

*Mini-Review*

# Androgen receptor functions in male and female reproduction

TAKAHIRO MATSUMOTO,<sup>1,2</sup> HIROTAKA KAWANO,<sup>1</sup> HIROKO SHIINA,<sup>1</sup>  
TAKASHI SATO<sup>1</sup> and SHIGEAKI KATO<sup>1,2\*</sup>

<sup>1</sup>Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, and <sup>2</sup>ERATO, Japan Science and Technology, Kawaguchi, Saitama, Japan

The androgen receptor (AR) is a ligand-dependent transcription factor involved in the regulation of many different physiological processes. Dysfunction of AR causes diverse clinical conditions, such as testicular feminization mutation (Tfm) syndrome and prostate cancer. However, the molecular basis of the AR in these disorders largely remains unknown, as a result of a lack of genetic models. Using conditional targeting technique with Cre-loxP system, we successfully generated null AR mutant (ARKO) mice. The ARKO males grew healthily, but they showed typical Tfm abnormalities. The ARKO

males exhibited late onset of obesity with impaired bone metabolism and sexual behaviors. No overt abnormality was found in female ARKO mice, but a premature ovarian failure-like phenotype was found with impaired folliculogenesis. Thus, androgen/AR system supports normal reproduction as well as normal female reproduction. (Reprod Med Biol 2007; 6: 11–17)

**Key words:** androgen receptor, androgen receptor knockout mouse, Cre-loxP system, testicular feminization mutation.

## FUNCTION OF NUCLEAR SEX STEROID RECEPTORS IN GENE REGULATION

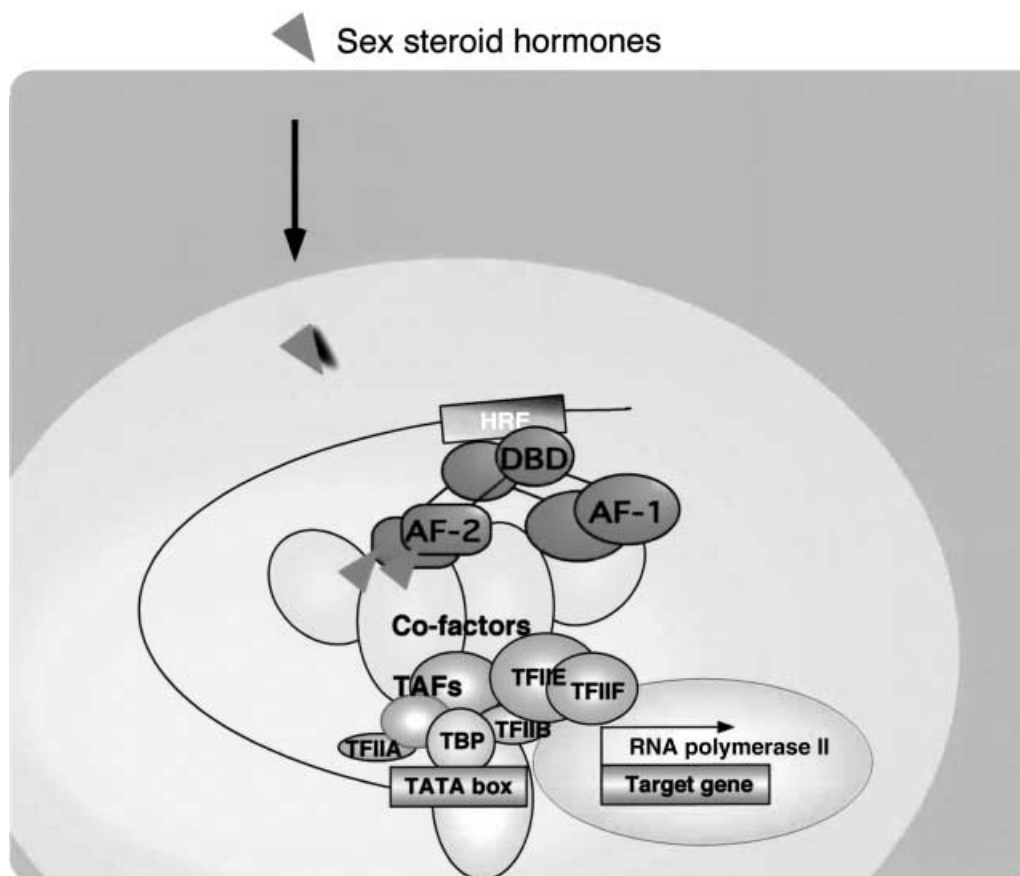
**S**EX STEROIDS EXHIBIT a wide variety of biological actions in physiological and pathological events.<sup>1,2</sup> Development of reproductive organ tumors, such as breast cancer and prostate cancer, often depends on the actions of sex hormones, but the molecular basis remains totally unknown. Nuclear sex hormone receptors (androgen receptor [AR] and estrogen receptors [ER]) are members of the nuclear hormone receptor superfamily and act as ligand-inducible transcription factors.<sup>3</sup> ER and AR form homodimers and bind specific DNA elements referred as hormone responsive elements (HRE) in the target gene promoters (Fig. 1). Members of the nuclear receptor (NR) gene superfamily serve as sequence-specific regulators in the promoters of their cognate target genes. Reflecting the spatio-temporal expression patterns of NR in animals, a wide variety of biological events are under the control of NR-mediated transcriptional regulations. Structurally, NR proteins

