LOCALIZATION OF ESTROGEN RECEPTORS IN MALE REPRODUCTIVE TISSUES AND SPERM CELLS – A REVIEW*

R. Krejčířová, P. Postlerová, R. Rajmon

Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Veterinary Sciences, Prague, Czech Republic

The effect of endogenous estrogens on the male reproductive tissues and development of gametes is an essential for the reproductive success. Estrogens affect the target cells via estrogen receptors (ERs) by both genomic and non-genomic pathways. The ER localization in the testis, epididymis, and sperm cells is a key to understanding the effect of estrogens on the sperm development, maturation, and function. The ER detection in male reproductive tissues and sperm cells at different development stages is described in representative mammalian species (human, mouse, rat, horse, and pig), in which the ER localization has been most described. According to various authors the ER occurrence in the male reproductive tissues and spermatozoa is quite distinct. Discrepancy in the published results is probably caused either by the application of different tissue preparation methods, or the choice of specific antibodies. Inconsistent findings should be subjected to further investigation to better understand the role of ERs in the male gamete development and mammalian reproduction

ERα, ERβ, GPER, testes, epididymis, germ cells

DE GRUYTER OPEN

doi: 10.2478/sab-2018-0034
Received for publication on December 14, 2017
Accepted for publication on January 25, 2018

INTRODUCTION

Estrogens represent one of the major groups of hormones that have the ability to regulate reproductive processes in both females and males and thus affect their fertility. Their effect on the organism is very varied depending on the targeted tissue. However, some substances from the external environment also have estrogenic effects similar to those of endogenous estrogens. Environmental estrogens, xeno- and phytoestrogens, are so-called endocrine disruptors with the ability to bind estrogen receptors (ERs) and affect physiological processes in the body. They can act through ERs agonistically or antagonistically and display either estrogenic or anti-estrogenic effects. They also have the ability to influence the reproduction process and gametogenesis (Shemes, Shore, 2012). Although estrogens were previously considered to be female sex hormones, they play also an indispensable role in the development and activity of male reproductive organs (Luconi et al., 2001; 2002). Estrogens play an important role in spermatogenesis, sperm maturation, capacitation, acrosome reaction, and fertilization (Pelletier, El-Alfy, 2000; Lukoseviciute et al., 2005; Kotula-Balak et al., 2012). High levels of estrogens disrupt the spermatogenesis, while the low ones have a positive effect (Carreau et al., 2011). Spermatozoa are also affected by estrogens during their transport and deposition in the female reproductive tract (Dostalova et al., 2017).

* Supported by the Internal Grant Agency of the Czech University of Life Sciences Prague (CIGA) (Project No. 20162027).

The effect of estrogens is determined by the presence of estrogen receptors (ERs) in the cells of targeted tissues to which they are able to bind, thus initiating the cellular responses (Carpino et al., 2004b; Chimento et al., 2010a, b). Detection and localization of ERs is essential for understanding the effect of estrogens on the organism (Razandi et al., 2004). The molecular mechanism of ER activity is linked with their cell localization. Beside the cytosol and cell nucleus, these receptors are also found in the plasma membrane or endoplasmic reticulum (Razandi et al., 2004). Classic steroid hormone signaling includes nuclear and cytosolic receptors (Levin, 2009). After binding the hormone, the receptor binds to the DNA regulating gene transcription and subsequently affects the cell activity (Beato et al., 1996). While the genomic effects of estrogens via classic ERs have been explained satisfactorily, the estrogen non-genomic effect via membrane receptors proceeds through a different molecular mechanism, as yet unexplained (Pedram et al., 2007). The non-genomic manner of action is very fast in comparison with the genomic one and runs for several seconds to a few minutes (Gruber et al., 2002; Heldring et al., 2007).

At present, three ERs are known in somatic cells. The first two are the classic estrogen receptors ERα (ESR1 according to the new nomenclature) and ERβ (ESR2 according to the new nomenclature) that mediate the effects of estrogens in genomic (nuclear) signaling, but appear to be involved in the non-genomic pathway as well (Whiting et al., 2000). Later, a membrane-associated G protein-coupled estrogen receptor 1 (GPER-1, also referred as GPER or GPR30) was described (Filardo et al., 2007). This estrogen receptor is associated with rapid non-genomic signaling out of the nucleus (Wang et al., 2014).

