Environmental and developmental origins of ovarian reserve

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BACKGROUND: Oocyte number is established early in life before a gradual loss of this ovarian reserve during reproductive life until oocyte availability becomes limiting at the menopause. Although there is a large genetic component to the ovarian reserve achieved before birth, other influences including the maternal endocrine and nutritional milieu, and environmental factors may represent important developmental determinants. Environmental and nutritional factors may also modify the downward trajectory of ovarian reserve in adult life. The combination of these early and later life influences has the potential to lead to diminished ovarian reserve, compromising fertility in later reproductive years and altering age at natural menopause.

METHODS: Literature searches of the ISI Web of Knowledge database were carried out using the main terms ‘ovarian reserve’ and ‘menopause’ and other terms encompassing a variety of factors with potential effects on ovarian reserve. The various searches were inspected manually and the relevant papers selected for critical analysis and interpretation.

RESULTS: Evidence was identified supporting the view that elevated prenatal androgens have an adverse effect on the early establishment of ovarian reserve, although the implications for ovarian reserve in the polycystic ovary syndrome (which may also be programmed through prenatal androgen exposure) remain uncertain. Recent evidence is cited suggesting that effects of maternal nutrient restriction on ovarian reserve may also involve changes in prenatal androgen exposure. A general rationale is developed through examination of evidence which emphasizes the roles of the aryl hydrocarbon receptor (AHR) and the estrogen receptor (ER) systems in ovarian reserve modulation. Because of their similarity to the natural ligands, many environmental compounds have the ability to bind to these receptors (albeit at lower affinities) and thereby have the potential to influence either the initial setting of ovarian reserve during development or the trajectory of ovarian reserve during adult life. For example, exposure to compounds in cigarette smoke may accelerate loss of ovarian reserve in smokers leading to diminished ovarian reserve, earlier age at last child and earlier menopause. Socioeconomic factors are clearly associated with age at natural menopause, with correlations with economic status and education level. However, such effects in western societies are in general small, and the underlying mechanisms remain unclear.

CONCLUSIONS: Exposure to many environmental compounds, particularly to those that leach from plastics and other synthetic materials, is commonplace in modern societies to the extent that many are found at measurable concentrations in body fluids within most of the population.

† These authors have made an equal contribution to this work.
Relating fluid levels of individual compounds to parameters reflecting ovarian reserve in selected populations appears to be an effective way forward and, indeed, some early-stage findings do show some cause for concern. There is a pressing need for the development of practical advice enabling women to minimize their intake of AHn/ER ligands, perhaps through dietary/cosmetic choices or improved food packaging.

**Key words:** ovarian reserve / nutrition / prenatal androgens / environmental / socioeconomic

### Introduction

As women continue to delay childbearing, and the contribution of ovarian ageing as a determinant of fertility outcomes increases, interest in the genetic, environmental and developmental determinants of ovarian reserve has grown. The term ‘ovarian reserve’ refers to the functional potential of the ovary and reflects the number and quality of oocytes within it. Many women with diminishing ovarian reserve will seek in vitro fertilization (IVF) to treat their age-related subfertility, and since ovarian reserve is, in turn, one of the main determinants of success, much effort is put into assessing an individual’s reserve status. Most IVF programmes now routinely perform some measurement of ovarian reserve in order to guide patient selection, prognosis and stimulation regimen (Broekmans et al., 2009).

The consequences of diminished ovarian reserve are potentially serious, not only for fertility but also to general health through early menopause. In recent years much effort has been expended on increasing our understanding of the genomic mechanisms, which regulate ovarian ageing and which underlie the variation in the age at natural menopause (ANM; reviewed by Voorhuis et al., 2010). For example, genome-wide association studies have identified a number of genetic loci significantly associated with ANM (He et al., 2009; Perry et al., 2009; Stolk et al., 2009, 2012). However, there is growing evidence that early developmental conditions as well as adult exposures may impact on ovarian reserve and hence long-term fertility and reproductive health. Understanding the mechanisms involved may provide additional insights into the regulation of ovarian reserve and suggest potential interventions which could modulate the rate of ovarian ageing. In this article, the developmental and environmental factors which may affect ovarian reserve are reviewed.

### Methodology

The database used was the ISI Web of Knowledge (Thomson Reuters). Firstly, the general term ‘ovarian reserve’ was used and the most recent 500 references inspected. Combinations of ‘ovarian reserve’ and the following separate terms were sought: ‘smoking’, ‘androgen’, ‘fetal development’, ‘nutrition’, ‘bisphenol A’, ‘environmental agents’, and ‘endocrine disruptors’. The general combination of ‘menopause AND age’ was then used and, again, the most recent 500 inspected. Combinations of ‘menopause AND age’ with ‘smoking’, ‘obesity’, ‘endocrine disruptors’, ‘environmental agents’, ‘nutrition’ and ‘andro- gen’ were then explored. More specific searches using the combined terms ‘pregnatal AND androgen AND polycystic’, ‘androgen AND fetal programming’ and ‘estrogen AND ovarian reserve’ were also employed. The various searches undertaken were inspected manually and relevant papers selected.

### Background

#### Ovarian reserve and fertility

The ovarian reserve reflects primarily the number of primordial follicles, which are non-growing follicles (NGFs), together with follicles recruited into the later pre-antral and antral stages of development ultimately capable of ovulation. During development, primordial germ cells (PGCs) migrate from the hind gut to the genital ridge and increase in number through mitosis. Eventually these PGCs, now called oogonia, enter meiosis and become integrated into primordial follicles where their development is arrested during the first meiotic division. The consensus view is that this meiotic arrest now sets an initial limit to ovarian reserve at this stage of development and that neo-oogenesis does not occur in adult life. In recent years this concept has been challenged by evidence supporting an element of replenishment of follicle numbers by post-natal oocyte production from germline stem cells in the ovary or bone marrow (Johnson et al., 2004; Zou et al., 2009). However, this concept remains controversial.

Extensive germ cell death occurs during early development as oocytes become assembled into the primordial follicle pool (Baker, 1963; Pepling and Spradling, 2001). Indeed, assembly of oocytes into primordial follicles is important for their continued survival (Hirshfield, 1991; Fulton et al., 2005). These mechanisms result in a peak number of 6–7 million primordial follicles at around 18 weeks of gestation which reduces to ~1–2 million at birth through apoptosis. This establishes and fixes ovarian reserve available in individual women before birth (Skinner, 2005). Subsequent decline in oocyte numbers continues through follicular atresia and apoptosis (Baker, 1963; Vaskivuo et al., 2001). By puberty, ~300 000 oocytes remain and then further loss throughout reproductive life leads to a virtual exhaustion of follicle numbers by the age of the menopause where less than 1000 will remain (Faddy et al., 1992). Follicular loss occurs through apoptosis during the transition from primordial to primary follicles, at later pre-antral and antral stages, and as a result of ovulation during menstrual cycles after sexual maturity. The progressive reduction in oocyte numbers throughout reproductive life is accompanied by an associated decline in oocyte quality (Broekmans et al., 2007). For example, it is thought that ‘trisomy 21 oocytes’ (i.e. those capable of giving rise to trisomy 21 children with Down syndrome) tend to remain in the ovarian reserve of older women as these oocytes have a slower rate of elimination through apoptosis (Hulten et al., 2010). It is proposed that the rising proportion of trisomy 21 oocytes within the oocyte reserve of older women contributes to the observed increased incidence of Down syndrome births for these women.

A number of models have been proposed which describe the rate of follicular loss with ageing. A recent study (Wallace and Kelsey, 2010) constructed a model of ovarian reserve from conception to menopause that best fits the histological evidence. This model predicts a maximum mean NGF population of ~300 000 per ovary established by birth with a 95% prediction interval of 35 000 to >2 million. Assuming similar decay characteristics, low or high values for original ovarian reserve predicted early or late menopausal ages, respectively.

