

Review

Roles of Androgen Receptor in Male and Female reproduction: Lessons from Global and Cell Specific Androgen Receptor Knockout (ARKO) Mice

XINCHANG ZHOU

From the Department of Otolaryngology, Iowa City, Iowa.

Correspondence to: Dr Xinchang Zhou, The Department of Otolaryngology, University of Iowa, 5270 CBRB, Iowa City, IA 52242

Telephone number: 319-335-6653

Fax number: 319-335-9890, E-mail: xinchang-zhou@uiowa.edu

Abstract

The androgen receptor (AR), a member of the nuclear receptor superfamily, is a ligand-dependent transcription factor involved in regulating expression of an array of androgen-responsive genes. AR-mediated androgen actions play the important roles in male and female reproductive development and function. AR mutations can cause a diverse range of diseases such as testicular feminization mutation (Tfm) syndrome, prostate cancer, Kennedy's disease. However, due to lack of genetic models, the molecular mechanisms involved in physiological and pathological effects of androgen /AR function in male and female reproductive health remains largely unknown. To get a better insight into the molecular working mechanisms of the AR, a global and several cell specific conditional knockout mouse models have been developed. These models are reviewed here and the phenotypes of the different cell specific ARKO mice are compared with those of the global ARKO mice.

Key words: nuclear receptor, conditional knockout, phenotype

Introduction

The androgen receptor (AR), a member of the nuclear receptor superfamily, plays important roles in male reproductive functions, as well as female folliculogenesis (Donath et al. 1997) and breast development (Liao et al. 2002). The AR gene comprises eight exons that encode a 110 kDa protein, which contains an N-terminal transactivation domain, a central DNA-binding domain, and a C-terminal ligand-binding domain (Chang et al. 1995). Testosterone and its more potent metabolite, dihydrotestosterone can bind AR, and the liganded AR form homodimers and interact with many coregulators to modulate androgen target genes (Heinlein et al. 2002). AR dysfunction causes a diverse range of clinical disorders, such as testicular feminization mutation (Tfm) syndrome, prostate cancer, and Kennedy's disease (La Spada et al. 1991; Griffin 1992; Quigley et al. 1995; Choong et al. 1998; McPhaul 1999). In addition to men, the *Tfm* syndrome has been described in other species, such as the dog (Schultz MG, 1962), the rat (Bardin et al. 1970), the mouse (Lyon et al. 1970, Goldstein et al. 1972), and the cat (Meyers-Wallen et al. 1989). However, the molecular basis of the AR function underlying these AR-related disorders remains to be elucidated due to the lack of stable genetic models.

Because male mice lacking a functional AR gene are expected to similar to *Tfm* male mice with infertility (Quigley et al 1995, McPhaul 1999), successfully disrupted AR gene essential for male reproduction must avoid its transmission to the next generation. Furthermore, as all *Tfm* model animals are genetically male, it is impossible to generate genetically AR gene mutation homozygous females (Matsumoto et al. 2003). Therefore, it is impractical to generate an ARKO mouse line by either breeding or classical gene KO methods. To generate ARKO mice, a cre-loxP strategy for conditional KO is necessary. The Cre-loxP system utilizes the expression of P1 phage Cre to catalyze the excision of DNA located between flanking loxP sites. This strategy differs from the standard targeted disruption procedure in that embryonic stem cells are generated in which the target segment is not disrupted but is flanked by loxP sites (floxed). The targeted gene thus functions normally, and mice can be bred to homozygosity for the targeted locus.

