

Distribution and changes in amounts of the androgen receptor in the pig uterus during the estrous cycle, early pregnancy and after treatment with sex steroids

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Abstract

Two experiments were performed to examine the expression of the androgen receptor (AR) gene in the pig uterus. In experiment 1, immunohistochemistry (IHC) was used to determine the distribution of the AR in uterine tissue of pigs when collected at the first day of estrus (day 0) and the mid-luteal phase (day 12) of the estrous cycle, or early pregnancy (day 12, $n=4$ gilts per group). In experiment 2, AR immunostaining and AR mRNA in uterine tissue were compared among ovariectomized gilts ($n=4$ per group) following treatment for 4 days with daily injections of: (1) progesterone (2 mg/kg bodyweight (BW)), (2) estradiol-17 β (E_2 , 2 μ g/kg BW), (3) E_2 plus progesterone (same dosages as 1 and 2 combined), (4) 5 α -dihydrotestosterone (DHT, 7 μ g/kg BW), or (5) vehicle (corn oil). Data were analyzed using ANOVA. In experiment 1, nuclear staining for AR in luminal and glandular epithelia was strong and did not differ in intensity between the two locations. Immunostaining of AR in the myometrium was less ($P<0.001$) intense than in the luminal and glandular epithelia. Nuclei of stromal cells contained AR immunostaining that varied in intensity from strong (mainly in subepithelial stroma) to weak or no staining. Stages of the estrous cycle or early pregnancy did not influence AR immunostaining in the endometrial epithelia and myometrium. In experiment 2, immunostaining of

AR in glandular and luminal epithelia and myometrium of ovariectomized gilts treated with vehicle or DHT was less ($P<0.05$) than in gilts treated with E_2 , progesterone, or E_2 plus progesterone. Immunostaining of AR did not differ between ovariectomized gilts treated with vehicle or DHT, or between gilts treated with E_2 , progesterone, or E_2 plus progesterone. In both experiments, intensity of AR immunostaining was greater in glandular epithelium located at the adluminal region compared with glandular epithelium located at the basal region of the endometrium. Competitive reverse-transcription PCR (RT-PCR) indicated a stimulatory effect ($P<0.01$) of E_2 on amounts of AR mRNA in whole endometrium. This increase in AR mRNA after E_2 treatment was not detected when E_2 was combined with progesterone. Endometrial AR mRNA was not influenced by DHT or progesterone relative to vehicle-treated gilts. In conclusion, immunoreactive AR is mainly present in luminal and glandular epithelia of the pig uterus and to a lesser extent in the myometrium, and does not change significantly during the estrous cycle or early pregnancy. Expression of the AR gene in the pig endometrium and myometrium appears to be regulated by E_2 and progesterone.

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Introduction

Androgens are considered male sex hormones, however they are also secreted and appear to have regulatory functions in females. Androgens in female mammals originate from the ovaries and adrenal glands. In pigs, androgens have been detected in follicular fluid and corpora lutea (Przala *et al.* 1984, Grant *et al.* 1989, Smith *et al.* 1992), however plasma concentrations of androgens during the porcine estrous cycle have been only partially described. Other sources of androgens such as elongating blastocysts (Fisher *et al.* 1985) and the placenta (Legrand

et al. 1984) could increase local amounts of androgens and perhaps influence specific physiological processes related to pregnancy.

The function of androgens in females is beginning to be elucidated. It has been demonstrated that treatment with androgens enhanced ovarian follicular development in pigs and primates (Cárdenas & Pope 1994, Vendola *et al.* 1998, Cárdenas *et al.* 2002), and altered epithelial cell proliferation and expression of the thioredoxin gene in the rat uterus (Armstrong *et al.* 1976, Sahlin *et al.* 1999, Weihua *et al.* 2002). The androgen receptor (AR), a transcription factor that belongs to the superfamily of nuclear receptors,

mediates the actions of the biologically active androgens, testosterone and 5 α -dihydrotestosterone (DHT) in target cells. The AR has been localized in the uteri of the rat, human, rhesus monkey and dog (Kimura *et al.* 1993, Pelletier *et al.* 2000, Vermeirsch *et al.* 2002). These previous experiments demonstrated some differences among species in relative content of AR in myometrial, stromal and epithelial cells. In the porcine uterus, the AR has been detected using immunoblot analysis (Koziorowski *et al.* 1984), however description of the cellular distribution of the AR has been only preliminary (Vale-Cruz *et al.* 2001). To date, there is little information about factors that regulate AR expression in the uterus and other female reproductive organs.

The objectives of the present experiments were: (1) to examine the distribution and relative amounts of the AR in the uterus of gilts during estrus, diestrus and early pregnancy, and (2) to determine changes in amounts of AR and AR mRNA in the uterus of ovariectomized gilts following administration of sex steroids.

Materials and Methods

Post-pubertal gilts (cross of European breeds) were exposed to boars once daily to detect estrus. Gilts that exhibited at least one estrous cycle were allocated randomly to the experimental treatments described below. Steroids (Sigma Chemical Co., St Louis, MO, USA) were dissolved in corn oil, and administered (i.m.) to gilts according to experimental protocols that were approved by the University Agricultural Animal Care and Use Committee.