can be divided into five domains, A–E<sup>4–6</sup> The highly conserved C domain acts as a DNA binding domain (DBD) which has two Zn finger motifs that recognize and stably bind to specific target DNA. The moderately conserved ligand binding domain (LBD) is mapped to the C-terminal E domain. The N-terminal A/B domain exhibits poor homology among NR but is responsible for ligand-induced transactivation, together with the LBD region in NR as interacting regions for the coactivator complexes. The autonomous activation function-1 (AF-1) located in the A/B domain is ligand-independent, whereas the AF-2 in the LBD is induced upon ligand binding.<sup>7–9</sup> Unliganded LBD appears to suppress the function of the A/B domain, whereas ligand binding to the LBD is thought to evoke the function of LBD and restore the A/B domain function through as yet undescribed intramolecular alteration of the entire steroid receptor structure.

## LIGAND-DEPENDENT TRANSCRIPTIONAL CONTROLS BY NUCLEAR RECEPTOR REQUIRES A NUMBER OF NUCLEAR COREGULATOR COMPLEXES

**L**IGAND-DEPENDENT AND -INDEPENDENT transcriptional control by NR requires the input of two types of coregulators with opposing functions.<sup>10</sup> It appears

\*Correspondence: Dr Shigeaki Kato, Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan. Email: uskato@mail.ecc.u-tokyo.ac.jp  
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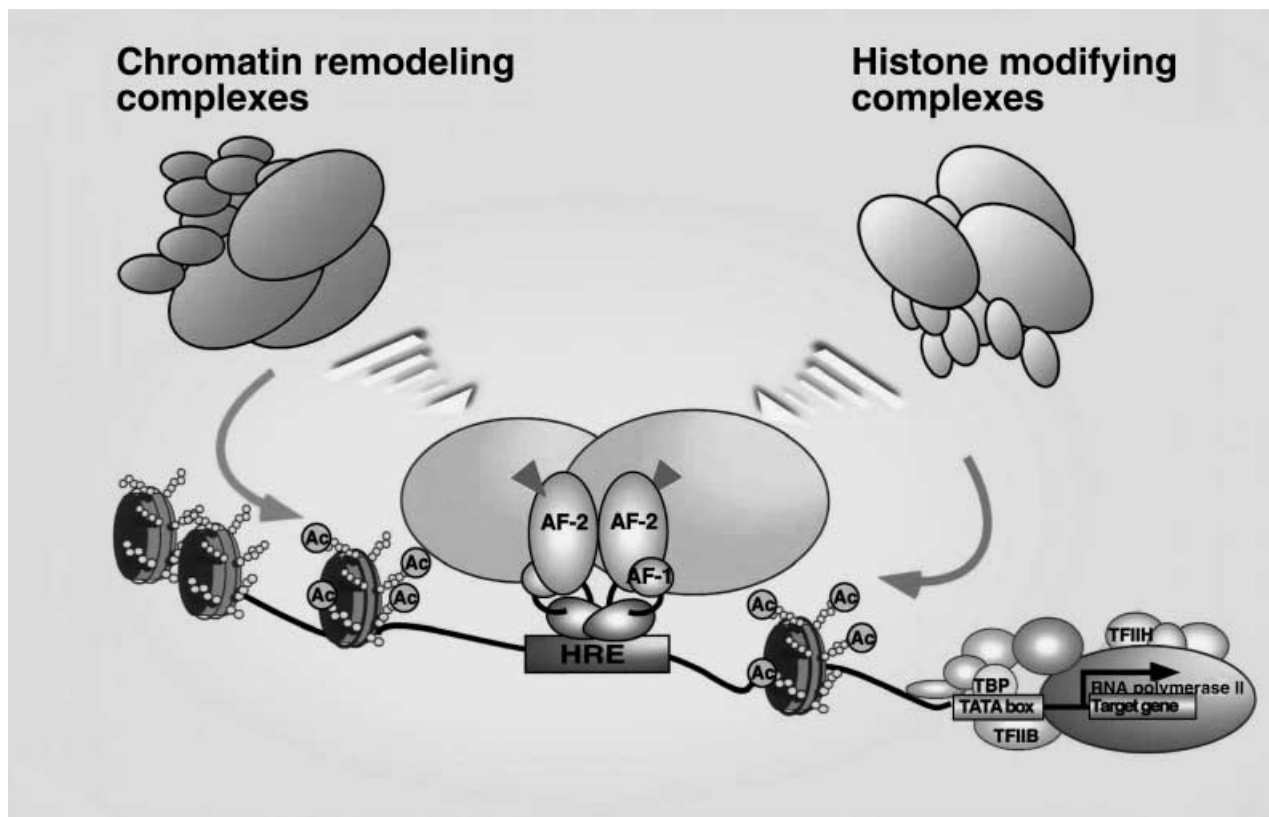
