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Androgen Receptor mRNA Expression in the Rhesus Monkey Ovary

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Immunocytochemical detection of androgen receptors (ARs) in several compartments of the macaque ovary, including the germinal epithelium, follicle, and corpus luteum, suggests a role for androgens in modulating ovarian function via the classical receptor-mediated pathway. To examine AR mRNA expression in the rhesus monkey ovary, total RNA was isolated from whole ovaries, the germinal epithelium–enriched cortical and medullary compartments of the ovary, and corpora lutea from early (d 3–5), mid (d 6–8), mid-late (d 10–12), and late (d 13–15) stages of the luteal phase of the menstrual cycle. RNA was also obtained from luteinized granulosa cells from monkeys receiving gonadotropin treatment to stimulate the development of multiple ovarian follicles. After reverse transcription of total RNA using oligo-dT as a primer, polymerase chain reaction (PCR) was used to amplify a unique 329 bp segment of the monkey AR hormone-binding region. Reverse transcriptase (RT)-PCR products of the expected size were detected in all ovarian and control tissues. Sequence analysis of the AR cDNA from the macaque ovary revealed 99% nucleotide homology and 100% predicted amino acid homology to the cDNA for the hormone-binding region of human AR. Northern analysis demonstrated the presence of a major AR mRNA species at 9.5 kb in corpus luteum, luteinized granulosa cells, and prostate, with additional bands detected in the corpus luteum and prostate at 7.9 and 3.4 kb, respectively. A sensitive RNase protection assay was used to examine AR mRNA levels in ovarian tissues and showed AR mRNA expression throughout the life-span of the corpus luteum. Thus, detection of AR mRNA in the primate ovary, including the periovulatory follicle and corpus luteum, supports the concept that these tissues are targets for receptor-mediated androgen action during the menstrual cycle.

Key Words: Androgen receptor; ovary; primate; menstrual cycle; corpus luteum; granulosa cell.

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Introduction

In addition to estrogens and progestins, the primate ovary synthesizes large amounts of androgens throughout the menstrual cycle. The two-cell model of steroidogenesis in the mammalian follicle supports the concept that androgens produced by the theca cells provide substrate for granulosa cell estrogen production *(1)*. A similar compartmentalization of steroidogenic enzymes and steroid synthesis apparently occurs in the primate corpus luteum *(2,3)*. High levels of androgens in follicular fluid *(4–6)* and androgen production by luteal cells *(7)* also support a possible autocrine or paracrine role for androgens to regulate the development and function of the primate follicle and corpus luteum.

The ovarian actions of androgens are thought to be mediated through classical nuclear androgen receptors (ARs). Administration of AR antagonists in vitro and in vivo can counteract androgen modulation of granulosa *(1)* and theca *(8)* cell steroidogenesis in rodents, suggesting that these effects of androgen are receptor mediated. High-affinity, low-capacity androgen-binding proteins have been identified in the ovaries of rats *(9)* and humans *(10)*. ARs were localized to every compartment of the monkey *(11)* and human *(12,13)* ovary by immunocytochemistry, including the granulosa and theca cell layers of small and preovulatory follicles, immature oocytes, the corpus luteum, and ovarian stroma cells. AR mRNA was detected in the granulosa, theca, and stromal cells of rat ovaries, with the

highest expression reported in the theca *(14)* and granulosa cells of preantral or early antral follicles *(15)*. In primates, AR mRNA expression was highest in the granulosa cells of small antral follicles of marmoset *(16)* and rhesus *(17)* monkeys. To our knowledge, the distribution and quantity of AR mRNA in the primate corpus luteum has not been reported.

The present study was designed to identify and quantitate AR mRNA expression in the ovary of the rhesus monkey, with particular attention to the corpus luteum. AR mRNA was detected in various compartments of the monkey ovary using the reverse transcriptase-polymerase chain reaction (RT-PCR), and a sensitive ribonuclease protection assay was used to examine changes in AR mRNA in the corpus luteum throughout the luteal phase of the menstrual cycle.

Results

Oligonucleotide primers were used to amplify AR cDNA from macaque ovarian and non-ovarian tissues. The nucleotide sequence for this region was identical to that previously published for macaque AR mRNA *(18)*. There was 99 and 96% homology between the nucleotide sequence for this portion of the macaque AR cDNA and the corresponding regions of the human and rat AR cDNAs, respectively. The amplified region corresponded to amino acids 790–898 of the human AR sequence *(19)*. The predicted amino acid homology between this portion of the macaque, human, and rat AR cDNAs was 100%.