Experiment 1

The objectives of this experiment were to determine the distribution and relative amounts of immunoreactive AR in the pig uterus during estrus, diestrus and day 12 of pregnancy. Eight gilts were hysterectomized on day 0 (first day of estrus) or 12 of the estrous cycle ($n=4$ per group). Four additional gilts were mated and hysterectomized on day 12 of pregnancy. Uterine horns of mated gilts were flushed with physiological saline to confirm pregnancy by observing elongating blastocysts. A small piece of uterus (about 1 cm² of surface area), dissected across the uterine tissue layers, was obtained from the middle portion of a uterine horn and fixed in 4% paraformaldehyde for 18 h at 4 °C. Tissue samples were embedded in paraffin, cut into 8 μ m sections and mounted on glass slides (Superfrost plus). Sections were stored at -20 °C until determination of AR by immunohistochemistry (IHC) as described below.

Experiment 2

The objectives of this experiment were to determine the effects of sex steroids on relative amounts of AR and AR

mRNA in uterine tissue of ovariectomized gilts. Four to five weeks after ovariectomy, gilts received daily i.m. injections of one of the following treatments during four consecutive days: (1) progesterone (2 mg/kg bodyweight (BW)), (2) estradiol-17 β (E₂, 2 μ g/kg BW), (3) progesterone plus E₂ (same doses as 1 and 2 combined), (4) DHT (7 μ g/kg BW), and (5) corn oil (vehicle). Gilts were hysterectomized 24 h after receiving their last treatment. A small piece of uterus was excised, and then fixed, embedded in paraffin and sectioned as described in experiment 1 for determination of AR by IHC. An additional sample of endometrium (approximately 200 mg) was dissected from the middle portion of a uterine horn, frozen in liquid nitrogen and then stored at -80 °C. Total RNA was isolated from endometrial tissue using TRI Reagent (MRC, Inc., Cincinnati, OH, USA) and stored at -80 °C for subsequent use in determination of AR mRNA by competitive reverse transcription-PCR (RT-PCR). Purity of RNA samples was verified by calculating the ratios of the absorbances at 260 and 280 nm. The absorbance at 260 nm was used to calculate the concentrations of RNA. The integrity of RNA was determined by estimating the 28s to 18s rRNA ratios following agarose gel electrophoresis under denaturing conditions.

Immunohistochemistry of AR

The procedure used for determination of pig AR by IHC has been recently described (Cárdenas & Pope 2002). The AR antibody, PA1-111A (Affinity Bioreagents, Inc., Golden, CO, USA) was produced against a 21-amino acid peptide that has 95% sequence homology with the N-terminus of the porcine AR (GenBank accession AF202775). Briefly, tissue sections were deparaffinized, hydrated and subjected to antigen retrieval by incubating in 0.01 M sodium citrate, pH 6.0, for 30 min at 95 °C. Tissue sections were treated with 0.3% hydrogen peroxide and then incubated in normal goat serum. Thereafter, sections were incubated with AR antibody (8 μ g/ml) for 18 h at 4 °C. This was followed by incubation in biotinylated goat anti-rabbit IgG and then in Vectastain ABC reagent (Vector Laboratories, Inc., Burlingame, CA, USA). Sections were incubated for 8 min with diaminobenzidine substrate solution containing nickel chloride to produce gray-black staining (Vector Laboratories). Adjacent tissue sections were processed as negative controls in which the primary antibody was pre-absorbed with neutralizing peptide, or the primary or secondary antibody was replaced with PBS. All sections for each experiment were processed simultaneously.

Sections were examined using a Zeiss Axioskop microscope. Digital images (8 bit, 256 tones of gray) of luminal epithelium, glandular epithelium (from the basal and adluminal regions of the endometrium) and myometrium were obtained at a magnification of 400 \times using a CCD camera attached to the microscope and Optimas software

(Media Cybernetics, Inc., Silver Spring, MD, USA). These images were used for densitometric analysis of AR immunostaining by outlining stained nuclei using a minimum threshold value of gray and determining the gray values (in pixels) of outlined nuclei using the ImageJ program (National Institutes of Health Internet page: <http://rsb.info.nih.gov/ij/>). The relative amount of AR in a cell type of a particular sample was represented by the AR staining intensity, which was estimated as the average gray value of the stained nuclei corrected for the gray value of the background.

