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New Insights into the Role of Androgens in Wolffian Duct Stabilization in Male and Female Rodents

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Androgen-mediated wolffian duct (WD) development is programmed between embryonic d 15.5 (e15.5) and 17.5 in male rats, and WD differentiation has been shown to be more susceptible to reduced androgen action than is its initial stabilization. We investigated regulation of these events by comparing fetal WD development at e15.5–postnatal d0 in male and female androgen receptor knockout mice, and in rats treated from e14.5 with flutamide (100 mg/kg/d) plus di-(n-butyl) phthalate (500 mg/kg/d) to block both androgen action and production, testosterone propionate (20 mg/kg/d) to masculinize females, or vehicle control. In normal females, WD regression occurred by e15.5 in mice and e18.5 in rats, associated with a lack of epithelial cell proliferation and increased apoptosis, disintegration of the basement membrane, and reduced epithelial cell height. Exposure to testosterone masculinized female rats including stabilization and partial differentiation of WDs. Genetic or chemical ablation of androgen action in males prevented masculinization and induced WD regression via similar processes to those in normal females, except this occurred 2–3 d later than in females. These findings provide the first evidence that androgens may not be the only factor involved in determining WD fate. Other factors may promote survival of the WD in males or actively promote WD regression in females, suggesting sexually dimorphic differences in the preprogrammed setup of the WD. (Endocrinology 150: 2472–2480, 2009)

Before sex determination and differentiation of gonads into either testes or ovaries, male and female fetuses have an identical urogenital system (1, 2), with reproductive target tissues reported to express the androgen receptor (AR) in both sexes (3). After functional differentiation of the fetal testis, the Leydig cells secrete testosterone (2, 4, 5). Testicular androgens bind to and activate the AR, which in turn drives masculinization, critical features of which include stabilization and differentiation of the wolffian duct (WD), prostate formation and expansion of the anogenital distance (AGD) (2, 6–8). Once the WD has been stabilized in males, it differentiates to form its adult derivatives, the epididymis, vas deferens, and seminal vesicles (9–11). Conversely, in females the ovary does not produce testosterone at this time, and so the WD degenerates (1, 2, 6). This is thought to be due to the lack of available ligand in the female rather than an inability to respond to androgens (12).

Previous studies have investigated androgen-dependent differentiation of the reproductive tract by examining the impact of blocking fetal androgen action either genetically (13) or chemically, using AR antagonists such as flutamide (11, 14) or compounds such as Di(n-butyl) phthalate (DBP), which reduces fetal testicular testosterone production (15–18). Recently, we discovered that the critical window for androgen action in ensuring both initial WD stabilization and later differentiation is between embryonic d 15.5 (e15.5) and e17.5 in rats (14). This window is just after the onset of fetal testicular testosterone production, and surprisingly several days either before the peak in testicular testosterone levels per testis (4, 19) or morphological differentiation of the WD (11, 14). These studies demonstrated that WD differentiation is more susceptible to reduced androgen action than is its initial stabilization (11, 14). However, in these studies it is possible that the treatment regime may not block AR-mediated signaling events completely because the flutamide was administered to the pregnant mother rather than directly to the fetus (11, 14), and endogenous testosterone levels are high in male fetuses during the last week of gestation, especially in the testis and WD (20). Therefore, it is possible that WD stabilization may proceed in the presence of lower levels of androgen action than that re...
quired for its differentiation or that WD stabilization and differentiation may be regulated by different mechanisms and/or additional factors.

The present study sought to gain additional insight into WD development by comparing normal WD stabilization in males with WD regression in males in which androgen action was chemically or genetically ablated and with females in which androgens are naturally absent (21, 22). Furthermore, because fetal urogenital tissues in the female express AR (3), we administered exogenous testosterone to pregnant dams to “masculinize” their female fetuses and examined the impact on WD stabilization/development. We demonstrate that exogenous testosterone can rescue and partially differentiate the WD in females, but, surprisingly, ablation of androgen action in males leads to WD regression 2–3 d later than in normal females.