**Figure 1** Nuclear sex hormone receptor controls expression of target genes in a hormone-dependent manner. Sex steroid hormones are thought to exert their physiological effects through transcriptional control by the cognate nuclear receptors (NR). NR recognize and bind with the specific recognition sites, termed hormone responsive elements (HRE). Hormone binding to NR induces association with general transcription factors (GTF) and the target genes are transcribed by RNA polymerase II (RNAPol II).

that most coregulators exist as multiprotein complexes. It is thought that three distinct classes of cofactors support NR transactivation, with two of these classes, CBP/p160 and GCN5/TRAP complexes, containing histone acetyltransferase (HAT) enzymes.<sup>11–15</sup> The other class, DRIP/TRAP complex, is a non-HAT coactivator complex.<sup>16–18</sup> The corepressor type complexes contain histone deacetylase (HDAC) enzymes, which along with NCoR/SMRT physically interact with NR through CoRNR motifs, and are thought to be functionally indispensable subunits in NR corepression complexes.<sup>10,15,19,20</sup> Although histone modification that is a result of HAT/HDAC activity in NR coregulator complexes, in cooperation with chromatin remodeling complexes, explains at least part of the mechanism of NR-mediated transcriptional controls, the molecular link between NR-mediated gene regulation and cell

cycle control remains elusive. In addition to such histone modifications, chromatin remodeling appears to be required (Fig. 2). Three subclasses of ATP-dependent chromatin remodeling complexes have been biochemically identified<sup>21–26</sup> and the SWI/SNIF-type complex is believed to support the ligand-induced transactivation function of NR.<sup>27–29</sup>

