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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

Table 1
Summary of receptor localization in the stallion testis presented as intensity of immunostaining[a].

Cell type	AR			ERα			ERβ		
	Pre	Peri	Post	Pre	Peri	Post	Pre	Peri	Post
Leydig	++	+++	+++	++	++	+++	+	++	++
Sertoli	++	+++	+++	+	++	–	++	+++	+++
Myoid	+++	+++	+++	–	–	+	–	–	-
Germ	–	–	–	++	++	++	++	–	-

[a] Intensity of immunostaining scored as negative (–), weak positive (+), moderate positive (++) or strong positive (+++) by two independent observers.

for 5 min and then allowed to cool to room temperature. Immunohistochemistry for ERα was performed on sections without antigen retrieval. Tissues were incubated overnight at 4 °C with rabbit anti-human AR (sc-816; 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) or mouse anti-human ERβ (MCA1974S; 1:40; Serotec, Raleigh, NC) or for 2 h at room temperature with rabbit anti-mouse ERα (sc-542; 1:250; Santa Cruz Biotechnology). Following primary antibody incubation, sections were incubated with species-appropriate biotinylated secondary antibody followed by an avidin–biotin horseradish peroxidase complex (ABC reagent). Immunostaining was visualized using 3-amino-9-ethylcarbazol (AEC; AR and ERα) or 3,3′-diamnobenzidine (DAB; ERβ) chromagen followed by counterstaining with Immunomaster Hematoxylin (American Mastertech, Lodi, CA) and evaluated by light microscopy using an Olympus BX6C microscope (Olympus America Inc., Center Valley, PA) equipped with a Photometrics Coolsnap Camera (Photometrics, Tuscon, AZ). 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. Intensity of immunostaining was scored as negative (–), weak positive (+), moderately positive (++) or strong positive (+++) as scored by two independent observers without knowledge of the type of receptor and age of animal.

3. Results

Androgen receptor (AR; Fig. 1 and Table 1) was expressed in the nucleus of Sertoli cells, Leydig cells and peritubular myoid cells of all age groups. Germ cells were negative for AR in all age groups.

Estrogen receptor alpha (ERα; Fig. 2 and Table 1) was expressed in the nucleus of Sertoli cells, germ cells and some interstitial/Leydig cells of pre- and peri-pubertal animals. Peritubular myoid cells were negative in these age groups. In post-pubertal animals, ERα was expressed in most Leydig cells and some germ cells. In contrast to the pre- and peri-pubertal animals, post-pubertal Sertoli cells were negative for ERα and peritubular myoid cells were positive for ERα.

Estrogen receptor beta (ERβ; Fig. 3 and Table 1) was expressed in the Leydig cell cytoplasm and in the Sertoli cell nuclei and cytoplasm of all age groups. Germ cells were positive for ERβ in pre-pubertal animals but were negative in peri- and post-pubertal animals. Peritubular myoid cells were negative for ERβ in all age groups.

Although there was quite a large age range in the post-pubertal group, there were no differences observed in the cellular localization of the AR, ERα and ERβ. Vacuoles observed in the lumen area of the peri-pubertal group were most likely due to the evolving formation of the lumen during puberty. Lipofuscin granules were present in the pre-pubertal and peri-pubertal ERα and AR negative control tissue. Lipofuscins are finely granular yellow-brown (reddish) pigment granules composed of lipid-containing residues of lysosomal digestion (oxidation of unsaturated fatty acids) found in developing tissue of young animals.

4. Discussion

The aromatase enzyme converts androgens to estrogens in the equine Leydig and Sertoli cells in an age dependent manner with more estrogens being produced from the Leydig cell as the stallion matures (Sipahutar et al., 2003; Hess and Roser, 2004). These steroid hormones have many endocrine actions in the stallion including regulation of the reproductive tract, feedback signaling to the hypothalamus and pituitary and effects on behavior (Roser, 2008; Janett et al., 2009). However, much less is understood regarding the potential paracrine and autocrine actions of these steroids within the equine testis. Paracrine action is when a hormone or factor acts on cells neighboring those from which it is produced while autocrine action is a hormone or factor acting on the cell that produces it (Roser, 2008). For testicular androgens and estrogens to act in a paracrine or autocrine manner their receptors would need to be present within the testis. Here we describe the cellular localization of AR, ERα and ERβ in the stallion testes throughout its life time thereby indicating that androgens and estrogens can act in a paracrine/autocrine manner within the developing and mature stallion testis.

In the present study, AR were expressed in the pre-pubertal, peri-pubertal and post-pubertal Leydig, Sertoli and peritubular myoid cells. This was not entirely consistent with the findings by (Bilinska et al., 2004). These authors reported that AR were localized in Leydig cells in immature stallions and Leydig cells, Sertoli cells and peritubular myoid cells in the mature stallion. Both studies do agree that AR were not present in germ cells. The adult findings are consistent with the adult rat and human (Sar et al., 1990) and the adult mouse (Zhou et al., 1996). In other species, localization of AR in germ cells has been controversial (Wang et al., 2009). An age dependent change in the location of AR in the equine testes was not observed suggesting that androgens in the stallion testis are important for initiation and maintenance of spermatogenesis and steroidogenesis via the Leydig, Sertoli and peritubular myoid cells but not the germ cells. An age dependent change in localization of AR in the pre-pubertal and pubertal pig was also observed but the changes were different than in the stallion (Kotula et al., 2000). In the pig, AR were only detected in Leydig cells in the pre-pubertal cells whereas in the mature pig, AR were located in Leydig, Sertoli and peritubular myoid cells; AR were not detected in germ cells of the pig (Kotula et al., 2000). These studies are consistent with the concept that androgens act to initiate and maintain spermatogenesis by way of a

