have more than one transcription factor regulating key genes (Toyoda *et al.*, 2009). A summary of these findings is shown in Fig. 2.

The transforming growth factor β superfamily, which includes *GDF9* and bone morphogenetic protein 15 (*BMP15*), is the largest family of growth factor ligands in mammals. They function in multiple developmental and physiological processes (Chang *et al.*, 2002). Oocyte-expressed *GDF9* is essential for granulosa cell proliferation and differentiation at the one- to two-layer follicle transition (Dong *et al.*, 1996). A key role of *GDF9* at this transition is to suppress inhibin-α and a secondary role is to stimulate the formation of a steroidogenically functional theca (Elvin *et al.*, 1999a, b). The *GDF9* promoter contains three *NOBOX*-binding elements (Choi and Rajkovic, 2006).

*Novel oocyte-enriched gene, G-protein receptor 149*

G-protein receptor 149 (*GPR149*) is a recently identified 'antifertility' gene, expressed in ovaries and oocytes, which is of particular interest. It was identified from searches of online databases for genes with expression patterns similar to *GDF9*. These genes were grouped by gene ontology into G protein-coupled receptors, three of which were identified as being of potential importance: *GPR3*, *GPR149* and *GPR175* (Mehlmann *et al.*, 2004; Aki *et al.*, 2008; Edson *et al.*, 2010).

Unique features of the *GPR149* receptor include a very long carboxy-terminal tail of 360 amino acids. Phylogenetic analysis of *GPR149* suggests that it is widely conserved in vertebrates (Edson *et al.*, 2010). *GPR149*-deficient mice have been generated and found to be viable and fertile (Edson *et al.*, 2010). *GPR149*-null females have increased fertility (large litter sizes, high litter frequency and prolonged time in oestrus). Increased fertility was secondary to ovulation, which was likely due to reduced follicular atresia and a corresponding increase in the number of antral follicles. Importantly, the number of antral follicles was not depleted in mature *GPR149*-null female mice, which suggests that the ovaries of *GPR149*-null females do not undergo premature ageing (Edson *et al.*, 2010).

Expression of the follicle-stimulating hormone receptor gene (*FSHR*) and *GDF9* is increased in *GPR149*-deficient mice. Elevated levels of *FSHR* increase cyclin D2 levels in granulosa cells and stimulate cell proliferation (Edson *et al.*, 2010). If *GPR149* is found to have a similar function in humans, *GPR149* antagonists may represent potential new fertility treatments.

*Cumulus expansion*

Cumulus cells are specialized cells that surround and nourish the oocyte. They have important roles in promoting cytoplasmic

**Table I** Gene mutations affecting gonadotrophin synthesis or actions and causing infertility.

| Gene | Phenotype | OMIM gene [OMIM infertility] |
|---|---|---|
| Bone morphogenetic protein 15 (*BMP15*) | Hypergonadotrophic ovarian failure (POF4) | 300247 [300510] |
| Bone morphogenetic protein receptor 1B (*BMPR1B*) | Ovarian dysfunction, hypergonadotrophic hypogonadism and acromesomelic chondrodysplasia | 603248 |
| Chromobox homolog 2, *Drosophila* polycomb class (*CBX2;* M33) | Autosomal 46,XY, male-to-female sex reversal (phenotypically perfect females) | 602770 (67) |
| Chromodomain helicase DNA-binding protein 7 (*CHD7*) | CHARGE syndrome and Kallmann syndrome (KAL5) | 608892 [612370] |
| Diaphanous homolog 2 (*DIAPH2*) | Hypergonadotrophic, premature ovarian failure (POF2A) | 300108 [300511] |
| Fibroblast growth factor 8 (*FGF8*) | Normosmic hypogonadotrophic hypogonadism and Kallmann syndrome (KAL6) | 600483 [612702] |
| Fibroblast growth factor receptor 1 (*FGFR1*) | Kallmann syndrome (KAL2) | 136350 [147950] |
| FSH receptor (*FSHR*) | Hypergonadotrophic hypogonadism and ovarian hyperstimulation syndrome | 136435 |
| FSHβ (*FSHB*) | Deficiency of follicle-stimulating hormone, primary amenorrhoea and infertility | 136530 [229070] |
| Forkhead box L2 (*FOXL2*) | Isolated premature ovarian failure (POF3) associated with BPES type I; *FOXL2* 402C → G mutations associated with human granulosa cell tumours | 605597 [608996] |
| Fragile X mental retardation 1 (*FMR1*) | Premature ovarian failure (POF1) associated with premutations | 309550 [311360] |
| GnRH receptor (*GNRHR*) | Hypogonadotrophic hypogonadism | 138850 |
| GnRH (*GNRH1*) | Normosmic hypogonadotrophic hypogonadism | 152760 (769, 770) |
| Kallmann syndrome 1 (*KAL1*) | Hypogonadotrophic hypogonadism and insomnia, X-linked Kallmann syndrome (KAL1) | 308700 |
| KISS1 receptor (*KISS1R;* GPR54) | Hypogonadotrophic hypogonadism | 604161 |
| LHβ (*LHB*) | *LHB* G102S mutations associated with infertility | 152780 |
| LH/choriogonadotrophin receptor (*LHCGR*) | Hypergonadotrophic hypogonadism (luteinizing hormone resistance) | 152790 |
| Nuclear receptor subfamily 0, group B, member 1 (*NROB1;* DAX1) | X-linked congenital adrenal hypoplasia with hypogonadotrophic hypogonadism; dosage-sensitive male-to-female sex reversal | 300473 [300200; 300018] |
| Nuclear receptor subfamily 5, group A, member 1 (*NR5A1;* SF1) | 46,XY male-to-female sex reversal and streak gonads and congenital lipoid adrenal hyperplasia; 46,XX gonadal dysgenesis and 46,XX primary ovarian insufficiency | 184757 (771) |
| Premature ovarian failure 1B (*POF1B*) | Hypergonadotrophic, primary amenorrhea (POF2B) | 300603 [300604] |
| Prokineticin (*PROK2*) | Normosmic hypogonadotrophic hypogonadism and Kallmann syndrome (KAL4) | 607002 [610628] |
| Prokineticin receptor 2 (*PROKR2*) | Kallmann syndrome (KAL3) | 607123 [244200] |
| R-spondin family, member 1 (*RSPO1*) | 46,XX, female-to-male sex reversal (individuals contain testes) | 609595 |
| Sex-determining region Y (*SRY*) | Mutations lead to 46,XY females; translocations lead to 46,XX males | 480000 |
| SRY-related HMB-box gene 9 (*SOX9*) | Autosomal 46,XY male-to-female sex reversal (campomelic dysplasia) | 608160 |
| Tachykinin 3 (*TAC3*) | Normosmic hypogonadotrophic hypogonadism | 162330 |
| Tachykinin receptor 3 (*TACR3*) | Normosmic hypogonadotrophic hypogonadism | 162332 |

Reproduced with permission from the Endocrine Society (Edson *et al.*, 2009). Because of space limitations, most cases associated with female-to-male sex reversal due to steroidogenesis defects, syndromes and chromosomal abnormalities are excluded from the table. The primary reference (in parentheses) is included for work not yet described in OMIM.

maturation of oocytes, which is necessary for pronuclear formation and subsequent developmental capability (Vanderhyden and Armstrong, 1989). Cumulus expansion involves production of a hyaluronic acid-rich extracellular matrix that surrounds the oocyte. In the periovulatory period, this expansion has several important roles including protection of the oocyte, aiding growth of the cumulus–oocyte complex, permitting oocyte capture by oviductial fimbria and enhancement of the fertilization ability of sperm.

The genes involved in the process of cumulus expansion include *GDF9* and *BMP15* with involvement of hyaluronan synthase 2 (*HAS2*), tumour necrosis factor-induced protein-6 (*TNFIP6*),

pentraxin 3 (*PTX3*), prostaglandin-endoperoxide synthase 2 (*PTGS2*) and gremlin 1 (*GREM1*) (Elvin *et al.*, 1999b, 2000; Varani *et al.*, 2002; Pangas *et al.*, 2004). The expression of these genes produces factors involved in the production of granulosa cells, promotion of glycolysis, stimulation of sterol biosynthesis and amino acid transport with bidirectional communication between the oocyte and cumulus cells (Dong *et al.*, 1996; Elvin *et al.*, 1999a, 2000; Yan *et al.*, 2001; Varani *et al.*, 2002; Pangas *et al.*, 2004). When the cumulus cells are removed (e.g. during ICSI procedures), supplying the oocyte with these substances may have benefit. Therefore, cumulus cells could potentially be used in IVF
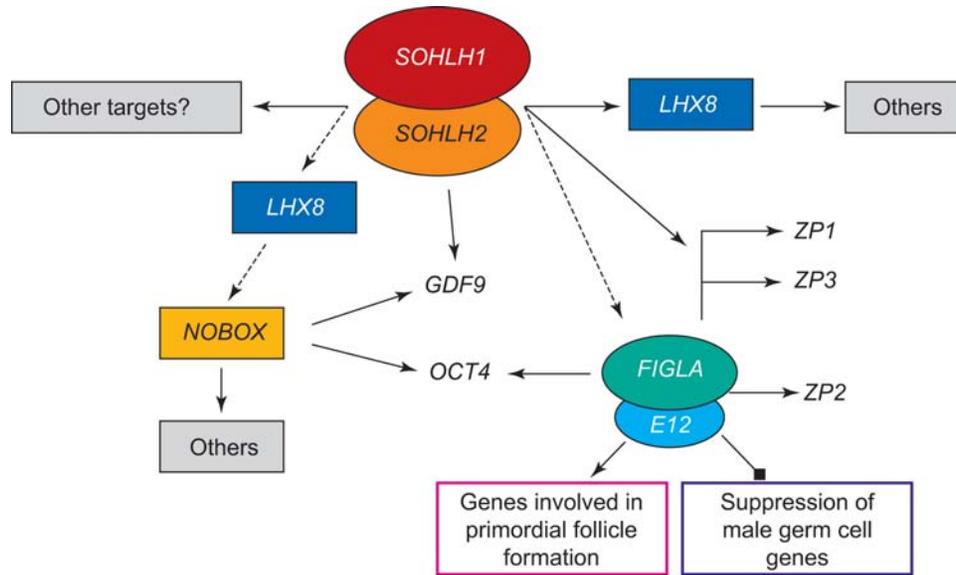
**Figure 2** Working model of the regulation of oocyte gene expression. As described in the text, a complex of *SOHLH1/2* is believed to be a master−master regulator of other master transcription factors (boxed or circled). Key downstream transcriptional targets (*GDF9*, *OCT4* and *ZP1−3*) are also shown.

programmes in co-culture with embryos to support development to the blastocyst stage.

Cumulus cell gene expression profiling has been used recently as a method of assessing oocyte quality and embryo development and is discussed later in this review.

## POI and poor response to ovarian stimulation

POI, which was known previously as premature ovarian failure, and follicular atresia may share several common mechanisms, including abnormal apoptosis, an abnormal number of primordial follicles and/or abnormal follicular maturation. Ovarian failure, whether natural or induced as a consequence of pathological insults, is driven by depletion of ovarian follicles through apoptosis (Tilly, 2001; De Vos et al., 2010).

The mean age at which the menopause occurs is 51 years and, with a range between 40 and 60 years, is relatively wide-ranging (Broekmans et al., 2009). Hence, exhaustion of the primordial follicle pool in some women takes 50% more time compared with others. Evidence suggests that all women are born with a similar number of follicles (Gosden and Faddy, 1994) and this number steadily dwindles throughout life as a result of atresia and recruitment towards ovulation (Gosden and Faddy, 1998). The rate of depletion of the follicular pool determines the age at which the menopause occurs and is thought to be controlled by multiple genes. Although only 1–2% of women undergo premature menopause, defined as before 40 years of age, studies in this population may provide important information regarding the genes involved in ovarian ageing and may help to predict better the age of menopause for individual patients (Torgerson et al., 1997; Goswami and Conway, 2005). Both infertility and poor response to ovarian hyperstimulation have been associated with subsequent early menopause in several studies (Farhi et al., 1997; Nikolaou et al., 2002; de Boer et al., 2003; Lawson et al., 2003; Broekmans

et al., 2009). Poor response to ovarian hyperstimulation is generally taken to be the retrieval of fewer than five oocytes and is associated with lower than average pregnancy rates (van der Gaast et al., 2006; Verberg et al., 2009).

The mean age of menopause is remarkably similar throughout the world and does not appear to have changed over time (Torgerson et al., 1997). There appears to be a strong link between mothers' and daughters' menopausal ages (Torgerson et al., 1997; van Asselt et al., 2004; Murabito et al., 2005) and twin studies suggest that the age of natural menopause is genetically linked (Snieder et al., 1998; Treloar et al., 1998). Other factors, including smoking and alcohol consumption, body mass index and parity, appear to play a minor role.

Aside from loss of fertility, POI underlies the emergence of an array of health problems in ageing women, including increased risks of cardiovascular disease and osteoporosis (Hartge, 2009; De Vos et al., 2010). In women who undergo bilateral oophorectomy before the natural menopause, with subsequent early reduction in oestrogen levels, evidence suggests an increased risk of cognitive impairment and neurological disorders such as Parkinson's disease (Rocca et al., 2007, 2008).

