Overexpression of progesterone receptor A isoform in mice leads to endometrial hyperproliferation, hyperplasia and atypia

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Abstract: A delicate balance in estrogen and progesterone signaling through their cognate receptors is characteristic for the physiologic state of the endometrium, and a shift in receptor isotype expression can be frequently found in human endometrial pathology. In this study, using a transgenic mouse model, we examined the mechanisms whereby alterations in progesterone receptor (PR) isotype expression leads to endometrial pathology. For an experimental model, we used transgenic mice (PR-A transgenics) carrying an imbalance in the native ratio of the two PR isoforms A and B (PR-A and PR-B) through the expression of additional A form and examined their uterine phenotype under different hormonal regimens, using various criteria. Uterine epithelial cell proliferation was augmented in PR-A transgenics and was abolished by PR antagonists. In particular, proliferative response to progesterone, independent of signaling through estrogen, was enhanced. Upon continuous exposure to estradiol and progesterone, the uteri in PR-A transgenics displayed gross enlargement, endometrial hyperplasia including atypical lesions, endometritis and pelvic inflammatory disease. Imbalanced expression of the two isoforms of PR in a transgenic model reveals multiple derangements in the regulation of uterine physiology, resulting in various pathologies including hyperplasias.

Key words: progesterone receptor / isotype / inflammation / hyperplasia / atypia

Introduction

The uterus is a major target tissue for estrogen and progesterone, and a coordinated action of these steroids is essential to maintain cellular replicative homeostasis. In particular, uterine cell proliferation is augmented by estradiol, which, in turn, is antagonized by progesterone (Martin et al., 1980; Graham and Clarke, 1997). Studies on estrogen receptor (ER) and progesterone receptor (PR) null mutant mice have provided direct evidence that both the positive effects of estradiol and negative effects of progesterone on uterine cell proliferation are mediated through their cognate receptors. As such, uterine growth is stunted in ER null mutant mice (Lubahn et al., 1993), whereas PR null mutant mice develop uterine hyperplasias (Lydon et al., 1995).

PR exists in two molecular forms commonly referred to as the ‘A’ (PR-A) and ‘B’ (PR-B) forms (Schneider et al., 1991; Graham and Clarke, 1997). It is known that (i) in the same cell, the PR-A and PR-B can have different functions, (ii) the activity of the individual form of the receptor can vary among different types of cells (Vegeto et al., 1993), (iii) depending on the cell, PR-A can either inhibit or enhance the activity of PR-B (Vegeto et al., 1993) and (iv) both PR-A and PR-B can modulate ERα- and estrogen-dependent gene expression (Chalbos and Galtier, 1994; Giangrande and McDonnell, 1999). Therefore, the ultimate responses to either estradiol and/or progesterone are dependent on the tissue/cell type in question and, in turn, dictated by the cross-talk between the two isoforms of PR and ERα. Indeed, studies on PR-A null mutant mice have shown that the PR-A and PR-B are functionally distinct with respect to their ability to antagonize estrogen action (Mulac-Jericevic et al., 2000). The isoform expression in the endometrium is temporally and spatially regulated. PR-A and PR-B are both expressed in the uterine stroma and myometrium and their expression levels depend on cyclic systemic hormone exposure (Mote et al., 2006). Stromal PRs have a special significance as they mediate the inhibitory effects of progesterone on estrogen-induced uterine epithelial proliferation.
illustrating the important stromal–epithelial interactions in uterine physiology (for review, see Cunha et al., 2004). In the luminal epithelial cells, PR-B is the sole isoform showing its significance for glandular secretion (Mote et al. 1999, 2006). As such, within the context of uterine cell proliferation, an imbalance in the expression and/or activities of the two isoforms of PR can have major consequences. Indeed, a predominant expression of either PR-A or PR-B is already evident in hyperplastic endometrium and therefore early during endometrial cancer (EC) development (Arnett-Mansfield et al., 2001). A number of studies on human EC specimens/cells have also revealed an imbalance in the expression of the two PR isoforms (Leslie et al. 1997; Kumar et al., 1998; Arnett-Mansfield et al. 2001). However, in the absence of in vivo studies, it is difficult to evaluate whether changes in the expression levels of the two isoforms of PR are an event accompanying the transformation of the endometrium or are responsible for the transformation. A need for evaluating the potential significance of an imbalanced expression of the PR isoforms to endometrial carcinoma is underscored by the fact that hyperplasias respond to treatment with progesterins far better than ECs (Quinn et al., 1985; Kumar et al., 1998; Thigpen et al., 1999).

