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Immunolocalization of estrogen receptor alpha, estrogen receptor beta and androgen receptor in the pre-, peri- and post-pubertal stallion testis

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ABSTRACT

In various species, androgens and estrogens regulate the function of testicular Leydig, Sertoli, peritubular myoid, and germ cells by binding to their respective receptors and eliciting a cellular response. Androgen receptor (AR) is expressed in Sertoli cells, peritubular myoid cells, Leydig cells and perivascular smooth muscle cells in the testis depending on the species, but its presence in germ cells remains controversial. Two different estrogen receptors have been identified, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ), and their localization and function in testicular cells varies depending on the species, developmental stage of the cell and type of receptor. The localization of AR in an immature and mature stallion has been reported but estrogen receptors have only been reported for the mature stallion. In the present study, the localizations of AR and ERα/ERβ were investigated in pre-pubertal, peri-pubertal and post-pubertal stallions. Testes were collected by routine castration from 21 horses, of light horse breeds (3 months–27 years). Animals were divided into the following age groups: pre-pubertal (3–11 months; $n = 7$), peri-pubertal (12–23 months; $n = 7$) and post-pubertal (2–27 years; $n = 7$). Testicular tissue samples were fixed and embedded, and the presence of AR, ERα and ERβ was investigated by immunohistochemistry (IHC) using procedures previously validated for the horse. Primary antibodies used were rabbit anti-human AR, mouse anti-human ERβ and rabbit anti-mouse ERα. Sections of each region were incubated with normal rabbit serum (NRS; AR and ERα) or mouse IgG (ERβ) instead of primary antibody to generate negative controls. Androgen receptors were localized in Leydig, Sertoli and peritubular myoid cells of all ages. Estrogen receptor alpha was localized in Leydig and germ cells of all ages but only in pre- and peri-pubertal Sertoli cells and post-pubertal peritubular myoid cells. Estrogen receptor beta was localized in Leydig and Sertoli cells of all ages but in only pre-pubertal germ cells and absent in peritubular myoid cells of all ages. Taken together, the data suggest that estrogen regulates steroidogenesis by acting through ERα and ERβ in the Leydig cells and promotes gametogenesis by acting through ERβ in the Sertoli cells and ERα in the germ cells. In contrast androgen receptors are not found in germ cells throughout development and thus are likely to support spermatogenesis by way of a paracrine/autocrine pathway via its receptors in Leydig, Sertoli and peritubular myoid cells.

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1. Introduction

In other species androgens and estrogens regulate the function of testicular cells by binding to their respective receptors and eliciting a cellular response (Dohle et al., 2003; Holdcraft and Braun, 2004; Sierens et al., 2005; Bilinska et al., 2006). Testosterone appears to be responsible for maintaining adequate blood-testis barrier function (Meng et al., 2005), inducing meiosis and postmeiotic development of germ cells (Dohle et al., 2003; Holdcraft and Braun, 2004) and inhibition of germ cell apoptosis (Singh et al., 1995). There also appears to be an androgen action on Sertoli cells to modulate gene expression, proliferation and differentiation (Dohle et al., 2003; Holdcraft and Braun, 2004). Androgens regulate steroidogenesis by regulating steroidogenic enzymes in Leydig cells (Hales et al., 1987; Shan et al., 1995).

Many of the biological actions of androgens are mediated by the androgen receptor (Lubahn et al., 1988; Chang et al., 1988a,b). Androgen and the AR play important roles in male spermatogenesis and fertility by way of a paracrine/autocrine system (Wang et al., 2009). There is general agreement that AR is expressed in Sertoli cells, peritubular myoid cells, Leydig cells and perivascular smooth muscle cells in the testis (Anthony et al., 1989; Sar et al., 1990; Kimura et al., 1993; Bremner et al., 1994; Vornberger et al., 1994; Zhou et al., 1996). The presence of AR in germ cells remains controversial. Several reports indicated that AR are present in germ cells (Warikoo et al., 1986; Kimura et al., 1993; Janssen et al., 1994; Vornberger et al., 1994; Zhou et al., 1996; Arenas et al., 2001; Solakidi et al., 2005; Aquila et al., 2007; Merlet et al., 2007), but other studies indicate that there is no staining in the germ cells (Galena et al., 1974; Grootegoed et al., 1977; Anthony et al., 1989; Bremner et al., 1994; Van Roijen et al., 1995; Suarez-Quian et al., 1999; Pelletier et al., 2000).