### Known causes of POI

In 70–90% of cases, the cause of POI in humans is unknown. Known causes include bilateral oophorectomy, chemotherapy, radiotherapy and Turner syndrome (TS; 45 X). Other known causes of POI include autoimmune disease [including autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy due to rare mutations in the *AIRE* (autoimmune regulator) gene (Ahonen et al., 1990)], pseudohypoparathyroidism, congenital disorders of glycosylation, galactosaemia, infections (e.g. herpes zoster and cytomegalovirus), syndromes [such as Fragile X (*FMR1* gene) (Murray et al., 1999) and blepharophimosis-ptosis-epicanthus inversus (BPES)] and isolated gene defects (Uda

**Table II** Human genes associated with ovarian insufficiency/failure.

| Acronym | Name |
| --- | --- |
| *AIRE* (Ahonen *et al.*, 1990) | Autoimmune regulator |
| *ATM* (Miller and Chatten, 1967) | Ataxia telangiectasia mutated |
| *BHLHB9* (De Vos *et al.*, 2010) | Basic helix−loop−helix domain-containing, class B, 9 |
| *BMP15* (Di Pasquale *et al.*, 2004) | Bone morphogenetic protein |
| *DACH2* (Prueitt *et al.*, 2002) | *Drosophila* dachsund |
| *DIAPH2* (Bione *et al.*, 1998) | Homologue *Drosophila* diaph |
| *FMR1* (Murray *et al.*, 1999) | Fragile X mental retardation 1 |
| *FMR2* (Murray *et al.*, 1999) | Fragile X mental retardation 2 |
| *XIST* (Sato *et al.*, 2004) | X inactivation transcript |
| *XPNPEP2* (Prueitt *et al.*, 2000) | Propyl aminopeptidase |
| *DAZL* (Tung *et al.*, 2006) | Deleted azoospermia |
| *DCM1* (Mandon-Pepin *et al.*, 2008) | Disrupted meiotic cDNA 1 |
| *EIF2B* (Fogli *et al.*, 2004) | Eukaryotic translation initiation |
| *ESR1* (Tung *et al.*, 2006) | Oestrogen receptor |
| *FIGLA* (Zhao *et al.*, 2008) | Murine factor germline alpha |
| *FOXL2* (Crisponi *et al.*, 2001) | Forkhead transcription factor |
| *FOXO1A* (Watkins *et al.*, 2006) | Forkhead box 01A |
| *FOXO3a* (Vinci *et al.*, 2008) | Forkhead box O3a |
| *FSHR* (Aittomaki *et al.*, 1995) | FSH receptor |
| *GALT* (Leslie *et al.*, 1992) | Galactose phosphate transferase |
| *GDF9* (Dixit *et al.*, 2005) | Growth differentiation factor 9 |
| *GPR3* (Kovanci *et al.*, 2008) | G protein-coupled receptor |
| *INHA* (Dixit *et al.*, 2004) | Inhibin A |
| *LHB* (Takahashi *et al.*, 1999) | LH beta |
| *MSH5* (Mandon-Pepin *et al.*, 2008) | MutS homolog 5 |
| *NOBOX* (Qin *et al.*, 2007) | Murine newborn ovary box |
| *NOGGIN* (Laissue *et al.*, 2007) | Binds and inactivates members of the transforming growth factor-β superfamily signalling proteins |
| *NR5A1* (SF-1) (Lourenco *et al.*, 2009) | Nuclear receptor subfamily 5 A1 |
| *NSB1* (Chrzanowska *et al.*, 2010) | Nijmegen breakage syndrome 1 |
| *PGRMC1* (van Dooren *et al.*, 2009) | Progesterone receptor membrane component-1 |
| *POLG* (Luoma *et al.*, 2004) | Mitochondrial DNA polymerase gamma mutations |
| *TGFBR3* (Dixit *et al.*, 2006) | Tumour growth factor receptor |
| *WT1* (Chun *et al.*, 1999) | Wilms Tumor 1 |

*et al.*, 2004). Known isolated gene defects involved in POI are listed in Table II (Miller and Chatten, 1967; Ahonen *et al.*, 1990; Leslie *et al.*, 1992; Aittomaki *et al.*, 1995; Bione *et al.*, 1998; Chun *et al.*, 1999; Murray *et al.*, 1999; Takahashi *et al.*, 1999; Prueitt *et al.*, 2000, 2002; Crisponi *et al.*, 2001; Di Pasquale *et al.*, 2004; Dixit *et al.*, 2004, 2005, 2006; Fogli *et al.*, 2004; Luoma *et al.*, 2004; Sato *et al.*, 2004; Tung *et al.*, 2006; Watkins *et al.*, 2006; Laissue *et al.*, 2007; Qin *et al.*, 2007; Kovanci *et al.*, 2008; Mandon-Pepin *et al.*, 2008; Vinci *et al.*, 2008; Zhao *et al.*, 2008; Lourenco *et al.*, 2009; van Dooren *et al.*, 2009; Chrzanowska *et al.*, 2010; De Vos *et al.*, 2010).

POI has been linked with several X chromosome-linked defects. Most women with TS have primary amenorrhoea, but some are fertile. Factors with a positive predictive value for fertility include mosaicism, normal FSH and anti-Müllerian hormone levels, and spontaneous menarche and puberty (Borgstrom *et al.*, 2009). *BMP15* has been described as the first gene on the X chromosome with a role in ovarian function (Layman, 2006). As discussed earlier, together with *GDF9*, *BMP15* plays an important role in oocyte−cumulus cell signalling (Layman, 2006; Gilchrist *et al.*, 2008) and it has been suggested that *GDF9* and *BMP15* mutations are involved in POI (Laissue *et al.*, 2006). A naturally occurring *BMP15* mutation in ewes causes increased ovulation rates, and twin and triplet births in heterozygotes, but primary ovarian failure in homozygotes (Galloway *et al.*, 2000). In humans, a natural heterozygous mutation in the pro-peptide region of the *BMP15* gene has been associated with hypergo-nadotrophic ovarian failure due to ovarian dysgenesis (Di Pasquale *et al.*, 2004). The mutation appeared to be associated with reduced granulosa cell growth. This suggests that *BMP15* is required for

human folliculogenesis. However, further studies are needed to elucidate the role of *BMP15* in humans.

Fragile X syndrome is caused by mutations of the *FMR1* gene in the distal part of the long-arm of the X chromosome. This occurs relatively frequently and is the most common cause of mental retardation, behavioural difficulties, autism and learning difficulties in boys. The syndrome is caused by an abnormal number of CGG repeats in the 5'-untranslated region of the gene. Typically, the number of repeats is fewer than 45, while abnormal genes may have more than 200, which causes *FMR1* gene inactivation. Women who have between 55 and 200 repeats (1 in 590 women) carry a premutation that causes altered ovarian function and POI (Wittenberger et al., 2007). Some studies have suggested, however, that there is no linear correlation between the number of repeats and POI, with the highest risk between 80 and 100 repeats (Sullivan et al., 2005; Ennis et al., 2006). Up to 28% of Fragile X premutation carriers (45–200 repeats) develop POI (Welt et al., 2004). Such women appear to have normal menstrual cycles, with slightly raised FSH levels (Welt et al., 2004).

*FOXL2* is involved in the development of granulosa cells and, in addition to female infertility, *FOXL2* mutations have been shown to cause eyelid/forehead dysmorphology (BPES) (Uda et al., 2004). Furthermore, studies have suggested that in a small proportion of patients with BPES, the genetic defect does not reside within the coding region of the *FOXL2* gene (De Baere et al., 2001) and incidences of POI with *FOXL2* mutations in patients without BPES have also been reported (De Baere et al., 2001; Harris et al., 2002).

Mutations in the *NR5A1* [FSH or steroidal factor 1 (SF-1)] gene, a key transcriptional regulator of genes involved in the hypothalamic–pituitary–steroidogenic axis, have also been associated with impairment of ovarian development and function (Lourenco et al., 2009). Such mutations are associated with POI and also with disorders of sex development in men. *NR5A1* is expressed in multiple cell types in the foetal, post-natal, prepubertal and mature ovary, in the adult adrenal cortex and in the Sertoli and Leydig cells of the testis (Lavorgna et al., 1991; Luo et al., 1994; Jeyasuria et al., 2004). The protein regulates the transcription of key genes involved in sexual development and reproduction, including *STAR* (encoding steroidogenic acute regulatory protein), *CYP17A1* (encoding 17-alphahydroxylase), *CYP11A1* (encoding cytochrome P-450 cholesterol side-chain cleavage), *LHB* (encoding the beta subunit of LH), *AMH* (encoding anti-Müllerian hormone), *CYP19A1* (encoding aromatase) and *INHA* (encoding inhibin-α subunit) (Shen et al., 1994; Keri and Nilson, 1996; Sugawara et al., 1997; Hanley et al., 2001; Gurates et al., 2003; Weck and Mayo, 2006; Mendelson and Kamat, 2007).

Women with *FSHR*-inactivating mutations develop multiple follicles in the early primordial, primary, secondary and pre-antral stages, but no antral or pre-ovulatory follicles (as their development is FSH-dependent) and such mutations are rare causes of POI (Aittomaki et al., 1995; Doherty et al., 2002). LH receptor mutations have also been associated with infertility. In a study of three sisters with an LH receptor mutation (two homozygous, one heterozygous), both homozygous women had normal menarche and regular menstrual cycles initially, but then experienced early oligomenorrhoea. The homozygous sisters underwent fertility treatment, which was unsuccessful in both cases. The heterozygous sister, however, had five natural pregnancies with two live births (Bruysters et al., 2008).

### Other candidate genes

Three types of study have been used to investigate the genetics of the natural menopause in humans; genome-wide linkage analysis, candidate gene-association studies and GWAS. Although GWAS have been used in many disease areas, until recently they had not been performed in reproductive medicine. Such studies need large numbers of patients and are expensive to perform, but can provide major breakthroughs in our understanding of disease aetiology.

Candidate genes for premature menopause include those involved in steroidogenesis, estradiol signalling and the cardiovascular system. A recent systematic review summarizes candidate genes and SNPs in association with the natural age at menopause and identified two genomic regions (19q13.42 and 20p12.3), containing two promising candidate genes [BR serine threonine kinase 1 gene (*BRKS1*) and maintenance complex component (*MCM*)] (Voorhuis et al., 2010).

Genome-wide linkage analysis studies have suggested the involvement of B-cell lymphoma 2 (*BCL2*) and gonadotrophin-releasing hormone 1 (*GnRH1*) as genes with suggestive linkage with the natural age of menopause (van Asselt et al., 2004; Murabito et al., 2005). The first genome-wide linkage search in familial POI identified a region on chromosome 5q14.1–q15 that may harbour a novel POI susceptibility gene (Oldenburg et al., 2008), and a recent GWAS identified *ADAMTS19* (5q31) as a possible candidate gene for POI (Knauff et al., 2009).

Other strategies to discover and study new candidate genes include the use of animal models. Inactivation of the proapoptotic *BAX* gene in mice has been shown to prolong ovarian function into advanced age (Perez et al., 2007) with minimization of many age-related health problems and, importantly, no increase in tumour incidence. Further studies are needed, however, to assess the importance of the *BAX* gene on ovarian function in humans.

aCGH analysis is able to detect submicroscopic chromosomal rearrangements with a high genomic resolution. This technique was used in a large cohort of Caucasian women with POI and identified eight copy number variations in eight different genes associated with POI. Five of these genes [*DNAH5* (dynein axonemal heavy chain), *NAIP* (neuronal apoptosis inhibitory protein), *DUSP22* (dual specificity phosphatases), *AKT1* (akt murine thymoma viral oncogene homolog 1) and *NUPR1* (nuclear protein 1)] are involved in reproduction; two are involved in reproductive diseases (*DNAH5* and *NAIP*), two in reproductive endocrinology (*DUSP22* and *NUPR1*) and one in folliculogenesis (*AKT1*). Thus, these genes represent potential candidate genes in POI (Aboura et al., 2009), but the results need to be confirmed in other studies.

Recently, four SNPs on chromosomes 19, 20, 6 and 5 have been associated with normal variation in menopausal age. They were identified through two GWAS and had a significant impact on the odds of having an early menopause in an independent cohort of women from the Breakthrough Generations Study. Thus, these genetic variants, which are associated with normal variation in menopausal age, are also significant risk factors for early menopause (Murray et al., 2011).

### Investigating POI

In the clinical setting, causative genes of POI are identified in very few cases. However, some syndromic causes of POI have physical manifestations that can provide clues to its aetiology; e.g. eyelid abnormalities

of patients with BPES and short stature in TS (Uda *et al.*, 2004; Borgstrom *et al.*, 2009). A family history of learning difficulties in boys suggests the presence of Fragile X syndrome and the presence of autoimmune disorders may suggest an immunological aetiology (Ahonen *et al.*, 1990; Murray *et al.*, 1999). When investigating POI, after obtaining informed consent, it is important to perform karyotyping and to screen for *FMR1* and *NR5A1* mutations as both have serious familial implications.

## Polycystic ovary syndrome

### Genetic factors in the aetiology of PCOS

PCOS is a complex, heterogeneous disorder of uncertain aetiology. Studies in animal models of PCOS, notably in the Rhesus monkey, have given rise to the hypothesis that PCOS has its origin in foetal life (Abbott *et al.*, 2002). In human females, exposure to excess androgen, at any stage from foetal development of the ovary to the onset of puberty, leads to many of the characteristic features of PCOS, including abnormalities of LH secretion and insulin resistance (Abbott *et al.*, 2002; Xita and Tsatsoulis, 2006). In human post-natal life, the natural history of PCOS can be further modified by factors affecting insulin secretion and/or action, most importantly, nutrition (Ciampelli *et al.*, 1999). There is strong evidence that genetic factors play an important part in the aetiology of PCOS (Franks and McCarthy, 2004; Franks *et al.*, 2006; Urbanek, 2007). The familial clustering of cases (Cooper *et al.*, 1968; Ferriman and Purdie, 1979; Givens, 1988; Hague *et al.*, 1988; Lunde *et al.*, 1989; Carey *et al.*, 1993), greater concordance in monozygotic compared with heterozygotic twins (Vink *et al.*, 2006) and heritability of endocrine and metabolic features of PCOS (Legro *et al.*, 1998, 2002; Franks *et al.*, 2008) all provide support for the involvement of genetic factors.