Materials and Methods

In vivo rat studies

Wistar rats were bred and maintained in our own animal house under standard conditions according to United Kingdom Home Office guidelines. Animals had access ad libitum to water and a soy-free breeding diet (SDS, Dundee, UK). Time matings were established, and the presence of a vaginal plug was taken as evidence of mating; this was defined as e0.5. A total of 38 pregnant dams were used for this study with dams randomly allocated to treatment groups. The natural regression studies were undertaken in rats that had not been dosed with any treatment (n = 6 litters). Control dams (n = 1) plus flutamide (100 mg/kg) (Sigma-Aldrich Corp., St. Louis, MO). Dams were then embedded in paraffin wax, sectioned (5 μm), and floated onto slides coated with 2% 3-aminopropyltriethoxy-silane (Sigma-Aldrich) and dried overnight at 50 C before histological analysis (see below). Representative WDs from at least three animals from at least three litters from the aforementioned groups of rats and mice were subsequently used for the studies detailed below. Histological analysis was performed on WD sections stained with hematoxylin and eosin, using standard protocols, and careful note was taken of any histological abnormalities.

Immunohistochemistry

Immunohistochemistry was performed on WDs recovered from mouse and rat fetuses using previously published standard avidin-peroxidase protocols and citrate antigen retrieval (11). WDs were stained for AR (1:50; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), laminin (1:100; Abcam plc, Cambridge, UK), and cleaved caspase 3 (1:200; Cell Signaling Technologies, Beverly, MA), phospho-histone H3 (1:1000; Upstate Biotechnology Inc., Lake Placid, NY), and pan-cytokeratin (1:200; Sigma-Aldrich). Cellular sites of expression were determined and slides photographed using a Provis AX70 (Olympus Optical, London, UK) microscope fitted with a Canon DS6031 camera (Canon Europe, Amsterdam, The Netherlands). To ensure reproducibility of results and allow accurate comparison of immunostaining between groups, sections of WDs from control and treated/knockout animals were processed in parallel on at least three occasions; sections of WDs from at least three animals in each group were run on each occasion. Appropriate negative controls were included, whereby the primary antibody was replaced by normal goat serum alone, to ensure that any staining observed was specific; none of the antibodies used showed other than minor nonspecific staining.

Epithelial cell height analysis

WD sections were immunostained for pan-cytokeratin as detailed previously to label clearly all epithelial cells. Sections were viewed using an Olympus BH-2 microscope fitted with a Prior automatic stage (Prior Scientific Instruments Ltd., Cambridge, UK). Image-Pro Plus version 4.5.1 with Stereoletter-Pro 5 plug-in software (Media Cybernetics UK, Wokingham, Berkshire, UK) was used to measure epithelial cell height. Using a ×63 objective, WD epithelial cell height was measured in every fifth epithelial cell per section. Only epithelial cells in which the nucleus could be clearly identified were measured, thus excluding from analysis any epithelial cells from treatment/knockout animals that were severely flattened or degrading.
Statistical analysis

Values have been expressed as means ± SEM. Data were analyzed using Fisher’s exact test (incidence of prostates and WDs) or one-way ANOVA (AGD and epithelial cell height), using GraphPad Prism version 4 (GraphPad Software Inc., San Diego, CA).

Results

Effectiveness of the rat models in manipulating masculinization endpoints (AGD and prostate formation)

Both AGD and prostate formation depend upon androgen action (8, 29, 30), and are commonly used markers of fetal androgen action. At e21.5, AGD in fetal male rats is approximately twice as long as in females, and at this age, elaborate prostatic bud branching can be identified in control male rat fetuses but not in females (Fig. 1). Fetal exposure to exogenous testosterone increased female AGD to a length comparable to control males and induced elaborate prostatic bud branching in female fetuses at e21.5. In contrast, masculinization was prevented at e21.5 in male rat fetuses exposed to DBP in combination with flutamide (DBP plus F), as evidenced by reduction of AGD to a length comparable to that in control females and complete prevention of prostatic bud branching (Fig. 1).

Timing of normal WD development in male and female rat fetuses

At e16.5–e17.5, a patent WD was readily identified in female rats lying medial to the Müllerian duct (MD); this WD began to regress in a cranio-caudal direction and, by e18.5, was barely identifiable upon gross examination (Fig. 2), with a patent lumen only visible at the caudal end. Conversely in males, the WD remained a simple straight duct throughout this period while the MD regressed between e17.5–e18.5 (Fig. 2). Therefore, a detailed investigation of WD regression and stabilization was undertaken in all subsequent studies at e18.5, when the WD has almost completely regressed in control females. Any experimen-
tal perturbation of the timing of WD regression should be readily identifiable at this age.

