COMMENTARY

Estrogen actions in the ovary revisited

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Abstract

Estrogens are synonymous with fertility and infertility in mammals. Our knowledge of the biological actions of estrogens, however, is incomplete. Three recent developments have thrown new light on the actions of estrogens in mammalian reproduction that will lead to a greater understanding of their functions. They are (a) the identification of a second estrogen receptor, called ERβ, (b) the identification of ligand-specific ER coactivators and (c) mouse models with targeted disruption of the genes encoding both ER and the aromatase enzyme. These models provide for the first time animals which are either unable to respond to endogenous or exogenous estrogens (ER 'knockouts'), or can respond to exogenous estrogen but do not make endogenous estrogen (aromatase 'knockout' or ArKO). Furthermore, the ArKO mouse has provided a model to study the effects on the ovary of exogenous estrogens of plant and synthetic origin that are of clinical relevance. The data show that estrogens are essential for fertility but not for survival after birth or for the formation of the reproductive tract. This commentary focuses on the roles of estrogen in folliculogenesis and in the maintenance of the ovarian somatic cell phenotype in the mouse. We also hypothesize that the ERα and ERβ may subserve the proliferative and differentiative actions of estrogen, respectively, within a follicle. In summary, estrogen is obligatory for normal folliculogenesis beyond the antral stage and for the maintenance of the female phenotype of the somatic cells within the ovaries. This clearly demonstrates a major role for sex steroids in somatic cell differentiation in the gonads of eutherian mammals and challenges the central paradigm that the ovary is the default gonad, arising due to the absence of testicular defining signals. Evidence is also provided for the plasticity of the adult female gonad. Understanding the mechanisms of estrogen actions will provide an insight into the regulation of reproductive disorders afflicting women today, notably ovarian dysfunction and the menopause.

Journal of Endocrinology (2002) **175,** 269–276

Introduction

Estrogens are synonymous with fertility and infertility in mammals. They were isolated originally as endocrine steroid hormones produced by the gonads and placenta that had negative and positive feedback influences on the hypothalamic–pituitary axis (Diczfalusy & Fraser 1998). It was established that estrogens could also have local influences on the uterus and within the gonads (Hisaw 1947). These properties of estrogen were exploited in the development of the contraceptive pill for women, which combines synthetic estrogens with a progestogen (Pincus *et al.* 1958). An estrogen receptor (ER) was subsequently identified in target cells and found to act as a nuclear transcription factor responsible for transmitting the genomic actions of estrogenic compounds (Jensen & DeSombre 1973).

Several observations, however, showed that our knowledge of the biological actions of estrogens was incomplete. Compounds developed and used as agricultural pesticides and for the plastics industry were shown to have estrogenic activity that could lead to infertility and tumors in animals including humans (Sharara *et al.* 1998). Treatment of pregnant women with diethylstilbestrol (DES) led to malformation of the reproductive tracts of their children, which included the development of vaginal cancer (Swan 2000). The actions of phytoestrogens used in hormone replacement therapy (Adlercreutz 1995) could not be explained in terms of known mechanisms of estrogen action. Finally, synthetic estrogens were observed to have diverse biological effects in different target tissues that were not explicable by the current knowledge of estrogen action through a single receptor (McDonnell *et al.* 2001).

Three recent developments have thrown new light on the actions of estrogens in mammalian reproduction. They are (a) the identification of a second estrogen receptor, called ERβ (Kuiper *et al.* 1996), (b) the identification