Classic estrogen receptors ERα and ERβ

Two subtypes of ERs belonging to the family of nuclear receptors are known: ERα (also referred to as ESR1 in the literature) and ERβ (referred to as ESR2) (Kumar et al., 2011). ERα and ERβ receptors may act as transcription factors or trigger extranuclear-initiated kinase signaling pathways (Kulkoyluoglu, Madak-Erdogan, 2016).

ERα and ERβ have a similar structure. Both are composed of distinct but functional interacting domains: the N-terminal domain, the DNA-binding domain, and the ligand-binding domain. It was found that the N-terminal domain of human ERα and ERβ shares less than 20% amino acid identity. This results in a relatively high specificity of effects on the targeted genes, depending on the ER subtype (Ogawa et al., 1998; Zhao et al., 2008). Their expression is tissue-specific and they have a different function (Grober et al., 2011; Kulkoyluoglu, Madak-Erdogan, 2016). Both subtypes of classic ERs have different ligand specificity and transcriptional activity (Acconcia, Kumar, 2006).

There is information on the localization of ERα and ERβ in the cell membranes, where these receptors participate in non-genomic signaling (Razandi et al., 2004; Levin, 2009). The membrane ERα appears to be very similar and possibly identical to the nuclear receptor (Razandi et al., 2002). Presumably, the classic receptors move to the plasma membrane; this process is determined by palmitoylation of ERs, allowing association of the ER with transport protein caveolin-1 and subsequent transfer to the caveolar region in the plasma membrane (Acconcia et al. 2003; Pedram et al., 2007, 2012), where the receptor can interact with many signaling proteins and activate G-protein subunits (Razandi et al., 2002; Kumar et al., 2007).

Localization of ERα and ERβ in the tissues of male reproductive organs. Classical estrogen receptors are not distributed in the male tissues evenly. Their highest number is found in the reproductive tract (Lombardi et al., 2001) and their localization has been described across mammalian species. The expression of both isoforms (ERα and ERβ) also differs. The occurrence of ERα is more specific, whereas ERβ is ubiquitous in the entire male reproductive tract (Eddy et al., 1996; Franca et al., 2005; Carreau, Hess, 2010). The distribution of both ER subtypes in various regions of the male reproductive tract is not only species-specific, but also differs within the species, for example, depending on age (Carpino et al., 2004b; Solakidi et al., 2005). The presence of ERs in male reproductive organs, namely the testis and epididymis, has been studied in several mammalian species, predominantly in small laboratory animals and humans. Additionally, the ERs in male reproductive tissues have been also described in farm animals, mostly in horses and pigs, in relation to the effect of endogenous estrogens on reproduction. Particularly in pigs, a high sensitivity to low doses of xenoestrogens was reported (Prelusky, 1994).

In the literature, the information on the distribution of ER isoforms has often been controversial. In reproductive tissues and spermatozoa of different species, ERs have been localized and detected using various polyclonal and monoclonal antibodies by immunohistochemistry and immunocytochemistry and by Western blot analysis. Nevertheless, the difference in the published results could have been caused also by either the application of different tissue preparation methods, or the choice of specific antibodies. Published results on localization of classical ERs in the testis, epididymis, and sperm cells of mouse, rat, human, stallion, and pig are summarized in the Table 1 (for the ERα detection) and Table 2 (for the ERβ detection).

Detection of ERα and ERβ in the testis. Data on the ERs in the testicular tissue is very different.

Table 1. Detection of ERα in testicular and epididymal tissues, germ cells, and spermatozoa in selected mammalian species

