**Molecular Human Reproduction, Vol.15, No.3 pp. 149–154, 2009**
Advanced Access publication on February 4, 2009 doi:10.1093/molehr/gap006

MHR Basic science of reproductive medicine

**NEW RESEARCH HORIZON** ***Invited Review***

# Androgen receptor's destiny in mammalian oocytes: a new hypothesis†

**Mo Li[1,2], Heide Schatten[3], and Qing-Yuan Sun[1,4]**

[1]State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Datun Road, Chaoyang, Beijing 100101, People's Republic of China [2]Graduate School, Chinese Academy of Sciences, Datun Road, Chaoyang, Beijing 100101, People's Republic of China [3]Department of Veterinary Pathobiology, University of Missouri-Columbia, Columbia, MO, USA

[4]Correspondence address. Tel/Fax: +8610-6480-7050; E-mail: sunqy@ioz.ac.cn or sunqy1@yahoo.com

**ABSTRACT:** Unlike the well-established roles of androgen and androgen receptor (AR) in males, the functions of this steroid and its receptor in the ovary are still unclear. For decades, androgen and AR have long been considered to play a negative (at least not a positive) role in mammalian oocyte maturation. However, recent studies by us and others showed their positive influence in promoting meiotic maturation. On the other hand, rapid non-genomic effects of androgens have been observed and are now generally accepted as contributing to the physiological effects of the steroids and their related receptors in somatic cells, and this has stimulated us to explore the complex roles of AR in the ovary. Based on the classic dogma and new findings, we collected evidence to propose that the expression of AR shifts from the oocytes to the theca cells and finally disappears in the oocytes during evolution. It is suggested that the non-genomic pathway involving androgen and AR in the mammalian oocytes, unlike somatic cells, cells will undergo elimination. The function of androgen and AR in promoting meiotic maturation may have been replaced gradually by gonadotrophins. Moreover, a possible relationship between AR and polycystic ovary syndrome is also discussed, which might provide a clue for the pathology of the disease.

**Key words:** meiosis / ovary / androgen receptor

## Recent findings and the controversy

In mammals, fully grown oocytes are arrested at the diplotene stage of the first meiotic prophase, which is termed as the germinal vesicle stage, and maturation of meiosis is triggered *in vivo* by a hormonal stimulus or removal from the inhibitory environment of follicles (Su *et al.*, 2003; Fan and Sun, 2004). As a member of the nuclear receptor superfamily encoded by an X chromosomal gene (Lubahn *et al.*, 1988), the androgen receptor (AR) plays pivotal roles in male developmental and physiological processes, particularly in sexual development and maturation, as well as the maintenance of reproductive organs and spermatogenesis. Classically, in common with other members of the nuclear receptor superfamily, AR functions as a ligand-inducible transcription factor. The binding of androgen to AR triggers receptor homodimerization, promoting the ability of AR to bind to its response element and recruit regulators to affect gene expression (Heinlein and Chang, 2002; Losel *et al.*, 2003). However, AR's functions in oocyte maturation remain unclear and are intensely controversial: for decades, androgens have long been believed to play negative or dispensable roles during meiotic maturation in mammals (Smith and Tenney, 1980; Eppig *et al.*, 1983; Schultz *et al.*, 1983; Anderiesz and Trounson, 1995) until they were recently shown to be involved in promoting oocyte maturation in the mouse (Gill *et al.*, 2004; Hammes, 2004; Jamnongjit *et al.*, 2005; Jamnongjit and Hammes, 2006). Furthermore, results from our laboratory showed that testosterone could potentially trigger porcine oocyte meiotic resumption, which is mediated by intra-oocyte AR, proto-oncogene protein kinase (SRC) and mitogen-activated protein kinase (MAPK) in the culture model containing low dose of hypoxanthine (HX) (Li *et al.*, 2008a). In contrast, by using follicle-enclosed oocytes and cumulus-enclosed oocytes (CEOs) of rat and mouse, another group questioned the meiosis-stimulating competence of androgens and the receptor (Motola *et al.*, 2007; Tsafriri and Motola, 2007). On the other hand, rapid non-genomic (or non-classical) effects of androgens have emerged and are now generally accepted as contributing to the physiological effects of the steroids and their related receptors in somatic cells (Cato *et al.*, 2002; Losel *et al.*, 2003), which again brings the elusive roles of androgen and AR in the ovary into sharp focus.