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of ligand-specific ER coactivators (Onate *et al.* 1995, McKenna *et al.* 1999), which may also show some ER isoform specificity (see McDonnell *et al.* 2000) and (c) mouse models with targeted disruption of the genes encoding for both ER (Lubahn *et al.* 1993, Krege *et al.* 1998, Couse *et al.* 1999*a*, Dupont *et al.* 2000), and for the aromatase enzyme (Fisher *et al.* 1998, Honda *et al.* 1998, Toda *et al.* 2001) responsible for the synthesis of estrogen. Importantly, these models provide for the first time animals which are either unable to respond to endogenous or exogenous estrogens (ER 'knockouts'), or can respond to exogenous estrogen but do not make endogenous estrogen (aromatase 'knockout' or ArKO). The data show that although estrogens are not obligatory for survival after birth, or for the formation of the reproductive tract, they are essential for normal fertility in both males and females. Furthermore, the ArKO mouse has provided a model to study the effects on the ovary of exogenous estrogens of plant and synthetic origin that are of clinical relevance. This commentary focuses on the roles of estrogen in folliculogenesis and in the maintenance of the ovarian somatic cell phenotype in the mouse.

Folliculogenesis

Follicles form in the ovary when developmentally arrested germ cells or oocytes recruit somatic follicular cells and organize into discrete 'resting' primordial follicles. This somatic cell investment maintains the oocyte in the diplotene stage of the first meiotic prophase, in the case of women, for up to 40–50 years. These follicles evolve through a complex series of proliferative and differentiative steps called folliculogenesis, to a structure and state where ovulation is feasible, or they succumb to atresia and die. Females have a finite number of 'resting' follicles at birth, which decrease thereafter. We still do not know what controls the reactivation of a few of these primordial follicles to enter the committed growth phase each day. There is important cross talk between the oocyte and the somatic cells (Canipari 2000) and between the somatic cells themselves. These autocrine and paracrine influences (Findlay 1993) regulate follicular growth and differentiation. The current evidence suggests that the preantral stages of committed follicles (primary and secondary) are dependent on local regulators, with gonadotropins having a facilitatory role. The reverse is true for the antral (tertiary and Graafian) follicles, where gonadotropins are essential for development, and locally produced hormones and growth factors play a facilitatory role (Findlay 1993, Scaramuzzi *et al.* 1993, Monget & Bondy 2000). Nevertheless, each follicle is considered to have a unique biological makeup that makes it different from every other follicle. In women it takes up to 6 months for a reactivated primary follicle to reach the ovulatory stage. This exceedingly rare event occurs in less than 1 in 100 follicles, with the remainder succumbing to atresia either via oocyte or granulosa cell apoptosis (Hsu & Hsueh 2000, Reynaud & Driancourt 2000).

Estrogen has acknowledged local intrafollicular actions (Hisaw 1947, Richards 2001). Estrogens act via two types of receptors, ERα and ERβ, and have direct proliferative and differentiative influences on follicle development, depending on the stage of folliculogenesis (Drummond & Findlay 1999). The ovary contains both ER subtypes, with a predominance of ERβ over ERα in granulosa cells. Despite an extensive amount of research on the production of estradiol-17β (E_2), the principal estrogen, by follicles, and the structure and functions of the respective ERs, the specific role(s) of E_2 and its receptors in serving the different actions of estrogen in the ovary have remained elusive (Richards 2001).

The actions of estrogen are intimately related to the actions of follicle-stimulating hormone (FSH) in folliculogenesis. FSH is a key driver of folliculogenesis. It is essential for the final growth of antral follicles (Kumar *et al.* 1997, Abel *et al.* 2000) and small growing follicles are responsive to but not dependent on FSH for growth. FSH, together with insulin-like growth factor-I (IGF-I) and E_2, stimulates the proliferation and differentiation of granulosa cells (Adashi *et al.* 1985, Zhou *et al.* 1997). The specific genes induced in granulosa cells by E_2 have not been identified, but it is clear that E_2 exerts a supporting role on FSH action. There are several candidate genes suspected of being regulated in granulosa cells by E_2. They include cyclin D2 (Richards 2001), inhibin α and inhibin βB (Charpentier *et al.* 2000). Studies on the influence of E_2 on gene expression in MCF-7 breast cancer cells revealed changes in a number of known and novel genes (Charpentier *et al.* 2000) that can now be investigated in granulosa cells.