As follicle numbers gradually decline with age, a sequence of reproductive events occurs, beginning with reduced fecundity and natural sterility, and progressing through menstrual cycle irregularity towards a complete cessation of menstruation at the menopause. This sequence
is depicted in Fig. 1, which shows data on ‘age at last child’ indicating onset of sterility (te Velde and Pearson, 2002), followed by ‘onset of cycle irregularity’ often marked by a 2–3 day shortening of menstrual cycle length (Treloar et al., 1967), which signals a period of increasing cycle irregularity culminating in completion of the menopause. The idea has developed that this sequence unfolds according to ‘fixed time intervals’ between the subsequent stages (te Velde and Pearson, 2002; Broekmans et al., 2009). In consequence, it can be envisaged that a lower trajectory of ovarian reserve (perhaps initiated before birth through lower reserve values) is likely to lead to earlier ‘age at last child’ as well as earlier ages for the subsequent events (depicted in Fig. 2). Similar earlier onsets of these events associated with declining reproductive life might be expected if accelerated loss of ovarian reserve was experienced during post-natal life because of adverse environmental or nutritional challenges (shown hypothetically in Fig. 2).

‘Developmental origins of health and disease’ hypothesis

It is well established that organ development during prenatal life is influenced by the prevailing intrauterine environment, and that adverse conditions during fetal life can lead to an increased risk of adult-onset diseases, such as type-2 diabetes and hypertension (Barker, 1995). The effects of this ‘fetal programming’ are likely to be wide ranging and may include alterations in the reproductive axis. A poor fetal environment has been associated with limitations in the development of both the endocrine pancreas (Fowden et al., 2005) and kidney (Schreuder et al., 2006), and this may be part of a more generalized ‘brain-sparing’ effect on abdominal organs that are compromised by reduced blood flow. Because of the close anatomical relationship between the ovary and the kidney during development and similarities in the derivation of the blood supply, it has been hypothesized that intratertine growth retardation may lead to a fall in the number of follicles developing within the ovary (examined by de Bruin et al., 2001). The development of the ‘Developmental origins of health and disease’ (DOHAD) hypothesis has been largely concerned with the availability of nutrients to the fetus as it develops and raises the question as to whether poor maternal nutrition could have an adverse effect on ovarian reserve. Casting the net wider, it is possible that circulating hormones during pregnancy can influence ovarian reserve. Also the potential effects of maternal exposure to environmental contaminants need to be considered (Mark-Kappeler et al., 2011). Further exposure during post-natal life and adulthood as well as social factors may also impact on ANM. The range of possible factors affecting ovarian reserve is summarized in Fig. 3, which sets the scene for an examination of evidence supporting each of these potential influences.

Factors influencing ovarian reserve

Intrauterine nutrition

Key evidence for the impact of intrauterine nutrition on ovarian reserve is summarized in Table I.

Maternal dietary composition has been shown to affect the development of the reproductive system in the fetus (Rhind, 2004). Some of these alterations may be mediated via changes in cellular proliferation in fetal ovaries, which may be regulated by numerous fetal and maternal factors related to the maternal diet (Lea et al., 2006). The question arises as to whether these regulatory mechanisms allow the level of maternal nutrition (perhaps at critical stages in gestation) to influence the establishment of the ovarian reserve in the fetus, with consequent later effects on ovarian reserve in adult life (consistent with the DOHAD hypothesis).

A number of studies have examined the effect of maternal diet on the development of follicles in the fetal ovary with little emphasis on potential longer term effects on adult ovarian reserve. Thus, studies on maternal feed restriction during pregnancy in sheep (Rae et al., 2001) showed a trend toward the development of smaller ovaries in the fetal lambs, with fewer advanced follicles without changing germ cell numbers. This finding was confirmed by a similar study in sheep (Murdoch et al., 2003) showing that a 50% dietary reduction during gestation led to a...
show poor (or delayed) follicular development without clear effects on overall oocyte numbers.

What are the effects of prenatal nutrient restriction on adult ovarian reserve? In sheep it appears that, indeed, there is a longer term impact of such restriction manifest as a lower ovulation rate in the adult (Rae et al., 2002), although measures of ovarian reserve were not applied in the study. In cows, an experimental model has been developed whereby maternal nutrient restriction during the first third of gestation leads to a range of alterations in female offspring including cardiovascular effects (consistent with the DOHAD model) without overall changes in birthweight and post-natal growth (Mossa et al., 2013). Importantly, these animals showed diminished ovarian reserve as measured by anti-Mullerian hormone (AMH), follicle stimulating hormone (FSH) and antral follicle count, over a broad range of ages up to 86 weeks. Moreover, the period of maternal nutrient restriction was concomitant with elevated levels of maternal testosterone. The possibility that fetal exposure to elevated androgens has the potential to negatively impact ovarian reserve will be examined later.

**Human studies**

If intrauterine growth were to have a clinically significant impact on follicular numbers, birthweight should be related to later measures of ovarian reserve. Evidence supporting this hypothesis derives from studies on young women born small for gestational age who were shown to have reduced ovarian volume, increased FSH and reduced ovulation rates (Ibanez et al., 2000; 2002a, b) suggesting poor ovarian reserve. However, anatomical analysis of human fetal ovaries does not suggest that slow fetal growth is associated with a smaller follicle pool size or accelerated depletion (de Bruin et al., 2001). Moreover, later studies which examined indicators of ovarian reserve in adolescent girls and young women found no significant reduction with earlier poor intrauterine growth (Hart et al., 2009) or low birthweight allowing for gestational age (Kerkhof et al., 2010; Lem et al., 2011). Also, a recent study showed no association between low birthweight and later development of ovulatory dysfunction in women (Shayeb et al., 2013).

An important end-point for studying the possible effects of intrauterine development on ovarian reserve is offered by using the ANM. Although one study comparing female twin pairs (Treloar et al., 2000) did not find evidence that lower birthweight was associated with earlier menopause, a recent report has re-examined this potential relationship using birthweight standardized for gestational age (Tom et al., 2010) and found that both extremes of birthweight (~2.5 kg or >4 kg) were indeed associated with an earlier age at menopause. In contrast, examination of a British birth cohort showed that ANM varied positively with duration of breastfeeding and weight at 2 years, but not with birthweight (Hardy and Kuh, 2002). This supported an earlier observation that low weight gain in infancy is associated with an earlier age at menopause (Hardy and Kuh, 2002). The importance of adequate lactation to support establishment of ovarian reserve during early post-natal growth has been confirmed by a study in rats where maternal malnutrition during lactation was shown to adversely affect follicular numbers (Ferreira et al., 2010).

**Prenatal endogenous androgens, development of polycystic ovary syndrome and associated effects on ovarian reserve**

Key evidence is summarized in Table II.

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**Table I** Key studies on the potential link between intrauterine nutrition and development of ovarian reserve.