Our laboratory has created transgenic mice (PR-A transgenics) carrying additional ‘A’ form of PR as transgene (Shyamala et al., 1998). In these mice, mammary glands, also an important target tissue for estrogen and progesterone, show developmental abnormalities; in particular, the epithelial cells exhibit several characteristics associated with transformed cells (Shyamala et al., 1998; Chou et al., 2003). Therefore, in the present studies, we examined the uterine phenotype of PR-A transgenics to determine the feasibility of these mice to serve as an experimental model for identifying the potential relationships between imbalanced expression of PR isoforms and genesis of endometrial hyperplasias.

Materials and Methods

Mice, treatment with steroids and tissue preparations

All mice used in these studies were of FVB strain. The generation of PR-A transgenic mice, which carry an imbalance in the normal ratio of the two forms of PR by overexpression of the A form, has been described previously (Shyamala et al., 1998). In brief, we used a binary transgenic system in which the GAL-4 gene, driven by the murine cytomegalovirus (CMV) promoter (CMV-GAL-4 mice), served as the transactivator of the PR-A gene, carrying four GAL-4-binding sites (UAS; UAS-PR-A mice). Crossing the CMV-GAL-4 mice with UAS-PR-A mice resulted in transgenic mice by homogenization in lysis buffer [50 mM Tris–HCl (pH 8.0), 125 mM NaCl, 1 mM sodium fluoride, 1 mM sodium orthovanadate, 10 mM sodium pyrophosphate and 1 mM phenylmethylsulfonyl fluoride] containing the following protease inhibitors: leupeptin, pepstatin, aprotinin, each at a final concentration of 1 μg/mL. The homogenates were sonicated, centrifuged at 110 g and the pellets were discarded. Protein concentrations in the supernatants (lysates) were determined by DC protein assay (Bio-Rad, Hercules, CA, USA). Aliquots of lysates equivalent to 20 μg of protein were subjected to electrophoresis through 8–16% SDS–PAGE gels and transferred to nitrocellulose membranes. The membranes were blocked with 10% non-fat powdered milk prior to treatment with the primary antibodies. Subsequently, the blots were washed and treated with appropriate secondary antibodies. Target proteins were normalized to β-actin for loading. Proteins were quantified with UN-SCAN-IT™ software version 6.0 (Silk Scientific Inc., Orem, UT, USA) on digitized protein bands of western blots.

Analysis for BrdU-, ERα- and PR-positive cells

BrdU-, ERα- and PR-positive cells were identified by immunohistochemistry. BrdU-immunohistochemistry was performed as described previously (Chou et al., 2003) using rat monoclonal anti-BrdU antibody (Harlan-Sera-Lab, UK). ERα- and PR-positive cell staining was carried out as described previously (Shyamala et al., 1997). Anti-ERα mouse monoclonal antibody 6F11 was used to detect mouse ERα (Novoceastra, UK). The anti-PR antibody used in these studies was generated by our laboratory (Shyamala et al., 1997). The antigen–antibody complexes were identified using Universal DAKO LSAB2-labeled streptavidin-biotin peroxidase kit (DAKO, Carpinteria, CA, USA). The sections were counterstained with Mayer’s hematoxylin solution (DAKO). After counterstaining, BrdU-, ERα- or PR-negative nuclei appeared purple-blue, whereas positive nuclei appeared dark brown.