## A hypothesis of AR's shift in ovarian follicles

New findings and remaining controversies have rekindled the question about AR in the ovary: in lower vertebrates, such as fish and frog,

†This paper was presented at the Beijing International Symposium on Reproductive Biology.

steroids (especially androgens) have been shown to undoubtedly be potent promoters during oocyte maturation (Smith and Ecker, 1971; Maller and Krebs, 1980; Smith and Tenney, 1980; Lutz *et al*., 2001; Gill *et al*., 2004; Hammes, 2004); in rodents, however, their competence appeared to be impaired to a certain extent not only because of the inconsistent competence of androgen reported by different laboratories (Gill *et al*., 2004; Hammes, 2004; Jamnongjit *et al*., 2005; Jamnongjit and Hammes, 2006; Motola *et al*., 2007; Tsafriri and Motola, 2007), but also because of the dominant roles of gonadotrophins in inducing meiotic maturation (Eppig, 1991; Fan and Sun, 2004). Even though, in addition to the ability to trigger meiotic reinitiation in denuded oocytes (DOs) (Gill *et al*., 2004), androgens have recently been reported to promote the maturation of oocyte–granulosa cell complexes and oocyte–cumulus cell complexes in mouse (Jamnongjit *et al*., 2005); interestingly, in the pig, a vertebrate higher in mammalian hierarchy compared with the mouse, AR can only mediate androgen-triggered meiotic resumption of DOs rather than CEOs in a culture model containing low dose of HX (Li *et al*., 2008a), which indicates that, according to the progressive evolution in vertebrates, the competence of AR appears to become increasingly weaker. In other words, during evolution, androgen and AR tend to be losing their function in the ovarian follicles.

Parallel to the indications mentioned above, numerous studies have shown a decreasing distribution of AR mRNA or protein expression from 'exterior' to 'interior' in the ovarian follicles of the individual species of mammals: in the mouse ovary, AR staining is observed in granulosa cells, thecal cells and stromal cells but not in oocytes (Cheng *et al*., 2002), although a recent report showed that AR also exists in oocytes (Gill *et al*., 2004). In the rat, AR mRNA displays intense staining in theca cells, whereas staining becomes moderate in granulosa cells (Hirai *et al*., 1994). In the sheep, which is a representative for other species, the expression of AR mRNA can be observed in granulosa and theca cells of follicles consisting of the oocyte surrounded by more than three layers of granulosa cells but not in follicles containing single or two layers of granulosa cells (Juengel *et al*., 2006), which is similar to the bovine ovary (Hampton *et al*., 2004). In the pig, intense AR immunostaining is present in the nuclei of granulosa cells of pre-antral and antral follicles, and the AR protein is mainly present in granulosa cells during the follicular phase (Cardenas and Pope, 2002). The expression of AR in the oocyte is much weaker compared with that in granulosa cells (Li *et al*., 2008a). In non-human primate ovaries, AR protein is most abundant in granulosa cells of pre-antral and antral follicles but not in oocytes. AR mRNA is expressed in granulosa and theca cells of pre-antral and antral follicles but not in oocytes (Weil *et al*., 1998). Finally, in humans, AR protein expression is observed at different stages in granulosa and theca cells but unequivocal or little in the oocyte itself (Suzuki *et al*., 1994; Walters *et al*., 2008). Through microarray and PCR analysis of oocyte cDNA, previous study did not detect mRNA of AR in human oocyte, suggesting that the mRNA level in a single oocyte was below the sensitivity of the array analysis and much lower than those of other genes in the oocyte (Wood *et al*., 2007). Together, the distributions of AR in different mammalian species appear to share the same trend, shifting from the inner (oocyte) to outer (granulosa or theca cells) in the follicle, though the distributions of AR in these species do not completely share the same pattern. Furthermore during development, the distribution exhibits a similar trend of decline: the amount of AR mRNAs in granulosa cells decreases as follicles develop to the preovulation stage (Tetsuka and Hillier, 1996; Slomczynska *et al*., 2001). AR protein in granulosa cells and the relative abundance of mRNA in whole ovarian total RNA are both down-regulated during follicular development (Tetsuka *et al*., 1995; Cheng *et al*., 2002).