<table>
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<tr>
<th>Study</th>
<th>Key findings</th>
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<tbody>
<tr>
<td>Raë et al. (2001)</td>
<td>In sheep, maternal feed restriction during pregnancy results in fewer ovarian follicles without changing oocyte numbers. Results suggest an inhibition of the transition from primordial to early primary follicles.</td>
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<tr>
<td>Da Silva-Buttkus et al. (2003)</td>
<td>Intrauterine growth restricted piglets (runts) showed increased numbers of primordial follicles, fewer primary and no secondary follicles compared with normals.</td>
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<tr>
<td>Mossa et al. (2013)</td>
<td>In cows, maternal nutrient restriction leads to lower ovarian reserve in female offspring.</td>
</tr>
<tr>
<td>Ibanez et al. (2000, 2002a, b)</td>
<td>Young women born small for gestational age were shown to have reduced ovarian volume and increased FSH.</td>
</tr>
<tr>
<td>De Bruin et al. (2001)</td>
<td>Analysis of human fetal ovaries does not support a smaller follicle pool size or accelerated depletion.</td>
</tr>
<tr>
<td>Cresswell et al. (1997)</td>
<td>Low weight gain in infancy, rather than low birthweight is associated with earlier menopause.</td>
</tr>
<tr>
<td>Treloar et al. (2000)</td>
<td>Comparing female twin pairs, no evidence found that lower birthweight was associated with earlier menopause.</td>
</tr>
<tr>
<td>Hardy and Kuh (2002)</td>
<td>Age at menopause varied with weight at 2 years but not birthweight.</td>
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23% fall in fetal ovarian weight without loss of germ cells. In pigs, intrauterine growth restriction was also associated with delayed follicular development, since more primordial follicles, fewer primary follicles and no secondary follicles were found in ‘runt’ piglets, which also demonstrated a lower ovarian mass (Da Silva-Buttkus et al., 2003). These studies on fetal nutrient restriction during pregnancy have therefore tended to
There is considerable evidence that fetal programming through androgens contributes to the development of polycystic ovary syndrome (PCOS) experienced in adult life (reviewed by Xita and Tsatsoulis, 2006). The impact of exposure of female fetuses to excess androgens has been studied in cases of ‘fetal androgen excess disorders’, where the subsequent development of a PCOS phenotype was observed even when androgen levels had been normalized after birth (Hague et al., 1990; Barnes et al., 1994). Further evidence derives from work on female Rhesus monkeys treated prenatally with exogenous androgens. Similarly, these monkeys developed clinical and biochemical features in adult life completely consistent with those observed in women with PCOS (Abbott et al., 2005).

**In utero** exposure to excess androgens may also influence ovarian reserve. In a study in sheep treated prenatally with testosterone from 30 to 90 days of gestation, a reduction in the total oocyte pool was observed at term (Steckler et al., 2005). A morphometric analysis of follicular distribution in this study showed that enhanced follicular recruitment had led to a decrease in the percentage of primordial follicles, a potential contributor to early reproductive failure. A similar study has been carried out in Rhesus monkeys where early prenatal androgenization was very effective in causing diminished ovarian reserve (Dumesic et al., 2009). This study was particularly telling in that a progressive fall in ovarian reserve with age (8.7–24.6 years) was assessed by measuring serum AMH. Not only was this fall accelerated after prenatal androgen treatment, but also IVF cycles carried out on these monkeys led to a greatly reduced yield of oocytes, which may also show a lack of developmental competence after fertilization (Dumesic et al., 2002).

The question arises as to whether women with PCOS experience adverse changes in ovarian reserve consistent with their prior exposure to elevated prenatal androgens. Evidence for a programming effect of in utero androgen exposure on ovarian reserve has already been discussed (Xita and Tsatsoulis, 2006) and it has been shown in a rat model that adult ovarian morphology, metabolic status and PCOS phenotype is dependent on the timing and level of androgen exposure in utero (Wu et al., 2010; Amalfi et al., 2012).

However, long-term follow-up of women with PCOS does not provide evidence of earlier menopause (reviewed by Nikolaou and Gilling-Smith, 2004) as would be expected under conditions of diminished ovarian reserve. Indeed, sustained fertility has been noted in women with PCOS during later reproductive years as measured by oocyte yield in IVF procedures (Mellembakken et al., 2011), and the stock of antral follicles as measured by ultrasound (Hudecova et al., 2009). Assessment of ovarian reserve through measurement of serum AMH reveals higher levels of this factor in women with PCOS (La Marca et al., 2009), although this may be explained (at least partially) by a higher level of AMH production per granulosa cell in PCOS patients (Pellatt et al., 2007) and the larger number of small follicles present. Microscopic analysis of follicles (including primordial and pre-antral stages) in ovarian biopsies from women with PCOS also revealed a substantial increase in overall follicular numbers, although the proportion of follicles at the primordial stage was decreased (Webber et al., 2003). It would appear that any initial effect of prenatal androgens on ovarian reserve through enhanced recruitment is masked by stockpiling of follicles in PCOS not only because of a lack of progress through ovulation but also an increased persistence of follicles in PCOS, which appear to have a longer lifespan, at least in culture (Webber et al., 2007).

Although some of the effects of androgens are mediated via specific androgen receptors (ARs), there is the potential for androgens to be converted to estrogens, through the action of aromatase, leading to effects that are mediated through estrogen receptors (ERs). From a wide physiological perspective, the actions of androgens and estrogens would generally be considered as being opposite leading to male and female phenotypes, respectively. However, the ovarian effects of fetal estrogen and androgen excess are remarkably similar (explained by Abbott et al., 2006), and are consistent with the notion that androgen action in these circumstances is a composite of direct and indirect actions through AR and ER, respectively. The separate contributions made by these two arms of androgen action have been studied in sheep where the effects of testosterone have been compared with those of dihydrotestosterone (DHT), which is a non-aromatizable androgen (Smith et al., 2009). In this study, prenatal testosterone was more effective than DHT in reducing ovarian reserve in females measured at 10 months of age, revealing an important role of estrogenic programming in this process. Moreover, more than half the ovarian reserve was lost in testosterone-treated animals at this time, providing a potential explanation for loss of cyclicity seen in these animals by 2 years of age as part of early ovarian ageing. Although this work on sheep provides a general blueprint for how prenatal androgen excess may be mediated via AR- and ER-mediated actions, the details of this balance remain largely unexplored in the other animal models used to study prenatal androgen excess (Abbott et al., 2006). Indeed, there appears to be little information on how this balance is manifest in the relevant primate models and in PCOS. The balance of receptors in the fetal ovary may vary according to stages of gonadal/follicular development. A lack of AR in primordial follicles (Horie et al., 1992) appears to rule out a direct action of androgens at this early stage, although direct action has been demonstrated in small (100–120 μm) follicles derived from mice, leading to increased follicular growth (Wang et al., 2001). The observed stimulatory action of androgens on follicular recruitment (e.g. Steckler et al., 2005) is therefore either mediated through ER or through AR at points beyond the primordial stage. It is

<table>
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<tr>
<th>Study</th>
<th>Key findings</th>
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<tr>
<td>Abbott et al. (2005)</td>
<td>Female rhesus monkeys treated prenatally with exogenous androgens develop features in adult life consistent with those observed in women with PCOS</td>
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<tr>
<td>Xita and Tsatsoulis (2006)</td>
<td>Evidence reviewed linking prenatal androgens with later development of PCOS</td>
</tr>
<tr>
<td>Steckler et al. (2005)</td>
<td>In sheep, in utero exposure to excess androgens causes a reduction in ovarian reserve through enhanced follicular recruitment</td>
</tr>
<tr>
<td>Dumesic et al. (2009)</td>
<td>In Rhesus monkeys, prenatal androgenization caused later development of decreased ovarian reserve, as measured through serum AMH and decreased oocyte yield in IVF cycles</td>
</tr>
<tr>
<td>Smith et al. (2009)</td>
<td>In sheep, evidence presented that much of the effect of prenatal androgens on ovarian reserve is through conversion to estrogens</td>
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PCOS, polycystic ovary syndrome; AMH, anti-Mullerian hormone.
possible that aspects of androgen action that involve mediation via ER may have relevance to the potential effects of environmental estrogens on ovarian reserve.

If prenatal exposure to androgens is important in the aetiology of PCOS, the question remains as to the source of prenatal androgens contributing to this increased exposure. One possibility is that higher fetal exposure derives from increased circulating androgens in pregnant women with PCOS (Sir-Petermann et al., 2002), where the normal protection through conversion to estrogens by placental aromatase may be compromised by higher insulin. Alternatively, the level of androgen production, availability (through changes in circulating sex hormone-binding globulin) or conversion through aromatase could be set genetically in the fetus (reviewed by Xita and Tsatsoulis, 2006). The potential for alteration of these control mechanisms through exposure to environmental chemicals with androgenic or anti-androgenic action is also worth considering.