RT-PCR Detection of AR mRNA in Ovarian and Nonovarian Tissues

RT-PCR product of the expected size (Fig. 1) representing AR mRNA was detected in ovarian tissues including whole ovary ($n = 4$), germinal epithelium ($n = 3$), and medullary compartments ($n = 3$), as well as in luteinized granulosa cells ($n = 1$) and the corpus luteum at mid luteal phase (data not shown; $n = 3$). AR mRNA was also detected in all nonovarian macaque tissues examined, including prostate, myometrium, spleen, kidney, thyroid, pancreas, and heart (Fig. 1; data not shown). Because RT-PCR as performed for these studies was not designed for quantitative comparisons, band intensity does not correlate with the amount of AR mRNA in various macaque tissues. AR mRNA was consistently absent when RT product prepared using water instead of RNA (Fig. 1) was included in the PCR and when RNA was used without treatment with RT (data not shown). The sequence of the PCR products obtained from macaque prostate, myometrium, and whole ovary were verified using restriction enzyme analysis. Digestion with *Bst*XI cleaved the AR product from all three tissues into predicted size fragments of 215 and 114 bp (data not shown). Amplification of a portion of the macaque β-actin mRNA in the same RNA samples used for the detection of AR mRNA demonstrated a product of the expected size (838 bp) in prostate, myometrium, whole ovary, and germinal epithelium and medullary compartments (data not shown), confirming the specificity of the primers used for reverse transcription and that the RNA used in these studies was intact prior to analysis.

Northern Analysis

Northern analysis demonstrated the presence of AR mRNA in monkey ovarian tissues (Fig. 2). A cDNA probe corresponding to a portion of the hormone-binding region of monkey AR recognized a major band at 9.5 kb in total RNA from monkey prostate, luteinized granulosa cells, and corpus luteum, with additional bands at 7.9 and 3.4 kb in corpus luteum and prostate, respectively. Smaller mRNA species may also be present in the prostate.

RNase Protection

Using the RNase protection assay, AR mRNA was detected in luteinized granulosa cells and corpora lutea at all stages of the luteal phase (Fig. 3). AR mRNA levels did not vary across the stages of the luteal phase. Luteal levels of the internal control mRNA cyclophilin did not appear to change across the luteal phase, consistent with previous studies *(20)*. However, cyclophilin levels per microgram of total RNA were apparently lower in luteinized granulosa cells than in luteal tissues.

Luteal Steroid Levels

Luteal tissue steroid levels were assessed to determine whether ligands for the AR were present in corpora lutea throughout the luteal phase of the menstrual cycle (Fig. 4). Luteal levels of androstenedione were lowest early in the luteal phase and rose to the highest levels by very late luteal phase ($p < 0.05$). Testosterone levels were below the limit of detection (5 pg/mL) in 21 of the 32 luteal tissue homogenates assayed. All samples had testosterone concentrations below 40 pg/mL (data not shown), and no trend toward changing testosterone across the luteal phase was noted. High intraluteal progesterone concentrations were maintained throughout the functional life-span of the corpus luteum, with declining luteal progesterone measured at the time of menstruation (very late luteal phase, $p < 0.05$). Estradiol levels remained unchanged across the luteal phase of the menstrual cycle.

Discussion

This study is the first to demonstrate the presence of AR mRNA in specific compartments of the rhesus macaque ovary and to assess monkey luteal AR mRNA levels. The portion of the macaque AR cDNA amplified by RT-PCR codes for a portion of the AR hormone-binding domain and shares significant nucleotide homology with the corresponding region of the human AR; the predicted amino acid sequence is identical between macaque and human AR in the region examined. Northern blotting confirmed a major AR mRNA species at 9.5 kb, with additional bands at 7.5