On the other hand, biosynthesis of androgens occurs in theca cells catalyzed by the enzyme of P450 17α-hydroxylase/17,20 ($P450_{17\alpha}$), and the synthesized androgens are transported to the granulosa cells where P450 aromatase ($P450_{arom}$) converts these androgens to estrogen and 17β-estradiol (Simpson, 2000; Kimura *et al*., 2007). Interestingly, the two important enzymes are expressed only in the theca and granulosa cells in the ovary, respectively (Wood and Strauss, 2002). In addition to the large amount of AR in theca and granulosa cells, these results imply a relatively complete system for the synthesis, metabolism and signal transduction of androgens independent of the germ cells, which is different from that in amphibians because the androgen production in the *Xenopus* ovary was shown to require oocyte as well as the surrounding follicular cells (Yang *et al*., 2003). Therefore, the AR in mammalian oocytes does not appear to be necessary for the ovary. Collectively, the distribution of AR in ovarian follicles of mammals appears to exhibit a microcosm of AR's shift during evolution which is illustrated in Fig. 1A.

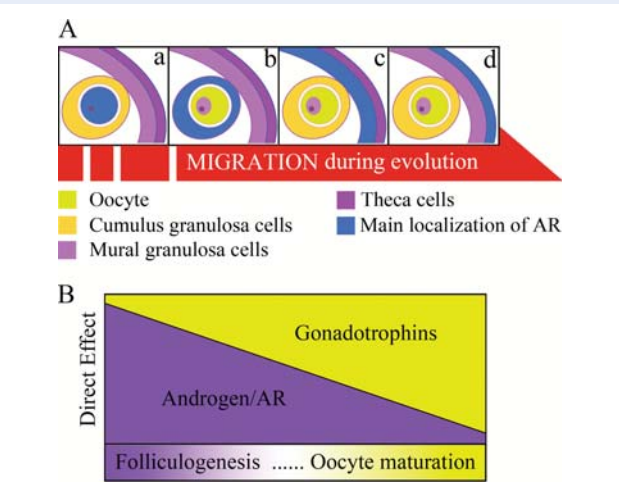


**Figure 1** (**A**) A hypothesized model of AR's shift in ovarian follicles during evolution of mammals. Typical mammalian ovarian follicles consist of at least four cell types. Each follicle contains an oocyte surrounded by cumulus granulosa cells. These cells are then bounded by outer mural granulosa cells, which in turn are surrounded by theca cells. In the early stages of evolution, AR was mainly located within the oocyte (a). As time evolves, the protein shifts to cumulus cells (b), mural cells (c) and then theca cells (d). (**B**) A hypothesized model of androgens and AR and gonadotrophins' direct effects on the ovary (except for indirect effects, e.g. gonadotrophins induce the synthesis of steroids and then affect follicle development). There might be a synergistic effect of steroids and gonadotrophins on the ovary. During the time of folliculogenesis, steroids appear to provide the major contribution, whereas gonadotrophins play critical roles during oocyte maturation.

## Androgen versus gonadotrophins in the ovary

Although the maturation promoting ability of androgen and AR in mammals is based on non-genomic effects, which is similar to that in amphibians, the fact that the level of androgen used in several studies (Gill *et al*., 2004; Hammes, 2004; Jamnongjit *et al*., 2005; Jamnongjit and Hammes, 2006; Li *et al*., 2008a) is higher than that *in vivo* raises doubts as to whether this non-genomic action is really necessary for mammalian oocyte maturation. Indeed, amphibians (lower vertebrates) and mammals (higher vertebrates) employ different mechanisms to complete meiotic maturation: during ovulation in amphibians, the induction of maturation is elicited through a direct effect of steroid hormones on the oocyte (Dettlaff, 1966; Smith *et al*., 1968; Smith and Ecker, 1971). In mammals, however, maturation mainly relies on the surrounding somatic cells rather than the oocyte itself (Su *et al*., 2003; Fan and Sun, 2004; Liang *et al*., 2007; Li *et al*., 2008b). Further supporting evidence comes from MAPK, which is a pivotal molecule for promoting maturation: in the amphibian ovary, activation of MAPK in the oocyte itself is necessary for meiotic resumption (Fabian *et al*., 1993); whereas in mammals, activation of MAPK in cumulus granulosa cells but not in the oocyte itself is required for maturation (Kalab *et al*., 1996; Su *et al*., 2002; Ohashi *et al*., 2003; Liang *et al*., 2007). Furthermore, lower vertebrates require their oocytes contribute to ovarian sex steroid production, whereas higher vertebrates, in which the ovarian volume primarily consists of follicle cells, may no longer need oocytes to fulfill this function (Yang *et al*., 2003).