Environmental exposures

It is well established that many chemicals present within the environment, as well as natural and artificial components of our diet, have the potential to interfere with the physiological role of hormones. These ‘endocrine-disrupting chemicals’ (EDCs) may interfere with hormone biosynthesis, signalling or metabolism (Diamanti-Kandarakis et al., 2009; Shanle and Xu, 2011). Many of these agents act as steroid receptor agonists and antagonists, particularly with regard to estrogenicity and androgenicity (Soto et al., 1994; Andersen et al., 2002; Shanle and Xu, 2011). EDCs can also interfere with the production of steroid hormones through specific effects on steroidogenesis (Chedrese and Feyles, 2001; Crellin et al., 2001), and can alter steroidal metabolism (Sanderson et al., 2000, 2002). The detection of residues of environmental agents in human serum, follicular fluid and seminal plasma (Younglai et al., 2002) underlines the importance and relevance of this area of study.

For the purposes of this review, it is useful to consider the effects of EDCs on both the ER and aryl hydrocarbon receptor (AHR) systems as ligand binding to these receptors has been shown to influence oogenesis (see sections below). ERs have relatively large binding pockets and have been described as relatively promiscuous in that they exhibit a broad specificity for ligands (Zenzes et al., 2000; Cooper et al., 1995). AHRs show a broad range of specificity enabling the binding of a diverse array of environmental chemicals (Shanle and Xu, 2011) and represent ‘open doors’ whereby exogenous hydrophobic ligands are able to gain access and influence processes involved in gametogenesis (Fig. 4). There is much cross talk between the AHR and ER systems through a variety of mechanisms to be discussed below once each receptor system is considered separately.

Interactions through the AHR system

It is well established that the smoking of cigarettes has an adverse impact on the reproductive health of women (Sharara et al., 1994; Hughes and Brennan, 1996; Shiverick and Salafia, 1999; Hruska et al., 2000; Mlynarcikova et al., 2005). This may include an adverse effect on ovarian reserve (key evidence is summarized in Table III). Smoking effects on ovarian reserve may involve the action of PAHs contained in cigarette smoke. The potential for PAHs to act through the AHR system in the ovary is underlined by studies which show that the AHR protein is abundantly expressed in granulosa cells and oocytes of mouse follicles at all stages

![Figure 4](image-url)  
**Figure 4** Mediation of the effects of endocrine-disrupting chemicals through the aryl hydrocarbon receptor and estrogen receptor systems.

<table>
<thead>
<tr>
<th>Study</th>
<th>Key findings</th>
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<tr>
<td>Harlow and Signorello (2000)</td>
<td>Evidence summarized indicating that smoking hastens the onset of the menopause by as much as 1–2 years</td>
</tr>
<tr>
<td>Sharara et al. (1994)</td>
<td>Smoking accelerates the development of diminished ovarian reserve as evidenced by poorer FSH responses during the ‘Clomiphene Citrate Challenge Test’</td>
</tr>
<tr>
<td>Cooper et al. (1995)</td>
<td>Active and passive smoking are associated with elevated FSH concentrations in women 38–49 years old</td>
</tr>
<tr>
<td>Zenzes et al. (1997)</td>
<td>Smoking reduces number of mature oocytes retrieved following ovulation induction in IVF cycles</td>
</tr>
<tr>
<td>El-Nemr et al. (1998)</td>
<td>Raised basal FSH and lower numbers of oocytes retrieved in IVF cycles in smokers compared with non-smokers</td>
</tr>
<tr>
<td>Fuentes et al. (2010)</td>
<td>Recent smoking is significantly associated with a decreased number of retrieved ova in IVF cycles</td>
</tr>
<tr>
<td>Waylen et al. (2009)</td>
<td>General review of the effect of smoking on the outcomes of ART. Poorer ovarian reserve is likely to be an important factor in lower pregnancy rates in smokers following ART</td>
</tr>
</tbody>
</table>

ART, assisted reproduction technologies.
of development (Robles et al., 2000). AHR-deficient mice that do not express the receptor protein accumulate a 2-fold higher number of primordial follicles compared with wild-type females at Day 4 post-partum because of a lack of oocyte apoptosis (Robles et al., 2000). Clearly AHR, acting through an as yet unidentified natural ligand, has the potential to regulate oocyte reserve during female gametogenesis. This concept now provides a mechanistic rationale for previous work which showed that exposure in utero to environmental compounds (now known to be capable of acting as AHR ligands) could interfere with oogenesis during critical periods of development thereby affecting the oocyte reserve at birth and subsequent fertility in later life (Mattison, 1982, 1983; Mattison et al., 1983, 1989).

More recent studies have expanded our appreciation of the intracellular mechanisms important in PAH-induced oocyte destruction. Apparently, activation of AHR causes the induction of Bax, as part of a group of pro-apoptotic cell death regulators, which leads to apoptosis (Matikainen et al., 2001; Pru et al., 2009). This PAH-induced pro-apoptotic mechanism has been shown to operate in a model system involving the conversion of human embryonic stem cells to primordial germ cells (Kee et al., 2010), and also applies to oocytes within human ovarian biopsies grafted into immunodeficient mice (Matikainen et al., 2001). Murine fetal ovaries cultured in the presence of PAHs showed extensive germ cell loss, which was prevented by a selective AHR antagonist (Matikainen et al., 2002). A further model involving mice exposed to PAHs during the prepregnancy and/or lactational periods showed a two-thirds depletion of oocyte reserve, which again was prevented with a specific AHR antagonist (Jurisicova et al., 2007). Clearly, these studies have uncovered an important pathway involved in the establishment of oocyte reserve.

Consistent with this potentially adverse effect on ovarian reserve is a report that maternal smoking during pregnancy is associated with earlier ANM of resulting daughters (Strohsnitter et al., 2001; Pru et al., 2009). This PAH-induced pro-apoptotic mechanism has been shown to operate in a model system involving the conversion of human embryonic stem cells to primordial germ cells (Kee et al., 2010), and also applies to oocytes within human ovarian biopsies grafted into immunodeficient mice (Matikainen et al., 2001). Murine fetal ovaries cultured in the presence of PAHs showed extensive germ cell loss, which was prevented by a selective AHR antagonist (Matikainen et al., 2002). A further model involving mice exposed to PAHs during the prepregnancy and/or lactational periods showed a two-thirds depletion of oocyte reserve, which again was prevented with a specific AHR antagonist (Jurisicova et al., 2007). Clearly, these studies have uncovered an important pathway involved in the establishment of oocyte reserve.

Maternal smoking will also involve exposure to other compounds in cigarette smoke with potentially harmful effects on ovarian development. For example, fetal and neonatal exposure of rats to nicotine results in ovarian dysfunction in adult female offspring (Holloway et al., 2006).

An attempt to measure directly the effects of maternal smoking on the number of fetal oogonia and somatic cells involved examining human first-trimester ovaries from fetuses legally aborted in relation to maternal smoking habits (Lutterodt et al., 2009). This study showed that although there was not a reduction in the number of oogonia with smoking, there was a significant decrease in the number of somatic cells with prenatal exposure to maternal smoking. Because oocytes cannot survive without enclosure in somatic cells, the authors concluded that the observed lack of somatic cells at this early stage could have long-range consequences on ovarian reserve and fertility later in life.

