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# Androgen receptor in the oviduct of the turtle, *Trachemys scripta*

Kyle W. Selcer[a,*], Stacy Smith[a], Jeffrey W. Clemens[a], Brent D. Palmer[b]

[a]*Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282, USA*
[b]*Department of Biology, University of Kentucky, Lexington, KY 40536, USA*

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**Abstract**

Circulating androgens reach high concentrations in females of some reptiles and amphibians. We are testing the hypothesis that androgens can act directly in female reptilian reproductive tissues, via the androgen receptor. In this study, we sought to determine if androgen receptors are present in the oviduct of the turtle, *Trachemys scripta*, using radioligand-binding assays and immunological assays. An androgen-binding site was detected in turtle oviductal cytosol and oviductal nuclear extract by radioligand binding assay, using $^{3}$H-dihydrotestosterone (DHT) as the ligand. This site was saturable ($B_{max}$=11 pmol/g tissue), had a high affinity ($10^{-10}$ M), and showed specificity typical of androgen receptors (DHT>testosterone, progesterone ⩾estradiol, cortisol). Western blotting using an anti-androgen receptor antibody revealed a band of immunoreactivity in oviductal cytosol at approximately 115 kDa, and a more prominent band at 50 kDa, possibly indicating a truncated form of the androgen receptor. Immunohistochemistry revealed crossreactivity of the androgen receptor antibody against oviductal glandular cells but not against oviductal luminal epithelial or muscularis cells. The presence of androgen receptor in the turtle oviduct suggests that androgens have a role in female reproduction and that their action can be mediated directly by androgen receptor.


*Keywords:* Androgen receptor; Female reproductive tract; Oviduct; Steroid hormone receptors; *Trachemys scripta*; Turtle

## 1. Introduction

Although androgens are typically associated with male reproduction, high concentrations of these sex steroid hormones are present in the blood of female nonmammalian vertebrates during certain periods of their seasonal reproductive cycles. Elevated blood androgen concentrations have been observed in females of a number of species of reptiles and amphibians, including the turtles *Sternotherus odoratus* (McPherson et al., 1982), *Chelonia mydas* (Licht et al., 1979), *Chrysemys picta* (Klicka and Mahmoud, 1977), *Caretta caretta* (Wibbels et al., 1990) as well the frogs *Rana pipiens* (Dubowsky and Smalley, 1993) and *Rana catesbieana* (Licht et al., 1983). In these species, androgen concentrations of females may substantially exceed estrogen concentrations and may approach androgen concentrations measured in males.

One possible explanation for high androgen concentrations in female nonmammalian vertebrates is that the androgens are serving as precursors for estrogen production. In this pathway, the effects of androgens would be mediated indirectly via the estrogen receptor after aromatization to estrogens. However, it has also been suggested that androgens may have direct effects mediated via the androgen receptor (Staub and DeBeer, 1997). The presence of androgen receptors in the oviduct would provide further evidence for a direct role of androgens. However, there are relatively few studies documenting androgen receptors in the oviducts of reptiles or amphibians.

Our long-term objective is to determine the role of androgens in reproduction of female nonmammalian vertebrates. We are using oviduct of the freshwater turtle, *Trachemys scripta* as a model system. The specific objective of the present study was to determine if androgen receptors are present in the oviduct of *T. scripta.* Presence of androgen receptors was evaluated by radioligand binding assays and by immunoassays (Western blots and immunohistochemistry).

* Corresponding author. Tel.: +1 412 396 5967; fax: +1 412 396 5907.
*E-mail address:* selcer@duq.edu (K.W. Selcer).

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## 2. Materials and methods

### 2.1. Animals

Adult female *T. scripta* were purchased from Lemberger Farms (Oshkosh, WI, USA) in May. Prior to collection of tissues, they were kept for several weeks in plastic swimming pools with heat lamps and rocks for basking and maintained under a 14:10 h light/dark cycle at 22 °C. Turtles were fed floating goldfish food and leaf lettuce ad libitum. Turtles were anesthetized by injection (i.m.) in the front legs with ketamine (80 mg/kg body weight). Turtles were killed by decapitation, after which oviductal tissues were harvested. The ovaries were removed and examined. All turtles contained several classes of yolked follicles in their ovaries, ranging from small (<1 mm) to large (>10 mm), which is typical of reproductively mature female *T. scripta*.

This study was approved and overseen by the Duquesne University Institutional Animal Care and Use Committee and all experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996).