Recent work has shed light on the mechanisms by which IGF-I and E_2 interact with FSH to influence granulosa cells. There is now evidence that in addition to its well characterized actions via the cAMP/protein kinase A (PKA) pathway, FSH stimulates phosphorylation of two closely related kinases that are downstream targets of the IGF-I/phosphatidylinositol 3-kinase (PI3-K)/ phosphatidylinositol-dependent kinase 1 (PDK1) pathway (Richards *et al.* 2002). These kinases are Sgk (serum and glucocorticoid-induced kinase) and protein kinase B (PKB) or Akt. These effects of FSH, which are independent of cAMP-dependent PKA, can be mimicked by forskolin, cAMP and IGF-I. Targets for PKB and Sgk include members of the forkhead (FOX) family of transcription factors, which are expressed in rodent ovaries (Richards *et al.* 2002). It has been shown that FSH and E_2 enhance expression of the gene for forkhead homolog of rhabdomysarcoma (FKHR) and its protein in granulosa cells of developing follicles (Richards *et al.* 2002). In the same study, E_2 also enhanced expression of other IGF-I pathway components and ERβ, indicating that E_2 and

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IGF-I may form an autocrine regulatory network within growing follicles. It has been suggested that FKHR expression may be linked to the proliferation of granulosa cells given that cells expressing FKHR were highly proliferative, expressed high levels of cyclin D2 (Robker & Richards 1998*a*) and ERβ (Sharma *et al.* 1999) and showed increased staining for proliferating cell nuclear antigen (PCNA)/BrdU (Robker & Richards 1998*b*). The targets of FKHR in granulosa cells are not yet known. The IGF-I/PDK1/PKB pathway is associated with cell survival, which suggests that FSH, enhanced by E_2, may have the capacity to influence cell survival, while at the same time, via PKA, FSH can impact on differentiation events in granulosa cells.

The advent of the ERα knockout (ERKO), ERβ knockout (BERKO), compound ERαβ knockout and ArKO mice offer an opportunity to define the actions of estrogen in folliculogenesis in a more exacting way. ERKO mice are acyclic, infertile and possess hyperemic ovaries devoid of corpora lutea (Couse & Korach 1999*a*). Folliculogenesis is arrested at the antral stage with large secondary follicles becoming cystic and hemorrhagic within 3 weeks of birth. The ovarian phenotype manifests in the presence of elevated luteinizing hormone (LH) levels. In contrast, BERKO females have small ovaries, some arrested follicular development and their fertility is compromised with reduced numbers of offspring per litter, consistent with the reduced number of corpora lutea observed (Krege *et al.* 1998). Gonadotropin levels are normal in these mice. Thus, the extent of follicular development is greater in BERKO than ERKO mice (Couse & Korach 1999*b*). The ERKO mice have a block in folliculogenesis at the early antral stage before the increase in granulosa cell proliferation (Hirshfield 1991), whereas the BERKO mice have antral follicles and can ovulate (Krege *et al.* 1998). We hypothesize therefore, that the proliferative actions of E_2 require ERα, whereas the differentiative (and antiproliferative) effects of E_2 are mediated principally by ERβ.

The hypothesis that ERα subserves the proliferative actions of E_2 has support from other experimental paradigms. BERKO mice show increased cell proliferation and an exaggerated response to E_2 in the uterus (Weihua *et al.* 2000), leading the authors to conclude that ERβ modulates the effects of ERα, and in addition, or as a consequence, has an antiproliferative function in the immature uterus. Studies on the prostate in BERKO mice suggest that activating ligands specific for ERβ could be important modulators of prostatic epithelial growth and differentiation (Weihua *et al.* 2001). Jarred *et al.* (2002) concluded that ERα is the predominant receptor mediating the mitogenic actions of estrogen in the mouse prostate, although they indicated that a role for ERβ remained to be defined. There is also evidence from studies on trabecular bone that ERβ acts in a repressive manner, possibly by counteracting the stimulatory action of ERα on bone formation (Windahl *et al.* 2001). Recently, Liu *et al.* (2002) described direct evidence for opposing actions of ERα and ERβ on cyclin D1 gene expression in HeLa cells. These authors concluded that ERβ may modulate the proliferative effects of ERα by blocking its action at the cyclin D1 gene or at other key pro-proliferative target genes.