The recently global AR knockout male mice showed *Tfm* syndrome, exhibiting infertility and female-typical external appearance with a blind end vagina and a clitoris-like phallus. Testes were located in

abdominal or inguinal region, and germ cell development was severely disrupted (Yeh et al. 2002, Matsumoto et al. 2003). The global female ARKO mice displayed subfertility with retarded development of mammary glands (Yeh et al. 2002, Yeh et al. 2003, Hu et al. 2004). These findings further emphasized the AR signaling necessary for male external and internal phenotypes, and female ovary and breast development. Because a gene alteration in the whole body may cause a complex phenotype so that it is hard to differentiate direct effects in a particular tissue or cell type from those secondary effects arising from a gene change in other cell types, it is necessary to use a cre-lox strategy in transgenic mice expressing Cre recombinase selectively in specific cells to generate a cell specific ARKO mouse model in which disruption of AR function is exclusively in a particular cell type. Here, we summarize the generation and phenotypes of both the global and cell-specific AR knockout (AR^{-y}) in germ cells (G-AR^{-y}), peritubular myoid cells (PM-AR^{-y}), Leydig cells (L-AR^{-y}), Sertoli cells (S-AR^{-y}), and prostate epithelial cell (PEARKO) mouse models to understand the consequences of AR loss in different cell types.

Generation of global and cell specific ARKO mice

To generate global or cell specific ARKO mice, the C57-B6/129/SvEv loxP-floxed AR mice were first produced (Yeh et al. 2002, Matsumoto et al. 2003) and then mated with the mice expressing Cre recombinase ubiquitously or selectively in specific cells. Global ARKO mice were generated by mating the floxed AR mice with the transgenic mice ubiquitously expressing Cre driven by the strong β -actin (ACTB)-promoter (Yeh et al. 2002), CMV promoter (Matsumoto et al. 2003), or PGK-promoter (Ophoff et al. 2009); Sertoli cell specific ARKO (S-AR^{-y}) mice were generated by mating the floxed AR mice with anti-Müllerian hormone (AMH)-Cre transgenic mice (Chang et al. 2004, De Gendt et al. 2004, Holdcraft et al. 2004); Leydig cell specific ARKO (L-AR^{-y}) mice were generated by mating the floxed AR mice with AMHR2-Cre transgenic mice (Xu et al. 2007); Peritubular myoid cell specific ARKO (PM-AR^{-y}) mice were generated by mating the floxed AR mice with Transgelin-Cre transgenic mice (Zhang et al. 2006); Germ cell specific ARKO (G-AR^{-y}) mice were generated by mating the floxed AR mice with Sycp1-Cre transgenic mice (Tsai et al. 2006); and prostate epithelial ARKO (PEARKO) mice are produced by the Cre recombinase under the control of the prostate epithelial cell-specific probasin gene promoter (Simanainen et al. 2007, Niu et al. 2008).

Phenotypes of global and cell specific ARKO mice

1. Phenotypes of global ARKO mice

Phenotypes of the global ARKO male mice. The early differentiation of male accessory sex organs is dependent on testosterone during early embryonic development when the 5 α reductase does not yet exist, and the major effect of testosterone directs the differentiation of male-specific Wolffian duct-derived internal genital structures at early embryonic stages, which include the epididymis, vas deferens, and seminal vesicles (Wilson 1981, Tong et al. 1996, Hutson et al. 1997). However, dihydroxytestosterone (DHT)-dependent development of the prostate and prostatic urethra occurs internally, and the differentiation of genital structures externalizes at later embryonic developmental stages, when the 5 α reductase responsible for conversion of testosterone to DHT is available (Quigley 1998). If the AR fails to activate its target genes in the presence of androgens during these critical stages, the severe defects in male sex differentiation and phenotype development will be caused. Global ARKO males (Fig. 1) had female-like appearance and were infertile, such as the short genito-anal distance, a vagina with a blind end, and a clitoris-like phallus, instead of a penis and scrotum. All of vas deferens, epididymis, seminal vesicle, and prostate were absent in ARKO males. However, no ovaries, fallopian tubes and uterus were observed, although small abdominal or inguinal testes were present, and are similar in Tfm mice or humans with complete androgen insensitivity syndrome (Yeh et al. 2002, Matsumoto et al. 2003). The external genitalia in male ARKO mice showed feminized appearance. The penis seemed microphallus and

the urethra showed hypospadias. The scrotum was poorly developed and looked like the labia majora in the female. Histological examination of the testes showed that spermatogenesis was severely arrested at pachytene spermatocytes. From these results, it was clear that AR was essential for the development of male reproductive organs and spermatogenesis, although it was not required for the formation of testis.