	Human	Mouse	Rat	Stallion	Boar
Testicular interstitial tissue/ myoid cells	N.D.	+ (35)	+ im (12)	+ ad (22), – im (22)	+ im (30), – im (25)
Leydig cells	– (6, 16, 31)	+ (16, 35)	+ ad (12, 23), + im (12)	+ ad (22), + SC im (22)	+ w ad (9, 17, 25), + im (30), – im (17, 25)
Sertoli cells	– (6, 16, 31)	– (16, 35)	+ ad (12), + im (12)	– ad (22), + im (22)	+ ad (9), + SC ad (17), – ad (25), + im (30), – im (17, 25)
Germ cells	+ SC (6, 8, 24), – (16, 31)	– (16, 35)	+ ad (23), + SC ad (12) – im (12)	+ SC ad (22), + im (22)	+ SC ad (25), + w ad (9), + SC ad (17), + SC im (17), – im (25)
E1 epithelial cells	+ w (31)	+ ad (7, 35), + im (35)	+ ad (34), ' + im (34)	+ ad (19), + SC im (19)	+ ad (9, 20, 21), + im (21)
E2 epithelial cells	+ w (31)	+ w ad (7, 35), + im (35)	+ ad (34), + im (34)	+ SC ad (19), + SC im (19)	+ ad (21), + w ad (9, 20), + im (21)
E3 epithelial cells	+ w (31)	+ w ad (7, 35), + im (35)	+ ad (34), + im (34)	+ SC ad (19), – im (19)	+ ad (9, 21), – ad (20), + im (21)
E1 sperm in lumen	N.D.	N.D.	N.D.	– (19)	+ (9)
E2 sperm in lumen	N.D.	N.D.	N.D.	– (19)	+ PA (9)
E3 sperm in lumen	N.D.	N.D.	N.D.	– (19)	+ (9)
Ejaculated sperm (im)	+ RC (26)	N.D.	N.D.	N.D.	N.D.
Ejaculated sperm (m) Head	+ ES, PA (33)	N.D.	– (34)	+ PA (2, 7)	– (27)
Ejaculated sperm (m) Midpiece	+ (1, 26), + w (8), – (31)	N.D.	+ (34)	+ (2, 7)	+ (27)
Ejaculated sperm (m) Flagellum	+ w (33)	N.D.	– (34)	+ (2, 7)	+ (9), + w (27)

E1 = caput epididymis, E2 = corpus epididymis, E3 = cauda epididymis, w = weak, SC = some cells, ad = adult animals, im = immature animals or spermatozoa, m = mature animals or spermatozoa, N.D. = not described, RC = residual cytoplasm, ES = equatorial segment, PA = post-acrosomal region;

(1) Aquila et al., 2004; (2) Arkoun et al., 2014; (6) Fietz et al., 2014; (7) Gautier et al., 2016; (8) Guido et al., 2011; (9) Gunawan et al., 2011; (12) Lucas et al., 2008; (16) Mäkinen et al., 2001; (17) Mutembei et al., 2005; (19) Parlevliet et al., 2006; (20) Pearl et al., 2007a; (21) Pearl et al., 2007b; (22) Pearl et al., 2011; (23) Pelletier et al., 2000; (25) Rago et al., 2004; (26) Rago et al., 2006; (27) Rago et al., 2007; (30) Ramesh et al., 2007; (31) Saunders et al., 2001; (33) Solakidi et al., 2005; (34) Zaya et al., 2012; (35) Zhou et al., 2002

Published results show that both classical ERs are more intensively expressed in adult testes compared to pre-pubertal tissue. However, this does not apply equally to all cell types. Generally, in Sertoli cells, the ERα expression decreases with age while that of ERβ increases (Hess et al., 2001; Hess, 2003; Carreau, Hess, 2010).

In *human*, the presence of both ERs has been reported in the germinal epithelium of seminiferous tubules in the testes even in the interstitial tissue, but the consistency in the published results is only partial. ERα was detected in human primary spermatocytes (Pentikainen et al., 2000) but not in the spermatogonia. Some authors reported their expression in spermatids (Pentikainen et al., 2000; Fietz et al., 2014). ERα is not present in Sertoli cells and Leydig interstitial cells (Mäkinen et al., 2001; Saunders et al., 2001; Fietz et al., 2014). The presence of the ERβ receptor has been demonstrated in human spermatogonia (Pentikainen et al., 2000; Mäkinen et al., 2001); especially, ERβ was detected in the nuclei of spermatogonia by Fietz et al. (2014). The same authors have also found ERβ in primary spermatocytes. This receptor was found to be expressed in round spermatids, but a negative result was documented in prolonged spermatids (Pentikainen et al., 2000; Mäkinen et al., 2001; Fietz et al., 2014). Some authors described the ERβ positivity in Sertoli cells,

Table 2: Detection of ERb in testicular and epididymal tissues, germ cells, and spermatozoa in selected mammalian species