This raises important questions about the mechanisms by which ERα and ERβ interact in response to agonist or antagonist stimulation. If both ERα and ERβ reside within the same cell type, there may be either homo- or heterodimerization, with heterodimerization believed to be a preferred option (see Drummond & Findlay 1999). In this model, it can be predicted that activation through a heterodimer of the ER would allow ERβ to exert its modulatory actions. If ERβ and ERα reside in different cell types and only form homodimers within those cell types, a modulatory action of ERβ on ERα would not be possible and a different model, one where paracrine factors are involved, becomes more plausible. The cellular distribution of ERα and ERβ will be an important determinant of E_2 action in the ovary. At present there are differences in the literature about the presence of ERα protein in granulosa cells, cells that contain abundant ERβ protein for example (Sar & Welsch 1999, Sharma *et al.* 1999). Similarly in rat prostate, low levels of ERα are present in the stroma but are not detectable in the epithelium (Cooke *et al.* 1997), whereas ERβ is localized in the epithelium (Kuiper *et al.* 1996).

The ArKO mouse (on an estrogen-free diet) has allowed us to define how far follicles can grow in the total absence of estrogen before becoming atretic (Fisher *et al.* 1998, Britt *et al.* 2000). As a consequence, we were able to describe the effect of hypoestrogenicity on the relative sizes of populations of different follicles (Britt *et al.* 2000). Female ArKO mice have undetectable levels of aromatase and estrogens whilst exhibiting high levels of serum testosterone, FSH and LH. These reproductive hormones have been implicated as playing crucial roles in various aspects of folliculogenesis. Not surprisingly, these ArKO mice are infertile with folliculogenesis arrested at the antral stage, and an absence of corpora lutea (Fig. 1). In 6-week-old ArKO mice, the ovaries contained an apparent increase in the numbers of secondary and antral follicles. The antral follicles that were present appeared morphologically atretic or prematurely luteinized as evidenced by the presence of pyknotic nuclei or cytoplasmic lipid droplets respectively. The ovaries exhibited an increasingly diffuse interstitium (with age) and contained increasing proportions of morphologically abnormal follicles containing cells resembling Sertoli cells (Fig. 1). The appearance of these abnormal follicles was delayed by phytoestrogens present in soy meal contained within the mouse chow (Britt *et al.* 2002*a*). Toda *et al.* (2001) also reported depletion of follicles and formation of hemorrhagic cysts in their ArKO mouse model at 5 months of age. They subsequently

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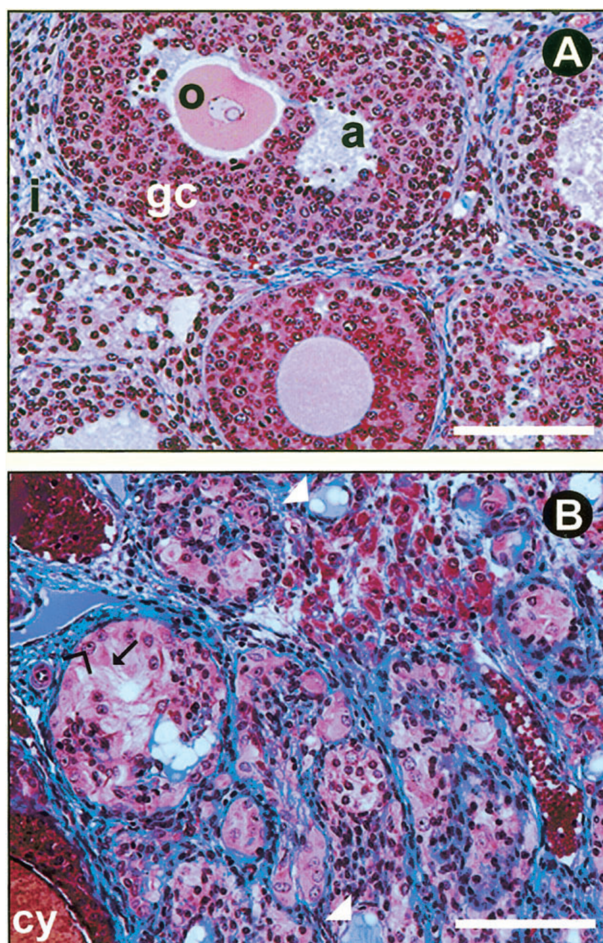