Western blotting of AR

Verification of AR binding by the AR antibody PA1-111A was performed by Western blotting. Endometrial tissue obtained from gilts on day 0 or 12 of the estrous cycle was transferred to lysis buffer (1/10 (w/v), 50 mM Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS, w/v), 10% glycerol (v/v), 5% beta-mercaptoethanol (v/v)) and homogenized for 30 s in a PowerGen 700 homogenizer (Fisher Scientific, Pittsburgh, PA, USA) set at medium speed. Aliquots of the homogenate were incubated at 95 °C for 10 min and then centrifuged at 14 000 g for 3 min. The supernatant was recovered and stored at -80 °C. Protein concentration was determined using the Bradford Reagent (Sigma Chemical Co.) and BSA as standard. Sample aliquots equivalent to 100 µg protein, and a mixture of pre-stained molecular weight standards were subjected to discontinuous SDS gel electrophoresis on 9% polyacrylamide (Gallagher 1999) using a Mighty Small vertical slab gel unit (Hoefer Scientific Instruments, San Francisco, CA, USA). Proteins were blotted onto a PVDF membrane (Immobilon-P, Millipore Corporation, Bedford, MA, USA) at 4 °C, overnight, using an electrophoretic transfer cell (Mini Trans-Blot, Bio-Rad Laboratories, Hercules, CA, USA) and transfer buffer (25 mM Tris base, 192 mM glycine, 10% methanol (v/v), pH 8.3). Detection of AR on the membrane was performed using the same kits described above for detection of AR by IHC. Membranes were incubated in TTBS (100 mM Tris.Cl, 150 mM NaCl, pH 7.5, 0.1% (v/v) Tween 20) supplemented with normal goat serum (1.5% v/v) for 2 h to block non-specific binding sites, and then treated with AR antibody (PA1-111A, 8 µg/ml in TTBS) at 4 °C for 20 h. Membranes were washed in TTBS and incubated in biotinylated goat anti-rabbit IgG (100 µl in 50 ml of TTBS) for 30 min at room temperature, washed again, and then incubated in a solution of avidin DH and biotinylated horseradish peroxidase (Vectastain ABC reagent, prepared in TTBS according to the manufacturer's instructions and further diluted to 1/5, and the concentration of NaCl increased to 0.5 M) for 30 min. For color development, the membranes were washed in TTBS and incubated in a solution containing the peroxidase substrate diaminobenzidine and nickel chloride. Membranes that served as

controls were not treated with either AR antibody or goat anti-rabbit IgG. Membranes were washed in water, allowed to dry and scanned for documentation.

Competitive RT-PCR of AR

The competitive RT-PCR of AR was performed using the GeneAmp RNA PCR Perkin Elmer kit (Roche Molecular Systems, Inc., Branchburg, NJ, USA) and homologous primers (forward, 5' AGC CTC TGG ACC AGT CAT TC 3'; reverse, 5' CAC CAT CTT CTG CCA GAG AC 3'). The RNA standard ('competitor') was constructed by introducing a 40 bp deletion in an AR product (242 bp) obtained using a previously described RT-PCR protocol (Cárdenas *et al.* 2002). The deletion was introduced by re-amplifying the AR product using the reverse primer given above and a modified forward primer (5' CAT ATT GAA GGC TAT GAG TGG CGT AGT GTG TGC C 3'). The resulting product was cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA) and then sequenced to verify the deletion and establish its orientation in the vector. The vector was linearized using NcoI enzyme and then used to synthesize the RNA competitor by run-off *in vitro* transcription using SP6 RNA polymerase (Riboprobe *In Vitro* Transcription System, Promega). The RNA was extracted from the reaction solution after digestion of the DNA template using RQ1 DNase (Promega). Synthesis of the AR mRNA competitor was verified by agarose gel electrophoresis and its concentration was determined by measuring the absorbance at 260 nm. The competitor was then divided into aliquots and stored at -80 °C.

For competitive RT-PCR, four reverse transcription reactions were prepared for each sample containing 250 ng of total RNA and different amounts of RNA competitor. The amounts of competitor RNA that produced an appropriate pattern of competition with wild-type RNA (Freeman *et al.* 1999) were determined in preliminary assays by testing different ranges of competitor, and were set at 0.25, 1, 4 and 16 pg (equivalent to 2.6, 10.5, 42.2 and 168.6 fmol respectively) per tube. First strand cDNA was synthesized using Maloney murine leukemia virus reverse transcriptase primed with random hexamers. Concentrations of the other components were those indicated by the kit manufacturer (see above). Tubes were incubated for 10 min at room temperature, 30 min at 42 °C and 5 min at 99 °C. PCR was performed using Taq DNA Polymerase, 1.5 mM MgCl₂ and 0.3 µM primers. Tubes were incubated for 1 min at 95 °C and then subjected to 25 cycles of 1 min at 95 °C, 45 s at 55 °C and 2 min at 72 °C. Cycling was followed by 7 min of incubation at 72 °C and then cooling to 5 °C. PCR products were separated by electrophoresis in 4.0% NuSieve 3:1 agarose gel, visualized using ethidium bromide and UV illumination, and then photographed. Intensity of cDNA bands were obtained by image analysis (ImageJ program) and

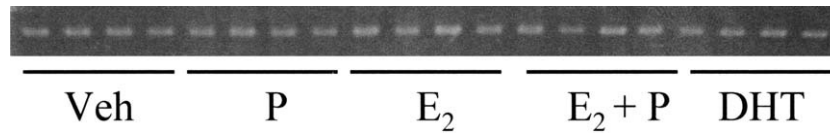


Figure 1 Ribosomal protein L-19 mRNA determined by RT-PCR in the endometrium of ovariectomized gilts (experiment 2) showing similar amounts of L-19 mRNA among samples. Veh, vehicle; P, progesterone.