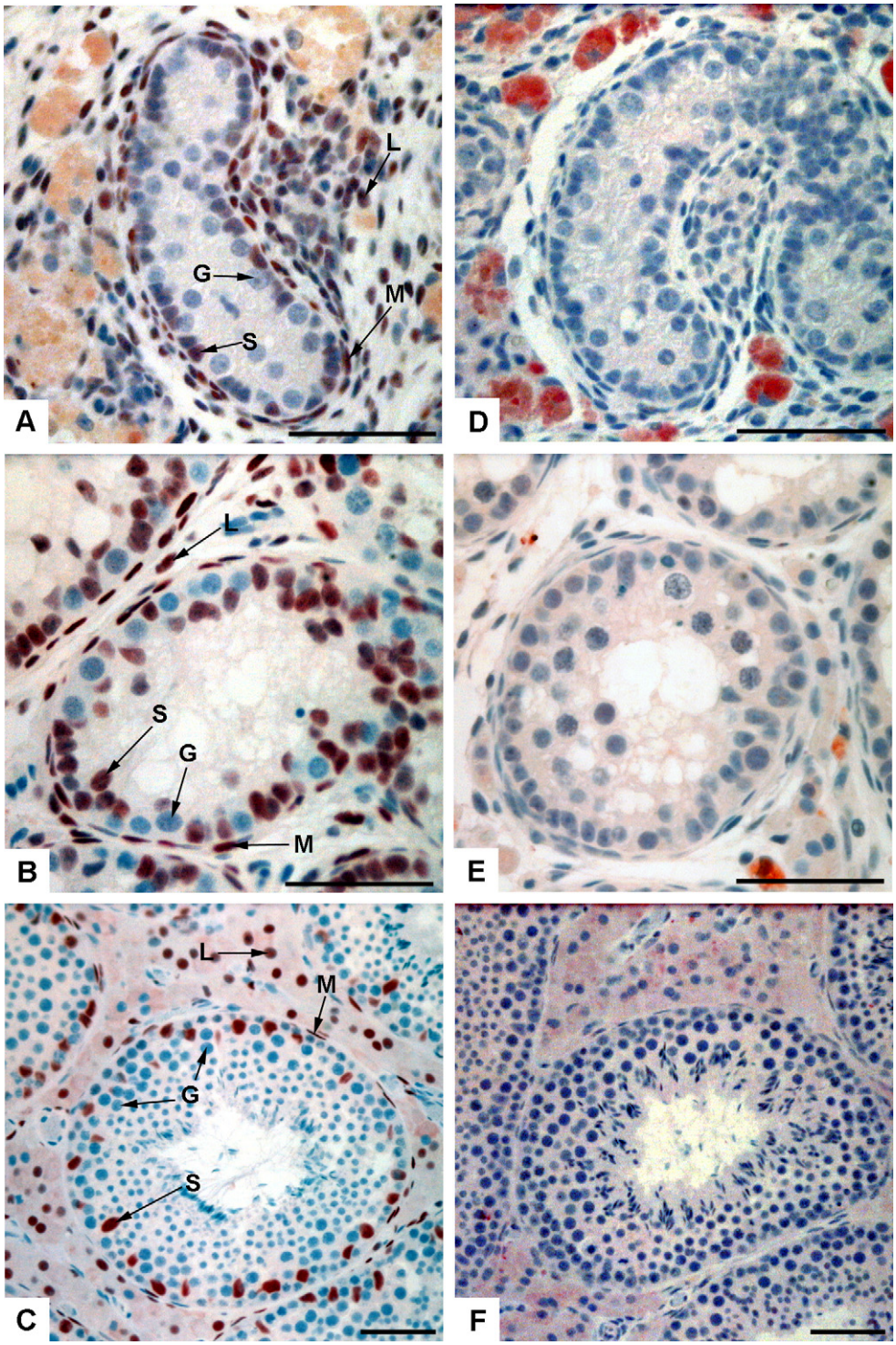

Fig. 1. Androgen receptor immunostaining in representative prepubertal (A), peripubertal (B) and postpubertal (C) testis of the stallion. No immunostaining is seen in the negative controls (D–F). S – Sertoli cell, L – Leydig cell, M – peritubular myoid cell, G – germ cell. Bars = 25 μm. Vacuoles in the lumen area were found in almost every peri-pubertal testicular tissue most likely due to the evolving formation of the lumen at this time. Lipofuscins were observed as reddish pigment granules in (A) pre- and (B) peri-pubertal negative controls.