### Genetic studies in PCOS

In the search for genes involved in the aetiology of PCOS, the focus has been on genes implicated in folliculogenesis, such as follistatin, genes involved in the androgen biosynthetic pathway (such as *CYP11A*, *CYP17* and *CYP19*) and those affecting insulin secretion or action [such as transcription factor 7-like 2 (TCF7L2), potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11), insulin receptor and peroxisome proliferator-activated receptor-γ (PPAR-γ)] (Franks and McCarthy, 2004; Gaasenbeek *et al.*, 2004; Escobar-Morreale *et al.*, 2005; Christopoulos *et al.*, 2008). However, despite the evaluation of more than 70 genes, the candidate gene approach has, to date, proved disappointing. Due to limited sample sizes, and genetic and phenotypic heterogeneity, findings have often been ambiguous or not reproducible. Even the few studies that have been sufficiently well powered to either identify or exclude candidate susceptibility loci have produced limited positive findings.

Two notable exceptions, for which there is convincing evidence for a role in PCOS, are the fibrillin-3 gene (*FBN3*) and the fat-mass and obesity-associated (*FTO*) gene loci. Evidence for linkage disequilibrium was observed at D19S884, a dinucleotide repeat marker on chromosome 19p13.2 (Urbanek *et al.*, 1999, 2005; Tucci *et al.*, 2001). Genotyping of the region around D19S884 subsequently identified *FBN3* as a likely candidate for a PCOS susceptibility gene.

The association of the *FTO* locus (on chromosome 16) with type 2 diabetes and obesity (Frayling *et al.*, 2007) led researchers to explore a role for this gene in the aetiology of PCOS. In a case–control study, an association was found between PCOS status and *FTO* genotype (Barber *et al.*, 2008). *FTO* variants that were known to predispose to obesity were also implicated in altered susceptibility to PCOS, confirming the mechanistic link between these conditions. *FTO* variants have also been shown to strongly influence the glucose intolerance component of the metabolic syndrome in patients with PCOS (Attaoua *et al.*, 2008). Based on the results from various studies of *FTO* in PCOS, the consensus is that the association is predominantly with obesity in women with PCOS, rather than PCOS *per se* (Barber *et al.*, 2008; Tan *et al.*, 2010; Wehr *et al.*, 2010).

Blastocysts derived from women with PCOS have markedly different transcriptome profiles than blastocysts from normal controls (Katz-Jaffe *et al.*, 2010); proteomic profiling identified 12 biomarkers that displayed significantly decreased expression in PCOS. It was postulated that these differences may, in part, explain the reduction in reproductive capacity of women with PCOS.

Recently, the role of microRNAs (miRNAs) has been studied in the pathogenesis of endometriosis and associated reproductive conditions [for review, see Ohlsson Teague *et al.* (2010)]. miRNAs are short (about 21 nucleotides), single-stranded RNAs that regulate gene expression at the post-transcriptional level (non-coding). Differences in miRNA profiles in transferable blastocysts from women with PCOS compared with those from donor fertile controls have been shown (McCallie *et al.*, 2010).

Although the search for candidate genes is bound to continue, it seems increasingly likely that GWAS (of the kind that has paid dividends in the search for genes in type 2 diabetes) is the approach that is most likely to uncover the genes contributing to PCOS. Although this would require substantial resources and very large case–control populations, the limitations of earlier studies may be overcome and new studies promise to be more informative. Indeed, results have been published recently of a GWAS in Han Chinese women with PCOS. A number of loci of interest were identified, including one close to the gene encoding the LH receptor (Chen *et al.*, 2011).

# Genetics of disorders of the female reproductive system

## Endometriosis

Endometriosis is an oestrogen-dependent condition that is characterized by the presence of endometrium-like tissue at ectopic sites such as the pelvic peritoneum and ovaries (Fig. 3). Pelvic pain and infertility are the most common features of endometriosis. There are several proposed hypotheses to explain the pathogenesis of endometriosis (Sasson and Taylor, 2008); the most widely accepted theory is that of retrograde menstruation. The mechanisms involved in the development of an endometriotic lesion in the pelvic peritoneum may include attachment, invasion into the mesothelium, and survival and proliferation of ectopic endometrial cells.

The basic underlying cause of endometriosis is likely to be multifactorial and involves interplay between several factors. The pathogenesis
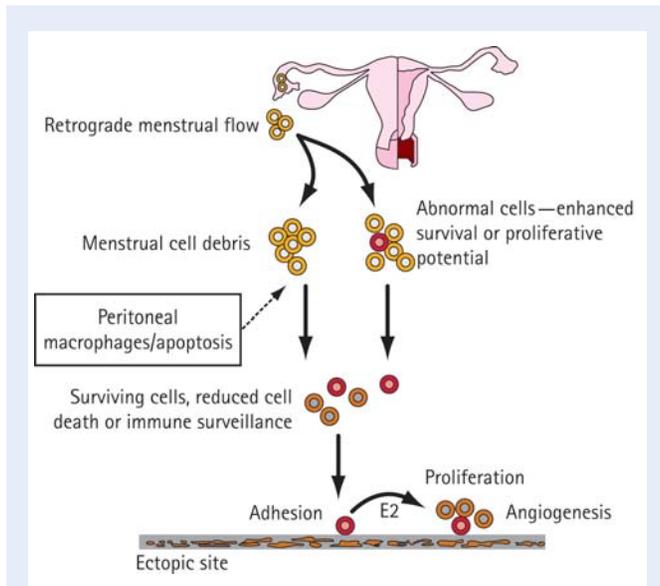
**Figure 3** A theoretical model of the development of endometriosis.

of endometriosis may, for example, involve retrograde menstruation in the context of an abnormal immune response and a genetic predisposition to developing endometriotic lesions; this may possibly occur after exposure to an unidentified environmental factor (Sasson and Taylor, 2008).

The study of endometriosis is complicated by a number of factors: these include the presence of different cell types within endometriotic lesions and the involvement of different pathogenic mechanisms in the formation of distinct types of endometriotic lesion (such as peritoneal, ovarian and rectovaginal) (Nisolle and Donnez, 1997). In addition, it may also be difficult to distinguish between cause and effect: differences in eutopic endometrium between patients with and without endometriosis, or in eutopic versus ectopic endometrium, may be the cause of this pathological condition or the result of another causative factor.

Although endometriosis can be treated surgically, recurrence of endometriotic lesions can occur. GnRH agonists and long-cycle oral contraceptives can also be used to manage the condition by suppressing ovulation and inducing a pseudo-menopausal state. Although many potential new agents, such as aromatase inhibitors, progesterone antagonists and immunomodulatory drugs, have been investigated, there is still a lack of suitable alternative pharmacological treatments (Guo, 2008; Huang, 2008; Vercellini et al., 2009). A better understanding of the pathogenesis of endometriosis may help to identify new pharmacological targets and facilitate the development of new treatments.

### Genetics of endometriosis

It has been proposed that endometriosis results from a series of multiple hits within target genes, in a mechanism similar to the development of cancer (Bischoff and Simpson, 2004). The initial mutation may be either somatic or heritable.

Endometriosis has long been recognized as having a familial association; data are consistent with a complex genetic basis (Malinak et al.,

1980; Simpson et al., 1980). Gene expression microarray studies have identified a number of candidate gene families that may be differentially regulated in endometriosis (Kao et al., 2003; Wu et al., 2006; Burney et al., 2007; Hull et al., 2008; Ohlsson Teague et al., 2009). A search for genetic polymorphisms associated with susceptibility to endometriosis has focused mainly on genes involved in inflammation, steroid hormone regulation, metabolism, biosynthesis, detoxification, vascular function and tissue remodelling (Tempfer et al., 2009). However, some polymorphisms have been investigated in only a single or a limited number of studies and, in some cases, conflicting results have been obtained. The majority of polymorphisms studied have, therefore, been found not to be associated with endometriosis. An evaluation of SNPs in 22 miRNAs that were differentially expressed in paired ectopic and eutopic endometria in women with endometriosis, however, identified two SNPs that were significantly associated with endometriosis-related infertility and disease severity: Wolf–Hirschhorn syndrome candidate gene 1 (WHSC1) alleles and solute carrier family 22, member 23 (SLC22A23) haplotypes (Zhao et al., 2011).

Two studies have shown differential expression of miRNAs in microarray analysis of eutopic and ectopic endometrial tissues (Pan et al., 2007; Ohlsson Teague et al., 2009). Although eight miRNAs were differentially expressed in both studies, the direction of dysregulation was not in agreement for any of these (Ohlsson Teague et al., 2010). A recent study using next-generation sequencing reported 22 dysregulated miRNAs (10 up-regulated miRNAs and 12 down-regulated miRNAs) in endometriomas compared with eutopic endometrium; miR-29c was implicated as a key up-regulated miRNA in endometriosis (Hawkins et al., 2011b). It is likely that specific miRNAs have a function in the pathophysiology of endometriosis, and the differences in results could be due to many factors, including the menstrual cycle phase at the time of biopsy. The putative roles of miRNAs in endometriosis and other reproductive diseases are described extensively in a recent review (Hawkins et al., 2011a).

A recent GWAS has identified a strong association signal for endometriosis at locus 7p152 (Painter et al., 2011). This locus is located in an intergenic region upstream of two plausible candidate genes, NFE2L3 and HOXA10.

### Epigenetic factors

Single molecular alterations in endometriosis may result in differential regulation of hormone metabolism (Attar and Bulun, 2006). The alterations may arise through epigenetic mechanisms such as DNA methylation. An example is transcriptional activation of SF-1 (NR5A1) in endometriotic stromal cells by hypomethylation of DNA (Xue et al., 2007). The presence of SF-1 in endometriosis and its absence in the endometrium are determined primarily by the methylation of its promoter. In endometriotic lesions, the presence of SF-1 may contribute to increased oestrogen production (Bulun et al., 2009).

### Stem cells: a new perspective

Stem cells located in the basal layer of the endometrium are thought to be responsible for cyclical tissue regeneration. Studies have been performed to isolate and characterize this cell population (Kato et al., 2007, 2010; Cervelló et al., 2010; Gargett and Masuda, 2010; Masuda et al., 2010). Such studies have identified a population of candidate endometrial stem cells in human endometrium; the cells

demonstrate multipotency and are different from other endometrial cell types. These cells have been termed side population cells (SP cells) as they exhibit a side population phenotype upon staining with Hoechst dye and flow cytometric analysis. SP cells have been demonstrated to proliferate and differentiate into various endometrial cell types *in vitro* and have unique angiogenic and migratory properties (Masuda *et al.*, 2010). Functional proof-of-concept studies have shown that human endometrium can be reconstructed in immunodeficient mice upon injection with human endometrial SP cells (Cervelló *et al.*, 2010).

It has been suggested that the retrograde menstruation of SP cells and subsequent implantation onto the surface of ectopic sites are responsible for the establishment of endometriotic lesions (Sasson and Taylor, 2008; Masuda *et al.*, 2010). It has also been hypothesized that an initial genetic mutation may cause aberrant behaviour of a sub-population of endometrial stem cells; clonal expansion of such cells may then occur and result in development of endometriosis (Gargett *et al.*, 2009). It is also conceivable that peritoneal cells could undergo de-differentiation back to endometrial cells, which take on an altered activity (Gargett *et al.*, 2009).

Studies of endometrial stem cells have provided a new focus for endometriosis research. Research on this subpopulation of endometrial cells could be of particular value; the findings may further our understanding of the pathogenesis of endometriosis and provide new therapeutic approaches.

# Predictors of oocyte quality and successful embryo development

Currently, many couples with fertility problems resort to IVF or ICSI when other treatments fail. Problems related to multiple pregnancies and poor embryo implantation are among the factors that may limit the success of IVF. The improvement of *in vitro* techniques, with sequential or co-culture systems, and subsequent reduction in the number of embryos transferred per cycle have ameliorated problems related to multiple pregnancy. However, implantation rates in IVF have not improved in the last decade (Nygren *et al.*, 2006; Andersen *et al.*, 2008). Any objective procedure that could assess the oocyte competence and potential for embryo implantation and development should increase success rates in assisted reproductive technology (ART).

In the study of oocyte competence, in recent years, the cumulus cell complex that surrounds and connects with the oocyte has received renewed attention. In a mouse model, this complex has been shown to play a central role in ovulation, and the capacity of the oocytes to support cumulus gene expression has been shown to be linked to the oocyte's developmental competence (Russell and Robker, 2007). Cumulus gene expression profiling therefore represents a possible means of identifying reliable biomarkers for oocyte quality and competence. It may also be useful to identify biomarkers for embryo development prediction.

## Cumulus gene expression profiling

Many studies suggest that cumulus cells: (i) coordinate follicular development with oocyte maturation, (ii) provide energy substrates for oocyte meiotic resumption, (iii) regulate oocyte transcription, (iv) promote nuclear and oocyte molecular maturation, (v) stimulate

amino acid transport and sterol biosynthesis, (vi) promote glycolysis and (vii) provide protection for the oocyte (Sugawara *et al.*, 1997; Elvin *et al.*, 1999b, 2000; Varani *et al.*, 2002; Sutton *et al.*, 2003; Pangas *et al.*, 2004; Su *et al.*, 2004; Eppig *et al.*, 2005; Diaz *et al.*, 2007; Gilchrist *et al.*, 2008). The vital supporting role of cumulus cells during *in vivo* and *in vitro* maturation has led many groups recently to focus research interest on cumulus cells. Isolated cumulus cells are relatively homogeneous with almost no contamination with other cells, whereas isolated granulosa cells, despite meticulous efforts, generally contain theca and blood cells: this is due to the methods employed for obtaining these cells. In addition, with the advent of the functional genomics and proteomics era, it has become possible to identify the transcriptome and proteome of cumulus cells using high-throughput technologies, such as microarray (Assou *et al.*, 2006) and high-resolution two-dimensional protein electrophoresis (Hamamah *et al.*, 2006). The current concept is that cumulus cells may constitute a reliable model for understanding oocyte quality and ovarian hyperstimulation protocol efficiency and may indirectly predict oocyte aneuploidy, embryo development and pregnancy outcomes (McKenzie *et al.*, 2004; Zhang *et al.*, 2005; Feuerstein *et al.*, 2007; van Montfoort *et al.*, 2008).