Phenotypes of the global ARKO female mice. Androgens are primarily produced by theca cells in response to luteinizing hormone (LH) in female ovaries. Although androgen receptors (AR) are expressed in granulosa cells, stromal cells (Schreiber et al. 1976, Hirai et al. 1994, Tetsuka et al. 1995), theca cells (Horie et al. 1992), and oocytes (Szoltys et al. 2000, Cardenas et al. 2002, Gill et al. 2004), the roles of AR-mediated actions in female reproductive physiology remains unclear. Previous studies have revealed that androgens played a positive role in the early stage of folliculogenesis (Vendola et al. 1998, Weil et al. 1999), but other studies (Vendola et al. 1999, Hickey et al. 2005) suggested that this effect could be mediated by way of growth factor signal pathways instead of the AR-mediated pathway. Global ARKO female mice (Fig. 2) appeared normal but had longer oestrous cycles and reduced fertility (Yeh et al. 2001, Hu et al. 2004, Shiina et al. 2006, Waters et al. 2007), evident in the delayed production of their first litter and the reduced average number of the pups per litter. Heterozygous females exhibited an age-dependent reduction in pups per litter compared with wild type females, indicating a significant gene dosage effect of AR on female fertility (Waters et al. 2007). The ovaries from mature ARKO females exhibited a marked reduction in the number of corpora lutea (Hu et al. 2004, Waters et al. 2007) and oocytes recovered from naturally mated ARKO females (Waters et al. 2007). Furthermore, the defective conformation of the cumulus cell-oocyte complex from the ARKO females implies a lower fertilization capability of the ARKO oocytes. However, early embryo development to the two-cell stage was unaltered (Waters et al. 2007). Global ARKO female mice also showed the retarded development of mammary glands (Yeh et al. 2003, Shiina et al. 2006) with reduced ductal branching in the prepubertal stages, and fewer Cap cells in the terminal end buds, as well as decreased lobuloalveolar development in adult females, and fewer milk-producing alveoli in the lactating glands, maybe due to the defects of insulin-like growth factor I-insulin-like growth factor I receptor and mitogen-activated protein kinase (MAPK) signals as well as estrogen receptor (ER) activity. In addition, AR-mediated actions also have a distinct role in the regulation of uterine development, because the loss of AR in ARKO female mice predisposes the uterus to aberrant uterine growth (Waters et al. 2009). Taken together, these data provide *in vivo* evidence showing that AR plays an important role in the normal development and functions of ovaries, uteruses and breasts in females.

2. Phenotypes of cell specific ARKO mice

Phenotypes of Sertoli cell selective ARKO mice. Sertoli cells are generally believed to be the primary mediators of the androgen regulation of spermatogenesis because they provide physical and nutritional support to the developing germ cells (Griswold 1998). The hypothesis was examined by three Sertoli cell specific ARKO (S-AR^{-/y}) groups (Chang et al. 2004, De Gendt et al. 2004, Holdcraft & Braun 2004a). In contrast to global ARKO mice, results from S-AR^{-/y} mice showed a normal external male appearance and have normal internal male genital tract development. S-AR^{-/y} males (Fig. 3) have fully descended testes but the size of the testis is markedly reduced. S-AR^{-/y} testis histologically showed a decrease in diameter of seminiferous tubules and reduced germ cell complement as compared with wild type male littermates. Poor germ cell differentiation with the majority of germ cell maturation ceasing at the diplotene primary spermatocyte stage was seen in the seminiferous tubules of S-AR^{-/y} mice (Chang et al. 2004). Occasionally, a few secondary spermatocytes occur, and very few round spermatids exist (De Gendt et al. 2004, Holdcraft & Braun 2004a). In agreement with the earlier studies in which