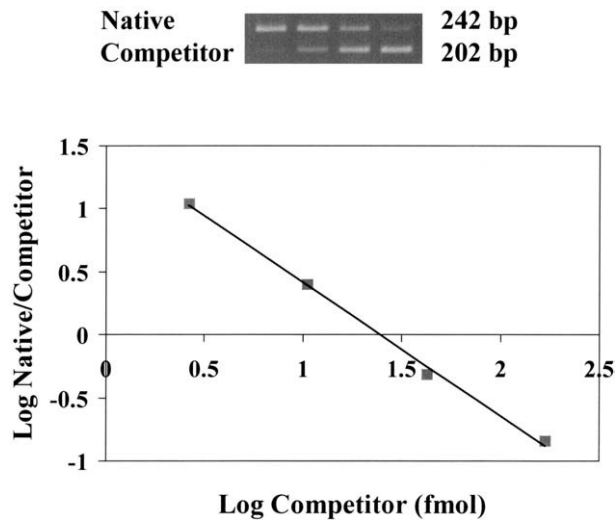


Figure 2 Determination of AR mRNA by competitive RT-PCR. Top, gel electrophoresis showing cDNA bands generated by native and increasing amounts of competitor AR mRNA in a representative sample. Bottom, regression analysis of the relationship between amounts of competitor (x -axis) and native/competitor ratio of band intensity (y -axis) performed to estimate the amounts of AR mRNA.

then plotted on logarithmic coordinates (amounts of competitor on the x -axis and native/competitor intensity ratio on the y -axis). The amount of AR mRNA in a sample was estimated as the amount of competitor equal to the amount of native RNA (native/competitor ratio of 1 equivalent to 0 in a log scale) determined using regression analysis. Slopes were not influenced by treatment ($P=0.36$) and RT-PCR of ribosomal protein L19 (performed as described in Cárdenas & Pope 2002) confirmed the absence of major differences in RNA degradation between samples (Fig. 1). Competitive RT-PCR data of AR determination in a representative sample is presented in Fig. 2. Intra- and inter-assay coefficients of variation of AR mRNA in a sample that was repeated three times in two assays were <11.0% and 13.3% respectively.

Statistical analysis

The amounts of AR immunostaining in glandular epithelium at the basal and adluminal regions of the endometrium were compared using ANOVA for a split-plot design. AR immunostaining in the basal and adluminal

regions were averaged to obtain an overall mean value of AR immunostaining in glandular epithelium for subsequently comparing with luminal epithelium and myometrium using a split-plot ANOVA. Means were compared by using the pdiff option of SAS (SAS Institute Inc., Cary, NC, USA). The amounts of AR mRNA in the whole endometrium were analyzed by using a one-way ANOVA and means compared by Fisher's protected LSD tests. $P \leq 0.05$ was considered significant.

Results

Experiment 1: distribution of the AR in the pig uterus during the estrous cycle and early pregnancy

Immunostaining of the AR was detected in nuclei of various cell types of the pig uterus. AR immunostaining was relatively strong in luminal and glandular epithelia, and moderate in the myometrium. Endothelial cells and most nuclei of endometrial stroma exhibited moderate to weak, or no, AR immunostaining (Fig. 3). Some cells of the subepithelial stroma demonstrated relatively strong AR immunostaining. Immunostaining of AR in glandular epithelium was less ($P < 0.01$) intense at the basal compared with the adluminal region of the endometrium, and was not influenced by reproductive stage or the interaction of reproductive stage by location (Table 1 and Fig. 3). The intensity of AR immunostaining was not different between glandular and luminal epithelia, but was less ($P < 0.05$) in the myometrium compared with glandular and luminal epithelia (Table 2). Intensities of immunoreactive AR in glandular and luminal epithelia and myometrium were not influenced by reproductive stage ($P=0.6$), or by the interaction of stage by cell type ($P=0.33$, Table 2 and Fig. 3).

Results of Western blotting demonstrated that the AR antibody utilized for the IHC determinations specifically binds a protein of approximately 110 kDa (Fig. 4). A protein of approximately 65 kDa was stained weakly and nonspecifically. This nonspecific binding was not detected in the IHC procedure, suggesting that it may be intrinsic to membrane binding.