### Cumulus cells as biomarkers to assess oocyte quality, embryo competence and pregnancy outcome

Cumulus cells are typically discarded during classical IVF and ICSI. These cells are easily accessible and plentiful, which makes them an ideal material to use for the assessment of oocyte quality and embryo development potential. Thus, analysis of gene expression in cumulus cells may provide an indirect indication of the microenvironment in which the oocyte matures, and will help embryologists to better assess embryo quality. Currently, the most common ways of evaluating embryo quality non-invasively are by examining parameters such as morphology and cleavage rate. However, these approaches are subjective and lack precision. Several groups have used microarray technologies, reverse transcriptase PCR (RT–PCR) and quantitative RT–PCR analyses to study the association between cumulus cell gene expression profiles and oocyte competence, embryo quality and pregnancy outcome (Table III) (McKenzie *et al.*, 2004; Cillo *et al.*, 2007; Feuerstein *et al.*, 2007; Assou *et al.*, 2008, 2010; van Montfoort *et al.*, 2008; Anderson *et al.*, 2009; Hamel *et al.*, 2010; Wathlet *et al.*, 2011). In a new and indirect approach for predicting embryo quality and pregnancy outcome, gene expression signatures have been identified recently by Assou *et al.* (2008, 2010) using transcriptomic data of cumulus cells. This non-invasive approach is based on the level of expression of potential biomarkers in cumulus cells (indicators of successful pregnancy) to assess the potential and quality of the embryo.

### Cumulus cell gene expression analysis for optimization of ovarian hyperstimulation protocols

As cumulus cells respond to stimulation protocols with a distinct gene expression profile and are sensitive to changes in environmental conditions, it may be possible to identify the genes that are expressed in the follicular environment. The individual characterization of gene expression may provide important insights for the evaluation of the impact of ovarian hyperstimulation protocols used during IVF.

**Table III** Association of cumulus cell gene expression with embryo quality and pregnancy outcomes.

| Cumulus cell origin | Approaches | Biomarkers | Outcome | Reference |
|---|---|---|---|---|
| Individual oocytes | Microarray (50 chips) | Including PCK1, BCL2L11, NFIB and others | Predict embryo and pregnancy outcomes | Assou et al. (2008) |
| Individual oocytes | Microarray (16 chips) | CCND2, CXCR4, GPX3, HSPB1, DVL3, DHCR7, CTNND1, TRIM28 | Negatively associated with oocyte competence | van Montfoort et al. (2008) |
| Individual oocytes | RT−PCR | HAS2, GREM1 | Positively associated with oocyte developmental competence | Cillo et al. (2007) |
| Individual oocytes | RT−PCR | STAR, AREG, CX43, PTGS2, SCD1, SCD5 | Negatively associated with oocyte competence | Feuerstein et al. (2007) |
| Individual oocytes | qRT−PCR | HAS2, PTGS2, GREM1 | Positively associated with oocyte competence and embryo development | McKenzie et al. (2004) |
| Individual oocytes | qRT−PCR | GREM1, BDNF | Positive and negative predictors of embryo quality, respectively | Anderson et al., (2009) |
| Mural granulosa cells and individual oocytes | qRT−PCR | PGK1, RGS2, RGS3, CDC42 | Associated with pregnancy | Hamel et al. (2010) |
| Individual oocytes | qRT−PCR | SDC4, PTGS2, VCAN, activated leucocyte cell adhesion molecule, GREM1, TRPM7, ITPKA | Predict embryo development and pregnancy | Wathlet et al. (2011) |

Modified with permission from Assou et al. (2010). AREG, amphiregulin; BCL2L11, BCL-like protein 11; BDNF, brain-derived neurotrophic factor; CCND2, cyclin D2; CDC42, cell division cycle 42; CTNND1, catenin delta 1; CX43, connexin 43; CXCR4, chemokines receptor 4; DHCR7, 7-dehydrocholesterol reductase; DVL3, dishevelled dsh homolog 3; GPX3, glutathione peroxidase; GREM1, gremlin 1; HAS2, hyaluronic acid synthase 2; HSPB1, heatshock 27 kDa protein 1; ITPKA, inositol 1,4,5-trisphosphate 3-kinase A; NFIB, nuclear factor 1B; PCK1, phosphoenolpyruvate carboxykinase 1; PGK1, phosphoglycerate kinase 1; PTGS2, prostaglandin-endoperoxide synthase 2; qRT−PCR, quantitative real-time PCR; RGS2, regulator of G-protein signalling 2; RGS3, regulator of G-protein signalling 3; RT−PCR, real-time PCR; SCD1, stearoyl-co-enzyme A desaturase 1; SCD5, stearoyl-co-enzyme A desaturase; SDC4. syndecan 4; STAR, steroidogenic acute regulatory protein; TRIM28, tripartite motif-containing 28; TRPM7, transient receptor potential cation channel, subfamily M, member 7; VCAN, versican.

Using fertilized and non-fertilized oocytes resulting from specific ovarian hyperstimulation protocols, potential differences in the protein expression profiles of cumulus cells have been investigated (Hamamah et al., 2006). In cumulus cells from either fertilized or non-fertilized oocytes, the greatest degree of similarity (more than 85%) was in the protein expression profiles. The analysis of protein expression profiles of cumulus cells from follicles obtained with the same ovarian hyperstimulation protocol showed a strong correlation between protein expression profiles whether or not embryos had been produced successfully. More than 80% of proteins were expressed similarly between cumulus cells from fertilized versus unfertilized oocytes from a cycle in a single patient that used a long GnRH protocol with human menopausal gonadotrophin (HP-hMG). In contrast, when cumulus cell protein expression profiles were analysed in oocytes with the same outcome from the same patient, but originating from different ovarian hyperstimulation protocols, a significant difference was seen in the protein expression profiles. Comparing protein expression profiles in cumulus cells from fertilized oocytes from the same patient, but after GnRH agonist long protocols with HP-hMG compared with those with recombinant FSH, only 55% of the proteins showed similar mobility and expression levels. Comparison of two groups of patients indicated that dissimilarities in protein pattern between patients become very high, even when comparing the same stimulation protocol and oocyte fertilization outcome. These data from protein expression profiling of human cumulus cells suggest that there may be a correlation between the synthesis of specific cumulus cell proteins and the maturity and fecundity of the oocyte. The study reveals three important new findings with respect to human cumulus cell biology: (i) human cumulus cells have robust metabolic activity, (ii) the overall protein expression profiles are highly similar between cumulus cells from oocytes obtained using the same ovarian hyperstimulation protocol and (iii) there are significant differences in protein expression between cumulus cells from oocytes obtained under two different ovarian hyperstimulation protocols, even when the outcomes are the same (Hamamah et al., 2006).

*Cumulus cell gene expression analysis in PCOS and aneuploidy identification*

Cumulus cells play a major role in the control of oocyte metabolism and, therefore, it is likely that malfunction of these cells might play a role in PCOS. Recently, the gene expression profile of cumulus cells isolated from patients with PCOS has been studied (Kenigsberg et al., 2009). The different gene expression patterns of cumulus cells from lean and obese women with PCOS support the idea that in the two types of patient, the condition may have different pathophysiologies. Furthermore, in recent years, several groups have initiated research aimed at identifying non-invasive biomarkers of chromosome imbalance. Some investigators have detected characteristic transcriptional changes in the cumulus cells attached to aneuploid oocytes (Wells et al., 2008). This suggests that genes involved in the meiotic process in human oocytes are regulated by genes expressed in cumulus cells.

## 'Omics' technologies for embryo assessment

Accurate selection of the most appropriate embryo for SET has been a major aim for reproductive specialists in recent years. To date, the

only universally used method to score the potential development and implantation capability of the human embryo is morphological assessment. Therefore, there is a need for new adjunctive technologies for determining the best embryo for transfer and improvement of implantation rates. Successful embryo implantation requires endometrial receptivity, the development of a viable embryo and adequate bi-directional communication between the blastocyst and endometrium. The advent of the era of high-throughput 'omic' methodologies (genomics, transcriptomics, proteomics and metabolomics) has facilitated the study of such processes.

Some of these technologies may provide non-invasive methods for embryo evaluation in the future.

### Genomics

Preimplantation genetic screening (PGS) can be used during early stages of embryo development to assess aneuploidy. In some countries, PGS is used to maximize the chances of a successful pregnancy in certain patient subpopulations, such as those of advanced maternal age, or women with a history of recurrent implantation failure or miscarriage.

However, the use of PGS is associated with a number of potential drawbacks. Standard PGS methods utilize a Day-3 biopsy, in which one or two blastomeres are removed from the embryo, followed by FISH to detect chromosomal abnormalities. Therefore, the loss of embryos and a reduction in embryo quality are possible. Furthermore, there is a high rate of mosaicism in Day-3 embryos (which might lead to an incorrect diagnosis), and the survival of embryos after blastomere removal and subsequent cryopreservation has been found to be severely reduced (Joris *et al.*, 1999; Platteau *et al.*, 2006).

Several comprehensive chromosome analysis methods are now available, including aCGH, quantitative PCR and SNP microarrays (Johnson *et al.*, 2010). aCGH evaluates aneuploidy of each pair of chromosomes and results can be available within 24 h. Combined with single blastomere biopsy on Day-3 embryos, aCGH has been shown to be robust, with only 2.9% of embryos with no results, and associated with low error rates (1.9%) (Gutiérrez-Mateo *et al.*, 2011). Unlike SNP arrays, aCGH does not require prior testing of parental DNA; therefore, advanced planning and careful scheduling are unnecessary. A short CGH method has also been shown to detect more chromosomal aneuploidies than FISH and has been used successfully to achieve pregnancy (Rius *et al.*, 2011).

### Transcriptomics

Over the last decade, microarray studies have characterized the gene expression profile of the human endometrium in different physiological states, such as during decidualization, the window of implantation, endometriosis, the normal menstrual and stimulated cycles, and endometrial cancer (Horcajadas *et al.*, 2007; Haouzi *et al.*, 2009a, b). However, gene expression profiling studies of human embryos are limited, due to legal and ethical issues. Data on early mammalian development have been provided mainly by murine studies (Tanaka *et al.*, 2000; Hamatani *et al.*, 2004; Wang *et al.*, 2004; Jeong *et al.*, 2006).

Gene expression profile studies in cumulus and granulosa cells have been described earlier and are summarized in Table III. Such studies have identified candidate biomarkers for oocyte quality and competence (Cillo *et al.*, 2007; Hamel *et al.*, 2008), early embryo development (McKenzie *et al.*, 2004; van Montfoort *et al.*, 2008; Anderson *et al.*, 2009) and embryo quality and pregnancy outcome (Assou *et al.*, 2008; Hamel *et al.*, 2010). Furthermore, in mice, blastocyst gene expression profiles correlate with outcome, including successful implantation and pregnancy loss (Parks *et al.*, 2011). miRNA expression in human blastocysts is different in transferable blastocysts from infertile patients (male factor infertility and those from women with PCOS) compared with those from donor fertile controls, and it has been suggested that an association of aberrant miRNA profiles may exist with human infertility (McCallie *et al.*, 2010).

Transcriptomic studies may provide valuable information that could lead to the identification of biomarkers. For a comprehensive review, refer to Assou *et al.* (2011).

### Proteomics

Although not as developed as the genomics/transcriptomics methodologies, proteomic analysis of mammalian embryos is also emerging as a powerful assessment tool (Katz-Jaffe *et al.*, 2005; Shankar *et al.*, 2005). Analysis of the proteome of individual human embryos has the potential to provide novel biomarkers of good embryo development and implantation potential (Katz-Jaffe *et al.*, 2006a). Furthermore, by using bioinformatics to create networks linking proteins found to be differentially regulated between biological samples, functional pathways can be identified (Dominguez *et al.*, 2010). However, analysis of the proteome in a research setting requires extraction of proteins from lysed blastocysts, which limits the use of this technology in routine embryo assessment.

Analysis of the proteins contained in the surrounding embryo culture medium may provide a non-invasive method of using proteomic techniques to identify novel biomarkers of embryo viability (Katz-Jaffe *et al.*, 2006b). Domínguez *et al.* have used protein array analysis to characterize pooled spent sequential culture media prior to SET and to compare it with control media. A lower abundance of CXCL13, stem cell factor and macrophage-stimulating protein–α, but a higher abundance of soluble TNF receptor 1 was detected in the sequential culture media containing a blastocyst relative to the control (Domínguez *et al.*, 2008). Furthermore, blastocysts that went on to successfully implant were found to have a relatively lower abundance of CXCL13 and granulocyte-macrophage colony-stimulating factor in their culture media than those that did not implant; these proteins may be markers of successful implantation ability (Domínguez *et al.*, 2008).

Domínguez *et al.* have also used co-culture studies to explore the role of the endometrium in implantation. This technique involves endometrial biopsy, followed by separation and culture of the endometrial epithelial cells (EEC). A total of 32 proteins were found to be up- or down-regulated in blastocysts that went on to successfully implant and were co-cultured with EEC relative to those cultured in sequential media; of these proteins, interleukin 6 (IL-6) was found to be the most abundantly secreted in the EEC culture. Furthermore, the IL-6 concentration in sequential media from blastocysts that implanted was significantly lower than that of blastocysts that did not implant (Dominguez *et al.*, 2010). The proteomics of endometrial receptivity have also been studied by analysis of endometrial biopsies taken during the pre-receptive and receptive phases of the menstrual cycle (DeSouza *et al.*, 2005; Domínguez *et al.*, 2009; Haouzi *et al.*, 2009a, b). Two proteins found to be differentially expressed in pre-

receptive and receptive endometria were stathmin 1 and annexin A2 (down- and up-regulated in the receptive endometrium, respectively) (Domínguez et al., 2009). Support for the involvement of these proteins in endometrial receptivity was provided by the pre-receptive pattern of stathmin 1 and annexin A2 expression observed when an intrauterine device was used to model the non-receptive endometrium (Domínguez et al., 2009). In previous studies, a quantitative approach for proteomic assessment using isotope-coded affinity tags, affinity purification and online tandem mass spectrometry has been used to study differences between proliferative and secretory endometria (DeSouza et al., 2005). Only five proteins with significant differential expression were found; the glutamate NMDA receptor subunit zeta 1 precursor and FRAT1 were of greatest interest.