Figure 1 (A) The morphology of a wild-type mouse ovary: typical follicles consisting of growing oocytes (o) surrounded by layers of proliferating granulosa cells (gc). The granulosa cells possess the typical low cytoplasmic-to-nuclear ratio. In maturing follicles antral spaces (a) form within the granulosa cell layer. The interstitial region (i) is comprised of stromal and interstitial cells. (B) Morphology of a mouse ovary with a deletion in the aromatase gene (ArKO), at 16 weeks of age, maintained on a soy-free diet. The arrowhead and arrow depict the nucleus and cytoplasm respectively of somatic cells resembling Sertoli cells. These cells possess a high cytoplasmic-to-nuclear ratio with cytoplasmic extensions (arrow). Triangles signify lacunae, which have been identified as the remnants of primary follicles, presumably dying atretic follicles. Other follicles transform into hemorrhagic cysts (cy). The blue appearance of the interstitium in comparison with the wild-type ovary represents the increased deposition of collagen to these degenerating ovaries. Scale bar=0·1 mm. Adapted from Britt *et al.* 2002*a* with permission from FASEB Journal and all coauthors.

reported that the addition of bisphenol A, a zenoestrogen, to the diet prevented the formation of cysts and follicle loss, but failed to restore ovulatory capacity given that no corpora lutea were present (Toda *et al.* 2002).

In summary, these 'knockout' mouse models show that estrogen is obligatory for normal folliculogenesis beyond the antral stage.

Phenotype of ovarian somatic cells

The central dogma of mammalian sex determination states that testicular differentiation and subsequent male development occurs as a result of the sex-determining gene on the Y chromosome (Sry), which acts as a molecular switch in embryos having an XY chromosome constitution (Capel 1996). In non-mammalian vertebrates, alternative mechanisms of sex determination occur, which are dependent on factors in the external biological environment. These factors include temperature, and more importantly, aromatase activity (Desvages & Pieau 1992, Desvages *et al.* 1993). The role of estrogen in gonadal differentiation and maintenance of the ovarian phenotype of somatic cells in eutherian mammals has not been clearly established (Wilson *et al.* 1981, Pieau *et al.* 1994). Accounts of natural and experimental differentiation of male somatic cells have been reported in XX gonads under conditions of variable estrogen deficiency. Seminiferous tubule-like structures have been observed in freemartin cattle (Jost *et al.* 1973), following premature oocyte death (Hashimoto *et al.* 1990), and in XX gonads of W/W^v mutant mice, and busulphan-treated rats (Merchant 1975, Merchant-Larios & Centeno 1981). They have also been observed in fetal rodent ovaries transplanted into male hosts (Buyse 1935) and in aging rodents (Engle 1946, Crumeyrolle-Arias *et al.* 1976). The transdifferentiation of ovarian somatic cells to a testicular phenotype in these examples has been ascribed to the absence of viable oocytes (Hashimoto *et al.* 1990, McLaren 1991), rather than to the involvement of steroid hormones. A role for estrogen in the phenotype of the somatic cell is suggested in mice deficient in both types of functional ER (ERαβKO) (Couse *et al.* 1999, Dupont *et al.* 2000). In this model, seminiferous tubule-like structures develop in the gonads with concomitant oocyte loss and expression of Sox9 and MIS, genes associated with testis formation. These compound knockouts also exhibit ectoplasmic specialisations between the Sertoli-like cells (Dupont *et al.* 2000), which are indicative of a male phenotype. The inability of estrogen to transduce a genomic signal in these animals, however, precludes replacement studies to investigate the role of estrogen in the development and maintenance of female somatic cells within XX ovaries.