In our previous study, we found that only the AR in oocytes but not in somatic cells can exert positive effects on promoting maturation of porcine oocytes (Li *et al*., 2008a), indicating that, different from the conventional meiotic promoters (such as follicle stimulating hormone (FSH), luteinizing hormone (LH), or epidermal growth factor-like factors) in mammals, androgen and AR employ an amphibian mode to achieve maturation. This action of androgen and AR uncovers an interesting possibility from the viewpoint of evolution: it is possible that when mammals first appeared on earth, AR was abundantly expressed in oocytes and played more important roles than gonadotrophins. Under the pressure of evolution, higher vertebrates developed additional systems (i.e. effect of gonadotrophins on the surrounding granulosa cells) to trigger meiotic resumption. Making steroid production was sufficient but not necessary for oocyte maturation (Jamnongjit and Hammes, 2006; Motola *et al*., 2007; Tsafriri and Motola, 2007). Because of the powerful capability of FSH and LH that is in place now (Vermeiden and Zeilmaker, 1974; Eppig, 1991; Fan and Sun, 2004) over the long period of time, androgens may have undergone a shift from dominancy to co-operation and to decline in 'controlling' the oocyte maturation compared with gonadotrophins. Androgens would make their main contribution during the early stages of follicular growth (Fig. 1B). For example, during preantral follicular development, androgen was evidenced to promote the primary to secondary follicle transition (Yang and Fortune, 2006). This proposition was also supported by a previous study in the primate ovary (Vendola *et al*., 1998). Furthermore, in *Xenopus*, the ovaries contain all of the enzymatic machinery necessary for the conversion of sex steroid precursors to androgens independent of gonadotrophins (Yang *et al*., 2003), but in mammals, the gonadotrophins are indispensable for steroidogenesis (Seger *et al*., 2001; Jamnongjit *et al*., 2005; Kimura *et al*., 2007). Various female AR-deficient mouse models have provided evidence for the role of AR in mammalian ovaries. Although homozygous $Ar^{Tfm}$ female mice exhibited increased follicle atresia and reduced follicle numbers, AR-mediated androgen action was qualitatively not essential for ovulation, mating, pregnancy or lactation (Lyon and Glenister, 1974; Walters *et al*., 2008). By deleting different exons of Ar by the Cre/LoxP system to generate mutant ARs in female mice, investigators showed important roles of AR in females including fertility, estrous cycles, ovarian gene expression and ovarian health, but these $Ar^{-/-}$ mice simultaneously exhibited normal follicle populations at least up to 16 week of age (Yeh *et al*., 2002; Hu *et al*., 2004; Shiina *et al*., 2006; Walters *et al*., 2007). Especially in the $Ar^{EX3-/-}$ mouse (deleting exon 3 of AR), no change in follicle growth rates and in granulosa or theca cell proliferation was observed. Fertilization and progression to the 2-cell stages are also normal (Walters *et al*., 2007, 2008). Therefore, it is still unclear whether the phenotypes in $Ar^{-/-}$ individuals are due to the direct deletion of AR or indirect consequences related to other genes or proteins, which are critical for oocyte development and maturation.

On a physiological level, the major role of androgen in the ovary may possibly serve as estrogen precursors (Kimura *et al*., 2007) but not to bind to the declined AR to trigger non-genomic effects. Therefore, different from the non-classical effect in somatic cells, the action of androgen and AR in the mammalian oocytes challenges us to think as a 'facing out' rather than 'starting' role. The maturation promoting the ability of androgen and AR might be left as a 'curtain call', with a declining role and replacement by gonadotrophins. As evolution proceeds, the AR might disappear in mammalian oocytes.

## Relationship between AR and polycystic ovary syndrome

If our hypothesis holds true, in mammals, at the current time AR should be mainly localized in granulosa and theca cells rather than in the oocyte. The degenerative but still existent AR in the oocyte itself which has not been eliminated completely by evolution appears unnecessary or even harmful to the female individual. For example, in humans, polycystic ovary syndrome (PCOS) is a disorder that affects ~5–10% of women in reproductive ages. This disease is characterized by increased androgen levels, anovulatory infertility, insulin resistance and hyerinsulinemia (Ehrmann, 2005; Wood *et al*., 2007). However, the definite mechanism leading to PCOS is still unsolved. Generally, the premature development of follicles in PCOS ovaries is thought to be due to the elevated levels of androgen (Drummond, 2006). However, if the direct pathology is not completely due to the unnecessary androgen, but partially due to the redundant AR in the oocyte, which might mediate signaling pathways to influence ovulation, PCOS could still occur. Based on the studies by others and us, we hypothesize that the relatively large amount of AR in granulosa (mural and cumulus) and theca cells could prevent excess androgen entering the oocyte, which ensures follicles to grow normally. In contrast, if dysfunctional or functionless