Positive cells were scored by counting randomly taken images of at least seven stained sections per animal (n ≥ 3). According to the image, cells were identified as luminal epithelial, glandular epithelial or stromal, and an average of 1500 nuclei per animal were counted and scored as positive or negative by a blinded investigator.

cDNA synthesis and quantitative reverse transcriptase–polymerase chain reaction analysis

Total cellular RNA was extracted using ToTally RNA isolation kit (Ambion, Austin, TX, USA) according to the protocol provided by the manufacturer. For cDNA synthesis, 6 μg of total RNA, prepared as described earlier, was treated with DNase I, to remove any contaminating genomic DNA, and then used for reverse transcriptase (RT)-coupled cDNA synthesis using oligo-(dT)15 primers and Superscript II (Life Technologies, Bethesda, MD, USA). The RT reaction was performed at 42°C for 50 min, followed by heating at 70°C for 10 min. The resultant cDNA was used 42°C for 50 min, followed by heating at 70°C for 10 min. It was either used immediately for quantitative RT–polymerase chain reaction (PCR) or stored at −20°C for later use.

For PCR, the primers for various genes (lactoferrin, amphiregulin, SOX4) were selected using Primer Express (Perkin-Elmer Applied Biosystems, Foster City, CA, USA), which selected optimized primer sequences for this system. PCR reactions were performed using the ABI Prism 7700
sequence of each primer set, optimal experimental conditions were established and standard curves were generated using serially diluted samples. The amount of transcripts in each sample was calculated from the standard curve and normalized to β-actin gene, run as an internal control.

Statistical analysis
At least three animals per treatment group were analyzed. Slides were scored by two blinded investigators. Groups were compared using t-test or Mann–Whitney test depending on their distribution. Probability distributions were calculated using the χ² square test. Statistical significance was established at P < 0.05.

Results

PR-A transgenics show a constitutive expression of PR-A in uterine epithelial cells
In order to overexpress PR-A isotype, we utilized a binary transgenic system in which the GAL-4 gene, driven by the murine CMV promoter (CMV-GAL-4 mice), served as the transactivator of the PR-A (Shyamala et al., 1998). To verify that the transgenic animals we generated show an overexpression of the actual PR-A isotype, we performed a western blot using homogenized uteri at estrous/metestrous. PR-A is the 94-kDa truncated form of the 116-kDa protein PR-B (Supplementary Material, Fig. S1). Intact wildtype and intact PR-A transgenics express both PR isotypes with a relative dominance of the A-form. Quantification of PR-A showed an ∼1.15-fold higher expression of PR-A in the intact transgensics compared with wildtype animals. Whereas ovariectomy almost abandoned PR expression in wildtype animals, PRs were still expressed in the PR-A transgenics (Supplementary Material, Fig. S1). This demonstrated that the transgenic animals showed a constitutive expression of the PR-A which results in a mild overexpression. Immunohistochemical staining of PR-A expression in ovariectomized PR-A transgenics was positive for glandular epithelial and stromal but not luminal epithelial cells (data not shown).

Uterine epithelial cell proliferation is augmented in PR-A transgenics
To assess the proliferative status of the uterus in PR-A transgenics, intact mice were administered with BrdU and the tissues were analyzed for BrdU-positive cells by immunohistochemistry. As expected, in the uteri of wildtype mice, BrdU-positive cells were detected in both luminal and glandular epithelial cells at diestrus/proestrous (Fig. 1A), and the epithelium was virtually devoid of BrdU-positive cells at estrous/metestrous (Fig. 1C). Similarly, in the uteri of PR-A transgenics, the number of BrdU-positive cells was higher at diestrus/proestrous when compared with estrous/metestrous (Fig. 1B and D). However, in comparison with wildtype mice, there was a significant increase in BrdU-labeling index in both luminal and glandular epithelial cells of PR-A transgenics (Fig. 1, compare A and C; B and D). The BrdU-labeling indices (combined data from all stages of estrous cycle) in the various uterine cell types, in both genotypes, are also shown in Fig. 1E.