androgens/AR play an important role in the Sertoli cell maturation rather than Sertoli cell proliferation, however, some studies (De Gendt et al. 2004, Tan et al. 2005) showed that adult S-AR^{-y} mice develop nearly normal numbers of Sertoli cells as compared with wild type male control, indicating that the effect of androgens on the number of Sertoli cells is not mediated by the AR signal pathway (De Gendt et al. 2004, Tan et al. 2005). More detailed analysis of the S-AR^{-y} testes revealed that androgen, acting through Sertoli cells AR, regulates the microenvironment of seminiferous epithelium by influencing a broad spectrum of gene changes in Sertoli cells (Denolet et al. 2006, Wang et al. 2006, Eacker et al. 2007). These results showed that loss of Sertoli cell specific AR function could impair the normal supportive function for movement of developing germ cells, the junction complex formation and basement membrane development of Sertoli cells or the functional integrity of the blood-testis barrier, and Sertoli cell nursery functions for developing germ cells (Wang et al. 2006, Denolet et al. 2006). Surprisingly, development and function of the adult generation of Leydig cells was disturbed not only in ARKO but also in S-AR^{-y} mice (De Gendt et al., 2005). Leydig cell number in S-AR^{-y} testis was normal on day 12, but was reduced by more than 40% at later ages and the Leydig cell size appeared to be increased. However, Leydig cell number in global ARKO mice was reduced by up to 83% at all ages and Leydig cell size did not increase beyond day 12. Immunohistochemistry and quantitative RT-PCR for Leydig cell-specific markers confirmed that steroidogenic function per Leydig cell was increased in S-AR^{-y} mice but decreased in global ARKO mice. The altered Leydig cell number and function in the S-AR^{-y} testis might be due to expression changes of platelet-derived growth factor-A and oestrogen sulfotransferase. The epididymis size in S-AR^{-y} mice was decreased by 63% of wild type size (Wang et al. 2006), and S-AR^{-y} mice failed to impregnate the wild-type female mice due to absence of mature sperm production (Chang et al. 2004, De Gendt et al. 2004, Holdcraft & Braun 2004a). These S-AR^{-y} mice studies above (Chang et al. 2004, De Gendt et al. 2004, Holdcraft & Braun 2004a, Tan et al. 2005, Denolet et al. 2006, Wang et al. 2006, Eacker et al. 2007) clearly demonstrated that AR function in Sertoli cells is essential for the maintenance of fully competent Sertoli cell functions and the appropriate hormone levels to support the completion of meiosis I during spermatogenesis.

Phenotypes of Leydig cell selective ARKO mice. Testicular testosterone produced by the Leydig cells is essential for qualitatively and quantitatively complete spermatogenesis and development of the male phenotype. Long-term intratesticular testosterone withdrawal causes the failure of progression of round spermatid to elongated spermatids, particularly during stages VII and VIII of the spermatogenic cycle (O'Donnell et al. 1994). The AR is also expressed abundantly in the mature Leydig cells. Knocking out the AR selectively in Leydig cells (L-AR^{-y}) by Amhr2 driven Cre strategy (Tsai et al. 2006, Xu et al. 2007) resulted in mice with decreased size of testis and epididymis, reduced serum levels of testosterone and increased serum levels of LH. The histology of adult L-AR^{-y} mice testes showed a decrease in diameter of seminiferous tubules, and germ cell development arrested predominantly at the round spermatid stage. There were no further differentiated mature elongated spermatids or spermatozoa throughout the L-AR^{-y} testis. Consequently, L-AR^{-y} mice were infertile because no sperm existed in the epididymis. Compared with wild type male mice, the decreased size of testis in L-AR^{-y} mice was mainly due to the defect of Leydig cell steroidogenic function subsequently affecting Sertoli cell functions to support postmeiotic germ cells, while the decrease in epididymis size in L-AR^{-y} mice could be due to the lower serum testosterone levels and no mature sperm production. The decreased synthesis of testosterone by Leydig cells and lower levels of serum testosterone reduced feedback on the hypothalamus and pituitary and thus LH levels were elevated. It is important to note that the AR knockout in Leydig cells in this model was not complete. S-AR^{-y} mice also had consequences for the development of normal Leydig cell numbers with a normal or increased Leydig cell size, while the size of the Leydig cells in ARKO mice was reduced (De Gendt et al. 2005). The L-AR^{-y} mice studies revealed that AR function in Leydig cells is essential for the maintenance of appropriate Leydig cell steroidogenic function and subsequent affect on Sertoli cell functions to support spermiogenesis, the final differentiation of round spermatids to mature elongated spermatids (Xu et al. 2007).