Increasing evidence over the last decade has established the importance of estrogen in regulating testicular function (Abney, 1999; O'Donnell et al., 2001; Hess and Carnes, 2004). Estrogen has been implicated in spermatogonial stem cell division (Miura et al., 1999), initiation and maintenance of spermatogenesis (Ebling et al., 2000), and as a germ cell survival factor (Pentikainen et al., 2000).

Two different estrogen receptors have been identified; estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). It appears that both ER subtypes have similar affinities for estradiol (Kuiper et al., 1997). The localization of estrogen receptors in testicular cells varies depending on the species, developmental stage of the cell and type of receptor (Abney, 1999; O'Donnell et al., 2001; Hess and Carnes, 2004). In the boar, ERα mRNA and ERα immunoreactivity have been localized in spermatogonia and primary spermatocytes, whereas ERβ mRNA and ERβ immunoreactivity were localized in Sertoli cells (Rago et al., 2004; Lekhkota et al., 2006). However, in other species such as the rat, human, non-human primates, dog, cat and marmoset, ERα and ERβ immunoreactivity was found not only in spermatogonia, spermatocytes and Sertoli cells, but also in spermatids, Leydig cells and peritubular myoid cells and these locations appeared to change with age in some reports (van Pelt et al., 1999; Pelletier and El-Alfy, 2000; Pelletier et al., 2000; McKinnell et al., 2001; Saunders et al., 2001; Nie et al., 2002; Zhou et al., 2002).

The roles of androgen and estrogen in the stallion testis remain largely unknown. Previous results from our laboratory demonstrated that the intratesticular concentration of testosterone was relatively unchanged throughout development whereas, intratesticular estradiol concentrations increased with age (Stewart and Roser, 1998; Parlevliet et al., 2006) suggesting estrogen may be involved in testicular development and mature testicular and epididymal function. Active immunization of colts against estrogens resulted in increased testis size and sperm production after puberty (Thompson and Honey, 1984) but the mechanism by which this occurs is unclear. The cellular localization of androgen receptors in the immature and mature stallion has been demonstrated, but the estrogen receptors in the stallion testis have only been reported in the mature animal (Bilinska et al., 2004, 2005, 2006; Hejmej et al., 2005). In the present study, the cellular localization of androgen and estrogen receptors was investigated in pre-pubertal, peripubertal and post-pubertal stallions. Age related changes in the localization of the steroid receptors in the testis, would suggest a dynamic autocrine/paracrine role of these steroids in regulating testicular function during reproductive maturation.

2. Materials and methods

Testes were collected by routine castration from a total of 21 horses, of light horse breeds, ranging in age from 3 months to 27 years. Animals were divided into the following age groups: pre-pubertal (3–11 months; $n = 7$), peri-pubertal (12–23 months; $n = 7$) and post-pubertal (2–27 years; $n = 7$). The sources of these horses were the Animal Science Horse Barn, the Center for Equine Health, the UC Davis Veterinary Medical Teaching Hospital (VMTH) and various private owners. Animal Use Protocols for routine castration at the various sites were approved by the Animal Use Committee at UC Davis. Testicular tissue samples were prepared as described previously (Hess and Roser, 2004). Portions of the testis from each animal were fixed in 4% paraformaldehyde overnight followed by 24 h in PBS and then serially dehydrated in ethanol.

The presence of ERα, ERβ and AR in the testes was investigated by immunohistochemistry (IHC) using antibodies and procedures previously validated for the horse (Parlevliet et al., 2006). Using Western blots we previously demonstrated that these antibodies bound to proteins of appropriate size from stallion epididymal tissue (ERα – 65 kDa; ERβ – 60 kDa; AR – 109 kDa) confirming they were specific for ERα, ERβ and AR (Parlevliet et al., 2006). Additionally, these antibodies have been used successfully by other research groups to immunolocalize ERα, ERβ and AR in the reproductive tract, including the testis, of other species (Saunders et al., 2000, 2002; Omoto et al., 2005; Tan et al., 2005; Li et al., 2006; Kipp et al., 2007; Boukari et al., 2009).

Tissue was paraffin embedded and sectioned at a thickness of 5 μm. Antigen retrieval for AR and ERβ was performed by placing slides in Coplin jars in a steamer and heating to 93 °C. Slides were kept at 93 °C