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Estrogen inhibits caudal progression but stimulates proliferation of developing müllerian ducts in a turtle with temperature-dependent sex determination

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ABSTRACT

Müllerian duct development appears to be similar in many vertebrate groups, and previous studies have shown that this development is estrogen sensitive. For example, embryonic exposure to diethylstilbestrol (DES) in humans and mice, and estrogen exposure in chickens, can have multiple, usually adverse, effects on müllerian duct differentiation and growth. The current study investigates 17β-estradiol's effects on müllerian duct development in a reptile, the turtle *Trachemys scripta*. In *T. scripta*, normal müllerian duct development proceeds cranially to caudally over developmental stages 15 to 21. To ascertain 17β-estradiol's effect on this process, four groups of eggs were incubated at a female-producing temperature. Each group was treated with 50 μg of 17β-estradiol or a vehicle control at one of four stages (15, 17, 19, 21). The degree of müllerian duct development was assessed by examining gross morphology and histology. Results showed that estradiol-17β blocked development of the müllerian duct if applied before differentiation began. If applied afterwards, 17β-estradiol caused hypertrophy in the differentiated portion, but prevented further differentiation of the müllerian duct in more caudal regions. Therefore, reptilian müllerian ducts in *T. scripta* are estrogen sensitive and estrogen's effects may be similar to those reported for birds.

1. Introduction

Many vertebrates share a common genetic system for embryonic patterning that includes the reproductive tract. A common event during this development is müllerian duct differentiation. During embryonic development, the müllerian ducts differentiate in both males and females, but then regress in males (van Tienhoven, 1983). In females, müllerian ducts develop into the upper vagina, cervix, oviducts, and uterus.

Previous studies have found that müllerian duct development is estrogen sensitive. For example, in humans exposed to diethylstilbestrol (DES), an estrogen agonist, *in utero*, there is an increase in adenocarcinoma in the vagina (Herbst et al., 1971) and a high incidence of genital tract abnormalities that adversely affect reproductive capacity (Mably et al., 1992; Walters et al., 1993; Kaldas and Hughes, 1989). In a similar trend, mice exposed to DES *in utero* also have abnormally shaped reproductive tracts (Block et al., 2000).

Müllerian duct development in chickens also shows estrogen sensitivity (Hutson et al., 1982, 1985; MacLaughlin et al., 1983; Stoll et al., 1987). For example, exogenous estrogen treatment has been shown to prevent müllerian duct regression in male and female embryos (Hamilton, 1961; Hutson et al., 1982, 1985; Stoll et al., 1987, 1990, 1993). Estrogen treatment also causes agenesia, blocking of müllerian duct differentiation, when estrogen is applied prior to or during the early stages of müllerian duct development (Stoll et al., 1987, 1993). Additionally, DES has been shown to stimulate müllerian duct hypertrophy in chicken embryos (Hutson et al., 1982).

Estrogen effects on müllerian ducts are also seen in reptiles. In the turtle, *Trachemys scripta*, müllerian ducts normally develop and regress in a fashion similar to that reported for birds (Wibbels et al., 1999). Studies examining steroid hormone effects on sex determination in that turtle noted müllerian duct deficiencies (Wibbels and Crews, 1992). Additionally, estrogen and norethindrone stimulated müllerian duct hypertrophy in alligator embryos (Austin, 1989, 1991; Lance and Bogart, 1991). The goal of the current study is to examine the effects of exogenous 17β-estradiol treatments on normal müllerian duct development in *T. scripta*, a turtle exhibiting temperature-dependent sex determination (Wibbels et al., 1991; Wibbels et al., 1999).

2. Materials and methods

Freshly laid *T. scripta* eggs were obtained commercially (Kliebert/Clark Turtle and Alligator Farm, Hammond, LA, USA). Because this study was designed to examine the effects of 17β-estradiol on normal müllerian duct differentiation in females, all eggs were incubated at (31 °C), a temperature known to be 100% female-producing (Bull et al., 1982; Crews et al., 1991; Wibbels et al., 1991). After approximately one week, all eggs were candled to verify that they were at similar stages

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of development. From that point forward, embryonic development was monitored by dissecting two eggs approximately every two days. Specific developmental stages were determined based on criteria established by Greenbaum (2002).

Several dosages of 17β-estradiol were evaluated in previous studies, and 50 μg of 17β-estradiol had the greatest effect on the oviducts (Crews et al., 1989; Wibbels and Crews, 1992). Therefore, eggs in the experimental groups were treated with 50 μg 17β-estradiol dissolved in 5 μL of ethanol at various stages during embryonic development. Control eggs received 5 μL of ethanol. All treatments were placed on the surface of the eggshell. Previous studies indicate that this is an effective method for delivering steroid hormones to developing *T. scripta* eggs (Crews et al., 1991; Wibbels and Crews, 1992).