	Human	Mouse	Rat	Stallion	Boar
Testicular interstitial tissue/ myoid cells	+ (31)	+ (35)		– im and ad (22)	+ im and ad (25, 30)
Leydig cells	+ (31), – (6, 16)	+ (35), – (16)	+ ad (12)	+ im (22), + ad (22)	+ ad (10, 17, 30), – ad (25), + im (25, 30), – im (17)
Sertoli cells	+ (6, 31), – (16)	+ (16, 35)	+ ad (12, 23)	+ im (22), + ad (22)	+ ad (10, 17), – ad (25), + w im (25, 30), – im (17)
Germ cells	+ SC (6, 8, 16, 24, 31)	+ (16, 35)	+ ad SC (12)	+ im (22), - ad (22)	+ w ad (10, 30), + SC ad (17, 25), + im (17, 25)
E1 epithelial cells	+ (31)	+ (35)	+ ad (7, 34), + im (34)	+ im (19), + ad (19)	+ ad (4, 10, 20, 21), + im (21), – im (4)
E2 epithelial cells	+ (31)	+ (35)	+ ad (7, 34), + im (34)	+ im (19), + ad (19)	+ ad (4, 10, 20, 21), + im (21), + w im (4)
E3 epithelial cells	+ (31)	+ (35)	+ ad (7, 34), + im (34)	+ im (19), + ad (19)	+ ad (4, 10, 20, 21), + im (21), – im (4)
E1 sperm in lumen	N.D.	N.D.	N.D.	– (19)	+ (10)
E2 sperm in lumen	N.D.	N.D.	N.D.	– (19)	+ AR (10)
E3 sperm in lumen	N.D.	N.D.	N.D.	– (19)	+ (10)
Ejaculated sperm (im)	+ flagellum, RC (26), – head (26)	N.D.	N.D.	N.D.	N.D.
Ejaculated sperm (m) Head	– (26)	N.D.	N.D.	+ PA (2)	+ AR (27)
Ejaculated sperm (m) Neck	– (26)	N.D.	N.D.	– (2)	– (27)
Ejaculated sperm (m) Midpiece	+ (8, 31), – (26)	N.D.	N.D.	+ (2)	– (27)
Ejaculated sperm (m) Flagellum	+ (1, 8, 26), + w (31)	N.D.	N.D.	+ (2)	– (27)

E1 = caput epididymis, E2 = corpus epididymis, E3 = cauda epididymis, w = weak, SC = some cells, ad = adult animals, im = immature animals or spermatozoa, m = mature animals or spermatozoa, N.D. = not described, RC = residual cytoplasm, ES = equatorial segment, AR = acrosomal region, PA = post-acrosomal region;

(1) Aquila et al., 2004; (2) Arkoun et al., 2014; (4) Carpino et al., 2004; (6) Fietz et al., 2014; (7) Gautier et al., 2016; (8) Guido et al., 2011; (10) Gunawan et al., 2012; (12) Lucas et al., 2008; (16) Mäkinen et al., 2001; (17) Mutembei et al., 2005; (19) Parlevliet et al., 2006; (20) Pearl et al., 2007a; (21) Pearl et al., 2007b; (22) Pearl et al., 2011; (23) Pelletier et al., 2000; (24) Pentikäinen et al., 2000; (25) Rago et al., 2004; (26) Rago et al., 2006; (27) Rago et al., 2007; (30) Ramesh et al., 2007; (31) Saunders et al., 2001; (34) Zaya et al., 2012; (35) Zhou et al., 2002

but Leydig cells were reported as negative (Fietz et al., 2014). In contrast, both Sertoli and Leydig cells were indicated by Mäkinen et al. (2001) as negative.

In *murine* testes, Mäkinen et al. (2001) detected the ERα receptor only in the nuclei of Leydig cells. The other cells were negative. Zhou et al. (2002) showed ERα-positive staining in Leydig cells and, in addition, in some peritubular myoid cells; germ cells of all stages and Sertoli cells were negative. The ERβ receptor was detected in mouse spermatogonia, at all stages of spermatocytes (except those in the meiotic division), in Leydig and Sertoli cells, and also in most peritubular myoid cells (Zhou et al., 2002). Similar results were reported by Mäkinen et al. (2001), who showed positive labeling for ERβ at the developmental stages of spermatic cells from spermatogonia to spermatocytes, as well as in the nuclei of Sertoli cells. In adult *rats*, ERα was detected in the entire testicular tissue, especially in germ cells, particularly in the cytoplasm of round spermatids and spermatocytes, as well as in Leydig cell nuclei (Pelletier et al., 2000). Lucas et al. (2008) demonstrated positive ERα labeling in Sertoli and Leydig cell nuclei, and in some peritubular myoid cells of 15-day-old rats; however, the germ cells were reported as negative. In adult rats, the same group of authors described ERα positivity only in Sertoli cells and some spermatids. ERβ was localized in the nuclei of cells on the pe-

riphery of rat seminiferous tubules, likely in Sertoli cells (Pelletier et al., 2000). Lucas et al. (2008) documented ERβ receptor to be positive in Sertoli and Leydig cells and in some germ cells.