**Ability of exogenous testosterone to stabilize and differentiate the WD in female rats**

At e18.5 and e21.5, WDs were present in all females exposed in utero to testosterone, with 87% of them showing some degree of coiling at e21.5; however, this coiling was less extensive than that observed in WDs of control, age-matched males (Fig. 2 and Table 1). These “stabilized” WDs in females persisted into adulthood but never developed as fully as the equivalent “epididymal” organ in males (data not shown). Exposure to flutamide (100 mg/kg) in combination with testosterone (20 mg/kg) pre-vented WD stabilization in females, and, in this treatment group, no WDs were identified in any female examined at e21.5 (data not shown). These data confirm that females can respond to androgens, and that testosterone alone can stabilize and partially differentiate the WD in females; this experimental masculinization can be prevented by flutamide exposure. There was no obvious effect of exogenous testosterone exposure on the gross morphology of WDs in males at either e18.5 or e21.5, i.e. epididymal coiling was not initiated any earlier or to any greater extent than in control males (data not shown).

**Impact on WD development of ablating androgen action in male rats**

Treatment with DBP plus F did not induce any gross WD abnormalities in males at e18.5 (Fig. 2), and a complete and patent WD was observed in all e18.5 fetuses examined (Fig. 2 and Table 1). In contrast, at this age the WD in control females had completely regressed (Fig. 2). However, exposure to DBP plus F resulted in the loss of all WD structures in 25% of males at e20.5 (data not shown) and in all male fetuses by e21.5 (Fig. 2 and Table 1), with a patent lumen only apparent in the residual caput segment of the WD, whereas the rest of the WD appeared as a remnant mesenchymal-like structure (Fig. 2). Thus, although DBP plus F resulted in the absence of the WD, the timing of its regression (e20.5–e21.5) was 2–3 d later than occurred in normal females (e18.5) (Table 1).

**WD regression in ARKO mice**

In WT male mice, the WD could clearly be identified as a simple straight duct at e15.5, but by e16.5, some coiling could be identified in the future caput epididymis (Fig. 3). By the day of birth (pnd0), the future epididymis was highly coiled. Conversely in WT females, the WD had regressed almost completely by e15.5, with a patent lumen only visible at the caudal end (Fig. 3), lying medial to the MD. In contrast, in ARKO males a complete WD could still be readily identified at e15.5 (Fig. 3 and Table 1). By e16.5, the WD had started to regress in ARKO males, but a patent lumen could still be identified in the caput by gross inspection (Fig. 3). At pnd0 the WD had almost completely regressed, with a patent lumen only apparent in the residual caput segment of the WD, whereas the rest of the WD appeared as a remnant mesenchymal-like structure. Therefore, complete ablation of AR signaling in ARKO mice resulted in an absence of the WD by birth, but its regression (around e16.5) occurred at approximately 2 d later than occurred in normal females (Table 1).

**Apoptosis, proliferation, and morphology of the epithelium in WDs from male and female fetuses**

Analysis of apoptosis, cell proliferation, and epithelial degeneration was undertaken in WDs recovered from male and female fetuses because these cellular processes have been suggested to

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**TABLE 1. Summary of the incidence of WD regression and the histological processes involved**

<table>
<thead>
<tr>
<th>Mouse</th>
<th>WD regressed</th>
<th>Apoptosis epithelia</th>
<th>Proliferation</th>
<th>Epithelia</th>
<th>Stroma</th>
<th>BM interrupted</th>
<th>Epithelial height reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>e15.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT male</td>
<td>0% (0/5)</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARKO male</td>
<td>0% (0/5)</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>100% (6/6)</td>
<td>N/A</td>
<td>N/A</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e16.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT male</td>
<td>0% (0/16)</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARKO male</td>
<td>30% (3/10)</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>100% (17/17)</td>
<td>N/A</td>
<td>N/A</td>
<td>✓</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Rat**