The ArKO mouse, however, has allowed us to assess the role played by steroid hormones in the sexual differentiation of mammals. In the absence of a Y chromosome (or Sry region), estrogen-deficient ArKO female mice develop masculinized gonads (Table 1; Britt *et al.* 2001, 2002*a*). Cells morphologically resembling testicular Leydig cells were present within the interstitial regions of

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Table 1 Ultrastructural characteristics of somatic cells in the ovaries of wild-type mice (WT), and mice with a deletion in the aromatase gene (ArKO) in comparison with wild-type testes

	Testis WT	Ovary ArKO	Ovary WT
Tubular/follicular region			
High cytoplasmic-to-nuclear ratio	Yes	Yes	No
Tripartite nucleoli	Yes	Yes	No
Cytoplasmic extensions	Yes	Yes	No
Ectoplasmic specializations	Yes	Yes	No
Interstitial region			
Smooth endoplasmic reticulum			
Extensive	Yes	Yes	No
Whorls	Yes	Yes	No
Large cytoplasm	Yes	Yes	Occasionally
Annular nucleoli	Yes	Yes	No
Steroidogenic mitochondria	Yes	Yes	Yes
Lamellar cristae	No	No	Yes
Tubulo-vesicular cristae	Yes	Yes	No

Adapted from Britt *et al.* 2002*a*.

6-week-old ArKO ovaries. Their numbers increased as a function of age. At 16–18 weeks of age, few healthy preantral follicles were observed, and folliculogenesis was blocked at the primordial stage in several individuals. Detailed light microscopy identified abnormal follicles as seminiferous tubule-like structures filled with Sertoli-like somatic cells, apparently arising from the trans/re-differentiation of granulosa cells. There were specialized desmosome-like adherens junctions and Sertoli-cell specific, ectoplasmic specializations between these Sertoli-like cells (Table 1, Fig. 2). Espin, an actin bundling protein present in these ectoplasmic specializations, was detected in wild-type testis and ArKO abnormal follicles but was absent in follicles of wild-type ovaries. We also showed that consistent with the appearance of Sertoli cells, the ovaries of ArKO mice expressed levels of Sox9 mRNA similar to those observed in wild-type testis, whilst significantly higher than in wild-type ovaries. This extends recent observations (Couse *et al.* 1999) of increased Sox9 expression in ovaries of ERαβKO mice with similar Sertoli cell-like structures. Sox9 expression has also been associated with Sertoli cell development in '*oddsex*' mice (Bishop *et al.* 2000) and in female intersex chick gonads (Vaillant *et al.* 2001). Genetically female (XX) mice overexpressing Sox9 also contain Sertoli-like cells in their ovaries (Vidal *et al.* 2001). Furthermore, the increased