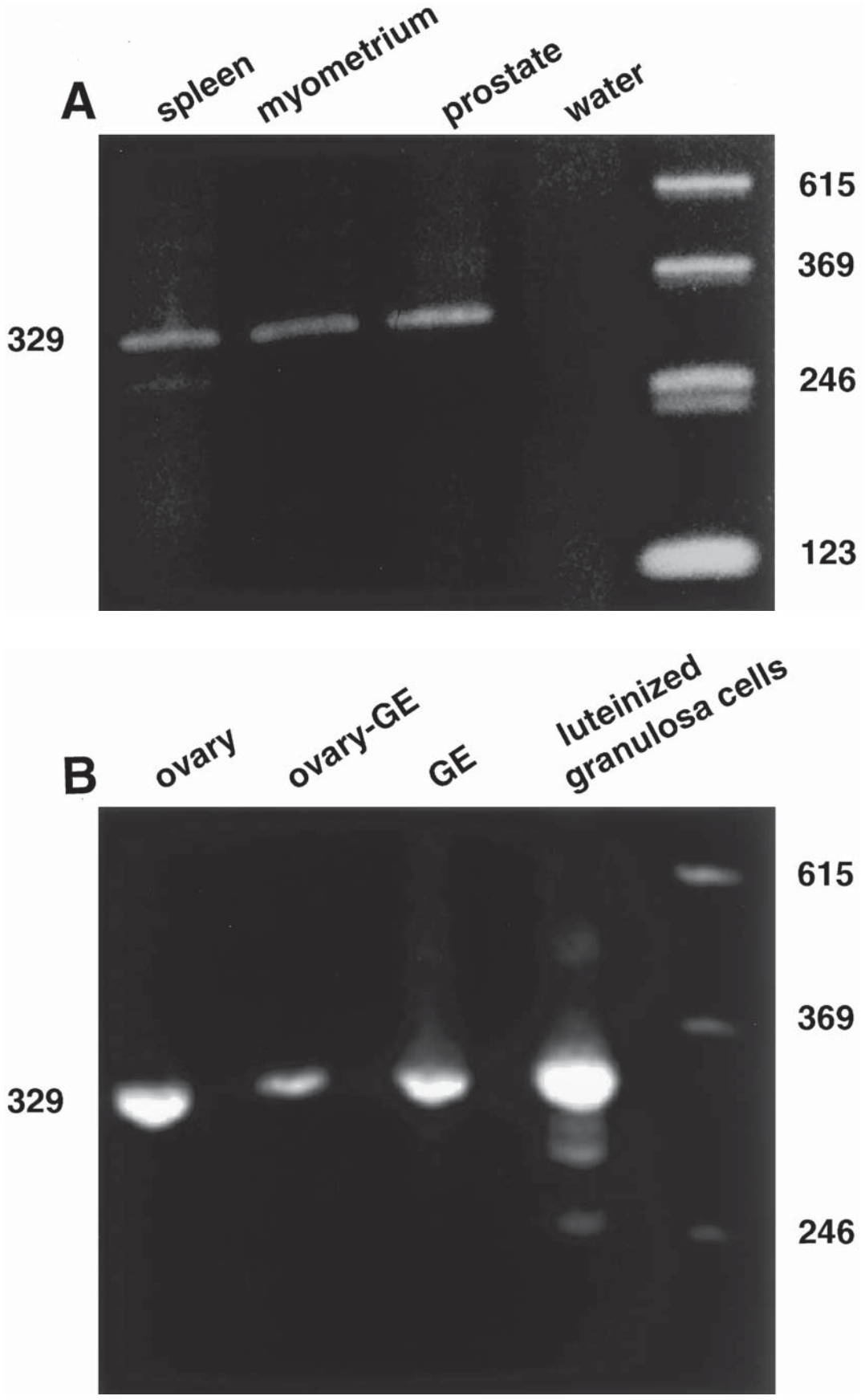

Fig. 1. Detection of AR mRNA in monkey tissues by RT-PCR. Reverse-transcription reactions included **(A)** water (negative control) or RNA from monkey spleen, myometrium, and prostate; **(B)** whole ovary, ovary without germinal epithelium (ovary-GE), germinal epithelium–enriched ovarian cortex (GE), and luteinized granulosa cells. All RT products were used for PCR amplification of monkey AR mRNA. Size markers (base pairs) are shown at the right, and the expected size of the PCR product (329 bp) is indicated at the left.

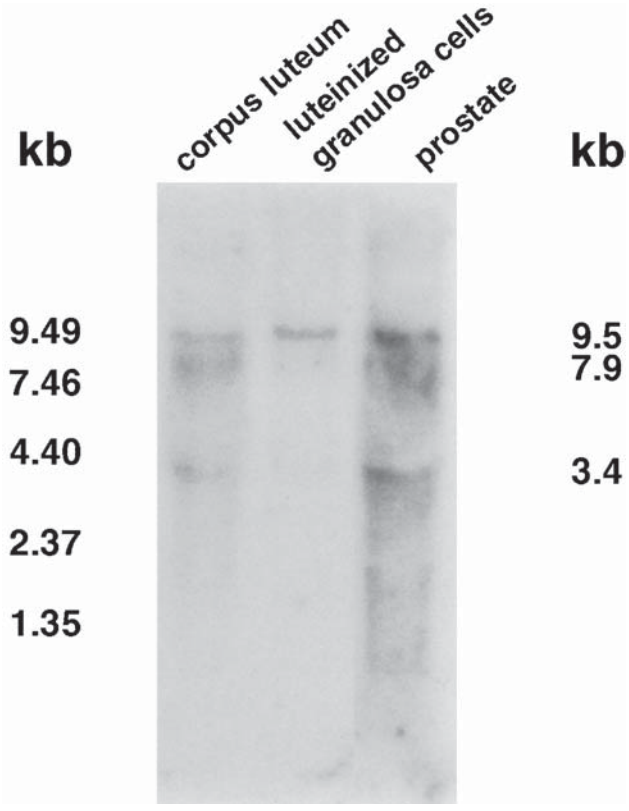

Fig. 2. Northern blot analysis of AR mRNA. Total RNA (50 μg) from monkey corpus luteum (luteal d 10), luteinized granulosa cells, and prostate were probed for AR mRNA as described in Materials and Methods. Distribution of size standards are shown at the left, and predicted sizes of AR mRNA species are shown at the right.