#### ANDROGEN RECEPTOR INACTIVATION BY GENE TARGETING WITH THE CRE-LOXP SYSTEM IN MICE

THERE WERE BASIC and technical difficulties in generating an AR knockout (ARKO) mouse (Fig. 3). The AR gene is located on the X chromosome, thereby existing as a single copy in 46, XY male<sup>30</sup> in which androgen exerts most profound effects. As male mice



**Figure 2** Coregulators support ligand-dependent transcriptional controls by nuclear receptors (NR) through chromatin remodeling and histone modifications. Ligand binding positively and negatively controls gene expressions of target genes by NR through switching of coregulators; most of them form histone modifying enzyme complexes, together with histone remodeling by ATP-dependent chromatin remodeling complexes.

lacking the AR gene are expected to show Tfm abnormalities with complete infertility,<sup>31–34</sup> successful targeted disruption of the AR gene (which is essential for reproduction), necessarily prohibits its transmission to the next generation. It is thus impossible to generate ARKO mouse line either in nature or by conventional gene targeting method. Furthermore, because all Tfm models are genetically male, it is simply impractical to generate a genetically female animal that is homozygous for the AR gene mutation.

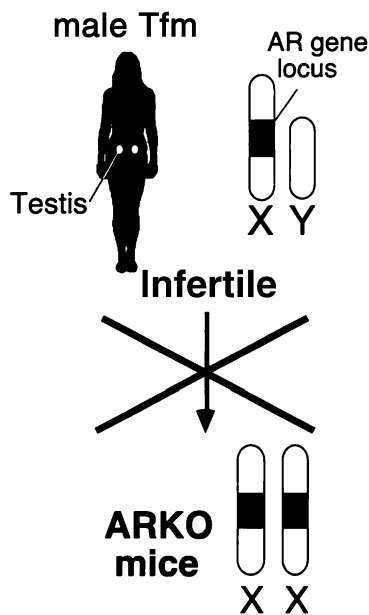
To avoid this problem, we applied a Cre-loxP system<sup>35</sup> to establish the ARKO mice line (Fig. 3). We first generated the floxed AR mice, in which the AR gene locus was flanked by loxP sites. The floxed AR mice were fully fertile and expressed AR protein normally. We then crossed them with mice expressing the Cre recombinase ubiquitously under the control of the CMV promoter, and obtained male and female ARKO mice at theoretical Mendelian frequency.<sup>36</sup>

#### FEMALE-TYPICAL APPEARANCE OF MALE ARKO MICE

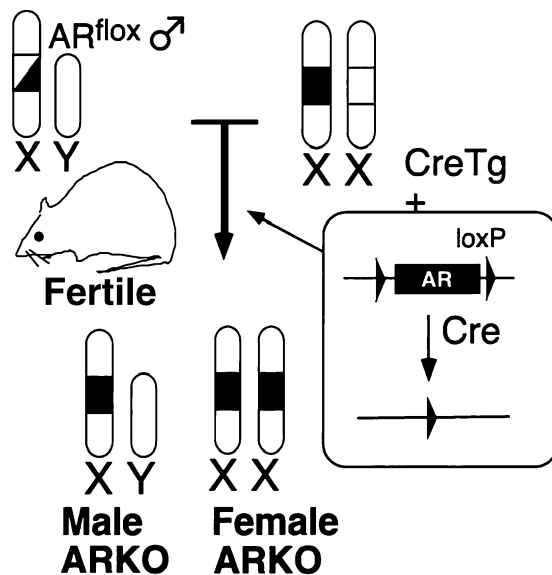
THE APPEARANCE OF the male and female ARKO and the wild-type mice are shown in Figure 4. The ARKO males exhibited female-typical external appearance, such as vagina with blind end, and clitoral-like phallus, instead of penis and scrotum.<sup>36</sup> Male reproductive organs including seminal vesicle, vas deferens, epididymis and prostate were absent in ARKO males. However, no ovary and uterus were observed, although they had inguinal small testes. The histological examination of the testes showed that spermatogenesis was severely arrested. From these results, it is clear that AR is not required for the formation of testis, but essential for development of male reproductive organs and spermatogenesis.