Phenotypes of peritubular myoid cell selective ARKO mice. Peritubular myoid cells are the AR positive cells surrounding the seminiferous tubules in the testis. These cells may play an indirect role in spermatogenesis through the control and maintenance of the Sertoli cell function as well as in the transport of spermatozoa through the tubular lumen by contractions of the seminiferous tubules (Maekawa et al. 1996, Romano et al. 2005). Indeed, the adult PM-AR^{-y} mice exhibited a decreased testis and the decreased epididymal sperm count compared with wild type males, but the fertility is normal (Zhang et al. 2006). Loss of functional AR in PM cells might impair smooth muscle contractility, Sertoli cell nourishing function, and the integrity of Sertoli cell junctions, consequently affecting testis sperm production and slowing down the germ cell movement (Zhang et al. 2006). However, a recent study on the peritubular myoid cell selective ARKO mice (Welsh et al. 2009) showed that all PTM-ARKO males were azoospermic and infertile, indicating that AR in PTM cells is essential for normal testis function, spermatogenesis, and fertility in males. The very different testicular phenotypes from the two research groups of PTM-ARKO mice were just caused by their usage of different smooth muscle-driven Cre lines (SM22-Cre and smMHC-Cre) on cell-specific ablation of testicular AR from mouse testes, because the SM22-Cre line reported by Zhang et al. did not alter AR expression in testicular PTM cells (Frutkin et al. 2006, Welsh et al. 2009), whereas the smMHC-Cre line used by Welsh et al. (2009) deleted AR from a proportion of PTM cells, although both lines deleted AR from smooth muscle cells surrounding testicular blood vessels. Therefore, the mild phenotypes reported by Zhang *et al.* in their PTM cell AR knockout (KO) might not truly reflect the importance of androgen action *via* PTM cells.

Phenotypes of germ cell selective ARKO mice. Unlike Sertoli cells, peritubular myoid cells, and Leydig cells ((Anthony et al. 1989, Sar et al. 1990, Kimura et al. 1993, Bremner et al. 1994, Vornberger et al. 1994, Zhou et al. 1996), whether germ cells express the AR by the localization of AR using antibodies is still controversial. Some studies indicated that AR is present in germ cells in different species (Kimura et al. 1993, Janssen et al. 1994, Vornberger et al. 1994, Zhou et al. 1996, Arenas et al. 2001), but other reports showed there is little AR staining in the germ cells (Galena et al. 1974, Grootegoed et al. 1977, Anthony et al. 1989, Bremner et al. 1994, Van Rooijen et al. 1995, Suarez-Quian et al. 1999, Pelletier et al. 2000). The testis of G-AR^{-y} mice showed similar testis size and normal spermatogenesis at every spermatogenic stage compared with wild type testis (Tsai et al. 2006). The G-AR^{-y} mice study clearly demonstrated that androgen/AR signals in germ cells do not have significant effects on sperm maturation and testis development.

Phenotypes of prostate epithelial and/or prostate stroma selective ARKO or AR knockdown mice. The prostate is an androgen-dependent organ during fetal organogenesis, through pubertal development when the prostate completes structural and functional maturation, and in later life when prostate cells remain androgen sensitive during evolution of prostate disease (Cunha et al. 2004, Heinlein et al. 2004). Androgens stimulate proliferation and restrain apoptosis of prostate cells. Prostate diseases during later life are among the major causes of death, disability, and health costs in developed countries. Compared with the global ARKO mice exhibiting the abolished prostate development, the prostate epithelial AR knockout (PEARKO) mice (Simanainen et al. 2007, Niu et al. 2008, Simanainen et al. 2009) showed prostate development with normal branching morphogenesis but lobe-specific decrease in prostate weight and hindered structural and functional differentiation of the mature prostate epithelium. The most striking change was increased proliferation and abnormal lesions of epithelial cells predominantly in the anterior lobe of PEARKO mice. These results indicated the vital role of stromal AR in postnatal prostate growth and structural differentiation and the requirement of epithelial AR in maintaining functional differentiation and proliferation of epithelial cells in a lobe-specific manner (Simanainen et al. 2007). The epithelial apoptosis in castration-induced prostatic involution did not significantly differ between control (intact AR) and PEARKO (only stromal AR) males, demonstrating that prostate epithelial involution following castration is mediated primarily via stromal AR-dependent apoptotic signals. The epithelial AR