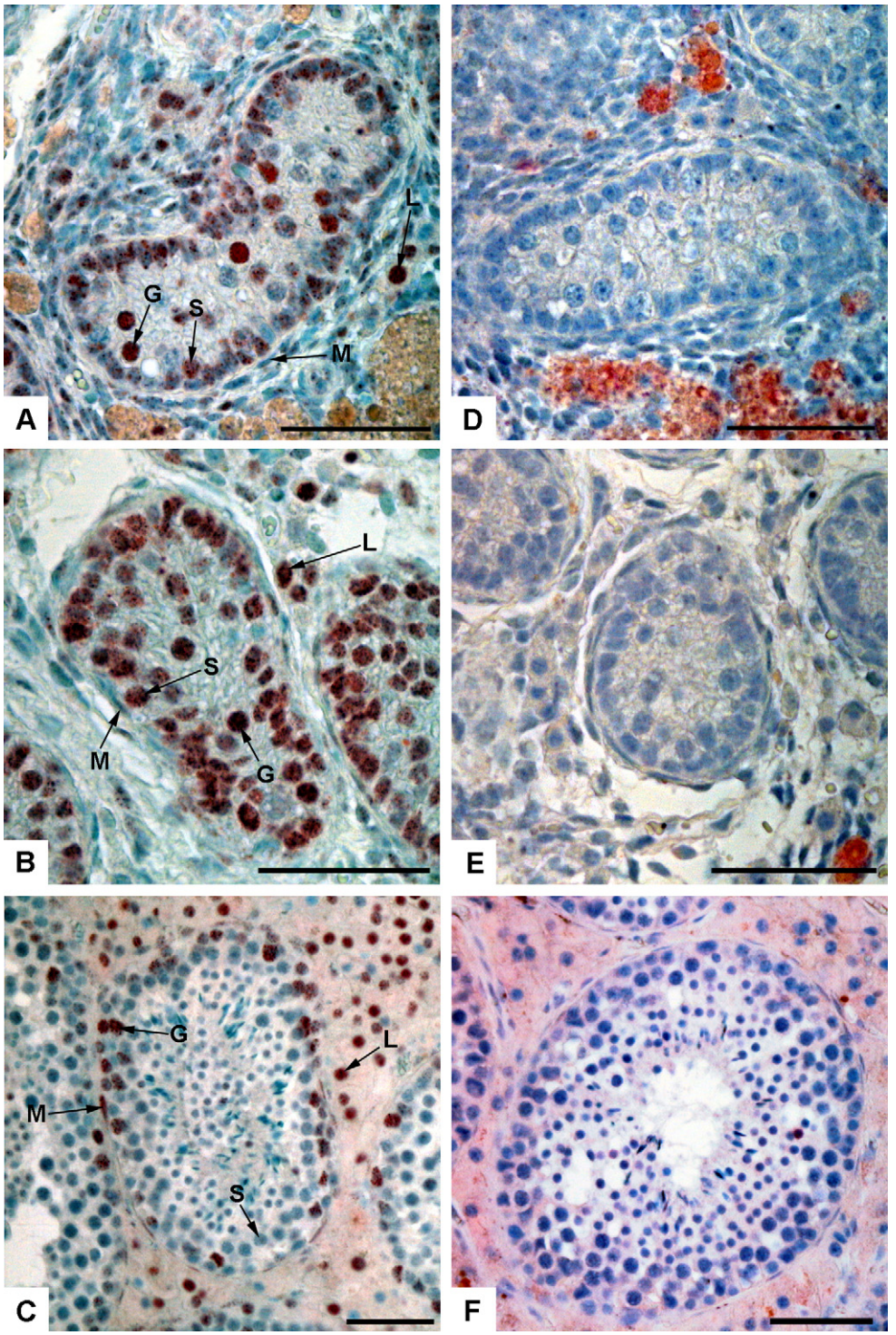

Fig. 2. Estrogen receptor alpha immunostaining in representative prepubertal (A), peripubertal (B) and postpubertal (C) testis of the stallion. No immunostaining is seen in negative controls (D-F). S – Sertoli cell, L – Leydig cell, M – peritubular myoid cell, G – germ cell. Bars = 25 μm. Vacuoles in the lumen area were found in almost every peri-pubertal testicular tissue most likely due to the evolving formation of the lumen at this time. Lipofuscins were observed as reddish pigment granules in (A) pre- and (B) peri-pubertal negative controls.