Estimation of plasma hormone levels in the ARKO males showed markedly lowered androgens as well as a luteinizing hormone, but the estradiol level was almost

## (a) Conventional method or Tfms



## (b) Cre-loxP system



**Figure 3** Strategy for generating ARKO mice line. (a) The androgen receptor (AR) gene is located in the X chromosome and male Tfm animals are infertile in any case, so the mutated AR gene cannot be transmitted to the next generation. (b) In the first step, floxed AR mice that carry a functional, but loxP-flanked, AR gene are generated by introducing the loxP sites in the first exon of the AR gene by homologous recombination in embryonic stem cells. Next, by mating these mice with CMV-Cre transgenic mice, the AR gene is disrupted during embryogenesis.

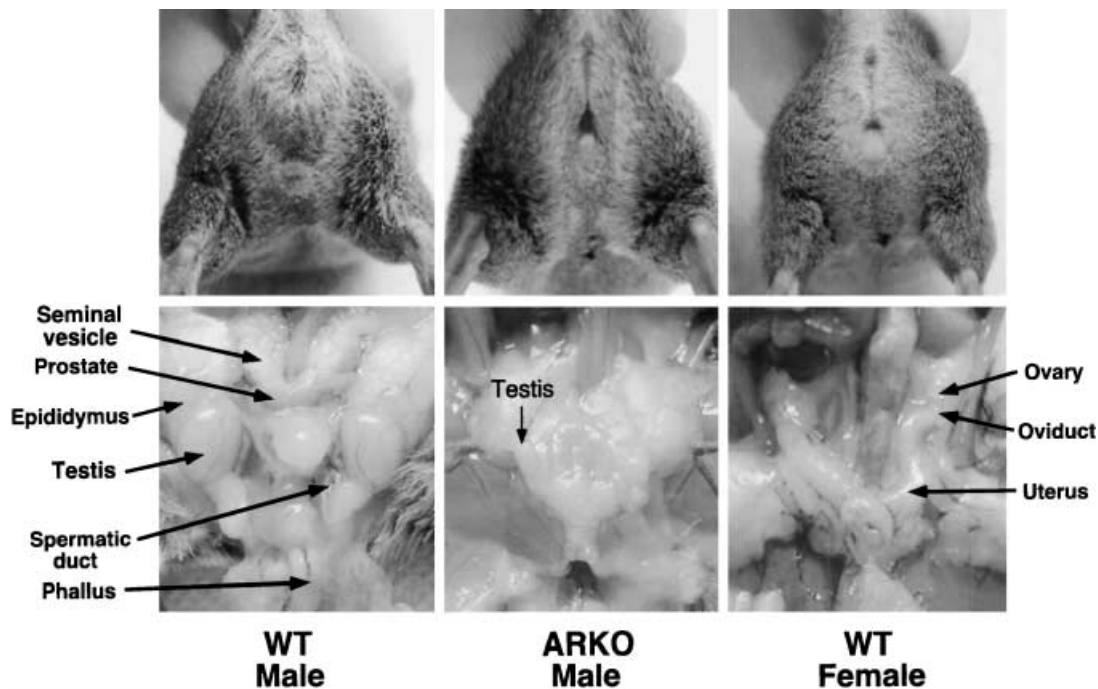
the same as that of the wild-type males.<sup>36</sup> These suggest that we can investigate the effect of androgens independently by using the ARKO mouse in that only the AR is disrupted while the estrogen receptors remain intact. Until 10 weeks-of-age, the ARKO males exhibited growth retardation, and the growth curve was indistinguishable from that of the wild-type female littermates. However, thereafter, the rapid increase in the growth of ARKO males was observed, and until 12 weeks, the bodyweights of the ARKO males exceeded those of the wild-type male littermates.<sup>37</sup> The late onset of the drastic increase in the growth curve in the ARKO males was understandable, as clear obesity developed (Fig. 5a). Significant increases of wet tissue weights in subcutaneous, infrarenal and intraperitoneal white adipose tissues (WATS) were observed at 30 weeks-of-age (Fig. 5b). In contrast, no significant alteration in serum lipid parameters and food intake was observed. No obesity was seen in female ARKO mice.