communications exist between granulosa cells or granulosa cells and the oocyte, physiologically unnecessary androgen will enter the oocyte and bind to the remnant AR, triggering a series of non-genomic actions, provoking some follicles which should undergo atresia, to overcome the physiological barrier, and form dysfunctional or functionless follicles in the ovary. In female rhesus monkeys prenatally androgenized with testosterone, large polyfollicular ovaries develop which resemble polycystic ovaries found in women with PCOS (Abbott *et al*., 2005; Drummond, 2006). Previous studies also provided indirect evidence that androgen-augmented development of the pre-antral follicle in a culture system for whole mouse follicles (Murray *et al*., 1998). Similarly, an increased diameter of *in vitro* cultured follicles of immature mice was induced by androgen treatment, and the stimulatory effects of testosterone, androstenedione and dihydrotestosterone were abrogated by an AR antagonist (Wang *et al*., 2001). Moreover, fetal estrogen deficiency results in impaired oocyte and follicle development, immature and abnormal adult ovaries, and excessive ovarian stimulation from endogenous gonadotrophins ultimately generating hemorrhagic follicles. However, androgen deficiency, without accompanying estrogen deficit, has little apparent impact on ovarian development (Abbott *et al*., 2006). Evidence for the negative role of androgens also comes from PCOS patients treated with the anti-androgen, flutamide. After 6 months of treatment, ovulation was restored (De Leo *et al*., 1998). By analyzing the global gene expression profiles between normal and PCOS human oocytes, a previous study showed 374 significantly different genes in mRNA abundance in PCOS oocytes and 68 of these differentially expressed genes contained putative AR and/or peroxisome proliferating receptor γ binding sites (Wood *et al*., 2007), suggesting the close relationship between intra-oocyte AR and PCOS. Collectively, these findings imply some potentially but unnecessarily stimulatory effects of androgen and AR in ovary. Figure 2 represents a possible relationship between the unnecessary androgen and abnormal ovulation. On the other hand, using AR-knockout mice by the Cre-loxP system, Sato *et al*. (2004) evoked typical features of testicular feminization abnormalities in males. In contrast, no obvious abnormality is initially observed in females (Kimura *et al*., 2007), which further implies that AR may not play key roles in the ovary. A similar view also comes from a recent review showing that the major role of androgen and AR in the mammalian ovary may just be to maintain oocyte and follicle health rather than oocyte maturation (Tsafriri and Motola, 2007). It is premature to speculate about the destiny of AR in the mammalian follicles. However, the hypothesis presented here might reveal a potential trend which will play a prelude to the complicated and paradox receptor in meiotic cell cycles.

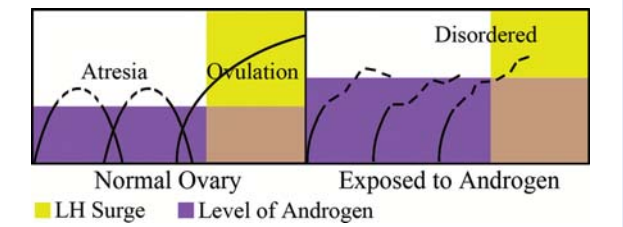


**Figure 2** Low concentration of androgen exists in ovary and makes weak contributions to oocyte maturation and ovulation. During this time, most of the follicles undergo atresia and only the mature follicles (oocytes) are selected and ovulate. If oocytes or follicles are exposed to the extra androgen that can trigger a series of unnecessary events (perhaps rapid non-genomic actions) within the follicles (oocytes), multiple follicles are promoted to undergo abnormal development. The mechanism of ovulation will be disturbed.

## Funding

This work was supported by the National Basic Research Program of China (2006CB944001, 2006CB504004), and Knowledge Innovation Program of the CAS (KSCX2-YW-R-52).

## References

Abbott DH, Barnett DK, Bruns CM, Dumesic DA. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Hum Reprod Update* 2005;**11**:357–374.

Abbott DH, Padmanabhan V, Dumesic DA. Contributions of androgen and estrogen to fetal programming of ovarian dysfunction. *Reprod Biol Endocrinol* 2006;**4**:17.