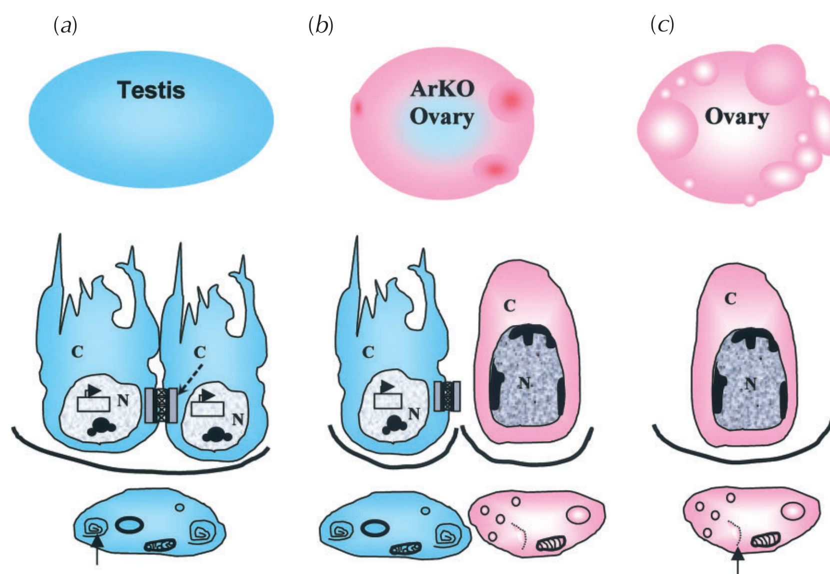

Figure 2 Schematic diagram depicting the ultrastructural characteristics of the somatic cells of the gonad. (A) Testes possess Sertoli cells within the seminiferous tubules of the testes. The Sertoli cells have a high cytoplasmic (C)-to-nuclear (N) ratio and homogeneous chromatin distribution. Mature cells possess defined, and in some cases tripartite nucleoli. The nucleus also expresses Sox9. Between adjacent Sertoli cells are fence-like cell-specific junctions called ectoplasmic specializations (dashed arrow). These junctions possess immunopositive espin protein. The Leydig cells are present in the interstitial area. They characteristically contain whorls of endoplasmic reticulum (arrow), tubulo-vesicular mitochondrial cristae, annular nuclei (bold oval) and some lipid. (B) The gonads of the female ArKO mice possess both the ovarian (C) and testicular (A) somatic cells. (C) Granulosa cells of the ovary possess a small cytoplasm (C)-to-nuclear (N) ratio with heterogeneous chromatin distribution. The interstitial cells possess lipid (small circles), endoplasmic reticulum (arrow) and lamellar mitochondrial cristae.

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collagen deposition observed in the ArKO ovaries correlates with the regulation of the collagen type 1 by Sox9 (Ng *et al.* 1997).

Estradiol replacement in 7-week-old ArKO female mice, for 3 weeks, prevented the onset of the male phenotype of somatic cells (Britt *et al.* 2002*a*). Toda *et al.* (2001) reported that ArKO mice treated with estrogen from 4 weeks of age reversed the accumulation of smooth endoplasmic reticulum in luteinized interstitial cells. However, these cells were not definitively identified and the authors did not comment on the appearance of Sertoli-like cells in the ArKO ovary. We have shown that these luteinized interstitial cells are ultrastructurally similar to Leydig cells and that estrogen replacement ameliorated their appearance, in addition to suppression/reversal of the appearance of the tubular structures containing Sertoli-like cells.

This data confirms and extends observations in ERαβKO mice in which Sertoli-like cells with similar properties were observed in the ovaries (Couse *et al.* 1999, Dupont *et al.* 2000). These compound knockouts are not estrogen-free and it is possible that there remains an influence of estrogen via some, as yet unidentified, ER or through a non-genomic action of E_2. Our observation of Sertoli-like cells in the ovaries of ArKO mice on a phytoestrogen-free diet unequivocally shows that these cells develop in the complete absence of estrogen (Fig. 2). The presence of cells morphologically resembling Leydig cells in the interstitium of ArKO ovaries is a novel observation not previously reported in ER mutant mice.

A major role for sex steroids in the somatic cell differentiation of ovaries of eutherian mammals has been demonstrated by studies of the ArKO mouse ovary. These findings also challenge the central paradigm that the ovary is the default gonad arising due to the absence of testicular defining signals. Estrogen is clearly required for the female-type phenotype of the somatic cells within the mammalian ovaries. Evidence for the plasticity of the adult female gonad has been provided.

Androgen action in models of hypoestrogenicity

The possibility remains that some or all of the ovarian phenotype in these mouse models of hypoestrogenicity is due to inappropriate actions of androgens on the ovary. This could be mediated through either elevated levels of serum androgens as in the ArKO mice (Fisher *et al.* 1998), or inappropriate expression of the androgen receptor (AR) as in the BERKO mice (Cheng *et al.* 2002).