It is well accepted that tobacco smoking during adult life (often starting during teenage years) leads to an early onset of the menopause in women by as much as 1–2 years (Harlow and Signorello, 2000; Gold et al., 2001). Confirmation that smoking is a significant independent risk factor for early ANM has been provided by a recent meta-analysis of 11 studies (Sun et al., 2012). Because the menopause is associated with the exhaustion of the ovarian follicular pool, it is suggested that the early onset with smoking indicates a tendency for ovarian follicular reserve to fall with smoking so that the point of exhaustion comes earlier. Evidence for this has been provided by indirect measures of ovarian reserve, such as circulating levels of FSH. One study on women aged 38–49 years showed that basal FSH levels were 66% higher in active smokers than in non-smokers and 39% higher in passive smokers than in non-smokers (Cooper et al., 1995). Analogous evidence obtained using the ‘Clomiphene citrate challenge test’ also showed that smoking hastens the depletion of ovarian reserve (Sharara et al., 1994). Two meta-analyses (Augood et al., 1998; Waylen et al., 2009) have confirmed that smoking has an adverse effect on the clinical outcomes of assisted reproduction technologies (ART). This is partly explained by decreased numbers of retrieved oocytes obtained in treatment cycles of smokers (Zenzes et al., 1997; El-Nemr et al., 1998; Fuentes et al., 2010). Using AMH as a measure of ovarian reserve, several studies report lower serum AMH in active (as opposed to former or passive) smokers (Plante et al., 2010; Freour et al., 2008) consistent with impaired oocyte recovery in IVF cycles (Freour et al., 2008). A longitudinal study has confirmed that women who smoke experience a more rapid fall in AMH values during later reproductive years associated with an earlier age at final menstrual period (Sowers et al., 2010).

We have examined earlier in the review how an adverse influence on ovarian reserve (such as that elicited by smoking) may impact not only on the ANM, but also the ‘age at last child’ (summarized in Fig. 2). Using perinatal data collected on 50 082 births between 2002 and 2010 within Southampton, UK, we were able to examine directly this possibility by comparing the ‘age at last child’ for groups of smokers and non-smokers (Macklon et al., 2011). We established that, indeed, on average smokers had their last child several years earlier than non-smokers (mean ages in years: never smoked, 30.74 ± 5.19; up to 10 cigarettes per day, 26.61 ± 5.78; 10–20 per day, 26.36 ± 5.43; >20 per day, 28.02 ± 6.7). The difference between smokers and non-smokers remained significant (P < 0.05) after allowing for differences in socioeconomic factors including level of educational attainment. Although unknown confounders may have influenced these findings, they are consistent with the concept that smoking leads to a lower trajectory of ovarian reserve depletion. Not only does this lead to an earlier menopause (as evidenced by others), but also brings forward preceding events, such as ‘age at last child’, which in turn will be a factor of decreased fertility as ovarian reserve ebbs away.

The mechanisms underlying smoking toxicity on reproductive and ovarian function are complex because of the considerable array of circulating metabolites associated with inhalation of tobacco smoke (Hoffmann and Hoffmann, 1997). Some of these will interact directly with the gamete pool through receptors such as AHR. Other mechanisms have been postulated including an effect of smoking on ovarian vascularization. Higher amounts of soluble vascular endothelial growth factor receptor-1 secreted in smokers may result in decreased angiogenesis and reduced oocyte maturation (Motejlek et al., 2006).

**Interactions with the ER system**

We have seen how the broad specificity of the ER-binding pocket provides an ‘open door’ to a range of chemicals in our diet and in the environment allowing their access to sensitive control mechanisms concerned with
Estrogenic effects on the development of multi-oocyte follicles

During embryonic development, oocytes initially form in clusters called ‘nests’. In rodents these break down after birth to yield individual oocytes surrounded by granulosa cells (the primordial follicles), and as part of this process about two-thirds of the original oocytes are lost through apoptosis (Pepling, 2012). It is proposed that falling steroid hormone levels (particularly estrogens) after birth act as a trigger for nest breakdown as a range of estrogens and progesterone are able to inhibit nest breakdown experimentally both in vivo and in vitro (Chen et al., 2007). Such inhibition leads to an increase in multi-oocyte follicles (MOFs), which are thought to be the remnants of nests which have undergone incomplete breakdown. Development of MOFs has also been demonstrated after administration of DES to neonatal mice (Iguchi et al., 1990), and similar effects shown for genistein (Jefferson et al., 2002) and BPA (Suzuki et al., 2002). Such effects of DES and genistein are thought to be mediated predominantly through ER-beta (Jefferson et al., 2002; Kim et al., 2009).

There are important implications of early estrogen exposure for oocyte reserve. Thus, neonatal rats treated with estradiol develop ovaries containing fewer primordial follicles (Sotomayor-Zarate et al., 2011). Also, mice exposed in utero to DES show a decreased capacity for reproduction with lower numbers of oocytes, which have a greater tendency to degenerate (McLachlan et al., 1982). This tallies with a reduction in the average ANM for daughters born to mothers who took DES during pregnancy (Hatch et al., 2006; Steiner et al., 2010). The case for the lower affinity dietary estrogens, such as genistein, is less clear. It is well established that MOFs, generated as a result of neonatal treatment of rodents, persist into adulthood (Cimafranca et al., 2010; Losa et al., 2011) and are a clear sign that these estrogens can fundamentally change the course of oocyte development. Moreover, these effects appear to manifest at levels of neonatal genistein exposure consistent with levels experienced by infants fed on soy formula (Cimafranca et al., 2010). However, mice treated neonatally with genistein appear to show normal fertility in adulthood (Cimafranca et al., 2010), and blastocysts generated from oocytes from genistein-treated mice show full developmental competence if transplanted into pseudo-pregnant controls (Jefferson et al., 2009). Interpretation of the effect of BPA on MOF formation is complicated by the observation that a range of effects of BPA occur at levels of the compound that are below the level necessary for stimulation of MOF generation. For example, BPA may increase primordial follicle activation (and thereby reduce primordial numbers) at levels ineffective in changing MOF generation (Rodriguez et al., 2010). A similar depletion of ovarian reserve, through increased follicular recruitment, has been observed in lambs treated neonatally with BPA (Rivera et al., 2011).

The importance of these mechanisms involving estrogens and MOF formation in humans is open to question as primordial follicle formation occurs at ∼4–5 months of gestation in our species at a time when circulating estradiol is rising. Perhaps local conditions in fetal ovaries at this time may involve a fall in estradiol or progesterone and that this is the trigger for primordial follicle formation (Pepling, 2012). A potential mechanism here is that steroid-binding proteins, such as α-fetoprotein, which bind estrogen and circulate during pregnancy, may serve to sequester estrogen and thereby lower free estrogen levels in the fetal ovary (Mizejewski, 2004). Studies on BPA exposure during pregnancy in rhesus monkeys (Hunt et al., 2012), where circulating estrogen levels are maintained during pregnancy as in humans, does confirm that increased MOF generation can occur in response to BPA exposure during the critical phase of primordial follicle formation in this species.

Certainly, MOFs are present within the human ovary (Gougeon, 1981) and may be retrieved during IVF procedures (Dandekar et al., 1988). From work in rhesus monkeys exposed to BPA (Hunt et al., 2012) it would...
appear that some oocytes found as part of MOFs may be present in very sub-optimal circumstances, perhaps trapped within the granulosa layer of follicles or in structures where they remain unenclosed. Clearly, such oocytes may not always be equivalent to those in uniovular follicles in terms of their ability to ovulate, fertilize and develop. Overall, evidence suggests that early estrogen exposure, perhaps through diet or environmental exposure, has the potential to influence the prevalence of MOFs in the adult ovary and thereby affect fertility in the longer term.

**BPA and the development of meiotic abnormalities in oocytes**

Key evidence relating to this aspect of BPA action is summarized in Table IV. As mentioned above, BPA has a complex interaction with the various ER systems in cells. Recognition that BPA action includes effects on meiosis derives from observations on mice accidentally exposed to BPA leaching from plastic materials in their cages and water bottles (Hunt et al., 2003). Remarkably, this low level of exposure to BPA was sufficient to cause an increase in aneuploidy in oocytes. Further work suggested that BPA disrupts meiotic spindle formation, centrosome dynamics and chromosomal alignment and segregation (Can et al., 2005; Lenie et al., 2008), as well as meiotic arrest (Eichenlaub-Ritter et al., 2008). Recent studies on human oocytes in vitro have confirmed that BPA has toxic effects on critical processes in meiosis, such as pairing synopsis and recombination, as well as oocyte survival (Brieno-Enriquez et al., 2011).