Experiment 2: amounts of AR and AR mRNA in the uterus of ovariectomized gilts treated with sex steroids

Uterine cell types that contained AR in ovariectomized gilts treated with sex steroids were the same as those

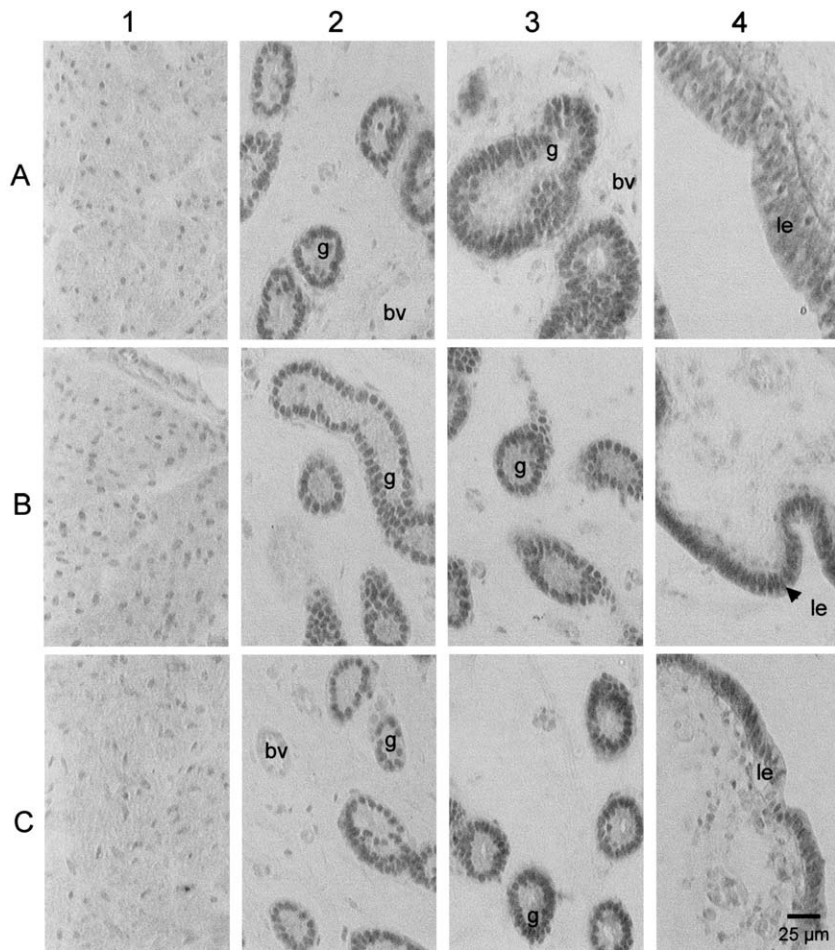


Figure 3 Immunohistochemical detection of the AR in the uterus of gilts at estrus (A), day 12 of the estrous cycle (B) and day 12 of pregnancy (C). 1, myometrium; 2, basal endometrium; 3, adluminal endometrium; 4, luminal epithelium. g, endometrial gland; le, luminal epithelium; bv, blood vessel.

Table 1 Intensity (mean gray value) of AR immunostaining in glandular epithelium at the basal (GEB) and the adluminal (GEL) regions of the endometrium in gilts during the estrous cycle and early pregnancy^a

	GEB	GEL
Stage^b		
Estrus (day 0)	66.6 ± 3.8	75.9 ± 3.8
Luteal phase (day 12)	77.0 ± 3.8	81.4 ± 3.8
Pregnancy (day 12)	64.5 ± 3.8	81.8 ± 3.8
Mean	69.3 ± 2.2 ^c	79.7 ± 2.2 ^d

^aValues are least-squares means ± s.e., n=4 gilts per stage.

^bNo effect of physiological stage ($P=0.32$) or stage × location ($P=0.28$).

^{c,d}Means differ ($P<0.01$).

Table 2 Intensity (mean gray value) of AR immunostaining in the uterus of gilts during the estrous cycle and early pregnancy^a

	Myometrium	Glandular epithelium	Luminal epithelium
Stage^b			
Estrus (day 0)	42.9 ± 3.5	71.2 ± 3.5	74.5 ± 4.0
Luteal phase (day 12)	43.8 ± 3.5	79.2 ± 3.5	85.6 ± 3.5
Pregnancy (day 12)	44.4 ± 3.5	73.1 ± 3.5	69.7 ± 4.0
Mean	43.7 ± 2.0 ^c	74.5 ± 2.0 ^d	76.6 ± 2.2 ^d

^aValues are least-squares means ± s.e., n=4 gilts per stage.

^bNo effect of stage ($P=0.6$) or the interaction of stage by type of tissue ($P=0.33$).

^{c,d}Means having different superscripts differ (main effect of type of tissue, $P<0.001$).

described for intact gilts in experiment 1 (Fig. 5). Intensities for immunoreactive AR in the uterus of ovariectomized gilts was influenced by sex steroid treatment ($P=0.03$) and cell type ($P=0.001$), but there was no

treatment by cell type interaction ($P=0.67$). AR immunostaining in glandular and luminal epithelia and in the myometrium was greater ($P<0.05$) in gilts treated with E_2 ,