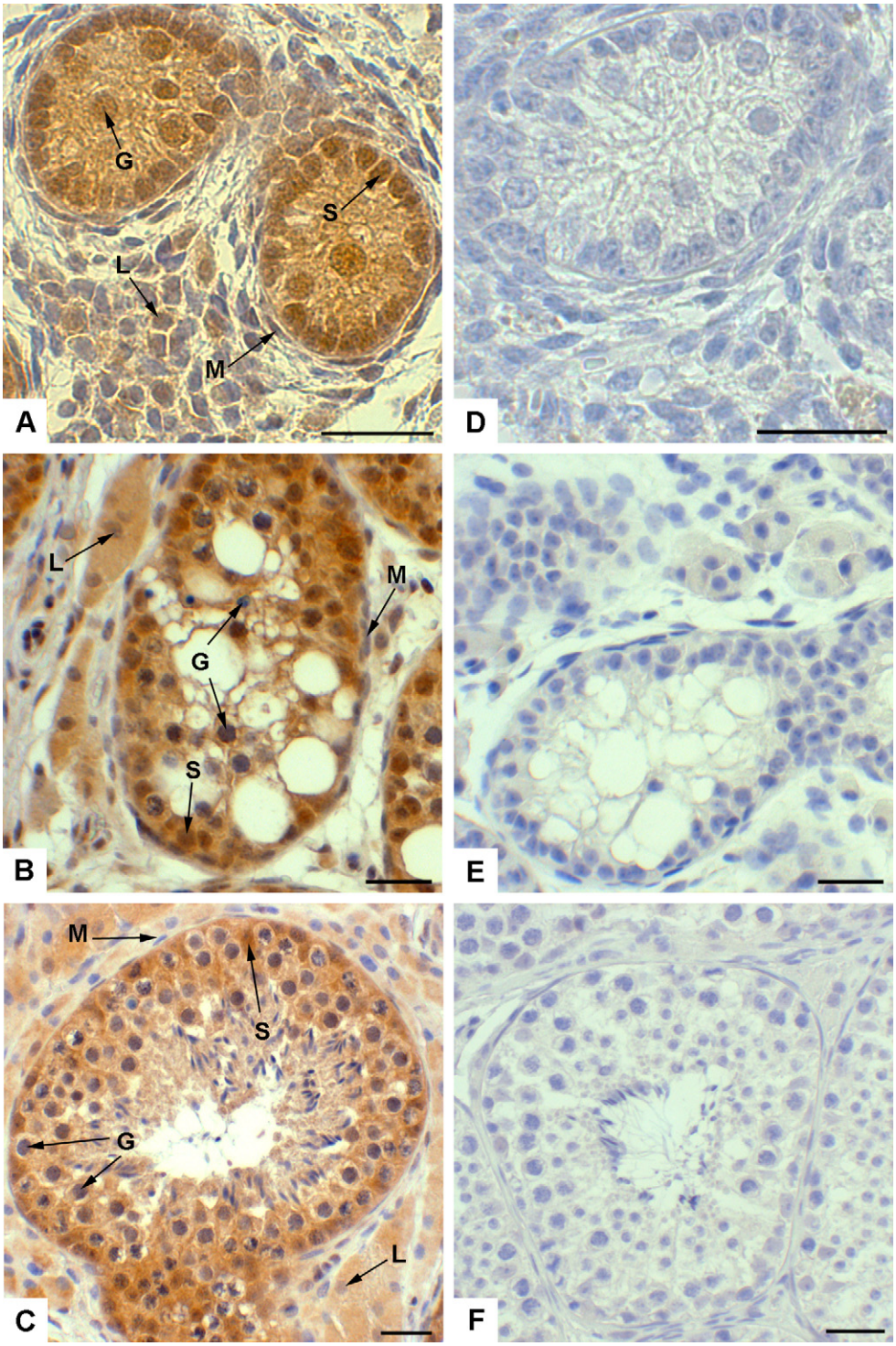

Fig. 3. Estrogen receptor beta immunostaining in representative prepubertal (A), peripubertal (B) and postpubertal (C) testis of the stallion. No staining is seen in the negative controls (D-F). S – Sertoli cell, L – Leydig cell, M – peritubular myoid cell, G – germ cell. Bars = 25 μm. Vacuoles in the lumen area were found in almost every peri-pubertal testicular tissue most likely due to the evolving formation of the lumen at this time.

paracrine/autocrine system. It is interesting to note that intra-testicular testosterone concentrations in the stallion did not change throughout development (Stewart and Roser, 1998) suggesting that changes in testicular function during puberty may be, in part, a function of the number and location of AR in the testes and not the amount of hormone. Because stallions did not have AR located in germ cells, the effects of androgens on spermatogenesis may be

of a paracrine nature involving Sertoli or Leydig cells. Leydig cell specific AR knockout mice also have an altered expression of several key steroidogenic enzymes (Xu et al., 2007). Testosterone has been demonstrated to regulate the genes required for *de novo* cholesterol synthesis in Leydig cells (Eacker et al., 2008). These data suggest that testosterone in the stallion acts as a paracrine factor regulating germ cell development and an autocrine factor regulating steroid hormone synthesis from the Leydig cell.

In the pre-pubertal and peri-pubertal stallion, ERα was expressed in some Leydig cells, Sertoli cells and germ cells (spermatogonia) but not in peritubular myoid cells. However, localization of the receptors changed in the post-pubertal stallion with the expression of ERα in most Leydig cells, germ cells as well as peritubular myoid cells but not in Sertoli cells. In contrast (Bilinska et al., 2005) in the mature stallion, demonstrated that immunostaining for ERα was restricted to Leydig cells. Age dependent changes in localization of the ERα have been observed in other species (O'Donnell et al., 2001; Mutembei et al., 2005; An et al., 2008; Gonzalez-Moran et al., 2008; Otsuka et al., 2008). Perhaps in the pre-pubertal and peri-pubertal stallion, ERα is required to initiate spermatogenesis by inducing mitosis of spermatogonia, enhancing steroids from the Leydig cells and growth factors from the Sertoli cells (Roser, 2008). Once an adult, estrogen, via ERα, and AR may regulate the peritubular myoid cells to drive spermatocytes into the lumen (Maekawa et al., 1996) and to also produce paracrine factors such as peritubular myoid substance (Pmods) which regulates Sertoli cell function (Skinner and Fritz, 1986). Expression of ERα in the Leydig cells changes little with age except for an apparent increase in the number of ERα positive cells in post-pubertal animals. This is consistent with the observation that the Leydig cell population increases with age (Johnson and Neaves, 1981). An increase in post-pubertal Leydig cells is consistent with an increase in estrogen concentration in testicular tissue (Stewart and Roser, 1998; Parlevliet et al., 2006) given that estrogen is produced by Leydig cells in the stallion (Eisenhauer and Roser, 1995). Interestingly, estrogen appears to inhibit Leydig cell development in mice (Abney, 1999). If estrogen acts similarly in the stallion testis, then the lower concentrations of estradiol observed in younger stallions (Stewart and Roser, 1998) would be required to allow for Leydig cell development. Estrogen has been suggested to be key to establishing Sertoli cell function (O'Donnell et al., 2001) and it could do this through ERα and ERβ during development and through ERβ in post-pubertal stallions.