Endometrial receptivity has also been studied by other groups using non-invasive techniques. Boomsma et al. (2009) have used a multiplex immunoassay to analyse endometrial secretions, aspirated immediately prior to embryo transfer during IVF. Using this approach, cytokine profiles predictive of implantation and clinical pregnancy have been identified (Boomsma et al., 2009). The data obtained provide support for associations between MCP-1 and IP-10 levels with implantation, and IL-1β and TNF-α levels with clinical pregnancy (Boomsma et al., 2009).

Recent studies have investigated discriminating signatures between individual euploid and aneuploid blastocysts. Microdrops of spent IVF culture medium from individual blastocysts of transferable quality were processed and analysed by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry to determine a blastocyst secretome fingerprint. Each individual blastocyst was then subjected to CGH for comprehensive analysis of all chromosomes (Fragouli et al., 2008). Of the 14 aneuploid blastocysts analysed, nine had a single chromosomal aneuploidy and five had chaotic changes involving more than two chromosomes. Secretome fingerprints from individual blastocysts identified protein signatures that allowed discrimination between euploid and aneuploid chromosomal constitutions (Katz-Jaffe et al., 2008).

### Metabolomics

Metabolites represent the end products of cell regulatory processes and can be used to study the response of biological systems to genetic, nutritional and environmental influences; the metabolome (i.e. the complete set of small molecule metabolites found within a biological sample) therefore provides a good indicator of cellular activities.

High-resolution nuclear magnetic resonance (NMR) has been used to analyse the metabolic profile of follicular fluid samples from oocyte donors and has identified metabolites that could be useful as biomarkers of the follicular maturation state (Pinero-Sagredo et al., 2010). NMR spectroscopy has also been used for compositional analysis of other areas of the reproductive tract, including cervical mucus, ovarian tissue, fallopian tubes and uterine matter (Baskind et al., 2011). Incorporation of such techniques into female fertility research may be valuable for understanding subfertility and for predicting outcomes of assisted conception treatments (Baskind et al., 2011).

Metabolomic technology is being used in the development of a new method for aneuploidy detection. This approach is based on the assumption that an embryo with a missing or extra chromosome may have modified metabolism. Given the drawbacks of the use of

PGS to detect chromosomal abnormalities, alternative methods would be of value. Analysis of the global metabolome of the embryo (using a combination of ultra-performance liquid chromatography and mass spectroscopy) to analyse low-molecular-weight molecules in spent culture media allows study of the embryo without invasive techniques (unlike PGS). By correlating metabolomic profiles with the results of subsequently performed PGS, it has been possible to differentiate between embryos with normal and abnormal chromosome numbers, and even classify embryos according to their chromosomal anomaly (Sanchez-Ribas et al., 2008). Although this technique may be limited by the low quantity of metabolites in spent culture media, preliminary results are promising and encourage further development of this non-invasive tool for embryo assessment.

Aneuploidy screening represents a unique and valuable application of metabolomics. Analysis of the metabolomic profile of embryo culture media using infra-red techniques is also being explored as an embryo assessment strategy (Seli et al., 2007, 2010; Scott et al., 2008).

### Translating 'omics' technologies to the ART clinic

As with any ART procedure, the easier the technique, the more likely that it can be used to produce reliable results in routine procedures in ART clinics. Most ART laboratories have, or have easy access to, enzyme-linked immunosorbent assay platforms for routine hormone testing. However, few laboratories have sophisticated equipment for performing quantitative PCR or mass spectrometry. If the cost of this equipment falls over time, or as more focused assays become available, one or more of these technologies may become used routinely in clinical practice. Another possibility is that as procedures become routine and as key genes, proteins or small molecules are identified that correlate with pregnancy outcomes, clinical samples (cumulus cells, media from embryo cultures or blastomeres) could be collected and stored in specialized solutions or containers, and testing could be performed at a commercial facility. Such a process could allow cost-effective performance of the assays and standardization of the technologies. In addition, this practice could allow selection of high-quality oocytes based on cumulus cell profiles after the oocytes have been vitrified. Under such circumstances, there would not be immediate time pressures to obtain the results and oocytes could be assessed before transfer in a subsequent cycle. In the future, it is likely that a combination of multiple technologies will be used with the goal of obtaining a healthy newborn on the first attempted embryo transfer.

## Conclusions

In conclusion, our knowledge of ovarian genetics is rapidly expanding and this review provides an important update of those genes identified to have a role in infertility, particularly POI. To date, the search for a candidate gene for PCOS has produced limited positive findings despite involvement of numerous genes. In the field of endometriosis, it has been proposed that the condition results from a series of multiple hits within target genes and gene expression microarray studies have identified a number of candidate gene families that may be differentially regulated. The members of the Fifth EVAR Workshop Group agreed that the way forward in advancing the knowledge of genes involved in reproduction and fertility-associated pathologies was through GWAS involving large numbers of patients. As GWAS are

expensive to conduct, it was suggested that pooling of data from different research groups may be the way forward.

Future challenges for genomics and genetic screening of patients include identification of high-throughput strategies and how to apply findings to patients being seen in the ART clinic. The identification of high-quality embryos in ART remains a priority in order to select those with high potential development and implantation capability for transfer. Gene expression profiling of cumulus cells and proteomic and metabolomic technologies show promise for non-invasive oocyte and embryo assessment.

## Authors' roles

B.C.J.M. Fauser: developed the Fifth EVAR Workshop scientific programme, participated as a Chairman, was involved in drafting the article and revising it critically for important intellectual content and approved the final version of the manuscript. K. Diedrich: participated in the Fifth EVAR Workshop as a Chairman, was involved in drafting the article and revising it critically for important intellectual content and approved the final version of the manuscript. P. Bouchard, F. Domínguez, M. Matzuk, S. Franks, S. Hamamah, C. Simón, D. Ezcurra and C.M. Howles: participated in the Fifth EVAR Workshop, were involved in drafting the article and revising it critically for important intellectual content and approved the final version of the manuscript. P. Devroey: was involved in the planning of the Fifth EVAR Workshop and was involved in drafting the article and revising it critically for important intellectual content and approved the final version of the manuscript.

## Acknowledgements

## Funding

## Conflict of interest

B.C.J.M. Fauser has received fees and grant support from the following companies: Andromed, Ardana, Ferring Pharmaceuticals, Genovum, Glycotope, Merck Serono, MSD, Organon, Pantarhei Bioscience, Philips, PregLem, Schering, Schering-Plough, Serono and Wyeth. C.S. is an International Scientific Advisor for Merck Serono S.A., Geneva, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany. D. Ezcurra and C.M. Howles are employees of Merck Serono S.A., Geneva, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany.

## References

Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome—a hypothesis. *J Endocrinol* 2002;**174**:1–5.

Aboura A, Dupas C, Tachdjian G, Portnoi MF, Bourcigaux N, Dewailly D, Frydman R, Fauser B, Ronci-Chaix N, Donadille B et al. Array comparative genomic hybridization profiling analysis reveals deoxyribonucleic acid copy number variations associated with premature ovarian failure. *J Clin Endocrinol Metab* 2009;**94**:4540–4546.

Ahonen P, Myllarniemi S, Sipila I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med* 1990;**322**:1829–1836.

Aittomaki K, Lucena JL, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, Kaskikari R, Sankila EM, Lehvaslaiho H, Engel AR et al. Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell* 1995;**82**:959–968.

Aki T, Funakoshi T, Nishida-Kitayama J, Mizukami Y. TPRA40/GPR175 regulates early mouse embryogenesis through functional membrane transport by Sjogren's syndrome-associated protein NA14. *J Cell Physiol* 2008;**217**: 194–206.

Andersen AN, Goossens V, Ferraretti AP, Bhattacharya S, Felberbaum R, de Mouzon J, Nygren KG. Assisted reproductive technology in Europe, 2004: results generated from European registers by ESHRE. *Hum Reprod* 2008;**23**: 756–771.

Anderson RA, Sciorio R, Kinnell H, Bayne RA, Thong KJ, de Sousa PA, Pickering S. Cumulus gene expression as a predictor of human oocyte fertilisation, embryo development and competence to establish a pregnancy. *Reproduction* 2009; **138**:629–637.

Assou S, Anahory T, Pantesco V, Le Carrour T, Pellestor F, Klein B, Reyftmann L, Dechaud H, De Vos J, Hamamah S. The human cumulus–oocyte complex gene-expression profile. *Hum Reprod* 2006;**21**:1705–1719.

Assou S, Haouzi D, Mahmoud K, Aouacheria A, Guillemin Y, Pantesco V, Reme T, Dechaud H, De Vos J, Hamamah S. A non-invasive test for assessing embryo potential by gene expression profiles of human cumulus cells: a proof of concept study. *Mol Hum Reprod* 2008;**14**:711–719.

Assou S, Haouzi D, De Vos J, Hamamah S. Human cumulus cells as biomarkers for embryo and pregnancy outcomes. *Mol Hum Reprod* 2010;**16**:531–538.

Assou S, Boumela I, Haouzi D, Anahory T, Dechaud H, De Vos J, Hamamah S. Dynamic changes in gene expression during human early embryo development: from fundamental aspects to clinical applications. *Hum Reprod Update* 2011;**17**: 272–290.

Attaoua R, Ait El Mkadem S, Radian S, Fica S, Hanzu F, Albu A, Gheorghiu M, Coculescu M, Grigorescu F. FTO gene associates to metabolic syndrome in women with polycystic ovary syndrome. *Biochem Biophys Res Commun* 2008;**373**:230–234.

Attar E, Bulun SE. Aromatase and other steroidogenic genes in endometriosis: translational aspects. *Hum Reprod Update* 2006;**12**:49–56.

Barber TM, Bennett AJ, Groves CJ, Sovio U, Ruokonen A, Martikainen H, Pouta A, Hartikainen AL, Elliott P, Lindgren CM et al. Association of variants in the fat mass and obesity associated (FTO) gene with polycystic ovary syndrome. *Diabetologia* 2008;**51**:1153–1158.

Baskind NE, McRae C, Sharma V, Fisher J. Understanding subfertility at a molecular level in the female through the application of nuclear magnetic resonance (NMR) spectroscopy. *Hum Reprod Update* 2011;**17**:228–241.

Bione S, Sala C, Manzini C, Arrigo G, Zuffardi O, Banfi S, Borsani G, Jonveaux P, Philippe C, Zuccotti M et al. A human homologue of the *Drosophila melanogaster diaphanous* gene is disrupted in a patient with premature ovarian failure: evidence for conserved function in oogenesis and implications for human sterility. *Am J Hum Genet* 1998;**62**:533–541.

Bischoff F, Simpson JL. Genetic basis of endometriosis. *Ann N Y Acad Sci* 2004; **1034**:284–299.

Boomsma CM, Kavelaars A, Eijkemans MJ, Lentjes EG, Fauser BC, Heijnen CJ, Macklon NS. Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. *Hum Reprod* 2009;**24**:1427–1435.

Borgstrom B, Hreinsson J, Rasmussen C, Sheikhi M, Fried G, Keros V, Fridstrom M, Hovatta O. Fertility preservation in girls with turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab* 2009;**94**:74–80.

Broekmans FJ, Soules MR, Fauser BC. Ovarian ageing: mechanisms and clinical consequences. *Endocr Rev* 2009;**30**:465–493.

Bruysters M, Christin-Maitre S, Verhoef-Post M, Sultan C, Auger J, Faugeron I, Larue L, Lumbroso S, Themmen AP, Bouchard P. A new LH receptor splice mutation responsible for male hypogonadism with subnormal sperm production in the propositus, and infertility with regular cycles in an affected sister. *Hum Reprod* 2008;**23**:1917–1923.

Bulun SE, Utsunomiya H, Lin Z, Yin P, Cheng YH, Pavone ME, Tokunaga H, Trukhacheva E, Attar E, Gurates B *et al*. Steroidogenic factor-1 and endometriosis. *Mol Cell Endocrinol* 2009;**300**:104–108.

Burney RO, Talbi S, Hamilton AE, Vo KC, Nyegaard M, Nezhat CR, Lessey BA, Giudice LC. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. *Endocrinology* 2007;**148**:3814–3826.

Carey AH, Chan KL, Short F, White D, Williamson R, Franks S. Evidence for a single gene effect causing polycystic ovaries and male pattern baldness. *Clin Endocrinol (Oxf)* 1993;**38**:653–658.

Cervelló I, Gil-Sanchis C, Mas A, Delgado-Rosas F, Martinez-Conejero JA, Galan A, Martinez-Romero A, Martinez S, Navarro I, Ferro J *et al*. Human endometrial side population cells exhibit genotypic, phenotypic and functional features of somatic stem cells. *PLoS One* 2010;**5**:e10964.

Chang H, Brown CW, Matzuk MM. Genetic analysis of the mammalian transforming growth factor-beta superfamily. *Endocr Rev* 2002;**23**:787–823.

Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, Li Z, You L, Zhao J, Liu J *et al*. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. *Nat Genet* 2011;**43**:55–59.

Choi Y, Rajkovic A. Characterization of NOBOX DNA binding specificity and its regulation of Gdf9 and Pou5f1 promoters. *J Biol Chem* 2006;**281**:35747–35756.

Choi Y, Ballow DJ, Xin Y, Rajkovic A. Lim homeobox gene, lhx8, is essential for mouse oocyte differentiation and survival. *Biol Reprod* 2008;**79**:442–449.