Androgen action in the ovary is thought to be a two-stage process (Hillier & Tetsuka 1997). In committed preantral follicles, testosterone or dihydrotestosterone of thecal origin acts via AR located principally in the granulosa cells to facilitate proliferation and FSH action. Once antral follicles are formed, there is a decrease in expression of AR in granulosa cells and increased utilization of androgen as a substrate for aromatase activity. Maintenance of AR and failure to express aromatase in the granulosa cells of antral follicles is characterized by a high androgen-to-estrogen ratio in the follicular fluid (Carson *et al.* 1981, Maxson *et al.* 1985), and leads to atresia.

Ovaries of BERKO mice, which contain many atretic follicles and a block in folliculogenesis at the antral stage, express AR aberrantly (Cheng *et al.* 2002). Treatment of BERKO mice with the AR antagonist, flutamide, partially reversed the phenotype by restoring folliculogenesis but not ovulation. Cheng *et al.* (2002) concluded that expression of ERβ was necessary for the down-regulation of AR in antral follicles in order to prevent atresia and provide sufficient androgen substrate for conversion to estrogen by aromatase in granulosa cells.

AR levels have not been measured in the ERKO, αβERKO or ArKO models. Elevated levels of testosterone have been reported to circulate in ArKO female mice (Fisher *et al.* 1998), but, to our knowledge, androgen levels have not been reported in the other models of hypoestrogenicity. It is possible, therefore, that these elevated levels of testosterone in the absence of E_2 in ArKO mice are responsible for the increased numbers of primary follicles, some or all of the advanced atresia and the block in folliculogenesis observed in the ovaries (Britt *et al.* 2000). It is worth noting that estrogen replacement, either as phytoestrogens in the diet or as estradiol pellets, was sufficient to delay the onset of the phenotype or partially restore folliculogenesis (Britt *et al.* 2002*a*). This model would be consistent with an action of estrogen via ERβ to downregulate AR and so reduce atresia, as suggested by Cheng *et al.* (2002).

Other implications

In the absence of estrogen, the female-specific phenotype of the ovarian cells is lost and a more testicular type of appearance is noted (Britt *et al.* 2001, 2002*a*). This is important in the light of menopausal ovaries which also exist in a state of hypoestrogenism and the fact that testicular tissue has been reported to develop within genetically female ovaries as females age (Engle 1946). There are various reproductive disorders afflicting women today that occur as a result of ovarian dysfunction, and/or ovulatory disorders. An understanding of how estrogen functions provides insight into the hormonal regulation of such ovarian processes, and into the symptoms associated with menopause.

Low levels of dietary phytoestrogens (contained in most commercially available supplements offered to menopausal women) profoundly affect the ovary (Britt *et al.* 2001, 2002*a*) and the hypothalamo–pituitary–gonadal axis (Britt *et al.* 2002*b*). Maintenance on a soy-containing diet from conception is able to protect the otherwise hypoestrogenic ovary from ensuing testicular development in the ArKO mouse. Thus dietary soya meal, acting as an estrogen

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agonist, and exogenous estrogen treatment postponed the development of male-type cells by the ovary. This has considerable significance, particularly for women undergoing the menopausal transition. Finally, it is well known that Asian populations ingest more soya protein than inhabitants of the so-called 'Western continents' and have a significantly reduced incidence of both breast and prostate cancer. We have shown that this may, at least in part, be the result of a direct action of these estrogenic chemicals on the differentiative status of the cell.

Acknowledgements

The authors would like to thank Professor Evan Simpson and Drs Ann Drummond and Margaret Jones for their constructive comments. The financial support of the NH&MRC of Australia Program Grant (RegKey 983212; J K F) and Fellowship (RegKey 198705; J K F) is gratefully acknowledged.

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Received in final form 25 June 2002
Accepted 26 June 2002

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