| e18.5 |              |                     |              |           |        |                |                          |
| Control male | 0% (0/17) | X                   | ✓            | ✓         | X      |                |                          |
| DBP plus F male | 0% (0/32) | X                   | ✓            | ✓         | X      |                |                          |
| Control female | 100% (18/22) | ✓               | ✓            | ✓         | ✓      |                | ✓                        |
| T female | 0% (0/19) | X                   | ✓            | ✓         | X      |                |                          |
| e21.5 |              |                     |              |           |        |                |                          |
| Control male | 0% (0/37) | X                   | ✓            | ✓         | X      |                |                          |
| DBP plus F male | 100% (35/35) | ✓               | ✓            | ✓         | ✓      |                | ✓                        |
| Control female | 100% (32/32) | ✓               | ✓            | ✓         | ✓      |                | ✓                        |
| T female | 0% (0/30) | X                   | ✓            | ✓         | X      |                |                          |

Values are the number of animals the WD regressed in, out of the total number of animals examined. *Bold* values are different from age-matched control males. *Non-bold* values are the same as age-matched control males.
play a role in MD regression in males (5, 31–33) and in causing WD abnormalities in males exposed to antiandrogens during fetal life (11, 14). These results are summarized in Table 1. Note that in control females, little epithelium was present in the WD, therefore, the images shown in this paper were selected to show the phenotype of any persisting epithelium.

Apoptotic cells (positively immunostained for cleaved caspase 3) were noted in the epithelium of the WD from female rats at e17.5 (data not shown) and in the remnant WD epithelium at e18.5 (Fig. 4A); this was in contrast to age-matched control males in which the WDs were immunonegative for cleaved caspase 3. Similarly, apoptotic cells were rarely detected in either the epithelium or stroma of WDs from testosterone-exposed female rats or from testosterone-exposed female rats. Conversely, apoptotic cells were evident in the epithelium of the WD in control males (arrowhead). Apoptosis was rarely seen at e16.5 in WDs from WT or ARKO male mice. Occasional apoptotic cells can be seen in the residual epithelium still remaining in WDs from female mice at e16.5 (arrow). B. Demarcation of the basement membrane in WDs from male and female fetuses by immunostaining for laminin (brown) in the basement membrane. Note that laminin forms a defined “ring” at the basement membrane in control male WDs (arrow) and also in WDs from DBP plus F-exposed male rats, from ARKO male mice, and from testosterone-exposed female rats. Conversely, in normal females the WD basement membrane is disrupted and the epithelium flattened, leaving patches of diffuse laminin expression (arrowhead). Note that laminin forms a defined “ring” at the basement membrane of the MD in control females. Scale bar, 100 μm.
WD at all ages examined (e16.5–e21.5, data not shown). Similar patterns were seen in WT mice (data not shown). Mitotic cells were noted in the WD epithelium and stroma at e18.5 and e21.5 (data not shown) in female rats exposed to exogenous testosterone. Phospho-histone H3 positive cells were still evident in both the stromal and epithelial cell compartments of WDs from DBP plus F-exposed male rats at e18.5 and in ARKO male mice at e16.5 (data not shown). However, by e21.5 in DBP plus F-exposed rats and pnd0 in ARKO male mice, mitotic cells were rarely noted in the residual epithelium of WDs but could still be identified in the remnant stromal compartment (data not shown). Therefore, cell proliferation was evident in the stromal compartment of WDs from both males and females in all models examined, but variation was noted in epithelial cell proliferation between males and females (Table 1).

Immunostaining for laminin highlighted that the basement membrane around the WD epithelium was interrupted and incomplete in the regressing WD from control female rats at e16.5 compared with the defined “ring” of laminin evident in the basement membrane of WDs from control male rats at e16.5–e21.5 (data not shown) (Table 1). By e18.5 the WD in control female rats had almost completely regressed, leaving only patches of laminin staining where the epithelium had once been (Fig. 4B). Similar patterns were seen in WT mice. Exposure of rats to exogenous testosterone prevented this interruption to the basement membrane and resulted in a defined “ring” of laminin in the basement membrane of WDs from females at e18.5 (Fig. 4B) and e21.5 (data not shown). In contrast to normal females, exposure of male rats to DBP plus F did not interrupt laminin expression in the basement membrane of WDs at e18.5 (Fig. 4B), but by e21.5, laminin expression was poorly defined and was often absent (data not shown). Similarly, in e16.5 ARKO male mice, laminin expression in the basement membrane was comparable to that in WT littermates (Fig. 4B), but by pnd0 this laminin “ring” was absent (data not shown).