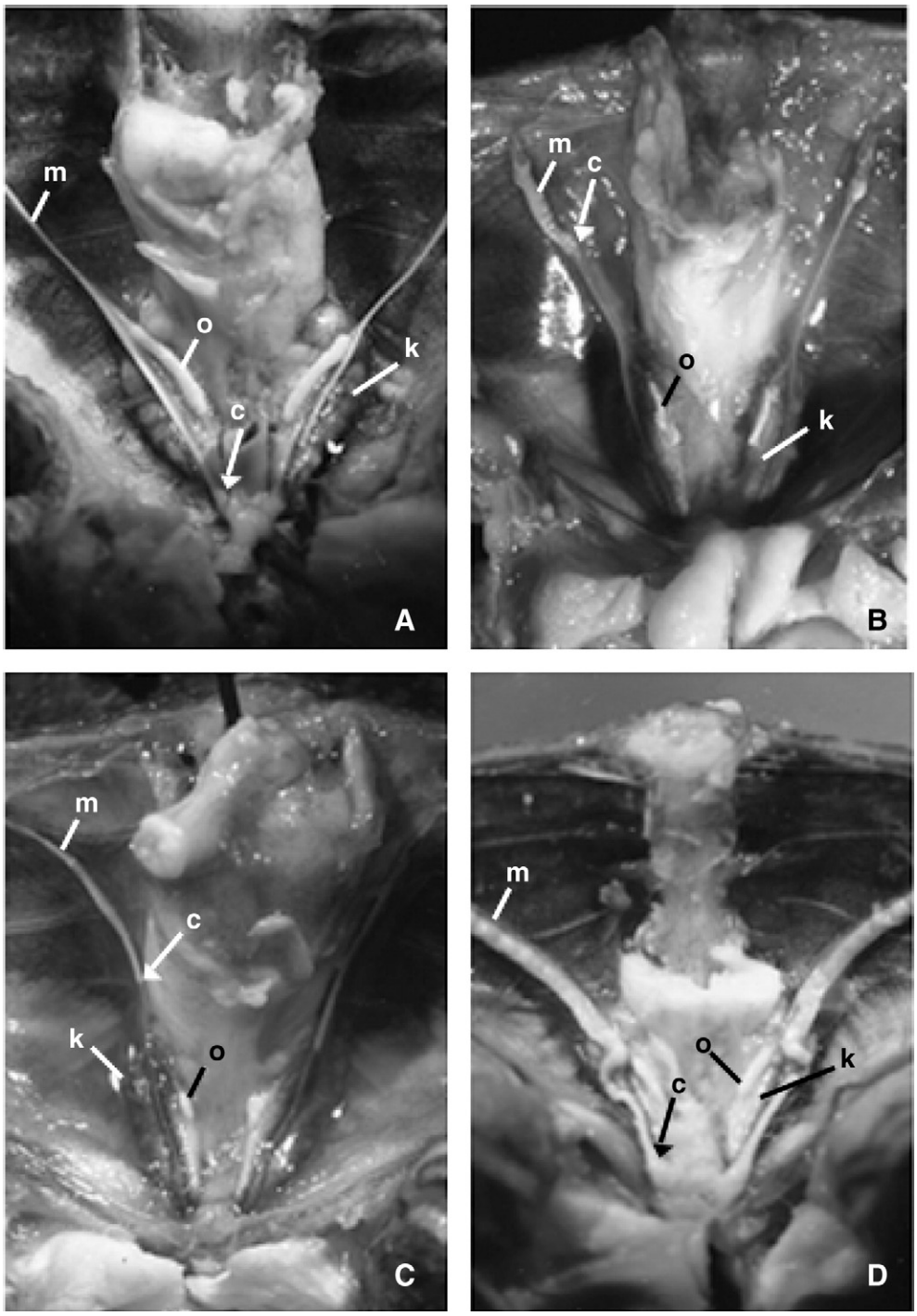

Fig. 1. External morphology of müllerian ducts, gonads, and kidneys of hatchlings from control eggs and from eggs treated with 50 μg of 17β-estradiol in 5 μL of ethanol. (A) Control hatchling that was treated with 5 μL of ethanol. (B) Hatchling from an egg treated with 17β-estradiol at stage 15 showing hypertrophy of the extreme cranial region of the müllerian ducts. A mesentery extends caudally from each hypertrophic region, but no müllerian duct is present caudally. (C) Hatchling from an egg treated with 17β-estradiol at stage 17, showing hypertrophic cranial region of müllerian duct extending to the anterior portion of the mesonephros, but the caudal region of the müllerian ducts are absent. D) Hatchling from an egg treated with 17β-estradiol at stage 21 showing full-length müllerian ducts that are hypertrophied. Embryonic staging criteria based on Yntema, 1968. m = müllerian ducts, o = ovary, k = kidney, c = caudal end of oviduct.