### IMPAIRED BONE METABOLISM AND SEXUAL BEHAVIORS IN MALE ARKO MICE

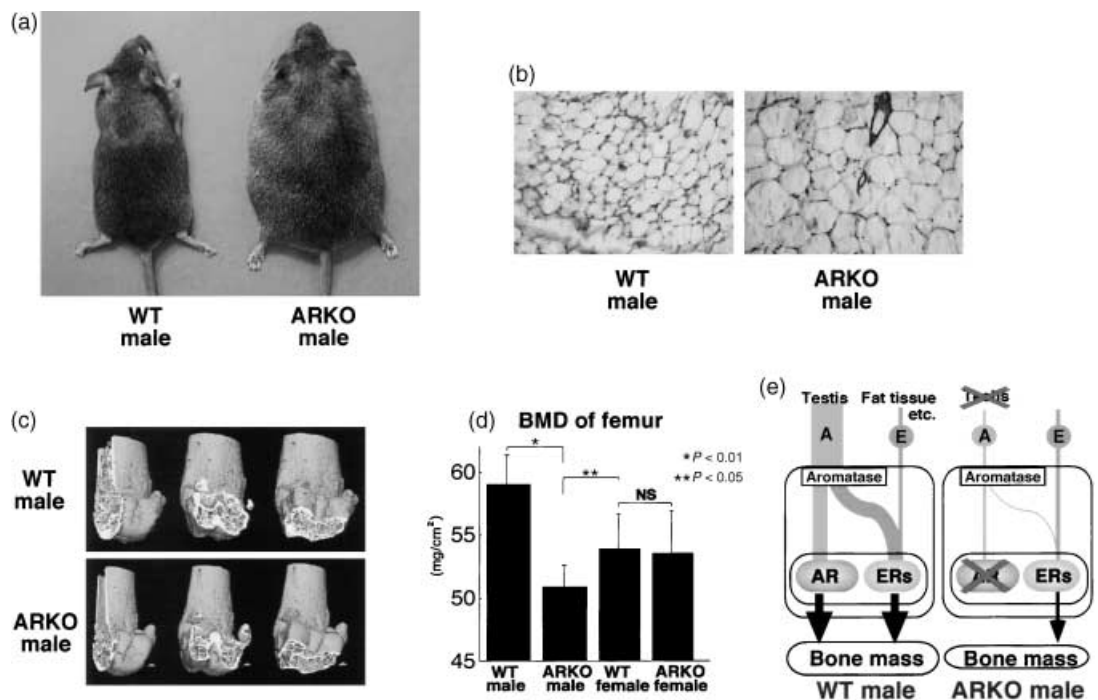
THE ARKO MALES showed a clear loss of bone mass with high bone turnover, when compared with the adult littermate males (Fig. 5c,d).<sup>38</sup> Such bone abnormality was not found in female ARKO mice, establishing that activated AR is essential for the male-type bone phenotype (Fig. 5e). Likewise, male-typical sexual behaviors were severely impaired by AR inactivation, but the sexual behaviors in female ARKO mice appeared normal.<sup>36</sup>

### PREMATURE OVARIAN FAILURE IN ARKO FEMALES

NO PHENOTYPIC ABNORMALITY was found in external and internal reproductive organs in ARKO females, but at 8 weeks, the females bore about half the normal number of pups per labor. At 40 weeks, the ARKO females became infertile.<sup>39</sup> Immunohistological analysis of normal ovaries detected significant expression of AR protein in growing follicles, and the ARKO ovaries at 8 weeks had impaired folliculogenesis with fewer numbers of follicles.<sup>39</sup> Folliculogenesis was absent in the ovaries of 40-week-old ARKO females, whereas the wild-type littermate females still had active ovarian function (Fig. 6). From these findings, together with the epidemiological study that an unknown gene(s) located on the X chromosome is responsible for a hereditary disorder called premature ovarian failure