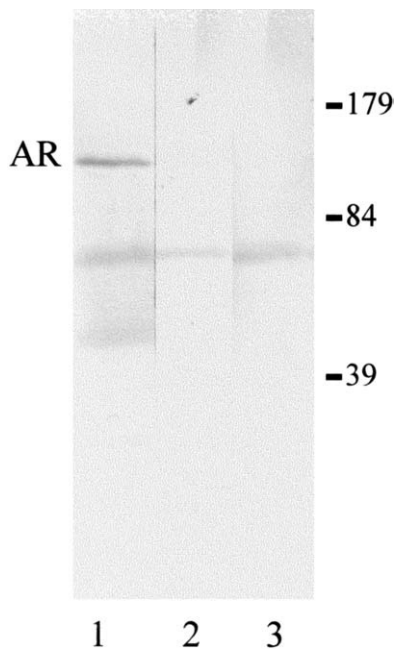


Figure 4 Verification of AR binding by Western blotting in pig endometrium. AR was detected as a band of approximately 110 kDa (lane 1). The weak staining of a band of approximately 65 kDa was non-specific. Lanes 2 and 3 are controls that were not treated with first or second antibody respectively. The apparent molecular weight of markers are given on the right in kDa.

progesterone, or E_2 plus progesterone relative to gilts treated with DHT or vehicle (Table 3 and Fig. 5). AR immunostaining in glandular epithelium was not different ($P > 0.05$) between gilts treated with DHT or vehicle, or between gilts treated with E_2 , progesterone, or E_2 plus progesterone (Table 3 and Fig. 5). Similar to experiment 1, AR immunostaining was less ($P < 0.01$) in the myometrium than in glandular or luminal epithelium (Table 3 and Fig. 5). Likewise, AR staining intensity in glandular epithelium was lesser ($P < 0.01$) in the basal compared with the adluminal region of the endometrium (91.3 ± 2.9 and 102.4 ± 2.9 respectively, see also Fig. 5G).

Amounts of AR mRNA in whole endometrium of ovariectomized gilts treated with progesterone, DHT, E_2 plus progesterone, or vehicle were not different ($P > 0.05$, Fig. 6), however gilts receiving these treatments had smaller ($P < 0.01$) amounts of AR mRNA in the whole endometrium compared with gilts administered E_2 (Fig. 6).

Discussion

The AR was detected in nuclei of most cell types of the pig uterus including epithelial, stromal, myometrial and endothelial cells. The greatest cellular concentration of the

AR was detected in glandular and luminal epithelia, and was approximately 40% less abundant in the myometrium. Other cell types exhibited variable and, in most instances, relatively low AR content. The relative abundance of AR in endometrial epithelia suggests that androgens might influence function(s), yet to be determined, in these cells. As in pigs, AR and its mRNA were detected in glandular and luminal epithelia, stroma and myometrium of the adult rat uterus (Hirai *et al.* 1994, Pelletier *et al.* 2000). In contrast, the AR was not detected in the uteri of immature rats (Weihua *et al.* 2002), suggesting associations of AR gene expression with postnatal uterine development. The relative distribution of the AR among uterine cell types appears to be different in women, monkeys and dogs compared with pigs and adult rats. In primates and dogs, immunostaining of AR was greater in endometrial stroma and myometrium than in glandular epithelium (Mertens *et al.* 1996, Slayden *et al.* 2001, Vermeirsch *et al.* 2002). Differences in cellular distribution of AR among species are probably associated with different roles of AR in the uterus and might also involve unique regulatory mechanisms of AR gene expression.

Immunostaining of AR in glandular epithelium was greater at the adluminal compared with the basal region of the endometrium and did not change during different reproductive stages or in ovariectomized gilts treated with sex steroids. This pattern of AR present in glandular epithelium has not been reported in other species and its biological significance is unclear. Uterine glands develop from the luminal epithelium by invagination (reviewed by Gray *et al.* 2001) and regions of cell proliferation are located at the apical and middle portions of glands (Tarleton *et al.* 1999, Taylor *et al.* 2000). There are indications from experiments in mice that DHT reduces cell death in endometrial epithelia (Terada *et al.* 1990). If androgens decrease cell death in the pig endometrium (to be confirmed), then increased AR expression in epithelium located at the upper part of uterine glands might help epithelial cell survival at a region where cell proliferation is reduced.

The amounts of AR in glandular and luminal epithelia or myometrium did not differ among gilts at estrus, diestrus or day 12 of pregnancy when the uterus is exposed to relatively high concentrations of E_2 , progesterone, or progesterone and E_2 (E_2 from elongating blastocysts) respectively. In support, no significant differences in amounts of immunoreactive AR were detected in endometrial epithelia or myometrium of ovariectomized gilts treated for 4 days with E_2 , progesterone, or E_2 plus progesterone in experiment 2. These observations indicate that in pigs, either estrogen or progesterone, or their combination, would induce AR in endometrial epithelia and myometrium. AR might be needed for some of the actions of E_2 and progesterone in the uterus. For instance, it has been proposed that induction of AR by E_2 in the rat uterus might amplify the effects of E_2 on epithelial cell