Christopoulos P, Mastorakos G, Gazouli M, Panidis D, Deligeoroglou E, Katsikis I, Papadias K, Diamandi-Kandarakis E, Creatsas G. Genetic variants in TCF7L2 and KCNJ11 genes in a Greek population with polycystic ovary syndrome. *Gynecol Endocrinol* 2008;**24**:486–490.

Chrzanowska KH, Szarras-Czapnik M, Gajdulewicz M, Kalina MA, Gajtko-Metera M, Walewska-Wolf M, Szufladowicz-Wozniak J, Rysiewski H, Gregorek H, Cukrowska B *et al*. High prevalence of primary ovarian insufficiency in girls and young women with Nijmegen breakage syndrome: evidence from a longitudinal study. *J Clin Endocrinol Metab* 2010;**95**:3133–3140.

Chun SY, McGee EA, Hsu SY, Minami S, LaPolt PS, Yao HH, Bahr JM, Gougeon A, Schomberg DW, Hsueh AJ. Restricted expression of WT1 messenger ribonucleic acid in immature ovarian follicles: uniformity in mammalian and avian species and maintenance during reproductive senescence. *Biol Reprod* 1999;**60**:365–373.

Ciampelli M, Fulghesu AM, Cucinelli F, Pavone V, Ronsisvalle E, Guido M, Caruso A, Lanzone A. Impact of insulin and body mass index on metabolic and endocrine variables in polycystic ovary syndrome. *Metabolism* 1999;**48**:167–172.

Cillo F, Brevini TA, Antonini S, Paffoni A, Ragni G, Gandolfi F. Association between human oocyte developmental competence and expression levels of some cumulus genes. *Reproduction* 2007;**134**:645–650.

Cooper HE, Spellacy WN, Prem KA, Cohen WD. Hereditary factors in the Stein–Leventhal syndrome. *Am J Obstet Gynecol* 1968;**100**:371–387.

Crisponi L, Deiana M, Loi A, Chiappe F, Uda M, Amati P, Bisceglia L, Zelante L, Nagaraja R, Porcu S *et al*. The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. *Nat Genet* 2001;**27**:159–166.

De Baere E, Dixon MJ, Small KW, Jabs EW, Leroy BP, Devriendt K, Gillerot Y, Mortier G, Meire F, Van Maldergem L *et al*. Spectrum of FOXL2 gene mutations in blepharophimosis-ptosis-epicanthus inversus (BPES) families demonstrates a genotype–phenotype correlation. *Hum Mol Genet* 2001;**10**:1591–1600.

de Boer EJ, den Tonkelaar I, te Velde ER, Burger CW, van Leeuwen FE. Increased risk of early menopausal transition and natural menopause after poor response at first IVF treatment. *Hum Reprod* 2003;**18**:1544–1552.

De Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. *Lancet* 2010;**376**:911–921.

DeSouza L, Diehl G, Yang EC, Guo J, Rodrigues MJ, Romaschin AD, Colgan TJ, Siu KW. Proteomic analysis of the proliferative and secretory phases of the human endometrium: protein identification and differential protein expression. *Proteomics* 2005;**5**:270–281.

Di Pasquale E, Beck-Peccoz P, Persani L. Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (BMP15) gene. *Am J Hum Genet* 2004;**75**:106–111.

Diaz FJ, Wigglesworth K, Eppig JJ. Oocytes determine cumulus cell lineage in mouse ovarian follicles. *J Cell Sci* 2007;**120**:1330–1340.

Dixit H, Deendayal M, Singh L. Mutational analysis of the mature peptide region of inhibin genes in Indian women with ovarian failure. *Hum Reprod* 2004;**19**:1760–1764.

Dixit H, Rao LK, Padmalatha V, Kanakavalli M, Deenadayal M, Gupta N, Chakravarty B, Singh L. Mutational screening of the coding region of growth differentiation factor 9 gene in Indian women with ovarian failure. *Menopause* 2005;**12**:749–754.

Dixit H, Rao KL, Padmalatha VV, Kanakavalli M, Deenadayal M, Gupta N, Chakrabarty BN, Singh L. Mutational analysis of the betaglycan gene-coding region in susceptibility for ovarian failure. *Hum Reprod* 2006;**21**:2041–2046.

Doherty E, Pakarinen P, Tiitinen A, Kiilavuori A, Huhtaniemi I, Forrest S, Aittomaki K. A novel mutation in the FSH receptor inhibiting signal transduction and causing primary ovarian failure. *J Clin Endocrinol Metab* 2002;**87**:1151–1155.

Domínguez F, Gadea B, Esteban FJ, Horcajadas JA, Pellicer A, Simon C. Comparative protein-profile analysis of implanted versus non-implanted human blastocysts. *Hum Reprod* 2008;**23**:1993–2000.

Domínguez F, Garrido-Gomez T, Lopez JA, Camafeita E, Quinonero A, Pellicer A, Simon C. Proteomic analysis of the human receptive versus non-receptive endometrium using differential in-gel electrophoresis and MALDI-MS unveils stathmin 1 and annexin A2 as differentially regulated. *Hum Reprod* 2009;**24**:2607–2617.

Dominguez F, Gadea B, Mercader A, Esteban FJ, Pellicer A, Simon C. Embryologic outcome and secretome profile of implanted blastocysts obtained after coculture in human endometrial epithelial cells versus the sequential system. *Fertil Steril* 2010;**93**:774–782.

Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 1996;**383**:531–535.

Edson MA, Nagaraja AK, Matzuk MM. The mammalian ovary from genesis to revelation. *Endocr Rev* 2009;**30**:624–712.

Edson MA, Lin YN, Matzuk MM. Deletion of the novel oocyte-enriched gene, Gpr149, leads to increased fertility in mice. *Endocrinology* 2010;**151**:358–368.

Elvin JA, Clark AT, Wang P, Wolfman NM, Matzuk MM. Paracrine actions of growth differentiation factor-9 in the mammalian ovary. *Mol Endocrinol* 1999a;**13**:1035–1048.

Elvin JA, Yan C, Wang P, Nishimori K, Matzuk MM. Molecular characterization of the follicle defects in the growth differentiation factor 9-deficient ovary. *Mol Endocrinol* 1999b;**13**:1018–1034.

Elvin JA, Yan C, Matzuk MM. Growth differentiation factor-9 stimulates progesterone synthesis in granulosa cells via a prostaglandin E2/EP2 receptor pathway. *Proc Natl Acad Sci USA* 2000;**97**:10288–10293.

Ennis S, Ward D, Murray A. Nonlinear association between CGG repeat number and age of menopause in FMR1 premutation carriers. *Eur J Hum Genet* 2006;**14**:253–255.

Eppig JJ, Pendola FL, Wigglesworth K, Pendola JK. Mouse oocytes regulate metabolic cooperativity between granulosa cells and oocytes: amino acid transport. *Biol Reprod* 2005;**73**:351–357.

Escobar-Morreale HF, Luque-Ramirez M, San Millan JL. The molecular-genetic basis of functional hyperandrogenism and the polycystic ovary syndrome. *Endocr Rev* 2005;**26**:251–282.

Farhi J, Homburg R, Ferber A, Orvieto R, Ben RZ. Non-response to ovarian stimulation in normogonadotrophic, normogonadal women: a clinical sign of impending onset of ovarian failure pre-empting the rise in basal follicle stimulating hormone levels. *Hum Reprod* 1997;**12**:241–243.

Ferriman D, Purdie AW. The inheritance of polycystic ovarian disease and a possible relationship to premature balding. *Clin Endocrinol (Oxf)* 1979;**11**:291–300.

Feuerstein P, Cadoret V, Dalbies-Tran R, Guerif F, Bidault R, Royere D. Gene expression in human cumulus cells: one approach to oocyte competence. *Hum Reprod* 2007;**22**:3069–3077.

Fogli A, Gauthier-Barichard F, Schiffmann R, Vanderhoof VH, Bakalov VK, Nelson LM, Boespflug-Tanguy O. Screening for known mutations in EIF2B genes in a large panel of patients with premature ovarian failure. *BMC Womens Health* 2004;**4**:8.

Fragouli E, Lenzi M, Ross R, Katz-Jaffe M, Schoolcraft WB, Wells D. Comprehensive molecular cytogenetic analysis of the human blastocyst stage. *Hum Reprod* 2008;**23**:2596–2608.

Franks S, McCarthy M. Genetics of ovarian disorders: polycystic ovary syndrome. *Rev Endocr Metab Disord* 2004;**5**:69–76.

Franks S, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. *Int J Androl* 2006;**29**:278–285.

Franks S, Webber LJ, Goh M, Valentine A, White DM, Conway GS, Wiltshire S, McCarthy MI. Ovarian morphology is a marker of heritable biochemical traits in sisters with polycystic ovaries. *J Clin Endocrinol Metab* 2008;**93**:3396–3402.

Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW *et al.* A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007;**316**:889–894.

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.

Galloway SM, McNatty KP, Cambridge LM, Laitinen MP, Juengel JL, Jokiranta TS, McLaren RJ, Luiro K, Dodds KG, Montgomery GW *et al.* Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nat Genet* 2000;**25**:279–283.

Gargett CE, Masuda H. Adult stem cells in the endometrium. *Mol Hum Reprod* 2010;**16**:818–834.

Gargett CE, Cervello I, Hubbard S, Simon C. Adult stem cells in the human endometrium. In Simon C, Pellicer A (eds). *Stem Cells in Human Reproduction: Basic Science and Therapeutic Potential (Reproductive Medicine & Assisted Reproductive Techniques)*. London: Informa Healthcare, 2009, 160–176.

Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. *Hum Reprod Update* 2008;**14**:159–177.

Givens JR. Familial polycystic ovarian disease. *Endocrinol Metab Clin North Am* 1988;**17**:771–783.

Gosden RG, Faddy MJ. Ovarian aging, follicular depletion, and steroidogenesis. *Exp Gerontol* 1994;**29**:265–274.

Gosden RG, Faddy MJ. Biological bases of premature ovarian failure. *Reprod Fertil Dev* 1998;**10**:73–78.

Goswami D, Conway GS. Premature ovarian failure. *Hum Reprod Update* 2005;**11**:391–410.

Guo SW. Emerging drugs for endometriosis. *Expert Opin Emerg Drugs* 2008;**13**:547–571.

Gurates B, Amsterdam A, Tamura M, Yang S, Zhou J, Fang Z, Amin S, Sebastian S, Bulun SE. WT1 and DAX-1 regulate SF-1-mediated human P450arom gene expression in gonadal cells. *Mol Cell Endocrinol* 2003;**208**:61–75.

Gutiérrez-Mateo C, Colls P, Sánchez-Garcia J, Escudero T, Prates R, Ketterson K, Wells D, Munné S. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. *Fertil Steril* 2011;**95**:953–958.

Hague WM, Adams J, Reeders ST, Peto TE, Jacobs HS. Familial polycystic ovaries: a genetic disease? *Clin Endocrinol (Oxf)* 1988;**29**:593–605.

Hamamah S, Matha V, Berthenet C, Anahory T, Loup V, Dechaud H, Hedon B, Fernandez A, Lamb N. Comparative protein expression profiling in human cumulus cells in relation to oocyte fertilization and ovarian stimulation protocol. *Reprod Biomed Online* 2006;**13**:807–814.

Hamatani T, Carter MG, Sharov AA, Ko MS. Dynamics of global gene expression changes during mouse preimplantation development. *Dev Cell* 2004;**6**:117–131.

Hamel M, Dufort I, Robert C, Gravel C, Leveille MC, Leader A, Sirard MA. Identification of differentially expressed markers in human follicular cells associated with competent oocytes. *Hum Reprod* 2008;**23**:1118–1127.

Hamel M, Dufort I, Robert C, Leveille MC, Leader A, Sirard MA. Genomic assessment of follicular marker genes as pregnancy predictors for human IVF. *Mol Hum Reprod* 2010;**16**:87–96.

Hanley NA, Rainey WE, Wilson DI, Ball SG, Parker KL. Expression profiles of SF-1, DAX1, and CYP17 in the human fetal adrenal gland: potential interactions in gene regulation. *Mol Endocrinol* 2001;**15**:57–68.

Hao J, Yamamoto M, Richardson TE, Chapman KM, Denard BS, Hammer RE, Zhao GQ, Hamra FK. Sohlh2 knockout mice are male-sterile because of degeneration of differentiating type A spermatogonia. *Stem Cells* 2008;**26**:1587–1597.

Haouzi D, Assou S, Mahmoud K, Tondeur S, Reme T, Hedon B, De Vos J, Hamamah S. Gene expression profile of human endometrial receptivity: comparison between natural and stimulated cycles for the same patients. *Hum Reprod* 2009a;**24**:1436–1445.

Haouzi D, Mahmoud K, Fourar M, Bendhaou K, Dechaud H, De Vos J, Reme T, Dewailly D, Hamamah S. Identification of new biomarkers of human endometrial receptivity in the natural cycle. *Hum Reprod* 2009b;**24**:198–205.

Harris SE, Chand AL, Winship IM, Gersak K, Aittomaki K, Shelling AN. Identification of novel mutations in FOXL2 associated with premature ovarian failure. *Mol Hum Reprod* 2002;**8**:729–733.

Hartge P. Genetics of reproductive lifespan. *Nat Genet* 2009;**41**:637–638.

Hawkins SM, Buchold GM, Matzuk MM. Minireview: the roles of small RNA pathways in reproductive medicine. *Mol Endocrinol* 2011a;**25**:1257–1279.

Hawkins SM, Creighton CJ, Han DY, Zariff A, Anderson ML, Gunaratne PH, Matzuk MM. Functional microRNA involved in endometriosis. *Mol Endocrinol* 2011b;**25**:821–832.

Horcajadas JA, Pellicer A, Simon C. Wide genomic analysis of human endometrial receptivity: new times, new opportunities. *Hum Reprod Update* 2007;**13**:77–86.