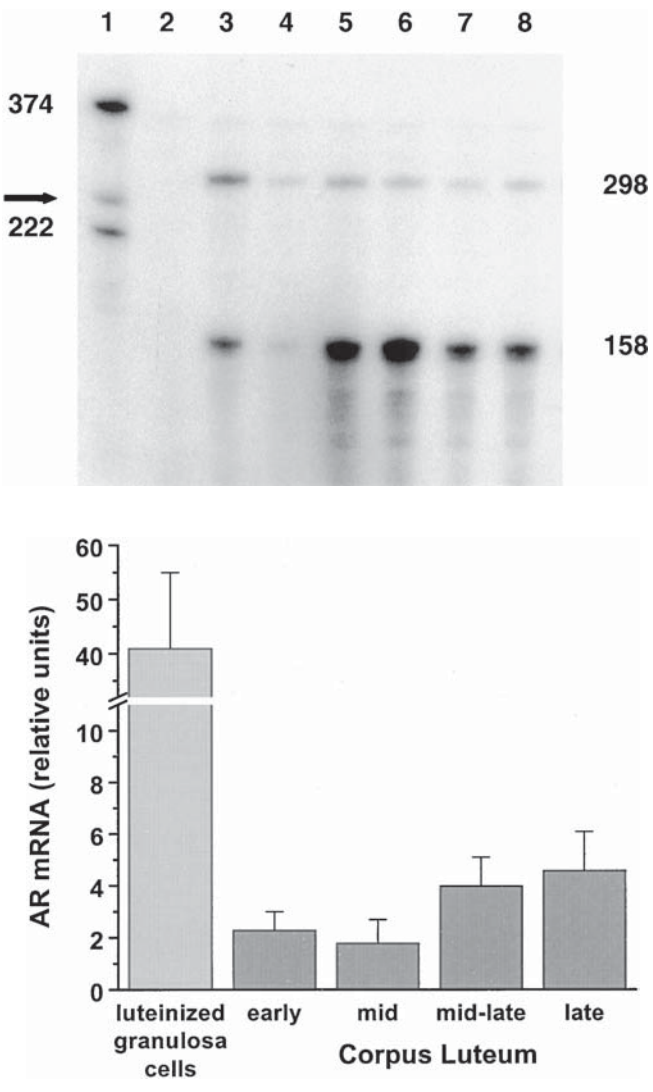

Fig. 3. RNase protection analysis of AR mRNA expression in luteinized granulosa cells and the corpus luteum throughout the luteal phase of the menstrual cycle. **(Top)** Lane 1, full-length AR mRNA (374 bases) and cyclophilin mRNA (222 bases) riboprobes; lane 2, tRNA alone; lane 3, ovarian RNA showing AR mRNA– (298 bases) and cyclophilin mRNA– (158 bases) protected fragments; lane 4, luteinized granulosa cell RNA; lane 5, early luteal RNA; lane 6, midluteal RNA; lane 7, mid-late luteal RNA; lane 8, late luteal RNA. Ten micrograms of monkey RNA were used in each of the samples shown in lanes 3–8. The arrow indicates the position of a product of AR mRNA riboprobe production that did not result in a protected fragment (data not shown). **(Bottom)** AR mRNA expression in luteinized granulosa cells ($n = 4$) and corpora lutea from early ($n = 4$), mid ($n = 5$), mid-late ($n = 4$), and late ($n = 4$) luteal phase. For each sample, the AR mRNA level was normalized to the cyclophilin mRNA level, and data are expressed as relative units. Data are expressed as mean ± SEM.

and 3.4 kb, consistent with data from the human *(19)*. AR mRNA was detected by RT-PCR in all macaque tissues and ovarian compartments examined, including the ovarian germinal epithelium, ovarian stroma, luteinized granulosa cells, and the corpus luteum. AR mRNA was detected in luteal total RNA at all stages, but levels did not change across the luteal phase. Detection of AR mRNA and androstenedione in periovulatory follicles and corpora lutea, together with earlier demonstration of the presence of AR protein *(11)* in these ovarian compartments, supports the concept that various tissues within the primate ovary are targets for local, receptor-mediated androgen actions during the menstrual cycle.