In *stallion*, ERα was found in germ and Leydig cells in pre-pubertal and post-pubertal individuals, and in Sertoli cells only in immature animals (Pearl et al., 2011). ERβ was localized in both Leydig and Sertoli cells in stallions of all age categories, in pre-pubertal animals also in germ cells. This receptor was missing in peritubular myoid cells (Pearl et al., 2011).

In *pigs*, there are very controversial results of the ER occurrence in the cells of testicular tissue and germ cells. Rago et al. (2004) found ERα to be negative in somatic and germ cells in non-adult boars (3 months of age). In adult animals (18 months of age), a positive signal of ERα was shown in some germ cells – weak in the spermatogonia, strong in spermatocytes, but negative in spermatids. Sertoli cells were also ERα negative; however, Leydig cells appeared to be slightly ERα positive (Rago et al., 2004). On the contrary, Gunawan et al. (2011) reported a strongly positive finding of ERα in Sertoli cell cytoplasm; a weak signal was detected in germ cells and Leydig cells. Another group of researchers (Mutembei et al., 2005) detected a prominent ERα signal in the spermatogonia and primary spermatocytes, weaker in other germ cells and in Leydig cells, and negative in the spermatozoa of adult boars. In immature animals, the spermatogonia and Leydig and Sertoli cells appeared to be negative. Ramesh et al. (2007) documented the presence of ERα in Sertoli cells of pre-pubertal boars. ERα was present in interstitial testicular cells at the same age of boars. Germ cells were slightly positive for ERα in all age categories, starting at three months of age (Ramesh et al., 2007).

The presence of ERβ in pre-pubertal boars was found in spermatogonia and Leydig cells; a negative or very weak positive signal was documented in Sertoli cells. In adult boars, the positivity was found at different stages of germ cells; a strong signal was detected in peritubular myoid cells, but Sertoli and Leydig cells were negative for the ERβ staining (Rago et al., 2004). Mutembei et al. (2005) detected the ERβ receptor in pre-pubertal boars only in spermatogonia; the Sertoli cells were negative. In adult animals, germ cells were positive for ERβ, with the exception of prolonged spermatozoa, as well as Sertoli and Leydig cells (Mutembei et al., 2005). Gunawan et al. (2012) found the ERβ antibody staining to be significantly positive not only in spermatogonia, primary spermatocytes, and spermatids, but also in the cytoplasm of Sertoli and Leydig cells. According to Ramesh et al. (2007), the rate of ERβ expression in Sertoli cells decreased with the growing age of boars. In interstitial cells, the incidence of ERβ was constant and independent of age. Germ cells were also slightly positive for ERβ in all age groups (Ramesh et al., 2007).

Detection of ERα and ERβ in the epididymis. ERα and ERβ have been identified in the epididymis of many mammalian species (Hess, Carnes, 2004). Changes in the concentration of steroids in the epididymis are related to animal age (Hess, 2003). Differences in the ER localization in various regions of the epididymis indicate the dynamic role of estrogens in the epididymal function and development (Parlevliet et al., 2006).

In *human*, Saunders et al. (2001) reported only a low presence of ERα in both the major and basal cells within the epididymal tube; ERβ was found in the nucleus of the epididymal epithelial cells and stromal cells.

In *rat*, ERα was detected in the nucleus as well as in the cytoplasm of epithelial cells of the epididymis (Zaya et al., 2012). Antibody staining was positive in both the nuclei and cytoplasm of epithelial cells of all segments of the adult epididymis. ERβ was found in the same epididymal regions.

In *mice*, ERα was present in epithelial cells especially in the caput, and more weakly in other parts of the epididymis (Zhou et al., 2002). Antibody labeling of ERβ was proved in epithelial cells of all three regions (head, body, and tail) of the epididymis (Zhou et al., 2002).