inactivation during postnatal prostate development sensitizes prostate epithelial cells to paracrine signaling mediated by stromal AR activity leading to indirectly androgen-induced epithelial hyperproliferation and formation of epithelial hyperplastic cysts by aromatizable androgens (Simanainen et al. 2009). The double prostate epithelium AR and stroma AR knocked down mice (Niu et al. 2008) further confirmed that the prostate stromal AR might play a more dominant role than the epithelial AR to promote epithelial proliferation.

Conclusions and future directions

The conditional knockout mice are a wonderful tool used to further unravel the molecular mechanisms of the roles of AR gene in male and female reproduction by global or cell specific ablations of AR gene in the body. From the results of the AR knockout models we can conclude that androgen and AR signal pathways in Wolffian ducts, genital tubercle, and urogenital sinus during embryonic developmental stage play a critical role in the differentiation and development of male internal and external genitalia, and AR has the differential roles in the different cells responsible for spermatogenesis, male fertility, and prostate structural and functional differentiation; AR also has an important role in normal ovary and breast development and functions in females. Activity of the AR plays an important role during at least three steps of spermatogenesis: progression through meiosis I, the transition from round to elongated spermatids, and the terminal stages of spermiogenesis (Holdcraft & Braun 2004b, Xu et al. 2007, Kerkhofs et al. 2009, Wang et al. 2009). However, there are still many questions that need to be answered, such as the exact molecular mechanisms for AR regulation of Sertoli cell, Leydig cell, PM cell proliferation and differentiation, and prostate epithelium and stromal cells. Loss of functional AR in Sertoli cells has also marked effects on Leydig cell development and function by pathways that have been only partially elucidated. It would also be of interest to determine that loss AR in how many types of cells in the body will be able to cause agenesis of male accessory genital organs as the global ARKO mice. The future studies include investigation of the detailed molecular mechanisms in cell-specific AR knockout male mice which possibly results in creation of a reversibly safe male contraceptive method and improvements in the treatment of infertility, hypogonadism and testicular dysgenesis syndrome, and generation of the cell specific ARKO female mice to be used to explore the relative contribution of different type of AR-expressed cells to androgen actions in female folliculogenesis and breast development.

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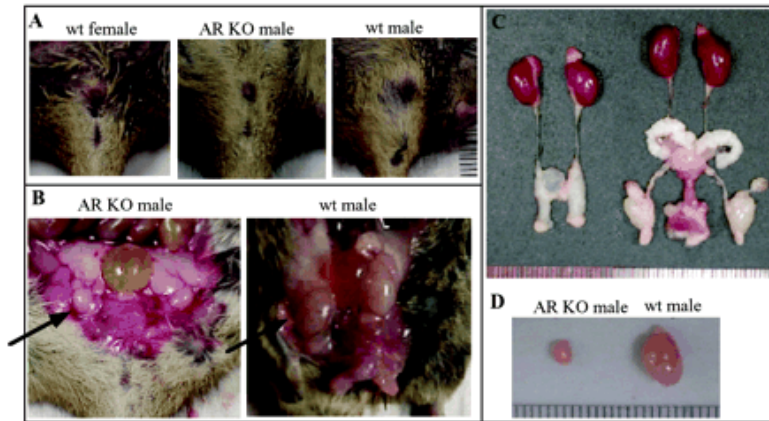
Figure Legends

Fig.1 Phenotype of 8-week-old male ARKO mice. Six 8-week-old male ARKO mice were killed. The results were always compared among siblings. (A) The external genitalia of male ARKO, wt male, and wt female. (B and C) The internal genitalia of male ARKO and wt male mice. Arrows in B identify the testis. (D) The testes of wt male and ARKO mice. Originally published in Yeh et al., 2002 (Copyright 2002, National Academy of Sciences, U.S.A).