Further evidence suggests that circulating BPA derived from maternal ingestion has the potential to pass to the fetal compartment and disrupt early oogenesis in utero (Susiarjo et al., 2007). In this study, pregnant mice in mid-gestation were treated with environmentally relevant doses of BPA and the effect on the fetal ovaries assessed. Oocytes within these ovaries displayed an array of meiotic abnormalities, and female offspring allowed to develop following this fetal exposure to BPA displayed a large increase in aneuploidy in their oocytes. Meiotic disturbance in fetal ovaries has also been demonstrated in rhesus monkeys following maternal exposure to BPA during a critical time in pregnancy coinciding with the onset of meiosis (Hunt et al., 2012). These actions of BPA on meiosis are mimicked in the fetal ovaries of mice made homozygous for a targeted disruption of ER-beta, and BPA has no additional action in these ER-beta null females (Susiarjo et al., 2007), implicating ER-beta in the mediation of the meiotic effects of BPA. A further study showed that perinatal exposure to environmentally relevant doses of BPA causes, over time, a marked reduction in the fertility and fecundity of mice (Cabaton et al., 2011), although it is not clear whether this effect is mediated by changes in ovarian reserve. Clearly, these studies raise a concern that exposure to BPA during development may have longer term influences on ovarian reserve and consequent fertility in female offspring.

**Estrogenic effects of BPA on granulosa cell viability**

Studies on BPA effects in culture have demonstrated a concentration-dependent inhibitory action on estradiol production by porcine (Mlynarcikova et al., 2005) and rat (Zhou et al., 2008) granulosa cells with a concomitant reduction in the expression of P450 aromata mRNA (Zhou et al., 2008). At least part of this inhibitory effect is mediated through decreased granulosa cell viability induced by BPA (Xu et al., 2002). Treatment with BPA increased apoptosis in granulosa cells in a time- and dose-dependent manner changing the balance of anti-apoptotic (measured as expression of bcl2) and apoptotic (measured as BAX signals) (Xu et al., 2002). As granulosa cell apoptosis is part of the process of follicular atresia (Hughes and Gorospe, 1991; Yu et al., 2004), BPA exposure might act by accelerating follicular atresia limiting the availability of mature oocytes. The basis of this effect may be the ability of BPA to act as an ER antagonist, although the interaction of BPA with the different sub-types of ER is complex (Hiroi et al., 1999).

**Relevance of BPA effects on oocytes to human fertility**

There is limited information on the relevance to the human situation of these studies describing potential effects of BPA on oocyte development, whether generated through fetal or adult exposure. However, in women receiving fertility treatment, urinary BPA concentrations have been shown to be inversely associated with the number of oocytes retrieved in IVF cycles and with peak estradiol levels (Mok-Lin et al., 2010). A further study investigated the relationship between serum BPA levels and IVF outcomes in women undergoing IVF treatment (Lamb et al., 2008), finding an inverse association between serum BPA and circulating estradiol as well as a trend towards a lower pregnancy rate in patients with higher BPA levels. Moreover, raised serum BPA has been associated with recurrent miscarriage (Sugiura-Ogasawara et al., 2005). Clearly, there is a need for further work to establish the reproductive consequences of BPA exposure in the human situation.

**Interaction of organochlorine pesticides with ER-alpha and ER-beta**

The organochlorine pesticides, most notably dichlorodiphenyltrichloroethane (DDT) and methoxychlor, show estrogenic activity through direct interaction with both ER-alpha and ER-beta (reviewed by Shanle and Xu, 2011). The estrogenic activity of DDT derives mainly from the o.p’-DDT isomer, which shows slight preference for ER-beta (Kuiper et al., 1998), while the estrogenic action of methoxychlor appears to be associated with its major metabolite, HPTE, which acts as an agonist for ER-alpha and an antagonist for ER-beta (Gaido et al., 1999).

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**Table IV** Key studies linking the exposure to BPA with development of meiotic abnormalities in oocytes.

<table>
<thead>
<tr>
<th>Study</th>
<th>Key finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunt et al. (2003)</td>
<td>Exposure of female mice to BPA released from damaged plastic caging material and water bottles causes increased aneuploidy in their oocytes</td>
</tr>
<tr>
<td>Can et al. (2005)</td>
<td>BPA induces delay in the meiotic cell cycle altering centrosome and spindle microtubular organization</td>
</tr>
<tr>
<td>Lenie et al. (2008)</td>
<td>Exposure of mouse follicular cultures to BPA causes meiotic abnormalities including changes in spindle formation</td>
</tr>
<tr>
<td>Mlynarcikova et al. (2005)</td>
<td>BPA causes alterations in steroid production by ovarian granulosa cells</td>
</tr>
<tr>
<td>Susiarjo et al. (2007)</td>
<td>Exposure of pregnant mice to environmentally relevant doses of BPA caused meiotic abnormalities in the oocytes within the fetal ovaries. The ovaries of the resulting female mice contained oocytes with increased rates of aneuploidy</td>
</tr>
<tr>
<td>Mok-lin et al. (2010)</td>
<td>BPA is detectable in the majority of women undergoing IVF and is inversely associated with the number of oocytes retrieved</td>
</tr>
</tbody>
</table>

BPA, bisphenol A.
A range of toxicological studies on organochlorine pesticides have been carried out in relation to reproduction (reviewed by Tiemann, 2008) and adverse effects on folliculogenesis and oocyte maturation demonstrated. Also, treatment of neonatal mice with methoxychlor (0.1, 0.5 or 1 mg i.p.) led to a dose-dependent decrease in ovulated eggs in adult life following ovulation stimulation (Eroschenko et al., 1997). This underlines the possibility that exposure to organochlorine compounds early in life could have a longer term influence on oocyte reserve. Whether such effects involve the mechanisms already discussed for non-pesticide estrogens, such as the generation of MOFs or perturbations of meiotic events, remains an open question.

It is difficult to estimate the potential effects of pesticide exposure on the fertility of women. Certainly, the general population is exposed to trace amounts of various pesticides used in agriculture and present within various foods. These may be absorbed and become detectable within bodily fluids. Trace quantities of organochlorine compounds (e.g. polychlorinated biphenyls (PCBs), the DDT metabolite known as DDE and hexachlorobenzene) are detectable in human follicular fluid (Trapp et al., 1984; Baukloh et al., 1985; Jarrell et al., 1993), and in serum where an association has been found with earlier ANM (Akkina et al., 2004). Particular occupational situations enable an evaluation of the impact of higher levels of pesticide exposure. Thus, occupational exposure of women who work in a greenhouse to pesticides has been shown to be associated with a reduction in fecundability (Abell et al., 2000) as measured by ‘time to pregnancy’. An association between general agricultural work history and female infertility has also been observed (Fuortes et al., 1997) which could be connected with pesticide exposure.

Another useful approach, providing insight as to the reproductive significance of trace amounts of organochlorine compounds found in bodily fluids, is to examine the relationship between levels of individual contaminants in follicular fluid (harvested as part of an IVF procedure) and parameters relating to IVF success. One such study found that follicular fluid levels of DDE (the most frequently detected pesticide residue, and at the highest levels detected) were associated with failed fertilization (Younglai et al., 2002), although a previous study had not found such an association (Jarrell et al., 1993). The potential significance of the trace quantities of contaminants found in follicular fluid is further highlighted by an observed enhancement of basal and FSH-stimulated aromatizing enzyme activity in human granulosa cells exposed in vitro to concentrations of DDE similar to those present in human follicular fluid (Younglai et al., 2004). This work raises the concern that enhanced estradiol production as well as being components of cosmetics and other personal care products (Kruger et al., 2008) allowing potential absorption through the skin.