The detection of ovarian AR mRNA in the present study parallels previous reports of AR protein in the primate ovary. In the present study, AR mRNA was detected in the medullary and germinal epithelium–enriched cortical compartments of the macaque ovary, confirming previous reports of AR protein in macaque germinal epithelium and stroma *(11)* and human stroma *(13)* using immunocytochemistry. AR mRNA was also detected in periovulatory follicles and corpora lutea. This is consistent with reports of strong immunocytochemical staining for AR in granulosa cells of periovulatory follicles and in large (presumably granulosa derived) luteal cells of young corpora lutea in human ovaries *(12,13)*. Immunostaining for AR was detected in luteal tissues of the rhesus monkey *(11)*, human *(12,13)*, marmoset *(16)*, and rat *(21)*, with most groups

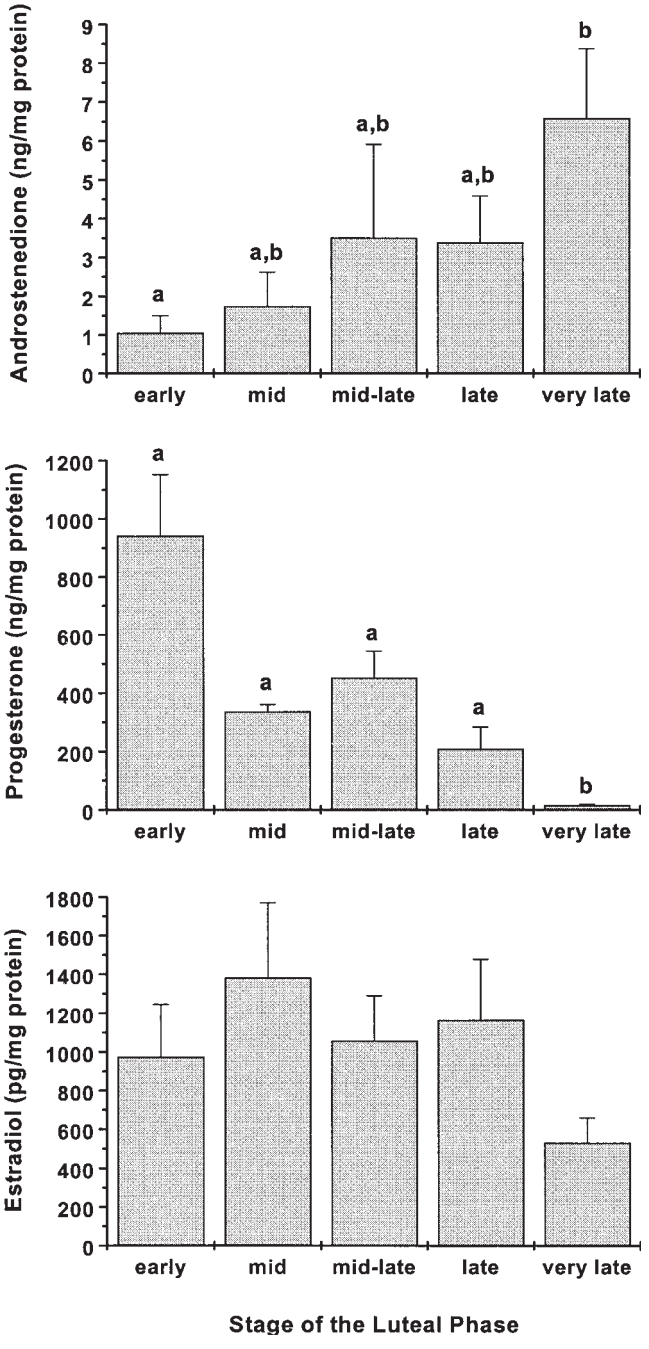

Fig. 4. Steroid levels in the corpus luteum during the menstrual cycle. Homogenates of luteal tissues collected at early ($n = 6$), mid ($n = 5$), mid-late ($n = 8$), late ($n = 6$), and very late ($n = 6$) luteal phase were assayed for **(top)** androstenedione, **(middle)** progesterone, and **(bottom)** estradiol. All steroid levels were normalized to homogenate protein concentrations. Testosterone levels were typically below the level of detection and did not change over the luteal phase (see text for details). Within each panel, groups with different superscripts are different; $p < 0.05$. Data are expressed as mean ± SEM.

reporting consistent staining across the luteal phase *(11,13,16)* in primate species. These data are consistent with the current findings of unchanging AR mRNA levels in monkey corpora lutea at all stages of the luteal phase and support the presence of AR expression in all ovarian compartments, including the periovulatory follicle and corpus luteum of the menstrual cycle in primates.