In *stallion*, Parlevliet et al. (2006) found varying presence of ERα using antibody detection in the epididymis depending on the age of animals and the epididymal part. In pre-pubertal animals, ERα was detected in the caput and corpus, but not in the cauda epididymis. In pubertal stallions, the occurrence of ERα was pronounced in all parts of the epididymis (including high levels of 17β-estradiol), indicating the essential role of this receptor in the testes during adolescence. In adult animals, ERα was shown in the cells of caput epididymis; in corpus and cauda epididymal tissues it was found in approximately 50% of the cells. According to Parlevliet et al. (2006), ERβ was present in the cells of the whole epididymis regardless of animal age.

In *boars*, Gunawan et al. (2011) detected ERα mainly in the caput and corpus epididymis, with lower intensity in the corpus part. In addition, Pearl et al. (2007a) reported the presence of ERα detected by antibodies mainly in the cells of caput tissue, less in the corpus. It is assumed that ERα plays an important role in the fluid reabsorption in this tissue (Gunawan et al., 2011). Pearl et al. (2007b) have shown the occurrence of ERα and ERβ in all parts of the boar epididymis. ERβ was localized in the main and basal cells of all three parts of the epididymis (Pearl et al., 2007a). Gunawan et al. (2012) documented the presence of ERβ in epithelial cells on histological sections of all parts of the boar epididymis. Carpino et al. (2004a) provided similar data in their work. In adult boars (18 months of age), anti-ERβ antibody stained the cells in all parts of the epididymis, but

in pre-pubertal boars at 2 months of age, positive antibody reaction was only very weak in epithelial cells of the epididymis, whereas the caput and cauda were negative.

ERα and ERβ in ejaculated spermatozoa. In recent years, expression of nuclear steroid receptors has opened new perspectives relating to the hormonal effect on sperm physiology (Aquila, De Amicis, 2014). A positive finding of ERs in sperm could mean that steroid hormones may affect the functional properties of spermatozoa (Kotula-Balak et al., 2012). Mature spermatozoa are considered transcriptionally inactive cells and able to translate the synthesized mRNA (Gur, Breitbart, 2015); therefore, it may be assumed that non-genomic rapid effects of estrogen occur in mature sperm (Dostalova et al., 2017).

Expression of ERα and ERβ (mRNA and protein) in *human* ejaculated sperm (Aquila et al., 2004) suggests that estrogens are able to modulate the spermatogenesis process starting from the inside of the testes to the sperm maturation after ejaculation (Aquila et al., 2004; Rochira et al., 2005; Carreau et al., 2011; Aquila, De Amicis, 2014). Data regarding the ER localization in ejaculated sperm are controversial in the literature. ERs are mainly observed in different parts of the sperm, but sometimes the regions of their coincidence overlap. In human sperm, Aquila et al. (2004) observed the ERα presence especially in the midpiece of the sperm using immunofluorescence. Rago et al. (2006) detected ERα in mature sperm only in the midpiece. For immature ejaculated sperm, this receptor was found only in the residual cytoplasm (Rago et al., 2006). Guido et al. (2011) indicated the presence of ERα only in the midpiece of spermatozoa. Solakidi et al. (2005) identified ERα in the area corresponding to the equatorial segment of the sperm head, the midpiece was negative, and only weak diffuse staining was observed in the flagellum.

In *human*, ERβ was detected by immunofluorescence only in the sperm flagellum, with predominant distribution in its proximal part (Aquila et al., 2004). According to these authors, ERα and ERβ receptors overlap in the proximal part of the flagellum. Rago et al. (2006) described the presence of ERβ in the entire tail of mature human sperm. In immature ejaculated spermatozoa, both ERβ and ERα were found in the residual cytoplasm, but also in the flagellum. However, quite different data may also be found in the literature. Lambard et al. (2004) identified ERβ mRNA in mature human sperm, but the ERβ protein was not found. Guido et al. (2011) described the ERβ presence in the middle part and in the flagellum. Solakidi et al. (2005) localized ERβ in the central part of the mitochondrial region and more weakly in the flagellum.

In *stallion*, localization of the ERα receptor in ejaculated sperm was detected by transmission electron microscopy (TEM) in the head, midpiece, and flagellum near the cell membrane (Arkoun et al., 2014). ERβ was also found in the sperm flagellum and post-acrosomal ring by immunofluorescent microscopy. Using the TEM method, this receptor was precisely localized in the sperm head, midpiece, and flagellum, associated with the plasma membrane (Arkoun et al., 2014).