Expression patterns and activity of ERα are not altered in the uteri of PR-A transgenics
In wildtype mice, uterine epithelial cell proliferation is dependent on signaling through estradiol/ERα (Cooke et al., 1997). Therefore, we examined whether the increase in uterine cell proliferation in PR-A transgenics was due to alterations in ERα expression. Analyses for ERα mRNA expression by real-time PCR did not reveal any detectable differences between the uteri of intact PR-A transgenic mice (3.34 ± 0.18 transcripts/β-actin) and their wildtype counterparts (3.1 ± 0.37 transcripts/β-actin). Expression patterns of ERα among the various cell types were also similar to that reported for wildtype mice (Gorski et al., 1968). As such, in the uteri of ovariectomized mice, ERα was detected in all cell types (Fig. 2Aa), which, upon treatment with estradiol, decreased in the stroma (Fig. 2Ab). Also, the profiles of ERα expression in the uteri of intact PR-A transgenics at proestrous closely resembled both the uteri of ovariectomized mice treated with estradiol (Fig. 2A, compare b and c) and the uteri of intact wildtype mice at proestrous (Fig. 2A, compare c and d). The overall distribution of ERα-positive cells (combined data from all mice independent of the stages of estrous cycle) in the various uterine cell types was also similar between the two genotypes (Fig. 2B).

Having found that ERα expression in the uteri of PR-A transgenics was similar to wildtype mice, we next examined whether there were alterations in the activity of ERα by analyzing for PR, a well-documented indicator of estrogenic response (Tibbetts et al., 1998). In ovariectomized wildtype mice, PR was detected predominantly in the epithelial cells and not in the stroma (Fig. 3A). Upon treatment with estradiol, there was an increase in stromal PR and a concomitant decrease in the epithelial cells (Fig. 3, compare A and B). These observations on uterine PR expression in FVB wildtype mice (used in the present studies) were in agreement with those reported previously for C57Bl wildtype mouse (Tibbetts et al., 1998). In ovariectomized PR-A transgenics, PR was detected in both epithelial and stromal cells (Fig. 3a), which, upon treatment with estradiol, decreased in the stroma (Fig. 3Ab). Also, similar to wildtype mice, there was an increase in stromal PR (Fig. 3, compare C and D). Estrogen treatment also led to a decrease in epithelial PR in PR-A transgenics, but it was still higher when compared with its wildtype counterpart. This was expected since the constitutive expression of PR in the uteri of PR-A transgenics also includes the expression of transgene. Regardless, these observations indicated that, in PR-A transgenics, the estrogenic response in the uterine stroma was intact.

In addition to promoting epithelial cell proliferation, estrogen also increases the expression of certain epithelial genes in the uterus. Among these, estrogenic regulation of lactoferrin (LF) is well characterized (Pentecost and Teng, 1987). Therefore, to examine whether the estrogenic response in the epithelial cells of PR-A transgenics was altered, we analyzed for LF gene expression. LF gene expression in the uteri of intact mice revealed that they were similar between the two genotypes (Fig. 4). Also, in both genotypes, ovariectomy reduced the steady-state levels of LF mRNA and this was reversed upon treatment with estradiol (Fig. 4). More importantly, when compared with wildtype mice, in the uteri of PR-A transgenic mice, there was no increase but a modest decrease in estradiol-dependent LF expression (P < 0.05). In contrast to estradiol, progesterone alone had no effect.
on LF expression but as shown previously (De Vivo et al., 2002), it inhibited the estradiol-dependent increase in LF transcripts; the extent of progesterone-dependent inhibition was similar between wildtype and PR-A transgenic mice (Fig. 4).