Androgens and perhaps other steroid hormones present in the follicle and corpus luteum may provide ligands for ovarian ARs. Androstenedione was present in the follicular fluid of the periovulatory follicle throughout the periovulatory interval, with concentrations of approx 87 n*M* before the ovulatory gonadotropin surge, and peak levels achieved 12 h after the administration of an ovulatory dose of human chorionic gonadotropin (hCG) *(4)*. Androstenedione was also measured in the corpus luteum throughout the luteal phase of the menstrual cycle (present study), with peak levels achieved at menses. Testosterone and its more potent metabolite dihydrotestosterone (DHT) were present in monkey follicular fluid *(6)*, but testosterone was not consistently detected in luteal tissues in the present study. Nonetheless, one cannot rule out that testosterone and DHT may be present in high concentrations in some androgen-producing cells to bind to and activate AR in luteal tissue. DHT is the most potent androgen in most androgen bioassays, followed by testosterone and androstenedione *(22)*; however, the very high concentrations of androstenedione in the follicle and corpus luteum may serve both as a ligand for androgen-stimulated functions and as a precursor for synthesis of testosterone and DHT in primate ovarian tissues. High levels of progesterone were also measured in rhesus macaque follicular fluid *(4)* and luteal tissues (present study), consistent with previous studies *(23,24)*. Although having 100-fold less affinity than testosterone for binding to AR *(22)*, high concentrations of progesterone are present in ovarian tissues and may successfully compete with androgens for binding to AR. Progesterone and the synthetic progestin medroxyprogesterone acetate can stimulate AR-dependent transcription *(25,26)*, suggesting that progestins in the ovary may block androgen action by binding to AR or, conversely, act as ligands to enhance AR transcriptional activity. Reports also suggest that estradiol can bind to AR *(22)*, although with low affinity, and induce AR transcriptional activity *(27)*. Therefore, it is possible that androstenedione, as well as other androgens, progestins, and estrogens, may regulate AR-mediated transcription in primate ovarian tissues.

Androgens may mediate intraovarian events related to folliculogenesis, oocyte maturation, and luteal function. Androgens differentially affect follicular development, oocyte maturation, and fertilization in rodents *(1)* and primates *(28–30)*, and chronic hyperandrogenism is associated with arrest of antral follicular development in women with polycystic ovarian syndrome *(31)*. However, the regulation of the primate corpus luteum by locally produced androgens is not well understood. Although few studies have examined the direct effects of androgens on the primate corpus luteum, the majority suggest that luteal progesterone synthesis is inhibited by androgens. Although

testosterone or DHT treatment in vitro of luteinized granulosa cells collected from a single woman was without effect *(32)*, exposure of human luteinized granulosa cells to testosterone during long-term culture in vitro antagonized hCG-stimulated progesterone and luteinizing hormone (LH) receptor production *(33)*. Similarly, hCG-induced progesterone production by human corpora lutea in vitro *(34)* and baboon corpora lutea in vivo *(35)* was inhibited by testosterone. In addition, androgens reportedly reduced luteal progesterone synthesis when administered to rats *(36)* and primates *(35)*, and luteal dysfunction has been associated with androgen excess in women *(34,37,38)*.

Interestingly, we noted a progressive rise in the intraluteal androgen:progesterone ratio over the course of the luteal phase, with increased androgen concentration correlating with luteolysis. In the pregnant rat, DHT induced luteolysis, resulting in termination of pregnancy *(39)*, supporting a role for androgens in the cessation of luteal function. While elevated androgens within the macaque corpus luteum during very late luteal phase may be a consequence of luteolysis, the hypothesis that androgens regulate luteal progesterone production and/or luteolytic events deserves further consideration.

In conclusion, we have demonstrated the presence of AR mRNA in various compartments of the macaque ovary, including luteinized granulosa cells and the corpus luteum. The presence of androstenedione in follicular fluid of the periovulatory follicle *(4,6)* and the corpus luteum (present study) supports the hypothesis that locally produced androgens modulate ovarian functions such as follicular growth and development, oocyte maturation, and luteal function through the classical receptor-mediated pathway.

Materials and Methods

Animals

The general care and housing of rhesus monkeys (*Macaca mulatta*) at the Oregon Regional Primate Research Center (ORPRC) were described previously *(40)*. Animal protocols and experiments were approved by the ORPRC Animal Care and Use Committee, and studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Adult females with regular menstrual cycles were checked daily for menses, and blood samples were obtained daily from unanesthetized monkeys by saphenous venipuncture from d 8 following the onset of menses. Serum was stored at –20°C. Day 1 of the luteal phase was defined as the first day of low serum estradiol (<100 pg/mL) following the midcycle estradiol surge *(41)*. Serum estradiol concentrations were measured by radioimmunoassay (RIA) by the Endocrine Services Laboratory, ORPRC *(42)*; intra- and interassay coefficients of variation (CVs) did not exceed 15%.