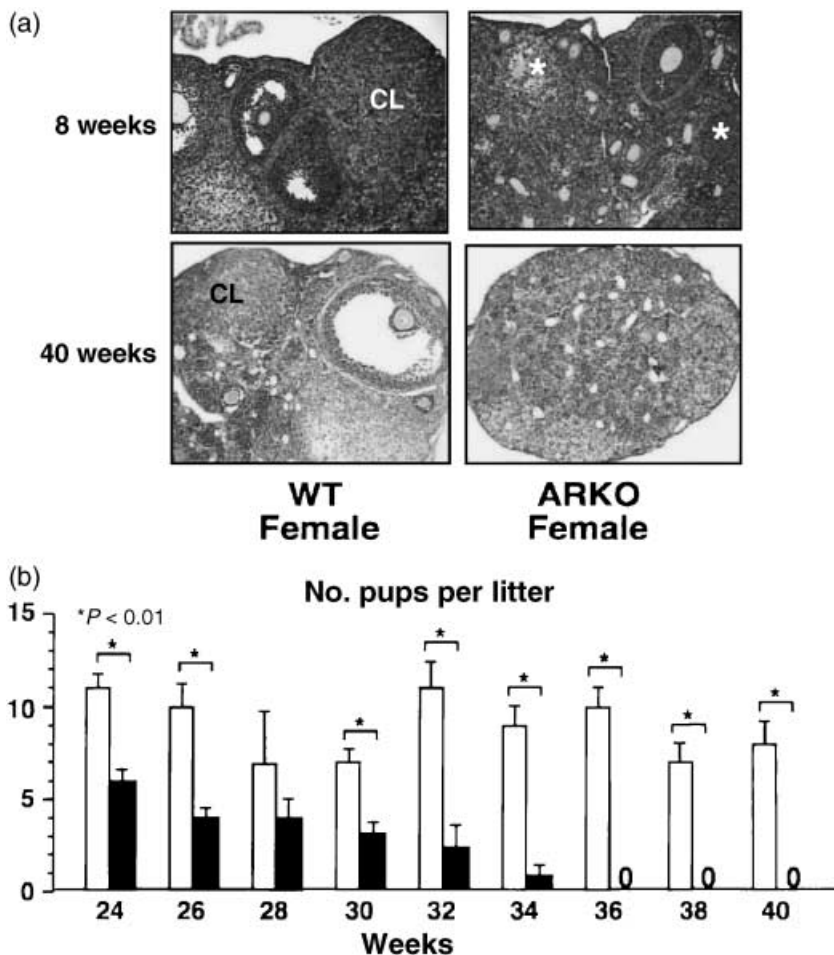


**Figure 4** The male ARKO mice were characterized by female-typical appearances, such as a clitoris-like phallus and a vagina with a blind end, but also the absence of internal male and female reproductive organs, except for the presence of an atrophic testis.



**Figure 5** Phenotypic features of male ARKO mice. (a) External appearance of 30-week-old male ARKO mice. (b) Subcutaneous white adipose tissues from 30-week-old male. (c) Three-dimensional computed tomography images of distal femora from representative 8-week-old male ARKO mice. (d) Bone loss in femur of male ARKO mice by BMD analysis. (e) Schema of skeletal sex hormone action.





**Figure 6** Premature ovarian failure in female ARKO mice. (a) Histology of ARKO ovary at 8 and 40 weeks-of-age. (b) Age-dependent reduction in the number of pups per litter in female ARKO mice.

(POF),<sup>40,41</sup> we believe that impaired androgen signaling might cause POF syndrome. To verify this, a clinical survey of POF patients is required to find a novel therapeutic clue.

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