The present study also suggests that estrogen actions in the stallion testes are mediated by way of ERβ in Leydig and Sertoli cells during pre-pubertal and peri-pubertal stages of development. Germ cells were positive for ERβ in pre-pubertal animal but were negative in peri- and post-pubertal stallions. Peritubular myoid cells were negative for ERβ in all age groups. Because ERα is not present in post-pubertal Sertoli cells, it is likely that the function of these cells is regulated by ERβ and AR. In contrast, it has been reported that ERβ staining was localized in Leydig, Sertoli and germ cells in post-pubertal stallions (Hejmej et al., 2005). Expression of ERs in the germ cells varies amongst other species (Hess and Carnes, 2004). Stallion germ cells expressed ERα in all age groups but ERβ only in pre-pubertal stages. This is clearly in contrast to the review by (O'Donnell et al., 2001) which reported that ERβ and not ERα was present in all types of germ cells in the rodent. However there are species–species and within-species differences due to age and methods (O'Donnell et al., 2001; Mutembei et al., 2005; An et al., 2008). ERα signaling has been demonstrated to be a germ cell survival factor for human germ cells by preventing apoptosis (Pentikainen et al., 2000) and αERKO mice have an increased number of apoptotic germ cells (Gould et al., 2007). Estrogen has also been implicated in spermatogonial stem cell division (Miura et al., 1999). βERKO mice have increased numbers of spermatogonial stem cells (Gould et al., 2007), therefore, it is possible that estradiol promotes germ cell division and survival in the stallion testis. Lack of ERβ in the adult stallion germ cells could lead to increased numbers of stem cells for spermatogenesis.

Taken together, these data suggest that estrogen regulates cell differentiation and 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 androgens likely activate the Leydig cells, peritubular myoid cells and Sertoli cells by way of AR in pre-pubertal, peri-pubertal and post-pubertal stallions suggesting that its role is to support the production of estrogen and other factors necessary for initiation and maintenance of spermatogenesis. It has previously been suggested that subfertility/infertility in the stallion is linked to abnormal hormone production (Roser, 2008). Subfertile and infertile stallions have similar intra-testicular concentrations of testosterone, estradiol, and estrogen conjugates when compared to fertile stallions (Stewart and Roser, 1998). It is therefore, interesting to hypothesize that subfertility/infertility may be related to problems of ER or AR expression or action rather than hormone production. The localization of ERα, ERβ and AR presented here suggest that estrogens and androgens likely act in an autocrine/paracrine manner to regulate testicular function in the developing and mature stallion. These results may provide a starting point for future investigations into the expression of ERs and AR in subfertile and infertile stallions.

References

Abney, T.O., 1999. The potential roles of estrogens in regulating Leydig cell development and function: a review. Steroids 64, 610–617.

An, K.W., Nelson, E.R., Jo, P.G., Habibi, H.R., Shin, H.S., Choi, C.Y., 2008. Characterization of estrogen receptor beta2 and expression of the estrogen receptor subtypes alpha, beta1, and beta2 in the protandrous black porgy (Acanthopagrus schlegeli) during the sex change process. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 150, 284–291.

Anthony, C.T., Kovacs, W.J., Skinner, M.K., 1989. Analysis of the androgen receptor in isolated testicular cell types with a microassay that uses an affinity ligand. Endocrinology 125, 2628–2635.

Aquila, S., Middea, E., Catalano, S., Marsico, S., Lanzino, M., Casaburi, I., Barone, I., Bruno, R., Zupo, S., Ando, S., 2007. Human sperm express a functional androgen receptor: effects on PI3K/AKT pathway. Hum. Reprod. 22, 2594–2605.

Arenas, M.I., Royuela, M., Lobo, M.V., Alfaro, J.M., Fraile, B., Paniagua, R., 2001. Androgen receptor (AR), estrogen receptor-alpha (ER-alpha) and estrogen receptor-beta (ER-beta) expression in the testis of the newt, Triturus marmoratus marmoratus during the annual cycle. J. Anat. 199, 465–472.

Bilinska, B., Hejmej, A., Gancarczyk, M., Sadowska, J., 2005. Immunoexpression of androgen receptors in the reproductive tract of the stallion. Ann. N. Y. Acad. Sci. 1040, 227–229.

Bilinska, B., Hejmej, A., Pawlak, M., Sadowska, J., Tischner, M., 2004. Immunoexpression of androgen receptors in testis of immature and mature stallions. Equine Vet. J. 36, 539–543.

Bilinska, B., Wiszniewska, B., Kosiniak-Kamysz, K., Kotula-Balak, M., Gancarczyk, M., Hejmej, A., Sadowska, J., Marchlewicz, M., Kolasa, A., Wenda-Rozewicka, L., 2006. Hormonal status of male reproductive system: androgens and estrogens in the testis and epididymis. In vivo and in vitro approaches. Reprod. Biol. 6 (Suppl. 1), 43–58.