Macaque Tissues

Ovaries, corpora lutea, and other macaque tissues as well as luteinized granulosa cells were removed from anesthetized monkeys during aseptic surgery. Whole ovaries and nonovarian tissues were obtained at autopsy from animals with health problems not related to the tissues obtained or from animals undergoing organ removal as a part of unrelated experimental protocols. An ovarian stimulation model developed for the collection of multiple mature oocytes for in vitro fertilization *(43)* was used to obtain luteinized granulosa cells from periovulatory follicles. Beginning at menses, rhesus monkeys received 60 IU of human follicle-stimulating hormone (hFSH) (Metrodin, Ares, Randolph, MA; d 1–6), followed by 60 IU of hFSH plus 60 IU of hLH (Pergonal, Ares; d 7–9) to stimulate the growth of multiple follicles. Animals received 1000 IU hCG (Profasi, Ares) on d 10, and follicles were aspirated 27 h after the hCG injection. After oocyte removal, granulosa cells were enriched from follicular aspirates on a 40% Percoll (Sigma, St. Louis, MO) gradient *(44)*. To obtain corpora lutea at specific stages of the luteal phase, tissues were surgically removed from monkeys in the early (post-LH surge d 3–5), mid (d 6–8), mid-late (d 10–12), late (d 13–15), and very late (d 16–17) luteal phase of spontaneous menstrual cycles.

Preparation of RNA

Total RNA was prepared immediately after tissue collection, or tissues and cells were frozen in liquid N_2 and stored at –80°C until preparation of RNA. RNA was isolated from fresh or frozen tissues using the cesium chloride ultracentrifugation method of Chirgwin and colleagues *(45)*, and RNA was prepared from luteinized granulosa cells using the acid phenol extraction method of Chomczynski and Sacchi *(46)*. RNA was stored at –20°C pending analysis.

Reverse Transcriptase Polymerase Chain Reaction

Total RNA samples (5 μg each) from macaque tissues and various compartments of the macaque ovary were reverse transcribed with 200 U of Moloney mouse leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, MD) in 5X RT buffer, 10 m*M* of each dNTP, 0.1 *M* dithiothreitol and 40 U of RNasin ribonuclease inhibitor (Promega, Madison, WI) using 2.5 μ*M* oligo $d(T)_{12–18}$ (Clontech, Palo Alto, CA) as the primer at 37°C for 2 h in a PCT Programmable Thermal Controller (MJ Research, Watertown, MA). The RT sample was either used immediately or stored at 4°C until use.

Amplification of AR cDNA employed oligonucleotide primers that bracketed a unique 329-bp segment of the AR hormone-binding domain corresponding to nucleotides 2899–2920 (sense 5'-TCTCAAGAGTTTGGATGGC) and 3205–3227 (antisense 5'-CTTTACTACCGTCTCTAGTAGAG) of the human AR cDNA (Clontech Laboratories) *(47)*. Sense and antisense primers to human β-actin mRNA (Clontech) were used in separate PCR reactions to

amplify an RT-PCR product of 838 bp. RT reaction products were combined with final concentrations of 1.5 m*M* $MgCl_2$; 2.5 U of *Taq* DNA polymerase (Promega); 10 m*M* each of dATP, dCTP, dGTP, and dTTP, and 1 μ*M* each of the 5' sense and 3' antisense primers in the supplied buffer to yield a final volume of 100 μL. The PCR cycles were carried out in the thermal controller at 92°C for 2 min, 55°C for 2 min, 72°C for 3 min, 92°C for 40 s, 55°C for 2 min, and 72°C for 3 min for a total of 35 cycles, and RT-PCR products were stored at 4°C. Water was included in some RT reactions instead of RNA, and this RT product was used for PCR as a negative control. RNA not treated with RT was also used as a negative control (data not shown). The RT-PCR products were separated on a 3:1 NuSieve (FMC, Rockland, ME): agarose gel and stained with ethidium bromide.

AR mRNA Sequence Verification

For restriction digest, RT-PCR products were incubated at 55 C for 4 h with 30 U of *Bst*XI (New England BioLabs, Beverly, MA) in buffer supplied by the manufacturer. Samples were electrophoresed as previously described. The RT-PCR product from macaque whole ovary was ligated into pCR II vector (TA Cloning System, Invitrogen, Carlsbad, CA) and transformed into competent *Escherichia coli*. The cDNA insert from one positive colony was sequenced using the dideoxy-chain termination reaction method (Sequenase Version 2.0 kit, United States Biochemical, Cleveland, OH).