Epithelial abnormalities were noted in the regressing WD of control females at all ages examined (e16.5–e18.5) in comparison to age-matched control male WDs. These included an apparent reduction in epithelial cell height and a narrowing or absence of a patent lumen at e18.5 (Fig. 5A). Exposure to exogenous testosterone prevented these abnormalities because the WD epithelium from exposed female fetuses was histologically comparable to WDs from age-matched control males at e18.5 (Fig. 5A) and e21.5 (data not shown). Exposure to DBP plus F did not result in reduced height of the WD epithelium in male fetuses at e18.5 (Fig. 5A) and e21.5 (data not shown), but by e21.5 the majority of these animals had very little epithelium evident, especially distal to the caput; any epithelium present was flattened with a grossly abnormal lumen (data not shown). Similar patterns were noted in WDs from male ARKO mice, whereby epithelial cell height was not reduced at e16.5, compared with WT littermates, and the lumen was patent (Fig. 5B). However, by pnd0, epithelium was rarely present in WDs from any ARKO male mice (data not shown). Quantitative measurement of epithelial cell height confirmed these histological observations (Fig. 5, A and B). No WD epithelium was present in female mice to measure at e16.5.

**Discussion**

Various studies have shown that androgens play a critical role in WD development (15, 17, 22, 34–36). The majority of studies used experimental impairment of fetal androgen action with subsequent evaluation of the males postnatally and demonstrated an absence of WD-derived tissues. The interpretation from these studies was that, in the absence of fetal androgen action, the WD regressed in males at the same time as in age-matched (normal) females. However, results from previous studies in rats by our group caused us to question this interpretation because we had shown that, at e18.5, an age when the WD has completely re-
pressed in normal females, WDs recovered from males exposed in utero to high concentrations of flutamide were comparable to those from control males (11). However, subsequent differentiation of the WD in these flutamide-exposed males was impaired by e21.5, and the WD subsequently degenerated during puberty. These results suggested that either WD stabilization can proceed in males in the presence of lower levels of androgen action than that required for subsequent differentiation or that WD stabilization is regulated by different mechanisms to those required for differentiation. We addressed this issue in the present studies using rodent models in which fetal androgen action was ablated that required for subsequent differentiation or that WD stabilization on androgens alone. Therefore, pregnant rats were sought a model in which more complete blockade of androgen action within the WD, but there is no obvious way to assess this directly. Therefore, to address this we examined WD regression in ARKO mice, a rodent model accepted to lack completely a functional AR due to a genetic mutation (28, 44). In ARKO male mice (n = 11 litters), as in DBP plus F-exposed male rats, WD regression is temporally delayed by approximately 2 d compared with normal littermate females. We also found similar results in tfm male mice, in which androgen action is genetically ablated due to a different inactivating mutation in the AR (n = 5 litters; unpublished data). Therefore, these studies confirmed our findings of delayed WD regression in males in two different rodent species using three different models to ablate androgen action.

It is not obvious why the WD should regress slower in males devoid of androgen action than in normal females, and raises the question of whether unknown factors could actively promote WD regression in females or whether factors other than androgens may help maintain the WD in males, particularly in the absence of androgen action. The precise source of these factors is unknown; they could be produced by the testis or be endogenous to the WD itself. This merits further investigation. However, this “maintenance” mechanism is clearly not sufficient to stabilize the WD long term in males because, in both the rat and mouse models lacking androgen action, the WD ultimately regresses by birth. This mechanism might serve to prolong survival of the WD in males to maximize its opportunity to respond to testosterone. Further investigations are required to identify the factor(s) involved, but possible candidates include inhibins and/or insulin-like factor 3 (Insl3). Body weight is not significantly different between that of ARKO or DBP plus F-exposed males and control females, therefore, we do not believe the differences in the timing of WD regression can be explained by growth rate (unpublished data) (28, 45). TfmrARKO mice provide rodent models for complete androgen insensitivity syndrome (CAIS), in which patients are genetically male but have a female phenotype (46–50). Most evidence from CAIS patients is derived from postnatal examination (51), with no definitive published evidence on the status of the fetal WD. Therefore, it is unclear whether in CAIS patients the WD fails to stabilize during early fetal life, as occurs in females, or whether the absence of WD structures in later life results from a “post-stabilization” degeneration of WD-derived tissues, as occurs in this study in rats exposed in utero to DBP plus F and in ARKO male mice.