Boukari, K., Meduri, G., Brailly-Tabard, S., Guibourdenche, J., Ciampi, M.L., Massin, N., Martinerie, L., Picard, J.Y., Rey, R., Lombes, M., Young, J., 2009. Lack of androgen receptor expression in Sertoli cells accounts for the absence of anti-Mullerian hormone repression during early human testis development. J. Clin. Endocrinol. Metab. 94, 1818–1825.

Bremner, W.J., Millar, M.R., Sharpe, R.M., Saunders, P.T., 1994. Immunohistochemical localization of androgen receptors in the rat testis: evidence for stage-dependent expression and regulation by androgens. Endocrinology 135, 1227–1234.

Chang, C.S., Kokontis, J., Liao, S.T., 1988a. Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science 240, 324–326.

Chang, C.S., Kokontis, J., Liao, S.T., 1988b. Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. Proc. Natl. Acad. Sci. U.S.A. 85, 7211–7215.

Dohle, G.R., Smit, M., Weber, R.F., 2003. Androgens and male fertility. World J. Urol. 21, 341–345.

Eacker, S.M., Agrawal, N., Qian, K., Dichek, H.L., Gong, E.Y., Lee, K., Braun, R.E., 2008. Hormonal regulation of testicular steroid and cholesterol homeostasis. Mol. Endocrinol. 22, 623–635.

Ebling, F.J., Brooks, A.N., Cronin, A.S., Ford, H., Kerr, J.B., 2000. Estrogenic induction of spermatogenesis in the hypogonadal mouse. Endocrinology 141, 2861–2869.

Eisenhauer, K.M., Roser, J.F., 1995. Effects of lipoprotein, equine luteinizing hormone, equine follicle-stimulating hormone, and equine prolactin on equine testicular steroidogenesis in vitro. J. Androl. 16, 18–27.

Galena, H.J., Pillai, A.K., Terner, C., 1974. Progesterone and androgen receptors in non-flagellate germ cells of the rat testis. J. Endocrinol. 63, 223–237.

Gonzalez-Moran, M.G., Guerra-Araiza, C., Campos, M.G., Camacho-Arroyo, I., 2008. Histological and sex steroid hormone receptor changes in testes of immature, mature, and aged chickens. Domest. Anim. Endocrinol. 35, 371–379.

Gould, M.L., Hurst, P.R., Nicholson, H.D., 2007. The effects of oestrogen receptors alpha and beta on testicular cell number and steroidogenesis in mice. Reproduction 134, 271–279.

Grootegoed, J.A., Peters, M.J., Mulder, E., Rommerts, F.F., Van der Molen, H.J., 1977. Absence of a nuclear androgen receptor in isolated germinal cells of rat testis. Mol. Cell Endocrinol. 9, 159–167.

Hales, D.B., Sha, L.L., Payne, A.H., 1987. Testosterone inhibits cAMP-induced de Novo synthesis of Leydig cell cytochrome P-450(17 alpha) by an androgen receptor-mediated mechanism. J. Biol. Chem. 262, 11200–11206.

Hejmej, A., Gorazd, M., Kosiniak-Kamysz, K., Wiszniewska, B., Sadowska, J., Bilinska, B., 2005. Expression of aromatase and oestrogen receptors in reproductive tissues of the stallion and a single cryptorchid visualised by means of immunohistochemistry. Domest. Anim. Endocrinol. 29, 534–547.

Hess, M.F., Roser, J.F., 2004. Immunocytochemical localization of cytochrome P450 aromatase in the testis of prepubertal, pubertal, and postpubertal horses. Theriogenology 61, 293–299.

Hess, R.A., Carnes, K., 2004. The role of estrogen in testis and the male reproductive tract: a review and species comparison. Anim. Reprod. 1, 5–30.

Holdcraft, R.W., Braun, R.E., 2004. Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. Development 131, 459–467.

Janett, F., Stump, R., Burger, D., Thun, R., 2009. Suppression of testicular function and sexual behavior by vaccination against GnRH (Equity) in the adult stallion. Anim. Reprod. Sci. 115, 88–102.

Janssen, P.J., Brinkmann, A.O., Boersma, W.J., Van der Kwast, T.H., 1994. Immunohistochemical detection of the androgen receptor with monoclonal antibody F39.4 in routinely processed, paraffin-embedded human tissues after microwave pre-treatment. J. Histochem. Cytochem. 42, 1169–1175.

Johnson, L., Neaves, W.B., 1981. Age-related changes in the Leydig cell population, seminiferous tubules, and sperm production in stallions. Biol. Reprod. 24, 703–712.

Kimura, N., Mizokami, A., Oonuma, T., Sasano, H., Nagura, H., 1993. Immunocytochemical localization of androgen receptor with polyclonal antibody in paraffin-embedded human tissues. J. Histochem. Cytochem. 41, 671–678.

Kipp, J.L., Kilen, S.M., Woodruff, T.K., Mayo, K.E., 2007. Activin regulates estrogen receptor gene expression in the mouse ovary. J. Biol. Chem. 282, 36755–36765.

Kotula, M., Tuz, R., Fr czek, B., Wojtusiak, A., Bilinska, B., 2000. Immunolocalization of androgen receptors in testicular cells of prepubertal and pubertal pigs. Folia Histochem. Cytobiol. 38, 157–162.

Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., Gustafsson, J.A., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863–870.