Anderiesz C, Trounson AO. The effect of testosterone on the maturation and developmental capacity of murine oocytes *in vitro*. *Hum Reprod* 1995;**10**:2377–2381.

Cardenas H, Pope WF. Androgen receptor and follicle-stimulating hormone receptor in the pig ovary during the follicular phase of the estrous cycle. *Mol Reprod Dev* 2002;**62**:92–98.

Cato AC, Nestl A, Mink S. Rapid actions of steroid receptors in cellular signaling pathways. *Sci STKE* 2002;**2002**:RE9.

Cheng G, Weihua Z, Makinen S, Makela S, Saji S, Warner M, Gustafsson JA, Hovatta O. A role for the androgen receptor in follicular atresia of estrogen receptor beta knockout mouse ovary. *Biol Reprod* 2002;**66**:77–84.

De Leo V, Lanzetta D, D'Antona D, la Marca A, Morgante G. Hormonal effects of flutamide in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 1998;**83**:99–102.

Dettlaff TA. Action of actinomycin and puromycin upon frog oocyte maturation. *J Embryol Exp Morphol* 1966;**16**:183–195.

Drummond AE. The role of steroids in follicular growth. *Reprod Biol Endocrinol* 2006;**4**:16.

Ehrmann DA. Polycystic ovary syndrome. *N Engl J Med* 2005;**352**:1223–1236.

Eppig JJ. Maintenance of meiotic arrest and the induction of oocyte maturation in mouse oocyte–granulosa cell complexes developed *in vitro* from preantral follicles. *Biol Reprod* 1991;**45**:824–830.

Eppig JJ, Freter RR, Ward-Bailey PF, Schultz RM. Inhibition of oocyte maturation in the mouse: participation of cAMP, steroid hormones, and a putative maturation-inhibitory factor. *Dev Biol* 1983;**100**:39–49.

Fabian JR, Morrison DK, Daar IO. Requirement for Raf and MAP kinase function during the meiotic maturation of Xenopus oocytes. *J Cell Biol* 1993;**122**:645–652.

Fan HY, Sun QY. Involvement of mitogen-activated protein kinase cascade during oocyte maturation and fertilization in mammals. *Biol Reprod* 2004;**70**:535–547.

Gill A, Jamnongjit M, Hammes SR. Androgens promote maturation and signaling in mouse oocytes independent of transcription: a release of inhibition model for mammalian oocyte meiosis. *Mol Endocrinol* 2004;**18**:97–104.

Hammes SR. Steroids and oocyte maturation—a new look at an old story. *Mol Endocrinol* 2004;**18**:769–775.

Hampton JH, Manikkam M, Lubahn DB, Smith MF, Garverick HA. Androgen receptor mRNA expression in the bovine ovary. *Domest Anim Endocrinol* 2004;**27**:81–88.

Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. *Endocr Rev* 2002;**23**:175–200.

Hirai M, Hirata S, Osada T, Hagihara K, Kato J. Androgen receptor mRNA in the rat ovary and uterus. *J Steroid Biochem Mol Biol* 1994;**49**:1–7.

Hu YC, Wang PH, Yeh S, Wang RS, Xie C, Xu Q, Zhou X, Chao HT, Tsai MY, Chang C. Subfertility and defective folliculogenesis in female mice lacking androgen receptor. *Proc Natl Acad Sci USA* 2004;**101**:11209–11214.

Jamnongjit M, Hammes SR. Ovarian steroids: the good, the bad, and the signals that raise them. *Cell Cycle* 2006;**5**:1178–1183.

Jamnongjit M, Gill A, Hammes SR. Epidermal growth factor receptor signaling is required for normal ovarian steroidogenesis and oocyte maturation. *Proc Natl Acad Sci USA* 2005;**102**:16257–16262.

Juengel JL, Heath DA, Quirke LD, McNatty KP. Oestrogen receptor alpha and beta, androgen receptor and progesterone receptor mRNA and protein localisation within the developing ovary and in small growing follicles of sheep. *Reproduction* 2006;**131**:81–92.

Kalab P, Kubiak JZ, Verlhac MH, Colledge WH, Maro B. Activation of p90rsk during meiotic maturation and first mitosis in mouse oocytes and eggs: MAP kinase-independent and -dependent activation. *Development* 1996;**122**:1957–1964.