**Progesterone-dependent gene expression is augmented in the uteri of PR-A transgenics**

In normal endometrium, signaling through estrogen/ERα promotes epithelial cell proliferation and this is antagonized by signaling through progesterone/PR (Martin et al., 1980; Graham and Clarke, 1997). However, progesterone can also promote proliferation by inducing specific growth factor expression (Graham and Clarke, 1997). Therefore, it was possible that, in comparison with wildtype mice, the ability of progesterone/PR to promote growth factor expression was enhanced in uterine epithelial cells in PR-A transgenics, resulting in hyperproliferation. If this were so, treatment with antiprogestins should have opposite effects on epithelial cell proliferation in the two genotypes. To this end, we treated intact wildtype and PR-A transgenics with the antiprogestin RU486 (mifepristone) and analyzed its effects on uterine epithelial cell proliferation. Mifepristone caused an increase in uterine epithelial cell proliferation in intact wildtype mice, presumably by counteracting the inhibitory effect of progesterone on estrogen/ERα-dependent proliferation (Fig. 1E). In contrast, treatment with mifepristone resulted in a vast decrease in BrdU-labeling index in the uterine epithelial cells of PR-A transgenics and was particularly striking in the glandular epithelial cells (Fig. 1E). A decrease in BrdU-labeling index in the epithelial cells was also seen upon treatment of intact PR-A transgenics with ZK 98299 (data not shown). These observations indicated that in the uteri of
PR-A transgenics, the growth-promoting effects of progesterone were more dominant than its growth inhibitory effects, which results in hyperproliferation.

Next, we examined whether, in the uteri of PR-A transgenics, there was an increase in progesterone/PR-dependent expression of growth factors by analyzing for amphiregulin. Amphiregulin was chosen as a marker because (i) it is regulated exclusively by progesterone in uterine epithelial cells, and (ii) the regulation of its expression by progesterone requires signaling through PR such that it is abolished upon treatment with antiprogestins (Das et al., 1995). Analyses for amphiregulin gene expression revealed that it was inducible by progesterone and not by estradiol in the uteri of ovariectomized PR-A transgenics. Furthermore, the increase in amphiregulin gene expression due to progesterone was ~5-fold higher in the uteri of PR-A transgenics when compared with wildtype mice (Fig. 5A).

To further verify whether progesterone-specific gene expression was augmented in the uteri of PR-A transgenics, we also analyzed for SOX4. SOX4 is a transcriptional modulator and its expression is regulated negatively by estradiol, which can be reversed by progesterone (Hunt and Clarke, 1999). Similar to wildtype mice, SOX4 gene expression was low in the uteri of ovariectomized PR-A-transgenics and did not increase upon treatment with estradiol.

**Figure 2** Analyses for immunoreactive ERα in the uterus of wildtype and PR-A transgenic mice. (A) Uteri from adult PR-A transgenic (a–c) and wildtype mice (d) were analyzed for ERα-positive cells by immunohistochemistry (arrows indicate ER-positive nuclei). (a) Ovariectomized mice; (b) ovariectomized mice treated with estradiol; (c) and (d) mice at proestrous. (B) The percentages of ERα-positive cells in various cell types.
However, when compared with the uterus of wildtype mice, there was approximately a 10-fold increase in SOX4 gene expression in the uteri of ovariectomized PR-A transgenics treated with progesterone (Fig. 5B).

**Figure 3** Analyses for immunoreactive PR in the uterus of wildtype and PR-A transgenic mice. Uteri from adult wildtype (A and B) and PR-A transgenic (C and D) mice were analyzed for PR-positive cells (brown stained nuclei in higher magnification insets) by immunohistochemistry, as described in text. (A and C) Ovariectomized mice; (B and D) ovariectomized mice treated with estradiol.