We can speculate about the implications of these complex AHR/ER interactions for oogenesis. It can be presumed that each individual female (whether in utero, as a neonate, child or adult) will experience a specific ‘AHR/ER ligand footprint’ within her specific environment, and this will depend on exposure to plasticizers, cigarette smoke, pesticides, cosmetics, dietary estrogens, etc. The effects of some of these potentially adverse exposures may be additive or even synergistic, whereas some exposures, particularly to phytoestrogens, may have a mitigating effect on the effects of other AHR/ER ligands.

**Interactions with the AR system**

We have already discussed how high levels of circulating, endogenous androgens during fetal life may adversely affect ovarian reserve. Aspects of this effect are mediated through ER interactions after androgen conversion to estrogen, concomitant with some direct androgenic action through the AR system, which is present in reproductive tissues including the ovary (Horie et al., 1992). Most environmental compounds capable of interacting with AR appear to have antagonistic activity (Roy et al., 2004; Kruger et al., 2008), however, making it unlikely that they would mimic or influence the agonist activity required for these adverse effects on ovarian reserve.

The question remains, therefore, as to whether lack of androgen action in females during fetal development (associated with exposure to environmental androgen antagonists) could represent a problem for establishment of ovarian reserve. Certainly, in the male, the possibility that AR antagonism through environmental exposure leads to disordered development of spermatogenesis has been entertained (Sharpe, 2010). Experimentally in females, the extreme case is offered by the ‘AR knockout’ mouse model (Hu et al., 2004), where ovarian fetal development remains relatively normal, although there are later effects on follicular development. We can conclude therefore that antagonism at the level of AR through environmental exposure is unlikely to influence the establishment of ovarian reserve during fetal development.

**Socioeconomic factors**

Using ANM as a proxy measure, it is possible to examine the influence on ovarian reserve of socioeconomic factors. A survey of literature on menopausal ages across the world (Palacios et al., 2010) reveals a remarkably constant value for ANM (median c. 50–53 years) within Europe and North America, although small regional differences may occur (e.g. southern versus north-eastern USA (McKnight et al., 2011). However, in poorer areas of Asia and Latin America, women with low socioeconomic status may have a substantially earlier ANM. For
example, the menopausal transition for Bolivian Movima women (a native American population) was reported to occur at an average age of 42.3 years (Castelo-Branco et al., 2005). A similar, early ANM has been reported for poorer women within a northern Indian rural community (Kapur et al., 2009), where the effect was offset with higher socioeconomic status. Certainly, an association between socioeconomic adversity and earlier ANM has been a consistent finding (Velez et al., 2009; Gold, 2011).

The question arises as to whether these 'geographical/socioeconomic' effects are mediated through alterations in diet. Certainly, severe caloric restriction appears to lower ANM as evidenced by a follow-up of females involved in the 1944–1945 Dutch famine (Elias et al., 2003), with exposure in childhood (from 2 to 6 years of age) leading to a fall in ANM of nearly 2 years. However, even this extreme dietary exposure led to a relatively modest change in ANM compared with the reduction experienced by women in poor, rural communities in Asia and Latin America (see above). Perhaps, the effects of life-long exposure to a poor diet are mediated by a combination of 'early life events' (either pre- or post-natal) and adult experience. These combined effects may lead to a marked reduction in ovarian reserve and substantially reduced ANM. One approach to unraveling the potentially separate effects of 'early life' and 'later life' exposure to nutritional and socioeconomic effects would be to study the effect of migration where early life is spent in one culture and adult life is spent in another. This has been examined for migration from the UK to Australia (Lee et al., 2004) where the transition appears to lead to a small increase in ANM. However, migrant populations are likely to be better educated and perhaps healthier than the remaining non-migrants, making data on the effects of migration difficult to interpret.

In order to understand further the mechanisms underpinning the effect of socioeconomic status on ANM, the role of fat deposition and distribution needs to be considered. It is well established that adipose tissue is a major site of conversion of androstenedione to estrone and that this process is positively related to body weight (Macdonald et al., 1978). The possibility that the extra estrogenic activity provided by estrone tends to delay ANM may explain an observed, positive effect of fat deposition on menopausal age (Akahoshi et al., 2002; Palmer et al., 2003). However, it is noteworthy that some studies have not

### Table V Summary of main findings linking developmental, nutritional and environmental factors with changes in ovarian reserve.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Research finding</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal androgens</td>
<td>In sheep, excess prenatal androgens caused a reduction in ovarian reserve</td>
<td>Steckler et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>In Rhesus monkeys, prenatal androgens caused the later development of decreased OR as measured through serum AMH or oocyte yield in IVF cycles</td>
<td>Dumesic et al. (2009)</td>
</tr>
<tr>
<td>AHR reception (smoking effects possibly through AHR)</td>
<td>In mice, AHR regulates OR during gametogenesis</td>
<td>Robles et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>In mice, exposure to PAHs during pre-pregnancy or lactational periods caused depletion of OR which was prevented with a specific AHR antagonist</td>
<td>Jurisicova et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Adult smoking is a significant independent factor for early ANM</td>
<td>Many studies</td>
</tr>
<tr>
<td></td>
<td>Recent female smoking associated with decreased number of retrieved oocytes in IVF cycles</td>
<td>Fuentes et al. (2010)</td>
</tr>
<tr>
<td>ER reception, MOF generation</td>
<td>In rodents, falling levels of steroid hormones trigger the breakdown of ‘oocyte nests’ to yield individual follicles. Inhibition of this process via ER reception led to development of MOFs</td>
<td>Chen et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Development of MOFs after administration of DES to neonatal mice, and similar effects shown for genistein and BPA</td>
<td>Several studies (see text)</td>
</tr>
<tr>
<td></td>
<td>In Rhesus monkeys, exposure of third trimester fetuses to BPA during time of follicle formation caused an increase in MOFs similar to that seen in rodents</td>
<td>Hunt et al. (2012)</td>
</tr>
<tr>
<td>ER reception, meiotic changes</td>
<td>Initial description in mice of meiotic effects in oocytes associated with the leaching of BPA from cages and water bottles</td>
<td>Hunt et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>In mice, female offspring previously exposed to BPA in fetal life showed large increases in aneuploidy in oocytes as a result of meiotic abnormalities. Effects shown to be through ER-B</td>
<td>Susiarjo et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>In Rhesus monkeys, meiotic disturbance shown in fetal ovaries following maternal exposure to BPA during critical time of pregnancy coinciding with the onset of meiosis</td>
<td>Hunt et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>In women, inverse correlation shown between urinary BPA levels and oocytes retrieved in IVF cycles</td>
<td>Mok-Lin et al. (2010)</td>
</tr>
<tr>
<td>Phthalates, PCBs</td>
<td>Levels of urinary phthalate metabolites shown to be associated with lower ovarian reserve parameters in women</td>
<td>Souter et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>In women, serum or urine PCBs shown to be associated with earlier ANM</td>
<td>Grindler et al. (2012)</td>
</tr>
<tr>
<td>Maternal nutrition</td>
<td>In cows, maternal nutrient restriction led to a lower ovarian reserve in female offspring</td>
<td>Mossa et al. (2013)</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>Socioeconomic adversity is associated with lower ANM. Mechanism unknown but likely to be nutritional</td>
<td>Many studies</td>
</tr>
<tr>
<td>Underweight/overweight</td>
<td>In general, diets rich in fat, protein and meat tend to delay ANM</td>
<td>Nagel et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Underweight women tend to start hormone replacement therapy earlier than normal weight women perhaps indicating an earlier ANM</td>
<td>Hardy et al. (2008)</td>
</tr>
</tbody>
</table>

*Abstract only at the time of writing.  
OR, ovarian reserve; AHR, aryl hydrocarbon receptor; ANM, age at natural menopause; MOF, multi-oocyte follicle; DES, diethylstilbestrol; BPA, bisphenol-A; PCB, phthalates/ polychlorinated biphenyl; PAHs, polycyclic aromatic hydrocarbons.*
found an association between BMI and ANM (summarized by Hardy et al., 2008). In general, diets rich in fat, protein and meat tend to delay ANM (Nagel et al., 2005). It is therefore reasonable to suspect that the very much reduced ANM in poorer, rural communities may be partially explained by the lack of fat deposition expected under such poor dietary conditions. This fits with the observation that underweight women in a UK cohort tended to start hormone replacement therapy earlier than normal weight women, perhaps indicating an earlier ANM (Hardy et al., 2008). It is also consistent with the ideas of Frisch (1987) who suggested that low levels of body fat within populations may lead to a block in menstrual cycling, causing both a delay in menarche and earlier ANM. However, studies on individuals within single populations have not, in general, shown an inverse correlation between the ages of menarche and menopause (van Noord et al., 1997).