Lekhkota, O., Brehm, R., Claus, R., Wagner, A., Bohle, R.M., Bergmann, M., 2006. Cellular localization of estrogen receptor-alpha (ERalpha) and -beta (ERbeta) mRNA in the boar testis. Histochem. Cell Biol. 125, 259–264.

Li, X., Strauss, L., Kaatrasalo, A., Mayerhofer, A., Huhtaniemi, I., Santti, R., Makela, S., Poutanen, M., 2006. Transgenic mice expressing p450 aromatase as a model for male infertility associated with chronic inflammation in the testis. Endocrinology 147, 1271–1277.

Lubahn, D.B., Joseph, D.R., Sullivan, P.M., Willard, H.F., French, F.S., Wilson, E.M., 1988. Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science 240, 327–330.

Maekawa, M., Kamimura, K., Nagano, T., 1996. Peritubular myoid cells in the testis: their structure and function. Arch. Histol. Cytol. 59, 1–13.

McKinnell, C., Saunders, P.T., Fraser, H.M., Kelnar, C.J., Kivlin, C., Morris, K.D., Sharpe, R.M., 2001. Comparison of androgen receptor and oestrogen receptor beta immunoexpression in the testes of the common marmoset (Callithrix jacchus) from birth to adulthood: low androgen receptor immunoexpression in Sertoli cells during the neonatal increase in testosterone concentrations. Reproduction 122, 419–429.

Meng, J., Holdcraft, R.W., Shima, J.E., Griswold, M.D., Braun, R.E., 2005. Androgens regulate the permeability of the blood-testis barrier. Proc. Natl. Acad. Sci. U.S.A. 102, 16696–16700.

Merlet, J., Racine, C., Moreau, E., Moreno, S.G., Habert, R., 2007. Male fetal germ cells are targets for androgens that physiologically inhibit their proliferation. Proc. Natl. Acad. Sci. U.S.A. 104, 3615–3620.

Miura, T., Miura, C., Ohta, T., Nader, M.R., Todo, T., Yamauchi, K., 1999. Estradiol-17beta stimulates the renewal of spermatogonial stem cells in males. Biochem. Biophys. Res. Commun. 264, 230–234.

Mutembei, H.M., Pesch, S., Schuler, G., Hoffmann, B., 2005. Expression of oestrogen receptors alpha and beta and of aromatase in the testis of immature and mature boars. Reprod. Domest. Anim. 40, 228–236.

Nie, R., Zhou, Q., Jassim, E., Saunders, P.T., Hess, R.A., 2002. Differential expression of estrogen receptors alpha and beta in the reproductive tracts of adult male dogs and cats. Biol. Reprod. 66, 1161–1168.

O'Donnell, L., Robertson, K.M., Jones, M.E., Simpson, E.R., 2001. Estrogen and spermatogenesis. Endocr. Rev. 22, 289–318.

Omoto, Y., Imamov, O., Warner, M., Gustafsson, J.A., 2005. Estrogen receptor alpha and imprinting of the neonatal mouse ventral prostate by estrogen. Proc. Natl. Acad. Sci. U.S.A. 102, 1484–1489.

Otsuka, S., Suzuki, M., Kamezaki, N., Shima, T., Wakatsuki, M., Kon, Y., Ohtaishi, N., 2008. Growth-related changes in histology and immunolocalization of steroid hormone receptors in gonads of the immature male green turtle (Chelonia mydas). J. Exp. Zool. A: Ecol. Genet. Physiol. 309, 166–174.

Parlevliet, J.M., Pearl, C.A., Hess, M.F., Famula, T.R., Roser, J.F., 2006. Immunolocalization of estrogen and androgen receptors and steroid concentrations in the stallion epididymis. Theriogenology 66, 755–765.

Pelletier, G., El-Alfy, M., 2000. Immunocytochemical localization of estrogen receptors alpha and beta in the human reproductive organs. J. Clin. Endocrinol. Metab. 85, 4835–4840.

Pelletier, G., Labrie, C., Labrie, F., 2000. Localization of oestrogen receptor alpha, oestrogen receptor beta and androgen receptors in the rat reproductive organs. J. Endocrinol. 165, 359–370.

Pentikainen, V., Erkkila, K., Suomalainen, L., Parvinen, M., Dunkel, L., 2000. Estradiol acts as a germ cell survival factor in the human testis in vitro. J. Clin. Endocrinol. Metab. 85, 2057–2067.

Rago, V., Maggiolini, M., Vivacqua, A., Palma, A., Carpino, A., 2004. Differential expression of estrogen receptors (ERalpha/ERbeta) in testis of mature and immature pigs. Anat. Rec. A: Discov. Mol. Cell. Evol. Biol. 281, 1234–1239.

Roser, J.F., 2008. Regulation of testicular function in the stallion: an intricate network of endocrine, paracrine and autocrine systems. Anim. Reprod. Sci. 107, 179–196.

Sar, M., Lubahn, D.B., French, F.S., Wilson, E.M., 1990. Immunohistochemical localization of the androgen receptor in rat and human tissues. Endocrinology 127, 3180–3186.

Saunders, P.T., Millar, M.R., Macpherson, S., Irvine, D.S., Groome, N.P., Evans, L.R., Sharpe, R.M., Scobie, G.A., 2002. ERbeta1 and the ERbeta2 splice variant (ERbetacx/beta2) are expressed in distinct cell populations in the adult human testis. J. Clin. Endocrinol. Metab. 87, 2706–2715.