### 2.2. Chemicals and reagents

[1, 2-$^3$H] Dihydrotestosterone (DHT) with a specific activity of 45.5 Ci/mmol was purchased from Dupont NEN (Boston, MA). Radioinert steroids (estrogen, progesterone, testosterone, cortisol, and dihydrotestosterone) were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). The buffers used for binding assays were as follows: TEMMG buffer (10 mM Tris, 1.5 mM EDTA, 1 mM monothioglycerol, 25 mM sodium molybdate, 10% (v/v) glycerol, pH 7.4), nuclear extraction buffer (TEMMG+0.5 M KCl), and washing buffer (10 mM Tris, 1.5 mM EDTA, 10% (v/v) glycerol, pH 7.4). Sephadex LH-20 was purchased from Pharmacia LKB Biotechnology (Piscataway, NJ, USA). Ecolume liquid scintillation cocktail was purchased from ICN Biomedicals (Costa Mesa, CA, USA).

For denaturing polyacrylamide gel electrophoresis (SDS-PAGE), the BioRad (Hercules, CA, USA) Mini Protean II electrophoresis system was used. The separating gels were 10% total acrylamide and the stacking gels were 4% total acrylamide. Sample buffer consisted of 62.5 mM Tris–HCl (pH 6.8), 20% glycerol, 2% SDS, and 5% β-mercaptoethanol (Fisher Scientific, Pittsburgh, PA, USA). For Western blots, Millipore Immobilon-P polyvinylidene difluoride (PVDF) membranes and Ponceau S stain were purchased from Sigma. Coomassie stain for SDS-PAGE consisted of 25% trichloroacetic acid, 10% methanol, and 0.1% R-250 Brilliant blue (Fisher). Destain for SDS-PAGE consisted of 8% acetic acid and 8% methanol. Western blotting buffers were the following: tank buffer (25 mM Tris, 192 mM glycine, 20% methanol), Tris–saline (50 mM Tris, 154 mM NaCl, pH 7.5), Tris–Tween (Tris–saline+0.05% Tween 20), and blocking reagent (5% nonfat dry milk in Tris–saline). The polyclonal (rabbit) androgen receptor antibody (PG-21) was purchased from Affinity Bioreagents (Neshanic Station, NJ, USA) and the secondary antibody (peroxidase-linked goat anti-rabbit IgG) was purchased from BioRad. Diaminobenzidine tablets were purchased from Sigma. Immun-Lite system was from BioRad. For immunocytochemistry, the antibody was AR N-20 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) was used. Fast DAB was the substrate (Sigma). Protease inhibitors (phenylmethyl–sulfonyl fluoride (PMSF), pepstatin, leupeptin, aprotinin, and bacitracin) were purchased from Sigma.

### 2.3. Tissue preparation

Oviductal tissues were stored at −80 °C prior to use. The upper half of the oviduct (proximate to the ovaries) was used for all experiments. Tissues were weighed, minced with scissors, diluted in TEMMG 1:10 (wt:vol), homogenized with a Tissue Tearor (Biospec Products; Racine, WI, USA) for 10 min, and then with a Kontes glass:glass homogenizer using 15 strokes of each pestle. The homogenate was centrifuged at 4 °C for 10 min at 1200×*g* to obtain a nuclear pellet. The supernatant was aspirated and then centrifuged for 1 h at 4 °C at 106,000×*g* in a Beckman XL-70 ultracentrifuge to obtain the cytosol fraction (supernatant). For the nuclear fraction, the nuclear pellet was resuspended in TEMMG, centrifuged for 10 min at 1200×*g*, the supernatant was aspirated, and the nuclear pellet was resuspended in TEMMG and centrifuged again at 1200×*g* for 10 min. The pellet was then resuspended in TEMMG+0.5 M KCl (1:5 original tissue wt/vol) and incubated for 1 h to extract the nuclear proteins, while being vortexed every 15 min. Centrifugation at 106,000×*g* was then performed for 1 h at 4 °C. The supernatant was used in binding assays and Western blots, and the pellet was discarded. For Western blot analysis, Centri/Por centrifuge concentrators (molecular mass cut-off 25,000 Da) were used to concentrate the proteins. Cytosol or nuclear extract (1 mL) was centrifuged in the concentrators for approximately 2 h at 2200×*g* to concentrate protein by a factor of 2.

### 2.4. Protein assays

The Coomassie protein assay (Bradford, 1976) was used for determination of soluble protein concentration in cytosol and nuclear extracts. Absorbances (620 nm) were determined using a microtiter plate reader (model 3550, BioRad). A BCA assay (Pierce, Rockford, IL, USA) was used for determination of protein concentration of turtle oviductal homogenates. Absorbances were read at 562 nm using a microtiter plate reader. Bovine serum albumin was used as the standard in both assays.