Looking at the other end of the weight spectrum, it is pertinent to examine whether ovarian reserve is influenced by obesity, an increasingly common feature of modern societies. Recent evidence shows that AMH levels are lower in obese women compared with the non-obese in late reproductive years (Freeman et al., 2007). Also, there is an association between obesity and poor ovarian response as measured during ART (Spandorfer et al., 2004). One likely interpretation of these data is that obesity leads to impaired follicular development (Freeman et al., 2007), a suggestion supported by observations that obese women experience some menstrual cycle disruption, such as longer cycles with shortened luteal phases (Santoro et al., 2004). It is an interesting possibility that obesity may, therefore, slow the rate of oocyte utilization and, if anything, have a delaying effect on the menopausal transition. Clearly, more research is needed to establish how obesity influences ovarian follicular function and reserve.

As well as a dietary explanation for socioeconomic or geographic influences on ANM in different populations, many authors have entertained the idea that differing levels of educational attainment may have some physiologic effect on ovarian function, and this has been reviewed recently (Canavez et al., 2011). Examining 29 published studies, this review concluded that there is a weak, positive association between educational attainment and ANM, although the level of significance of this effect varied between studies. Adjustment for confounding variables (such as smoking and diet) is always problematic in such studies, and makes interpretation of their results difficult. However, an observed link between cognitive ability in childhood and later ANM (Richards et al., 1999) appears to provide some extra support for the notion that educational attainment and ability are linked to the timing of the menopause.

**Conclusion**

A summary of the main findings relating developmental, nutritional and environmental factors to changing ovarian reserve is shown in Table V.

**Early life influences: nutrition**

The idea that nutritional influences in early life set initial levels of ovarian reserve (which later decay as adult life proceeds) is a concept which requires further investigation. However, the much earlier ANM experienced by many women in certain poorer, rural communities (Castelo-Branco et al., 2005; Kapur et al., 2009) requires an explanation which may involve an effect on ovarian reserve of inadequate nutrition, although the relative contributions of early versus later life influences is not clear.

**Early life influences: environmental**

A convincing case has been made for important effects of EDCs on ovarian reserve, and this is part of a wider picture of the effects of these chemicals on a variety of female reproductive functions and disorders (Crain et al., 2008). Studies on the estrogenic plasticizer BPA have clearly shown disrupting effects of this chemical on the process of meiosis in oocytes leading to aneuploidy (Can et al., 2005) and also to cell cycle arrest (Eichenlaub-Ritter et al., 2008). Such effects of BPA on meiosis are evident in the fetal ovaries of pregnant mice exposed to the chemical with resulting female offspring showing an increased incidence of compromised oocytes (Susiarjo et al., 2007). Recent work in rhesus monkeys (Hunt et al., 2012) has also confirmed earlier work in rodents suggesting that BPA exposure during pregnancy has the capability of increasing the level of MOFs within the ovaries of fetuses, where many oocytes remain unincorporated into primordial follicles.

There is good evidence that prenatal exposure to androgens contributes to the development of PCOS in adult life (Xita and Tsatsoulis, 2006), and also to a diminished ovarian reserve in rhesus monkeys (Dumesic et al., 2009) as evidenced by decreased oocyte recovery in an IVF programme. Some of these effects of androgens may be mediated by estrogenic action following conversion by aromatization (Smith et al., 2009). The observation that significant levels of BPA accumulate in amniotic fluid during pregnancy (Ikezuki et al., 2002) suggests that endocrine disruptors, such as BPA, would be available during pregnancy with perhaps the potential to influence androgen-related events important in establishing ovarian reserve.

**Post-natal/adult influences: nutrition**

Extreme dietary restriction in childhood, such as that associated with the 1944–1945 Dutch famine (Elias et al., 2003), appears to cause a fall in ANM which may be mediated by a decreased ovarian reserve. Similarly, the very much lower ANM experienced by women in certain rural, poorer communities may have a link to poor nutrition, although the balance of causation between prenatal, childhood and adult deprivation is not known (see above). At the other end of the nutritional spectrum, there is no evidence that excessive fat deposition in obesity leads to a loss of ovarian reserve, although fertility may be affected.

**Adult influences: environmental**

There is ample evidence that the smoking of tobacco causes a fall in ANM (Harlow and Signorello, 2000). The concept has been developed that smoking increases the normal rate of loss of ovarian reserve which occurs in adult life, and that this may account for the decreased numbers of retrieved oocytes in treatment cycles of smokers within ART programmes (Fuentes et al., 2010). Research on the mechanisms of action of components of tobacco smoke has focused on the pro-apoptotic effects of polycyclic aromatic hydrocarbons working via interaction with the AHR (Matikainen et al., 2002). It is possible that the lower trajectory of ovarian reserve in smokers influences the ‘age at last child’ anticipating the earlier arrival of the menopause. However, this is difficult to study as smoking behaviour is associated with social deprivation and lack of educational attainment, factors which, in themselves, tend to bring forward the ages at first and last pregnancy.

The disrupting effect of BPA on the meiotic maturation of oocytes within the ovaries of adult animals is well documented (Hunt et al.,
Exposure leads to increased rates of aneuploidy of oocytes as well as an increased likelihood of cell cycle arrest and cell death (see above).

The future direction of research

It is well established that many environmental compounds can be detected within the body fluids of the majority of individuals within populations today. For example, BPA is detectable in ~95% of urine samples collected from the general population (Calafat et al., 2005). Also, over 75% of individuals have detectable levels of circulating phthalate metabolites (Silva et al., 2005), with higher levels found in children (Koch et al., 2005). Clearly, this analytical methodology provides the potential for studying associations between body fluid levels of individual, or combinations of, environmental compounds or their metabolites, and parameters relating to oocyte reserve in populations of women. The inverse association between urinary BPA levels in women and number of oocytes retrieved in IVF cycles (Mok-Lin et al., 2010) has already been discussed. More recent work, at the time of writing published only in abstract form, has shown a trend towards lower antral follicle counts in women with higher levels of urinary phthalate metabolites (Souter et al., 2011). Also, serum or urine values of PCBs and phthalates above the 90th percentile in women appear to be associated with a >2-year reduction in the ANM (Grindler et al., 2012). Further reporting of these preliminary findings will be of considerable interest. Moreover, this general approach might lead to an examination of the effects of combinations of environmental compounds on oocyte reserve, which may be important bearing in mind the potential interactions between different receptor-induced mechanisms (e.g. between AHR and ER). Overall, these studies may, for the first time, provide a realistic indication of the level of threat to ovarian reserve posed by our ‘every day’ exposure to EDCs.

Further research might also focus on how environmental compounds gain access to physiological systems. For example, it is likely that details of the absorption of compounds inhaled as a result of cigarette smoking leads to some loss of ovarian reserve (at least in part through a mechanism involving AHR) and that this leads to a reduced ANM. Whether compounds, such as BPA or phthalates, leaching from plastics or made available through the use of cosmetics or other personal care products also lead to a significant effect on menopausal age is presently open to debate. Recent work attempting to find associations between body fluid levels of environmental compounds within groups of individuals and parameters related to ovarian reserve may pave the way for a greater understanding of this controversial area.

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Conflict of interest

None of the authors declare a conflict of interest in relation to this publication.

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