Huang HY. Medical treatment of endometriosis. *Chang Gung Med J* 2008;**31**:431–440.

Hull ML, Escareno CR, Godsland JM, Doig JR, Johnson CM, Phillips SC, Smith SK, Tavare S, Print CG, Charnock-Jones DS. Endometrial–peritoneal interactions during endometriotic lesion establishment. *Am J Pathol* 2008;**173**:700–715.

Jeong HJ, Kim HJ, Lee SH, Kwack K, Ahn SY, Choi YJ, Kim HG, Lee KW, Lee CN, Cha KY. Gene expression profiling of the pre-implantation mouse embryo by microarray analysis: comparison of the two-cell stage and two-cell block. *Theriogenology* 2006;**66**:785–796.

Jeyasuria P, Ikeda Y, Jamin SP, Zhao L, De Rooij DG, Themmen AP, Behringer RR, Parker KL. Cell-specific knockout of steroidogenic factor 1 reveals its essential roles in gonadal function. *Mol Endocrinol* 2004;**18**:1610–1619.

Johnson DS, Gemelos G, Baner J, Ryan A, Cinnioglu C, Banjevic M, Ross R, Alper M, Barrett B, Frederick J *et al.* Preclinical validation of a microarray method for full molecular karyotyping of blastomeres in a 24-h protocol. *Hum Reprod* 2010;**25**:1066–1075.

Joris H, Van den Abbeel E, Vos AD, Van Steirteghem A. Reduced survival after human embryo biopsy and subsequent cryopreservation. *Hum Reprod* 1999;**14**:2833–2837.

Joshi S, Davies H, Sims LP, Levy SE, Dean J. Ovarian gene expression in the absence of FIGLA, an oocyte-specific transcription factor. *BMC Dev Biol* 2007;**7**:67.

Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, Osteen K, Lessey BA, Giudice LC. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology* 2003;**144**:2870–2881.

Kato K, Yoshimoto M, Kato K, Adachi S, Yamayoshi A, Arima T, Asanoma K, Kyo S, Nakahata T, Wake N. Characterization of side-population cells in human normal endometrium. *Hum Reprod* 2007;**22**:1214–1223.

Kato K, Takao T, Kuboyama A, Tanaka Y, Ohgami T, Yamaguchi S, Adachi S, Yoneda T, Ueoka Y, Kato K *et al.* Endometrial cancer side-population cells show prominent migration and have a potential to differentiate into the mesenchymal cell lineage. *Am J Pathol* 2010;**176**:381–392.

Katz-Jaffe MG, Linck DW, Schoolcraft WB, Gardner DK. A proteomic analysis of mammalian preimplantation embryonic development. *Reproduction* 2005;**130**:899–905.

Katz-Jaffe MG, Gardner DK, Schoolcraft WB. Proteomic analysis of individual human embryos to identify novel biomarkers of development and viability. *Fertil Steril* 2006a;**85**:101–107.

Katz-Jaffe MG, Schoolcraft WB, Gardner DK. Analysis of protein expression (secretome) by human and mouse preimplantation embryos. *Fertil Steril* 2006b;**86**:678–685.

Katz-Jaffe MG, Fagouli E, Fillipovits J, Wells D, Schoolcraft WB. Relationship between the human blastocyst secretome and chromosomal constitution. *Fertil Steril* 2008; **90**:S80.

Katz-Jaffe MG, McCallie BR, Janesch A, Filipovits JA, Schoolcraft WB, Gardner DK. Blastocysts from patients with polycystic ovaries exhibit altered transcriptome and secretome. *Reprod Biomed Online* 2010;**21**:520–526.

Kehler J, Tolkunova E, Koschorz B, Pesce M, Gentile L, Boiani M, Lomeli H, Nagy A, McLaughlin KJ, Scholer HR et al. Oct4 is required for primordial germ cell survival. *EMBO Rep* 2004;**5**:1078–1083.

Kenigsberg S, Bentov Y, Chalifa-Caspi V, Potashnik G, Ofir R, Birk OS. Gene expression microarray profiles of cumulus cells in lean and overweight-obese polycystic ovary syndrome patients. *Mol Hum Reprod* 2009;**15**:89–103.

Keri RA, Nilson JH. A steroidogenic factor-1 binding site is required for activity of the luteinizing hormone beta subunit promoter in gonadotropes of transgenic mice. *J Biol Chem* 1996;**271**:10782–10785.

Knauff EA, Franke L, van Es MA, van den Berg LH, van der Schouw YT, Laven JS, Lambalk CB, Hoek A, Goverde AJ, Christin-Maitre S et al. Genome-wide association study in premature ovarian failure patients suggests ADAMTS19 as a possible candidate gene. *Hum Reprod* 2009;**24**:2372–2378.

Kovanci E, Simpson JL, Amato P, Rohozinski J, Heard MJ, Bishop CE, Carson SA. Oocyte-specific G-protein-coupled receptor 3 (GPR3): no perturbations found in 82 women with premature ovarian failure (first report). *Fertil Steril* 2008; **90**:1269–1271.

Laissue P, Christin-Maitre S, Touraine P, Kuttenn 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.

Laissue P, Christin-Maitre S, Bouchard P, Fellous M, Veitia RA. Mutations in the NOG gene are not a common cause of nonsyndromic premature ovarian failure. *Clin Endocrinol (Oxf)* 2007;**66**:900.

Lavorgna G, Ueda H, Clos J, Wu C. FTZ-F1, a steroid hormone receptor-like protein implicated in the activation of fushi tarazu. *Science* 1991;**252**: 848–851.

Lawson R, El-Toukhy T, Kassab A, Taylor A, Braude P, Parsons J, Seed P. Poor response to ovulation induction is a stronger predictor of early menopause than elevated basal FSH: a life table analysis. *Hum Reprod* 2003;**18**:527–533.

Layman LC. Editorial: BMP15—the first true ovarian determinant gene on the X-chromosome? *J Clin Endocrinol Metab* 2006;**91**:1673–1676.

Legro RS, Driscoll D, Strauss JF III, Fox J, Dunaif A. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. *Proc Natl Acad Sci USA* 1998; **95**:14956–14960.

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.

Leslie ND, Immerman EB, Flach JE, Florez M, Fridovich-Keil JL, Elsas LJ. The human galactose-1-phosphate uridyltransferase gene. *Genomics* 1992;**14**:474–480.

Liang L, Soyal SM, Dean J. FIGalpha, a germ cell specific transcription factor involved in the coordinate expression of the zona pellucida genes. *Development* 1997; **124**:4939–4947.

Lourenco D, Brauner R, Lin L, De Perdigo A, Weryha G, Muresan M, Boudjenah R, Guerra-Junior G, Maciel-Guerra AT, Achermann JC et al. Mutations in NR5A1 associated with ovarian insufficiency. *N Engl J Med* 2009;**360**:1200–1210.

Lunde O, Magnus P, Sandvik L, Hoglo S. Familial clustering in the polycystic ovarian syndrome. *Gynecol Obstet Invest* 1989;**28**:23–30.

Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* 1994;**77**:481–490.

Luoma P, Melberg A, Rinne JO, Kaukonen JA, Nupponen NN, Chalmers RM, Oldfors A, Rautakorpi I, Peltonen L, Majamaa K et al. Parkinsonism, premature menopause, and mitochondrial DNA polymerase gamma mutations: clinical and molecular genetic study. *Lancet* 2004;**364**:875–882.

Malinak LR, Buttram VC Jr, Elias S, Simpson JL. Heritage aspects of endometriosis. II. Clinical characteristics of familial endometriosis. *Am J Obstet Gynecol* 1980;**137**: 332–337.

Mandon-Pepin B, Touraine P, Kuttenn F, Derbois C, Rouxel A, Matsuda F, Nicolas A, Cotinot C, Fellous M. Genetic investigation of four meiotic genes in women with premature ovarian failure. *Eur J Endocrinol* 2008;**158**:107–115.

Masuda H, Matsuzaki Y, Hiratsu E, Ono M, Nagashima T, Kajitani T, Arase T, Oda H, Uchida H, Asada H et al. Stem cell-like properties of the endometrial side population: implication in endometrial regeneration. *PLoS One* 2010;**5**: e10387.

Matzuk MM, Lamb DJ. The biology of infertility: research advances and clinical challenges. *Nat Med* 2008;**14**:1197–1213.

McCallie B, Schoolcraft WB, Katz-Jaffe MG. Aberration of blastocyst microRNA expression is associated with human infertility. *Fertil Steril* 2010;**93**:2374–2382.

McKenzie LJ, Pangas SA, Carson SA, Kovanci E, Cisneros P, Buster JE, Amato P, Matzuk MM. Human cumulus granulosa cell gene expression: a predictor of fertilization and embryo selection in women undergoing IVF. *Hum Reprod* 2004; **19**:2869–2874.

Mehlmann LM, Saeki Y, Tanaka S, Brennan TJ, Evsikov AV, Pendola FL, Knowles BB, Eppig JJ, Jaffe LA. The Gs-linked receptor GPR3 maintains meiotic arrest in mammalian oocytes. *Science* 2004;**306**:1947–1950.

Mendelson CR, Kamat A. Mechanisms in the regulation of aromatase in developing ovary and placenta. *J Steroid Biochem Mol Biol* 2007;**106**:62–70.

Miller ME, Chatten J. Ovarian changes in ataxia telangiectasia. *Acta Paediatr Scand* 1967;**56**:559–561.

Murabito JM, Yang Q, Fox CS, Cupples LA. Genome-wide linkage analysis to age at natural menopause in a community-based sample: the Framingham Heart Study. *Fertil Steril* 2005;**84**:1674–1679.

Murray A, Webb J, Dennis N, Conway G, Morton N. Microdeletions in FMR2 may be a significant cause of premature ovarian failure. *J Med Genet* 1999;**36**:767–770.

Murray A, Bennett CE, Perry JR, Weedon MN, Jacobs PA, Morris DH, Orr N, Schoemaker MJ, Jones M, Ashworth A et al. Common genetic variants are significant risk factors for early menopause: results from the Breakthrough Generations Study. *Hum Mol Genet* 2011;**20**:186–192.

Nikolaou D, Lavery S, Turner C, Margara R, Trew G. Is there a link between an extremely poor response to ovarian hyperstimulation and early ovarian failure? *Hum Reprod* 2002;**17**:1106–1111.

Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril* 1997;**68**:585–596.

Nygren K, Andersen AN, Felberbaum R, Gianaroli L, de Mouzon J. On the benefit of assisted reproduction techniques, a comparison of the USA and Europe. *Hum Reprod* 2006;**21**:2194.

Ohlsson Teague EM, Van der Hoek KH, Van der Hoek MB, Perry N, Wagaarachchi P, Robertson SA, Print CG, Hull LM. MicroRNA-regulated pathways associated with endometriosis. *Mol Endocrinol* 2009;**23**:265–275.

Ohlsson Teague EM, Print CG, Hull ML. The role of microRNAs in endometriosis and associated reproductive conditions. *Hum Reprod Update* 2010;**16**:142–165.

Oldenburg RA, van Dooren MF, de Graaf B, Simons E, Govaerts L, Swagemakers S, Verkerk JM, Oostra BA, Bertoli-Avella AM. A genome-wide linkage scan in a Dutch family identifies a premature ovarian failure susceptibility locus. *Hum Reprod* 2008;**23**:2835–2841.

Painter JN, Anderson CA, Nyholt DR, Macgregor S, Lin J, Lee SH, Lambert A, Zhao ZZ, Roseman F, Guo Q et al. Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. *Nat Genet* 2011;**43**:51–54.

Pan Q, Luo X, Toloubeydokhti T, Chegini N. The expression profile of micro-RNA in endometrium and endometriosis and the influence of ovarian steroids on their expression. *Mol Hum Reprod* 2007;**13**:797–806.

Pangas SA, Jorgez CJ, Matzuk MM. Growth differentiation factor 9 regulates expression of the bone morphogenetic protein antagonist gremlin. *J Biol Chem* 2004;**279**:32281–32286.

Pangas SA, Choi Y, Ballow DJ, Zhao Y, Westphal H, Matzuk MM, Rajkovic A. Oogenesis requires germ cell-specific transcriptional regulators Sohlh1 and Lhx8. *Proc Natl Acad Sci USA* 2006;**103**:8090–8095.

Parks JC, McCallie BR, Janesch AM, Schoolcraft WB, Katz-Jaffe MG. Blastocyst gene expression correlates with implantation potential. *Fertil Steril* 2011;**95**: 1367–1372.

Perez GI, Jurisicova A, Wise L, Lipina T, Kanisek M, Bechard A, Takai Y, Hunt P, Roder J, Grynpas M et al. Absence of the proapoptotic Bax protein extends fertility and alleviates age-related health complications in female mice. *Proc Natl Acad Sci USA* 2007;**104**:5229–5234.

Pinero-Sagredo E, Nunes S, de Los Santos MJ, Celda B, Esteve V. NMR metabolic profile of human follicular fluid. *NMR Biomed* 2010;**23**:485–495.

Platteau P, Staessen C, Michiels A, Van Steirteghem A, Liebaers I, Devroey P. Which patients with recurrent implantation failure after IVF benefit from PGD for aneuploidy screening? *Reprod Biomed Online* 2006;**12**:334–339.

Prueitt RL, Ross JL, Zinn AR. Physical mapping of nine Xq translocation breakpoints and identification of XPNPEP2 as a premature ovarian failure candidate gene. *Cytogenet Cell Genet* 2000;**89**:44–50.

Prueitt RL, Chen H, Barnes RI, Zinn AR. Most X;autosome translocations associated with premature ovarian failure do not interrupt X-linked genes. *Cytogenet Genome Res* 2002;**97**:32–38.

Qin Y, Choi Y, Zhao H, Simpson JL, Chen ZJ, Rajkovic A. NOBOX homeobox mutation causes premature ovarian failure. *Am J Hum Genet* 2007;**81**:576–581.