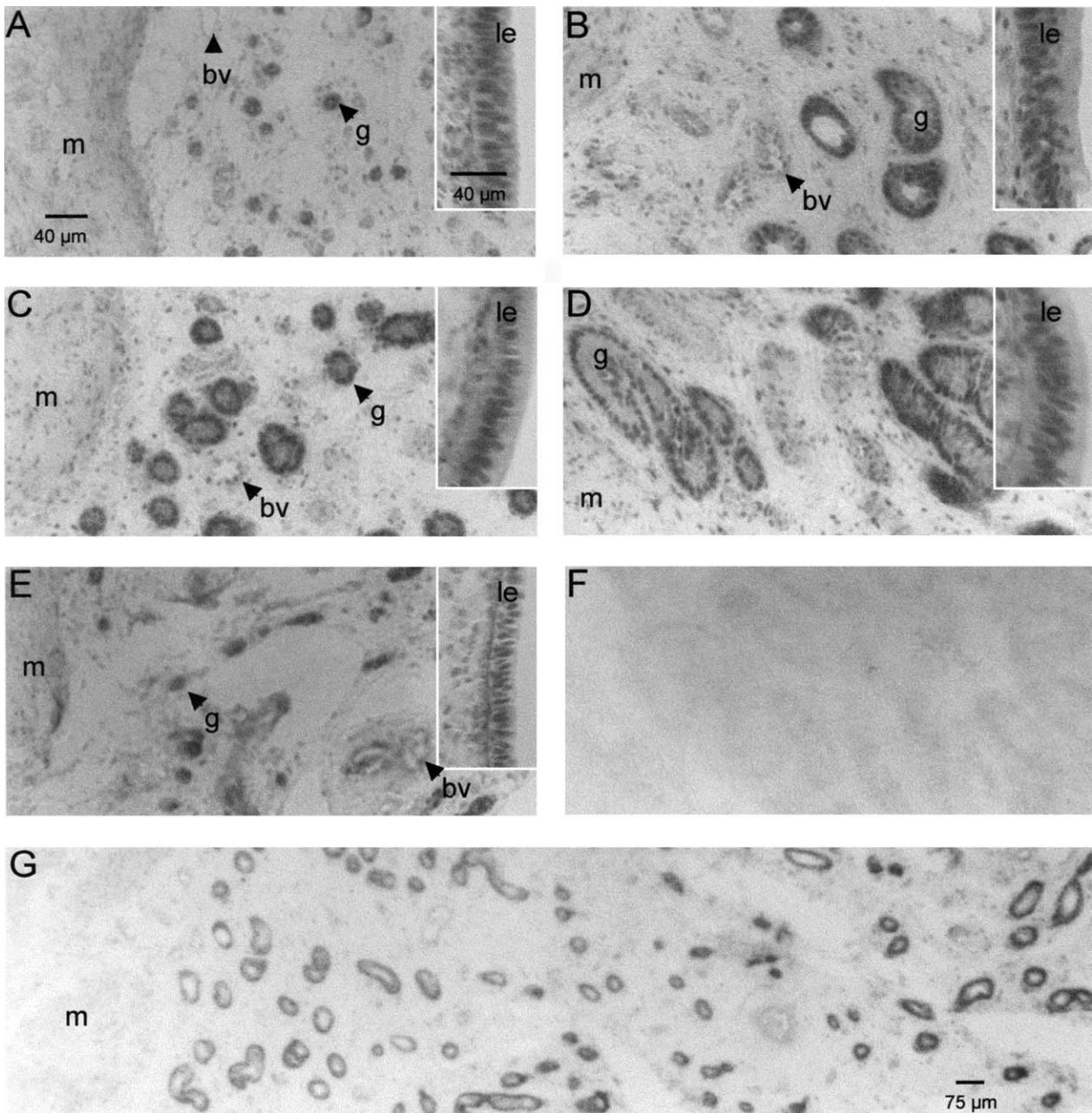


Figure 5 Immunohistochemical detection of the AR in the uterus of ovariectomized gilts treated with vehicle (corn oil, A), E₂ (B), progesterone (C), E₂ plus progesterone (D), or DHT (E). (F) Negative control (section adjacent to D in which AR antibody pre-incubated with immunizing peptide was used). (G) Section showing differential AR immunostaining across the endometrium of a gilt treated with E₂. Insets, luminal epithelium (le). m, myometrium; g, endometrial gland; bv, blood vessel; be, basal endometrium; ae, adluminal endometrium.

proliferation through induction of insulin-like growth factor by AR (Weihua *et al.* 2002).

Estradiol-17 β and progesterone, or their combination, upregulated AR in glandular and luminal epithelia and myometrium of ovariectomized gilts. Treatment of gilts with E₂ also produced an approximately twofold increase

in AR mRNA in whole endometrium relative to gilts that received vehicle or other steroid treatments. Upregulation of AR by E₂ observed in the present experiments agrees with results in rhesus monkeys and immature rats treated with E₂ (Slayden *et al.* 2001, Weihua *et al.* 2002). Progesterone did not affect the amounts of AR mRNA

Table 3 Intensity (mean gray value) of AR immunostaining in luminal and glandular epithelia and myometrium of ovariectomized gilts treated with sex steroids^a