Northern Analysis

Northern analysis was performed as previously described *(20)*. Total RNA prepared from monkey corpus luteum, luteinized granulosa cells, prostate, and spleen (50 μg each) as well as RNA size markers (Promega) were separated on a 1.2% agarose gel and transferred to Nytran membrane (Schleicher & Schuell, Keene, NH) by capillary action with 10X SSC (1.5 *M* sodium chloride and 0.15 *M* sodium citrate, pH 7.0). Blots were baked at 80°C for 2 h and stored at 4°C until use. For detection of AR mRNA, prehybridization and hybridization steps were performed in 50% formamide solution at 45°C, with 5×10^6 cpm/mL of AR cDNA ^{32}P radiolabeled probe prepared by random priming using a purchased kit (Boehringer Mannheim, Indianapolis, IN) added during hybridization. High stringency washes were performed using 0.5% SSC-0.1% sodium dodecyl sulfate at 45°C, and the blot was exposed to film (Kodak X-OMAT, Eastman Kodak, Rochester, NY) at –80°C.

AR mRNA RNase Protection Assay

A highly sensitive RNase protection assay *(18)* was used for detection of monkey AR mRNA. The AR cDNA was subcloned into a pGEM transcription vector (Promega), and ^{32}P radiolabeled riboprobes antisense to macaque AR and cyclophilin (internal control) mRNAs were prepared using a purchased kit (Promega). The AR mRNA riboprobe was 374 bases in length and yielded a protected fragment of 298 bases, whereas the cyclophilin mRNA riboprobe was 222 bases and yielded a protected fragment of 158 bases. Riboprobes antisense to AR mRNA (2×10^5 cpm/sample) and cyclophilin mRNA (1×10^5 cpm/sample) were hybridized at 50°C with total RNA from luteinized granulosa cells or luteal tissue (10 μg/sample; $n = 4$–5/group) and 15 μg of tRNA. A sample containing 25 μg of tRNA alone was used as a negative control. Ovarian RNA (10 μg) was included in every assay as a positive control and to allow comparison among assays. After exposure to RNase A (40 μg/mL; Boehringer Mannheim) and RNase T1 (2 μg/mL; Sigma) followed by proteinase K (140 μg/mL; Boehringer Mannheim), partially digested riboprobe fragments were separated by urea-polyacrylamide gel electrophoresis. Gels were dried and exposed to film for 4–15 h at –80°C. The relationship between the amount of ovarian total RNA (2–20 μg) and the intensity of the protected fragments was linear for both AR mRNA and cyclophilin mRNA over this range (data not shown).

Luteal Steroid Analysis

Portions of corpora lutea (2.2–9.8 mg wet wt; $n = 5$–8/stage) were frozen in liquid nitrogen and stored at –80°C. Tissues were then homogenized with 20 strokes of a glass:glass homogenizer in 1.0 mL of phosphate-buffered saline. A portion of each homogenate was assessed for protein concentration by the method of Bradford (Bio-Rad, Hercules, CA). For steroid analysis, homogenates were subjected to column chromatography before RIA for androstenedione *(48)*, testosterone *(48)*, progesterone *(49)*, and estradiol *(42)* concentrations. All steroids levels were normalized to protein concentrations of homogenates. The intra- and interassay CVs of variation for steroid RIAs were <15% for all assays.

Data Analysis

Autoradiograms of RNase protection assays were scanned using Photoshop 4.0 (Adobe Systems, Mountain View, CA), and densitometric analysis of bands representing AR mRNA and cyclophilin mRNA was performed using Image 1.40 (NIH, Research Services Branch, NIMH, Bethesda, MD), which compared band size and intensity to a standard curve and calculated the optical density (OD) of each band. The OD of the AR mRNA band was divided by the OD of the cyclophilin mRNA band, and the resulting ratio was expressed in arbitrary units. AR mRNA levels were compared using one-way analysis of variance (ANOVA). Linear regressions were performed using Origin (MicroCal Software, Northampton, MA). Data are expressed as mean ± SEM, and significance was assumed at $p < 0.05$.

Luteal levels of androstenedione and estradiol were assessed for heterogeneity of variance using Bartlett's test

and log transformed when necessary, followed by one-way ANOVA and Newman-Keuls test when indicated. Because no appropriate transformation could be found to eliminate heterogeneity of variance for the progesterone data, progesterone levels were assessed using the Kruswall-Wallis statistic followed by the Mann-Whitney test.

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