Rajkovic A, Pangas SA, Ballow D, Suzumori N, Matzuk MM. NOBOX deficiency disrupts early folliculogenesis and oocyte-specific gene expression. *Science* 2004;**305**:1157–1159.

Rius M, Daina G, Obradors A, Ramos L, Velilla E, Fernandez S, Martinez-Passarell O, Benet J, Navarro J. Comprehensive embryo analysis of advanced maternal age-related aneuploidies and mosaicism by short comparative genomic hybridization. *Fertil Steril* 2011;**95**:413–416.

Rocca WA, Bower JH, Maraganore DM, Ahlskog JE, Grossardt BR, de Andrade M, Melton LJ III. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology* 2007;**69**:1074–1083.

Rocca WA, Bower JH, Maraganore DM, Ahlskog JE, Grossardt BR, de Andrade M, Melton LJ III. Increased risk of parkinsonism in women who underwent oophorectomy before menopause. *Neurology* 2008;**70**:200–209.

Russell DL, Robker RL. Molecular mechanisms of ovulation: co-ordination through the cumulus complex. *Hum Reprod Update* 2007;**13**:289–312.

Sanchez-Ribas I, Dominguez F, Barr J, Castro A, Mato JM, Simon C. Metabolomic profile as a novel non-invasive tool for human embryo aneuploidy assessment. *Fertil Steril* 2008;**90**:S100.

Sasson IE, Taylor HS. Stem cells and the pathogenesis of endometriosis. *Ann N Y Acad Sci* 2008;**1127**:106–115.

Sato K, Uehara S, Hashiyada M, Nabeshima H, Sugawara J, Terada Y, Yaegashi N, Okamura K. Genetic significance of skewed X-chromosome inactivation in premature ovarian failure. *Am J Med Genet A* 2004;**130A**:240–244.

Scott R, Seli E, Miller K, Sakkas D, Scott K, Burns DH. Noninvasive metabolomic profiling of human embryo culture media using Raman spectroscopy predicts embryonic reproductive potential: a prospective blinded pilot study. *Fertil Steril* 2008;**90**:77–83.

Seli E, Sakkas D, Scott R, Kwok SC, Rosendahl SM, Burns DH. Noninvasive metabolomic profiling of embryo culture media using Raman and near-infrared spectroscopy correlates with reproductive potential of embryos in women undergoing in vitro fertilization. *Fertil Steril* 2007;**88**:1350–1357.

Seli E, Vergouw CG, Morita H, Botros L, Roos P, Lambalk CB, Yamashita N, Kato O, Sakkas D. Noninvasive metabolomic profiling as an adjunct to morphology for noninvasive embryo assessment in women undergoing single embryo transfer. *Fertil Steril* 2010;**94**:535–542.

Shankar R, Gude N, Cullinane F, Brennecke S, Purcell AW, Moses EK. An emerging role for comprehensive proteome analysis in human pregnancy research. *Reproduction* 2005;**129**:685–696.

Shen WH, Moore CC, Ikeda Y, Parker KL, Ingraham HA. Nuclear receptor steroidogenic factor 1 regulates the mullerian inhibiting substance gene: a link to the sex determination cascade. *Cell* 1994;**77**:651–661.

Simpson JL, Elias S, Malinak LR, Buttram VC Jr. Heritable aspects of endometriosis. I. Genetic studies. *Am J Obstet Gynecol* 1980;**137**:327–331.

Snieder H, MacGregor AJ, Spector TD. Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. *J Clin Endocrinol Metab* 1998;**83**:1875–1880.

Su YQ, Wu X, O'Brien MJ, Pendola FL, Denegre JN, Matzuk MM, Eppig JJ. Synergistic roles of BMP15 and GDF9 in the development and function of the oocyte–cumulus cell complex in mice: genetic evidence for an oocyte-granulosa cell regulatory loop. *Dev Biol* 2004;**276**:64–73.

Sugawara T, Kiriakidou M, McAllister JM, Kallen CB, Strauss JF III. Multiple steroidogenic factor 1 binding elements in the human steroidogenic acute regulatory protein gene 5'-flanking region are required for maximal promoter activity and cyclic AMP responsiveness. *Biochemistry* 1997;**36**:7249–7255.

Sullivan AK, Marcus M, Epstein MP, Allen EG, Anido AE, Paquin JJ, Yadav-Shah M, Sherman SL. Association of FMR1 repeat size with ovarian dysfunction. *Hum Reprod* 2005;**20**:402–412.

Sutton ML, Cetica PD, Beconi MT, Kind KL, Gilchrist RB, Thompson JG. Influence of oocyte-secreted factors and culture duration on the metabolic activity of bovine cumulus cell complexes. *Reproduction* 2003;**126**:27–34.

Suzumori N, Yan C, Matzuk MM, Rajkovic A. Nobox is a homeobox-encoding gene preferentially expressed in primordial and growing oocytes. *Mech Dev* 2002;**111**:137–141.

Takahashi K, Ozaki T, Okada M, Kurioka H, Kanasaki H, Miyazaki K. Increased prevalence of luteinizing hormone beta-subunit variant in patients with premature ovarian failure. *Fertil Steril* 1999;**71**:96–101.

Tan S, Scherag A, Janssen OE, Hahn S, Lahner H, Dietz T, Scherag S, Grallert H, Vogel CI, Kimmig R et al. Large effects on body mass index and insulin resistance of fat mass and obesity associated gene (FTO) variants in patients with polycystic ovary syndrome (PCOS). *BMC Med Genet* 2010;**11**:12.

Tanaka TS, Jaradat SA, Lim MK, Kargul GJ, Wang X, Grahovac MJ, Pantano S, Sano Y, Piao Y, Nagaraja R et al. Genome-wide expression profiling of mid-gestation placenta and embryo using a 15,000 mouse developmental cDNA microarray. *Proc Natl Acad Sci USA* 2000;**97**:9127–9132.

Tempfer CB, Simoni M, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: part II—endometriosis. *Hum Reprod Update* 2009;**15**:97–118.

Tilly JL. Commuting the death sentence: how oocytes strive to survive. *Nat Rev Mol Cell Biol* 2001;**2**:838–848.

Torgerson DJ, Thomas RE, Reid DM. Mothers and daughters menopausal ages: is there a link? *Eur J Obstet Gynecol Reprod Biol* 1997;**74**:63–66.

Toyoda S, Miyazaki T, Miyazaki S, Yoshimura T, Yamamoto M, Tashiro F, Yamato E, Miyazaki J. Sohlh2 affects differentiation of KIT positive oocytes and spermatogonia. *Dev Biol* 2009;**325**:238–248.

Treloar SA, Do KA, Martin NG. Genetic influences on the age at menopause. *Lancet* 1998;**352**:1084–1085.

Tucci S, Futterweit W, Concepcion ES, Greenberg DA, Villanueva R, Davies TF, Tomer Y. Evidence for association of polycystic ovary syndrome in caucasian women with a marker at the insulin receptor gene locus. *J Clin Endocrinol Metab* 2001;**86**:446–449.

Tung JY, Rosen MP, Nelson LM, Turek PJ, Witte JS, Cramer DW, Cedars MI, Reijo-Pera RA. Novel missense mutations of the Deleted-in-AZoospermia-Like (DAZL) gene in infertile women and men. *Reprod Biol Endocrinol* 2006;**4**:40.

Uda M, Ottolenghi C, Crisponi L, Garcia JE, Deiana M, Kimber W, Forabosco A, Cao A, Schlessinger D, Pilia G. Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. *Hum Mol Genet* 2004;**13**:1171–1181.

Urbanek M. The genetics of the polycystic ovary syndrome. *Nat Clin Pract Endocrinol Metab* 2007;**3**:103–111.

Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, Strauss JF III, Spielman RS, Dunaif A. Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. *Proc Natl Acad Sci USA* 1999;**96**:8573–8578.

Urbanek M, Woodroffe A, Ewens KG, Diamanti-Kandarakis E, Legro RS, Strauss JF III, Dunaif A, Spielman RS. Candidate gene region for polycystic ovary syndrome on chromosome 19p13.2. *J Clin Endocrinol Metab* 2005;**90**:6623–6629.

van Asselt KM, Kok HS, Putter H, Wijmenga C, Peeters PH, van der Schouw YT, Grobbee DE, te Velde ER, Mosselman S, Pearson PL. Linkage analysis of extremely discordant and concordant sibling pairs identifies quantitative trait loci influencing variation in human menopausal age. *Am J Hum Genet* 2004;**74**:444–453.

van der Gaast MH, Eijkemans MJ, van der Net JB, de Boer EJ, Burger CW, van Leeuwen FE, Fauser BC, Macklon NS. Optimum number of oocytes for a successful first IVF treatment cycle. *Reprod Biomed Online* 2006;**13**:476–480.

van Dooren MF, Bertoli-Avellab AM, Oldenburg RA. Premature ovarian failure and gene polymorphisms. *Curr Opin Obstet Gynecol* 2009;**21**:313–317.

van Montfoort AP, Geraedts JP, Dumoulin JC, Stassen AP, Evers JL, Ayoubi TA. Differential gene expression in cumulus cells as a prognostic indicator of embryo viability: a microarray analysis. *Mol Hum Reprod* 2008;**14**:157–168.

Vanderhyden BC, Armstrong DT. Role of cumulus cells and serum on the in vitro maturation, fertilization, and subsequent development of rat oocytes. *Biol Reprod* 1989;**40**:720–728.

Varani S, Elvin JA, Yan C, DeMayo J, DeMayo FJ, Horton HF, Byrne MC, Matzuk MM. Knockout of pentraxin 3, a downstream target of growth differentiation factor-9, causes female subfertility. *Mol Endocrinol* 2002;**16**:1154–1167.

Verberg MF, Macklon NS, Nargund G, Frydman R, Devroey P, Broekmans FJ, Fauser BC. Mild ovarian stimulation for IVF. *Hum Reprod Update* 2009;**15**:13–29.

Vercellini P, Somigliana E, Vigano P, Abbiati A, Barbara G, Crosignani PG. Endometriosis: current therapies and new pharmacological developments. *Drugs* 2009;**69**:649–675.

Vinci G, Christin-Maitre S, Pasquier M, Bouchard P, Fellous M, Veitia RA. FOXO3a variants in patients with premature ovarian failure. *Clin Endocrinol (Oxf)* 2008; **68**:495–497.

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.

Voorhuis M, Onland-Moret NC, van der Schouw YT, Fauser BC, Broekmans FJ. Human studies on genetics of the age at natural menopause: a systematic review. *Hum Reprod Update* 2010;**16**:364–377.

Wang QT, Piotrowska K, Ciemerych MA, Milenkovic L, Scott MP, Davis RW, Zernicka-Goetz M. A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo. *Dev Cell* 2004;**6**:133–144.

Wathlet S, Adriaenssens T, Segers I, Verheyen G, Van de Velde H, Coucke W, Ron EI R, Devroey P, Smitz J. Cumulus cell gene expression predicts better cleavage-stage embryo or blastocyst development and pregnancy for ICSI patients. *Hum Reprod* 2011;**26**:1035–1051.

Watkins WJ, Umbers AJ, Woad KJ, Harris SE, Winship IM, Gersak K, Shelling AN. Mutational screening of FOXO3A and FOXO1A in women with premature ovarian failure. *Fertil Steril* 2006;**86**:1518–1521.

Weck J, Mayo KE. Switching of NR5A proteins associated with the inhibin alpha-subunit gene promoter after activation of the gene in granulosa cells. *Mol Endocrinol* 2006;**20**:1090–1103.

Wehr E, Schweighofer N, Moller R, Giuliani A, Pieber TR, Obermayer-Pietsch B. Association of FTO gene with hyperandrogenemia and metabolic parameters in women with polycystic ovary syndrome. *Metabolism* 2010;**59**:575–580.

Wells D, Fragouli E, Bianchi V, Borini A, Patrizio P. Identification of novel non-invasive biomarkers of oocyte aneuploidy. *Fertil Steril* 2008;**90**:S35.

Welt CK, Smith PC, Taylor AE. Evidence of early ovarian aging in fragile X premutation carriers. *J Clin Endocrinol Metab* 2004;**89**:4569–4574.

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.

Wu Y, Kajdacsy-Balla A, Strawn E, Basir Z, Halverson G, Jailwala P, Wang Y, Wang X, Ghosh S, Guo SW. Transcriptional characterizations of differences between eutopic and ectopic endometrium. *Endocrinology* 2006;**147**:232–246.

Xita N, Tsatsoulis A. Review: fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *J Clin Endocrinol Metab* 2006;**91**:1660–1666.

Xue Q, Lin Z, Yin P, Milad MP, Cheng YH, Confino E, Reierstad S, Bulun SE. Transcriptional activation of steroidogenic factor-1 by hypomethylation of the 5′ CpG island in endometriosis. *J Clin Endocrinol Metab* 2007;**92**:3261–3267.

Yan C, Wang P, DeMayo J, DeMayo FJ, Elvin JA, Carino C, Prasad SV, Skinner SS, Dunbar BS, Dube JL et al. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol Endocrinol* 2001;**15**: 854–866.

Zhang X, Jafari N, Barnes RB, Confino E, Milad M, Kazer RR. Studies of gene expression in human cumulus cells indicate pentraxin 3 as a possible marker for oocyte quality. *Fertil Steril* 2005;**83**:1169–1179.

Zhao H, Chen ZJ, Qin Y, Shi Y, Wang S, Choi Y, Simpson JL, Rajkovic A. Transcription factor FIGLA is mutated in patients with premature ovarian failure. *Am J Hum Genet* 2008;**82**:1342–1348.

Zhao ZZ, Croft L, Nyholt DR, Chapman B, Treloar SA, Hull ML, Montgomery GW. Evaluation of polymorphisms in predicted target sites for micro RNAs differentially expressed in endometriosis. *Mol Hum Reprod* 2011;**17**: 92–103.