	Myometrium	Glandular epithelium	Luminal epithelium	Mean
Treatment				
Vehicle	48.6 ± 5.7	76.8 ± 5.7	90.5 ± 5.7	72.0 ± 5.6 ^d
Progesterone	72.3 ± 7.5	112.9 ± 7.2	100.4 ± 9.7	95.2 ± 8.0 ^c
E ₂	58.8 ± 5.7	104.8 ± 5.7	106.4 ± 5.7	90.0 ± 5.6 ^e
Progesterone+E ₂	65.5 ± 5.7	109.2 ± 5.7	107.5 ± 5.7	94.1 ± 5.6 ^e
DHT	46.3 ± 5.7	83.3 ± 5.7	89.5 ± 5.7	73.0 ± 5.6 ^d
Mean	58.3 ± 2.7 ^b	97.4 ± 2.7 ^c	98.9 ± 3.0 ^e	

^aValues are least-squares means ± s.e., n=4 gilts per treatment.

^{b,c}Main effect of type of tissue. Means having different superscripts differ (P<0.01).

^{d,e}Main effect of treatment. Means having different superscripts differ (P<0.05).

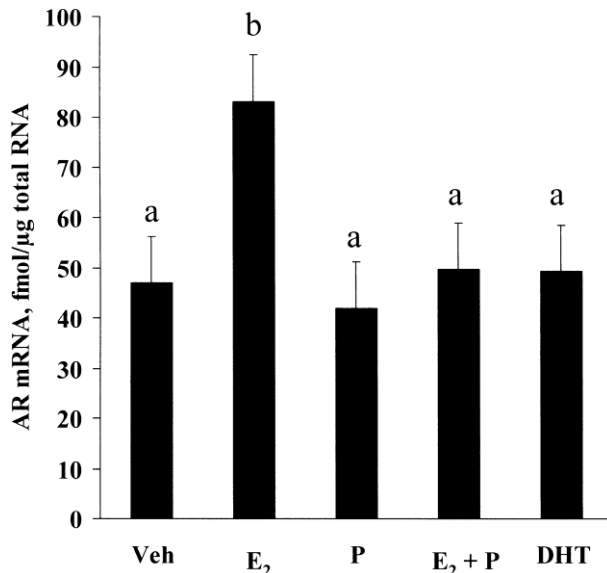


Figure 6 Mean concentrations of AR mRNA determined by quantitative RT-PCR in the endometrium of ovariectomized gilts (n=4 per group) treated for 4 days with sex steroids or vehicle. Bars having different letters differ (P<0.01). Error bars represent s.e. Veh, vehicle; P, progesterone.

and, instead, inhibited the effect of E₂ on AR mRNA. The effects of progesterone on AR mRNA in whole endometrium were not parallel to the positive effects of progesterone on AR protein. Whether this was due to the presence of multiple cell types in samples utilized for RT-PCR, or post-transcriptional regulation, remains to be elucidated. In rhesus monkeys, treatment with E₂ and progesterone increased (larger dose of progesterone) or decreased (smaller dose of progesterone) AR protein and/or mRNA in endometrial stroma compared with E₂ alone (Adesanya-Famuyiwa *et al.* 1999, Slayden *et al.* 2001). Inhibitory effects of progesterone on AR mRNA

were also observed in human endometrial stromal cells *in vitro* (Iwai *et al.* 1995). An inhibitory effect of progesterone on AR would be consistent with a decrease in uterine AR observed in primates during the secretory phase of the menstrual cycle (Mertens *et al.* 1996, Slayden *et al.* 2001). Together, these results indicate that progesterone decreases the amounts of AR mRNA, however the effects of progesterone on AR protein has varied between species or studies.

Unlike E₂ and progesterone, DHT (7 μg/kg BW) did not alter the amounts of AR protein in glandular and luminal epithelia or myometrium. Likewise, DHT did not alter the amounts of AR mRNA in whole endometrium. In reproductive organs of male rats and cultured endometrial stromal cells, androgens decreased the amounts of AR mRNA (Shan *et al.* 1990, Quarmby *et al.* 1990, Iwai *et al.* 1995) or increased the amounts of AR protein (Takeda *et al.* 1991, Prins & Birch 1993). It is important to note that the doses of androgens injected into male rats in the experiments cited above were greater (≥ 400 μg/kg) than the dose of DHT (7 μg/kg) used in the present study in female pigs. Females normally have lower plasma concentrations of androgens than males and the dose utilized was similar to a dose of testosterone previously shown to increase the plasma concentrations of this hormone from 0.2 to 1.2 ng/ml in gilts (Cárdenas & Pope 1994). It is possible that greater amounts of androgens or longer duration of treatment with androgens might upregulate AR in the uterus. For instance, long-term androgen treatment to women increased immunoreactive AR in myometrial and endometrial stroma (Chadha *et al.* 1994).

In conclusion, the AR was present in most cell types of the pig uterus but mainly in glandular and luminal epithelia. The amounts of AR were not significantly altered throughout the estrous cycle and early pregnancy. Estradiol-17β and progesterone exerted regulatory effects on AR gene expression in the porcine endometrium and myometrium.

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