Fig.2 Morphological comparison of ovaries from $AR^{+/+}$ and $AR^{-/-}$ females. (A and B) Ovaries from 4-week-old $AR^{+/+}$ and $AR^{-/-}$ females were histologically similar. (C) Statistical analysis of the number of the follicular compartments in $AR^{+/+}$ and $AR^{-/-}$ ovaries ($n = 2$). (D and E) The ovaries from sexually mature, 16-week-old $AR^{+/+}$ females, compared with their $AR^{-/-}$ counterparts. The granulosa layers in the antral follicles were locally thin (open arrowheads) in the $AR^{-/-}$ ovaries, whereas they were even in thickness in the $AR^{+/+}$ ovaries (arrows). An asterisk marks the zona pellucida remnants. (F) Statistical analysis of the number of the follicular compartments in $AR^{+/+}$ and $AR^{-/-}$ ovaries ($n = 3$ for each genotype). Statistical significance determined by using Student's unpaired and two-tailed t test is indicated. Representative sections are shown. P, primordial and primary follicle; PF, preantral follicle; APF, atretic primordial, primary, and preantral follicle; A, antral follicle; AF, atretic antral follicle; CL, corpus luteum. (Bar: 200 μ m.) Originally published in Hu et al., 2004 (Copyright 2004, National Academy of Sciences, U.S.A).

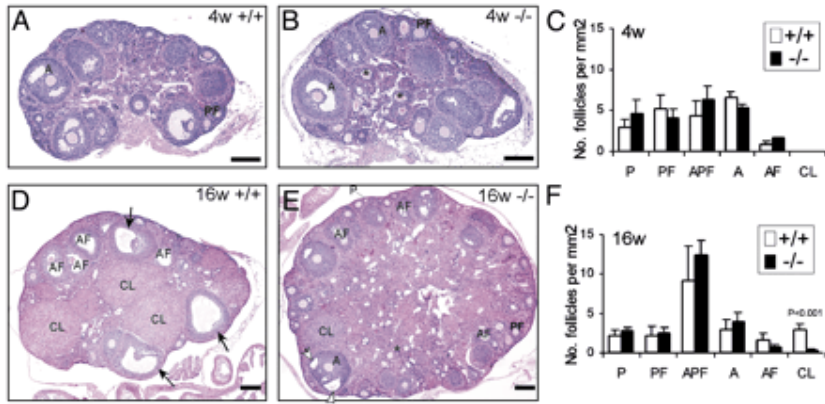
Fig. 3 Dissection of urogenital tracts of WT, ARKO, and SCARKO male mice at the age of 50 days. dd, ductus deferens; sv, seminal vesicles; t, testis; e, epididymis; ft, fat tissue. Originally published in DeGendt et al., 2004 (Copyright 2004, National Academy of Sciences, U.S.A).

Figure 1



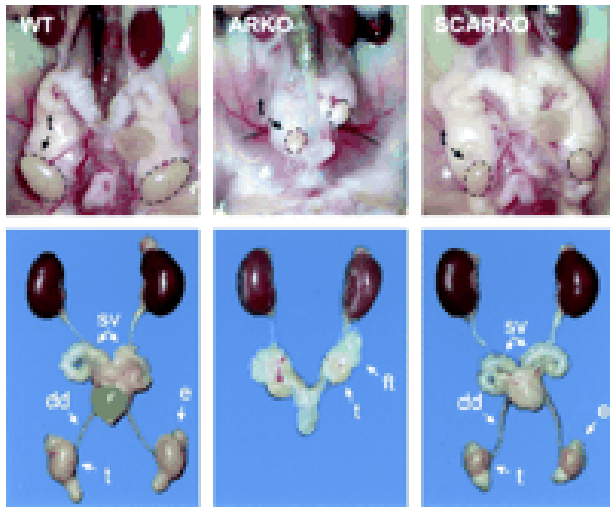
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Figure 2



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Figure 3



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