Kimura S, Matsumoto T, Matsuyama R, Shiina H, Sato T, Takeyama K, Kato S. Androgen receptor function in folliculogenesis and its clinical implication in premature ovarian failure. *Trends Endocrinol Metab* 2007;**18**:183–189.

Li M, Ai JS, Xu BZ, Xiong B, Yin S, Lin SL, Hou Y, Chen DY, Schatten H, Sun QY. Testosterone potentially triggers meiotic resumption by activation of intra-oocyte SRC and MAPK in porcine oocytes. *Biol Reprod* 2008a;**79**:897–905.

Li M, Liang CG, Xiong B, Xu BZ, Lin SL, Hou Y, Chen DY, Schatten H, Sun QY. PI3-kinase and mitogen-activated protein kinase in cumulus cells mediate EGF-induced meiotic resumption of porcine oocyte. *Domest Anim Endocrinol* 2008b;**34**:360–371.

Liang CG, Su YQ, Fan HY, Schatten H, Sun QY. Mechanisms regulating oocyte meiotic resumption: roles of mitogen-activated protein kinase. *Mol Endocrinol* 2007;**21**:2037–2055.

Losel RM, Falkenstein E, Feuring M, Schultz A, Tillmann HC, Rossol-Haseroth K, Wehling M. Nongenomic steroid action: controversies, questions, and answers. *Physiol Rev* 2003;**83**:965–1016.

Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS, Wilson EM. Cloning of human androgen receptor complementary DNA and localization to the X chromosome. *Science* 1988;**240**:327–330.

Lutz LB, Cole LM, Gupta MK, Kwist KW, Auchus RJ, Hammes SR. Evidence that androgens are the primary steroids produced by Xenopus laevis ovaries and may signal through the classical androgen receptor to promote oocyte maturation. *Proc Natl Acad Sci USA* 2001;**98**:13728–13733.

Lyon MF, Glenister PH. Evidence from Tfm-O that androgen is inessential for reproduction in female mice. *Nature* 1974;**247**:366–367.

Maller JL, Krebs EG. Regulation of oocyte maturation. *Curr Top Cell Regul* 1980;**16**:271–311.

Motola S, Popliker M, Tsafriri A. Are steroids obligatory mediators of luteinizing hormone/human chorionic gonadotropin-triggered resumption of meiosis in mammals? *Endocrinology* 2007;**148**:4458–4465.

Murray AA, Gosden RG, Allison V, Spears N. Effect of androgens on the development of mouse follicles growing *in vitro*. *J Reprod Fertil* 1998; **113**:27–33.

Ohashi S, Naito K, Sugiura K, Iwamori N, Goto S, Naruoka H, Tojo H. Analyses of mitogen-activated protein kinase function in the maturation of porcine oocytes. *Biol Reprod* 2003;**68**:604–609.

Sato T, Matsumoto T, Kawano H, Watanabe T, Uematsu Y, Sekine K, Fukuda T, Aihara K, Krust A, Yamada T *et al*. Brain masculinization requires androgen receptor function. *Proc Natl Acad Sci USA* 2004; **101**:1673–1678.

Schultz RM, Montgomery RR, Ward-Bailey PF, Eppig JJ. Regulation of oocyte maturation in the mouse: possible roles of intercellular communication, cAMP, and testosterone. *Dev Biol* 1983;**95**:294–304.

Seger R, Hanoch T, Rosenberg R, Dantes A, Merz WE, Strauss JF 3rd, Amsterdam A. The ERK signaling cascade inhibits gonadotropin-stimulated steroidogenesis. *J Biol Chem* 2001;**276**:13957–13964.

Shiina H, Matsumoto T, Sato T, Igarashi K, Miyamoto J, Takemasa S, Sakari M, Takada I, Nakamura T, Metzger D *et al*. Premature ovarian failure in androgen receptor-deficient mice. *Proc Natl Acad Sci USA* 2006;**103**:224–229.

Simpson ER. Role of aromatase in sex steroid action. *J Mol Endocrinol* 2000; **25**:149–156.

Slomczynska M, Duda M, Sl zak K. The expression of androgen receptor, cytochrome P450 aromatase and FSH receptor mRNA in the porcine ovary. *Folia Histochem Cytobiol* 2001;**39**:9–13.

Smith LD, Ecker RE. The interaction of steroids with Rana pipiens oocytes in the induction of maturation. *Dev Biol* 1971;**25**:232–247.