**Figure 4** Uterine lactoferrin gene expression in wildtype and PR-A transgenic mice. Uterus from wildtype (open bar) and PR-A transgenics (closed bar) intact or ovariectomized mice treated with saline (ovx) or estradiol (ovx+E) or progesterone (ovx+P) or estradiol and progesterone (ovx+EP) were analyzed for lactoferrin gene expression by quantitative PCR. The data are presented as transcript numbers (multiplied by $10^3$) normalized to β-actin transcripts and represent the average ± SEM of three experiments.

**Adult PR-A transgenics develop uterine abnormalities in response to exogenous estrogen and progesterone**

The fact that uterine epithelial cell proliferation was augmented in PR-A transgenics suggested that these uteri might have had a higher propensity for developing hyperplasias and other abnormalities.

Indeed, beginning at approximately 9 months of age (range 9–14 months), the uteri of PR-A transgenics displayed macroscopic and microscopic pathologies and in particular, pelvic inflammatory disease and endometritis. As a result, the uteri became enlarged owing to fluid retention, in turn, causing obstruction of the bladder and death (Fig. 6). This phenomenon was seen in >60% of transgenic mice continuously treated with estradiol and progesterone beginning at 10 weeks of age but only in 22% of treated age-matched wildtype mice (Table I, $\chi^2 = 3.86, P < 0.05$) showing the significantly higher susceptibility of PR-A transgenics for the development of this phenotype. Histological analyses (Fig. 7) revealed dilation of endometrial cavity and development of a serometra and infiltration of polymorphonuclear leukocytes. Some areas displayed reactive atypia but there was no evidence of neoplasias in any of the uteri examined.

**Discussion**

It is well documented that appropriate cellular responsiveness to progesterone is dependent on regulated expression and/or activities of the two isoforms of PR, namely the A and B forms. As such, alterations in the relative expression levels of the two isoforms can lead
to a derangement in uterine responsiveness to progesterone and, hence, epithelial cell proliferation (Shyamala et al., 1998). Alterations in the native ratio of PR-A and B have been documented in a number of histopathologic studies on human EC specimen and appear to be associated with the early phase of EC development (Cooke et al., 1997; Leslie et al., 1997; Shyamala et al., 1997; Chou et al., 2003). Moreover, EC patients negative for the expression of one or both of the PR isoforms have a shorter disease-free and overall survival (Saito et al., 2006). However, in the absence of in vivo studies, it is not possible to determine whether changes in the expression levels of the two isoforms of PR are an event accompanying the transformation of uterine epithelial cells or, in fact, are responsible for their transformation. Accordingly, the present studies were conducted using transgenic mice (PR-A transgenics) carrying an imbalance in the native ratio of A to B forms of PR. Indeed, as shown here, constitutive overexpression of PR-A leads to a hyperproliferative state in the luminal and glandular endometrium. A principal effect of progesterone in the uterus is to antagonize estrogen-dependent proliferation of epithelial cells (Martin et al., 1980; Graham and Clarke, 1997). The increased proliferation is not due to the inability of progesterone to antagonize estrogen-dependent epithelial proliferation. Rather, the observed phenotype appears to be a consequence of enhanced PR signaling since it is abolished upon treatment with mifepristone. Thus, our studies, as documented here, provide direct evidence that a regulated expression of the two isoforms of PR is critical for maintaining the homeostasis of epithelial cell proliferation. More importantly, they demonstrate that development of uterine abnormalities is a consequence of a misregulation in the relative expression of the two isoforms of PR and not vice versa.

In addition to antagonizing estrogen-dependent epithelial cell proliferation, progesterone/PR is also known to antagonize the pro-inflammatory effect of estrogen (Gorski et al., 1968). However, the development of sero-/pyometra in PR-A transgenics treated with exogenous estrogen and progesterone indicates that the ability of progesterone to inhibit inflammatory responses is jeopardized in PR-A transgenics.