Saunders, P.T., Millar, M.R., Williams, K., Macpherson, S., Harkiss, D., Anderson, R.A., Orr, B., Groome, N.P., Scobie, G., Fraser, H.M., 2000. Differential expression of estrogen receptor-alpha and -beta and androgen receptor in the ovaries of marmosets and humans. Biol. Reprod. 63, 1098–1105.

Saunders, P.T., Sharpe, R.M., Williams, K., Macpherson, S., Urquart, H., Irvine, D.S., Millar, M.R., 2001. Differential expression of oestrogen receptor alpha and beta proteins in the testes and male reproductive system of human and non-human primates. Mol. Hum. Reprod. 7, 227–236.

Shan, L., Hardy, D.O., Catterall, J.F., Hardy, M.P., 1995. Effects of luteinizing hormone (LH) and androgen on steady state levels of messenger ribonucleic acid for LH receptors, androgen receptors, and steroidogenic enzymes in rat Leydig cell progenitors in vivo. Endocrinology 136, 1686–1693.

Sierens, J.E., Sneddon, S.F., Collins, F., Millar, M.R., Saunders, P.T., 2005. Estrogens in testis biology. Ann. N. Y. Acad. Sci. 1061, 65–76.

Singh, J., O'Neill, C., Handelsman, D.J., 1995. Induction of spermatogenesis by androgens in gonadotropin-deficient (hpg) mice. Endocrinology 136, 5311–5321.

Sipahutar, H., Sourdaine, P., Moslemi, S., Plainfosse, B., Seralini, G.E., 2003. Immunolocalization of aromatase in stallion Leydig cells and seminiferous tubules. J. Histochem. Cytochem. 51, 311–318.

Skinner, M.K., Fritz, I.B., 1986. Identification of a non-mitogenic paracrine factor involved in mesenchymal–epithelial cell interactions between testicular peritubular cells and Sertoli cells. Mol. Cell. Endocrinol. 44, 85–97.

Solakidi, S., Psarra, A.M., Nikolaropoulos, S., Sekeris, C.E., 2005. Estrogen receptors alpha and beta (ERalpha and ERbeta) and androgen receptor (AR) in human sperm: localization of ERbeta and AR in mitochondria of the midpiece. Hum. Reprod. 20, 3481–3487.

Stewart, B.L., Roser, J.F., 1998. Effects of age, season, and fertility status on plasma and intratesticular immunoreactive (IR) inhibin concentrations in stallions. Domest. Anim. Endocrinol. 15, 129–139.

Suarez-Quian, C.A., Martinez-Garcia, F., Nistal, M., Regadera, J., 1999. Androgen receptor distribution in adult human testis. J. Clin. Endocrinol. Metab. 84, 350–358.

Tan, K.A., De Gendt, K., Atanassova, N., Walker, M., Sharpe, R.M., Saunders, P.T., Denolet, E., Verhoeven, G., 2005. The role of androgens in sertoli cell proliferation and functional maturation: studies in mice with total or Sertoli cell-selective ablation of the androgen receptor. Endocrinology 146, 2674–2683.

Thompson Jr., D.L., Honey, P.G., 1984. Active immunization of prepubertal colts against estrogens: hormonal and testicular responses after puberty. J. Anim. Sci. 59, 189–196.

van Pelt, A.M., de Rooij, D.G., van der Burg, B., van der Saag, P.T., Gustafsson, J.A., Kuiper, G.G., 1999. Ontogeny of estrogen receptor-beta expression in rat testis. Endocrinology 140, 478–483.

Van Roijen, J.H., Van Assen, S., Van Der Kwast, T.H., De Rooij, D.G., Boersma, W.J., Vreeburg, J.T., Weber, R.F., 1995. Androgen receptor immunoexpression in the testes of subfertile men. J. Androl. 16, 510–516.

Vornberger, W., Prins, G., Musto, N.A., Suarez-Quian, C.A., 1994. Androgen receptor distribution in rat testis: new implications for androgen regulation of spermatogenesis. Endocrinology 134, 2307–2316.

Wang, R.S., Yeh, S., Tzeng, C.R., Chang, C., 2009. Androgen receptor roles in spermatogenesis and fertility: lessons from testicular cell-specific androgen receptor knockout mice. Endocr. Rev. 30, 119–132.

Warikoo, P.K., Majumdar, S.S., Allag, I.S., Das, R.P., Roy, S., 1986. Interactions among motility, fertilizing ability, and testosterone binding on spermatozoa of bonnet monkey (Macaca radiata). Arch. Androl. 16, 135–141.

Xu, Q., Lin, H.Y., Yeh, S.D., Yu, I.C., Wang, R.S., Chen, Y.T., Zhang, C., Altuwaijri, S., Chen, L.M., Chuang, K.H., Chiang, H.S., Yeh, S., Chang, C., 2007. Infertility with defective spermatogenesis and steroidogenesis in male mice lacking androgen receptor in Leydig cells. Endocrine 32, 96–106.

Zhou, Q., Nie, R., Prins, G.S., Saunders, P.T., Katzenellenbogen, B.S., Hess, R.A., 2002. Localization of androgen and estrogen receptors in adult male mouse reproductive tract. J. Androl. 23, 870–881.

Zhou, X., Kudo, A., Kawakami, H., Hirano, H., 1996. Immunohistochemical localization of androgen receptor in mouse testicular germ cells during fetal and postnatal development. Anat. Rec. 245, 509–518.