Smith DM, Tenney DY. Effects of steroids on mouse oocyte maturation *in vitro*. *J Reprod Fertil* 1980;**60**:331–338.

Smith LD, Ecker RE, Subtelny S. *In vitro* induction of physiological maturation in Rana pipiens oocytes removed from their ovarian follicles. *Dev Biol* 1968;**17**:627–643.

Su YQ, Wigglesworth K, Pendola FL, O'Brien MJ, Eppig JJ. Mitogen-activated protein kinase activity in cumulus cells is essential for gonadotropin-induced oocyte meiotic resumption and cumulus expansion in the mouse. *Endocrinology* 2002;**143**:2221–2232.

Su YQ, Denegre JM, Wigglesworth K, Pendola FL, O'Brien MJ, Eppig JJ. Oocyte-dependent activation of mitogen-activated protein kinase (ERK1/2) in cumulus cells is required for the maturation of the mouse oocyte–cumulus cell complex. *Dev Biol* 2003;**263**:126–138.

Suzuki T, Sasano H, Kimura N, Tamura M, Fukaya T, Yajima A, Nagura H. Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: relationship to expression of steroidogenic enzymes. *Hum Reprod* 1994;**9**:1589–1595.

Tetsuka M, Hillier SG. Androgen receptor gene expression in rat granulosa cells: the role of follicle-stimulating hormone and steroid hormones. *Endocrinology* 1996;**137**:4392–4397.

Tetsuka M, Whitelaw PF, Bremner WJ, Millar MR, Smyth CD, Hillier SG. Developmental regulation of androgen receptor in rat ovary. *J Endocrinol* 1995;**145**:535–543.

Tsafriri A, Motola S. Are steroids dispensable for meiotic resumption in mammals? *Trends Endocrinol Metab* 2007;**18**:321–327.

Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. *J Clin Invest* 1998;**101**:2622–2629.

Vermeiden JP, Zeilmaker GH. Relationship between maturation division, ovulation and leuteinization in the female rat. *Endocrinology* 1974; **95**:341–351.

Walters KA, Allan CM, Jimenez M, Lim PR, Davey RA, Zajac JD, Illingworth P, Handelsman DJ. Female mice haploinsufficient for an inactivated androgen receptor (AR) exhibit age-dependent defects that resemble the AR null phenotype of dysfunctional late follicle development, ovulation, and fertility. *Endocrinology* 2007;**148**:3674–3684.

Walters KA, Allan CM, Handelsman DJ. Androgen actions and the ovary. *Biol Reprod* 2008;**78**:380–389.

Wang H, Andoh K, Hagiwara H, Xiaowei L, Kikuchi N, Abe Y, Yamada K, Fatima R, Mizunuma H. Effect of adrenal and ovarian androgens on type 4 follicles unresponsive to FSH in immature mice. *Endocrinology* 2001; **142**:4930–4936.

Weil SJ, Vendola K, Zhou J, Adesanya OO, Wang J, Okafor J, Bondy CA. Androgen receptor gene expression in the primate ovary: cellular localization, regulation, and functional correlations. *J Clin Endocrinol Metab* 1998;**83**:2479–2485.

Wood JR, Strauss JF 3rd. Multiple signal transduction pathways regulate ovarian steroidogenesis. *Rev Endocr Metab Disord* 2002;**3**:33–46.

Wood JR, Dumesic DA, Abbott DH, Strauss JF 3rd. Molecular abnormalities in oocytes from women with polycystic ovary syndrome revealed by microarray analysis. *J Clin Endocrinol Metab* 2007;**92**:705–713.

Yang MY, Fortune JE. Testosterone stimulates the primary to secondary follicle transition in bovine follicles *in vitro*. *Biol Reprod* 2006; **75**:924–932.

Yang WH, Lutz LB, Hammes SR. Xenopus laevis ovarian CYP17 is a highly potent enzyme expressed exclusively in oocytes. Evidence that oocytes play a critical role in Xenopus ovarian androgen production. *J Biol Chem* 2003;**278**:9552–9559.

Yeh S, Tsai MY, Xu Q, Mu XM, Lardy H, Huang KE, Lin H, Yeh SD, Altuwaijri S, Zhou X *et al.* Generation and characterization of androgen receptor knockout (ARKO) mice: an *in vivo* model for the study of androgen functions in selective tissues. *Proc Natl Acad Sci USA* 2002;**99**:13498–13503.

*Submitted on November 4, 2008; resubmitted on January 7, 2009; accepted on January 20, 2009*