Aged PR-A animals developed polypous hyperproliferative lesions with and without atypia. In atrophic uteri of post-menopausal women, generally a down-regulation of estrogen and PRs can be found (Mylonas et al., 2007). However, in a small series, Mylonas et al. (2007) recently demonstrated that there is a selective down-regulation of ERα, ERβ and PR-B with a subsequent higher expression of PR-A. This is remarkable considering the incidence of hyperplastic endometrial phenotype in some elderly women under combined hormone replacement.

**Figure 5** Amphiregulin and sox4 gene expression in the uteri of wildtype and PR-A transgenic mice. Amphiregulin (A) and sox-4 gene (B) expressions were analyzed in the uteri of ovariectomized wildtype (open bar) and PR-A transgenic mice (closed bar), treated with saline (ovx) or estradiol (ovx+E2) or progesterone (ovx+P) or both estradiol and progesterone (ovx+EP). The data are presented as transcript numbers (multiplied by 10³) normalized to β-actin transcripts and represent the average ± SEM of three experiments.

**Figure 6** Uterine abnormalities in PR-A transgenic mice. Beginning at 10 weeks of age, Tg (PR-A) and wildtype animals were treated with estradiol and progesterone continuously and were sacrificed when they developed overt anomalies or alternatively at 20 months of age if asymptomatic. This figure demonstrates massive fluid retention in uterine horns. Panels A–C show whole mounts demonstrating three representative examples of the gross dissections. Arrows point to cystically dilated uterine horns.
High-dose progesterone is frequently prescribed for endometrial hyperplasias and also for advanced cancers because of its growth-inhibiting effects on the endometrium. The reported response rates of receptor-positive ECs to MPA therapy are 70% compared with 16% in receptor-negative cases (Park, 1992). This shows that a number of receptor-positive patients especially with advanced disease do not respond. Considering the differential responses to progesterone therapy depending on the PR-A/PR-B ratio as demonstrated in our mouse model, it seems intriguing to investigate isoform distribution in primary tumors which still express PR and correlate these with outcome parameters after progesterone therapy. Together, our observations illustrate that an imbalanced PR isoform distribution can have multiple and complex consequences for endometrial physiology.

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**Declaration of authors.** All authors declare to have no competing personal or professional conflict of interest.

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**References**


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**Table 1:** Prevalence of uterine abnormalities in aged (>9 months) PR-A transgenics and wildtype animals with or without estrogen and progesterone treatment

<table>
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<th>PR-A E+P</th>
<th>PR-A Untreated</th>
<th>WT E+P</th>
<th>WT Untreated</th>
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<td>Animals with abnormalities</td>
<td>14 (60.1%)</td>
<td>2 (15.4%)</td>
<td>2 (22.2%)</td>
<td>1 (14.3%)</td>
<td>19 (100%)</td>
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<tr>
<td>Animals without abnormalities</td>
<td>9 (39.9%)</td>
<td>11 (84.6%)</td>
<td>7 (77.8%)</td>
<td>6 (85.7%)</td>
<td>33 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>23 (100%)</td>
<td>13 (100%)</td>
<td>9 (100%)</td>
<td>7 (100%)</td>
<td>52 (100%)</td>
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**Figure 7** This figure illustrates the corresponding histology. Panel A demonstrates a hyperplastic inflamed uterus. Panel D has a higher magnification of the endometrial cavity lined by a tall columnar hyperplastic epithelium with an underlying chronic inflammation. Panels B and C have dilated uterine cavities (arrows) with moderate acute endometritis. Higher magnification of panel B shows neutrophils invading the hyperplastic epithelium (E). The epithelium demonstrates a mild reactive atypia. In addition, a mixed inflammatory cell infiltrate extends into the underlying muscle consisting primarily of neutrophils, lymphocytes and plasma cells. Panels C and F also show dilated lumina with concomitant hyperplastic mucosal epithelium.

**Supplementary data**

Supplementary data are available at http://molehr.oxfordjournals.org/.