Because these findings in males question whether androgens are the only determining factor in WD stabilization, we investi-
gated the ability of exogenous fetal androgens to stabilize the WD in females. Exposure to exogenous maternal testosterone in rats can masculinize female offspring to varying degrees (25, 39, 52–55). However, some studies were unable to stabilize the WD, even in females in which prostates were readily identifiable (25).

In this study, male reproductive tissues were masculinized by exogenous maternal testosterone. This is a direct effect of androgens, rather than due to testosterone being aromatized to estrogen, because they could be blocked in female fetuses by combined exposure to testosterone plus flutamide. In our study, testosterone exposure not only stabilized the WD in all female rats examined but could even induce some degree of differentiation and compartmentalization, as evidenced by the initiation of “epididymal” coiling at e21.5. Interestingly, this coiling was never as pronounced as in control males. It may be that the dose of testosterone used in this study was not sufficient to fully stimulate coiling, or that the presence of the normal MD alongside the WD in females may physically prevent the “stabilized” WD from fully coiling, or that basic differences exist in the WD in females vs. males that prevent the WD in females from undergoing complete differentiation. Examination of testosterone-exposed females after birth showed that the stabilized female WD persisted postnatally and into adulthood, even though exposure to exogenous testosterone ceased at birth (data not shown). This suggests that patterning of the fetal WD is established early in reproductive development (e14.5–e17.5), and, once stabilized, the female WD persists postnatally. This is in contrast to males exposed to flutamide in utero, in which WD derivatives were present at birth but were usually absent by adulthood (11). This contrast further highlights fundamental differences between males and females, and merits further investigation.

Histological comparison of naturally regressing WDs in females with those from males deprived of androgen action revealed fundamental differences that support the view that the WD may be subtly different in males and females. First, apoptosis was observed in the WD epithelium from control females but not in WDs from control, ARKO, or DBP plus F-exposed males or in testosterone-exposed females. This is in agreement with previous studies in control female rats (5, 21, 56, 57). Second, cell proliferation was not observed in the WD epithelium of control females but was noted in both the epithelium and mesenchymal compartments of WDs from control and DBP plus F-exposed males, and in testosterone-exposed females at e18.5 and in ARKO males at e16.5. Third, our results suggest that during WD regression in females, epithelial cells lose their attachment to the basement membrane; these changes may be the trigger for apoptosis (58). Exposure to exogenous testosterone prevented these WD cellular abnormalities in females. WDs recovered from e18.5 males exposed to DBP plus F or e16.5 ARKO males showed no obvious histological abnormalities, whereas around the time of birth, similar abnormalities were noted to those observed in the regressing female WD at earlier ages. Together, these data demonstrate differences in the timing of and/or presence of the cellular processes observed in the WD epithelium in normal females compared with males. Furthermore, they highlight that exposure to exogenous testosterone induces “male” like changes in females but that deprivation of androgen action (genetically or chemically) cannot induce the cellular changes in males at the same age as they are seen in control females. These observations suggest that a similar mechanism for WD differentiation is operating in males and females in which androgen action is absent but that in males these changes occur several days later than in females.

In summary, the present study sought to gain new insight into WD development by examining the role for androgens in WD stabilization, rather than in later WD differentiation into its adult derivatives. These studies have shown that testosterone alone can stabilize and partially differentiate the WD in females but that genetically or chemically ablating androgen action in males does not induce WD degeneration at the same time as it occurs in females. This suggests either that factors other than androgens prolong the survival of the WD in males, possibly to maximize the opportunity for WD stabilization by endogenous fetal androgens, or that unknown factors actively promote WD regression in females. Although androgens are critical in WD stabilization and differentiation, these studies offer the first evidence that they may not be the only factor involved in dictating the fate of the WD in males. Our studies suggest there are fundamental differences in the preprogrammed setup of the WD in males compared with females and that the differential response in the fate of the WD may not depend solely on the presence or absence of androgens.

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