In *boar* ejaculated sperm, only Rago et al. (2007) have studied the ER localization. Their report described both types of nuclear ERs. The ERα receptor was detected in the mitochondrial part of the sperm flagellum. ERα expression could be related to estrogen regulation of sperm motility in the boars, as demonstrated in human and rat spermatozoa. The ERβ receptor was localized in the sperm acrosome region (Rago et al., 2007).

Estrogen receptor GPER

The G protein-coupled estrogen receptor (GPER/GPR30) is a 7-transmembrane receptor (Prossnitz et al., 2007; 2008) encoded by the *GPER1* gene (Otto et al., 2008). The GPER-binding domain was found on both the plasma membrane and intracellular membranes such as the endoplasmic reticulum and the Golgi apparatus (Filardo et al., 2007). Some authors consider this receptor to be primarily intracellularly distributed (Revankar et al., 2005); others even mention it as strictly cytosolic (Otto et al., 2008). Its intracellular localization corresponds to the assumption of permeability of ligands across the plasma membrane (Prossnitz et al., 2007; 2008). It is structurally unrelated to classic estrogen receptors ERα and ERβ (Wang et al., 2014). It is believed that the biological function of GPER could be associated with the type of cells and tissues in which it is found. Estradiol, as the predominant type of estrogen, binds to GPER with high affinity (Prossnitz et al., 2008) and mediates rapid effects, including the kinase activation, mobilization of intracellular calcium, and stimulation of intracellular cAMP (Cyclic adenosine monophosphate) production (Smith et al., 2016). It is considered to be a receptor involved only in the non-genomic signaling of estrogen action without the involvement of transcriptional mechanisms (Gruber et al., 2002).

Localization of GPER in the testis and epididymis. Data on the presence of estrogen receptor GPER/GPR30 in both the testes and epididymal tissue is relatively limited and often very controversial. Published results on the GPER localization in the male reproductive organs and sperm cells of representative animal species are summarized in the Table 3.

In the germ cells of adult male testis in *human*, GPER was found slightly expressed (Franco et al., 2011), or clearly identified (Chevalier et al., 2012). Rago et al. (2011) described Leydig and Sertoli cells as positive for GPER, but germ cells were negative. Fietz et al. (2014) demonstrated positive staining

Table 3. Detection of G protein-coupled estrogen receptor in testicular and epididymal tissues, germ cells and spermatozoa in selected mammalian species

	Human	Mouse	Rat	Stallion	Boar
Testicular interstitial tissue/ myoid cells	N.D.	N.D.	N.D.	N.D.	N.D.
Leydig cells	+ (5, 18, 28)	– (32)	– (13, 14)	N.D.	N.D.
Sertoli cells	+ (5, 18, 28), + w (6)	– (32)	+ (13, 14)	N.D.	N.D.
Germ cells	+ (5), + w (6), + SC (18), – (28)	+ (32)	– (13, 14)	N.D.	N.D.
E1 epithelial cells	N.D.	N.D.	+ ad w (15), – im (15)	N.D.	N.D.
E2 epithelial cells	N.D.	N.D.	+ ad (15), + w im (15)	N.D.	N.D.
E3 epithelial cells	N.D.	N.D.	+ ad (15), + im (15)	N.D.	+ im (11)
E1 sperm in lumen	N.D.	N.D.	N.D.	N.D.	N.D.
E2 sperm in lumen	N.D.	N.D.	N.D.	N.D.	N.D.
E3 sperm in lumen	N.D.	N.D.	N.D.	N.D.	N.D.
Ejaculated sperm (im)		N.D.	N.D.		
Ejaculated sperm (m) Head	– (29)	N.D.	N.D.	+ (7), – (2)	+ ES, AR (29), – N (29)
Ejaculated sperm (m) Neck	– (29)	N.D.	N.D.	+ (2, 7)	– (29)
Ejaculated sperm (m) Midpiece	+ (29)	N.D.	N.D.	+ (2, 7)	+ (29)
Ejaculated sperm (m) Flagellum	– (29)	N.D.	N.D.	+ (7), – (2)	– (29)

E1 = caput epididymis, E2 = corpus epididymis, E3 = cauda epididymis, w = weak, SC = some cells, ad = adult animals, im = immature animals or spermatozoa, m = mature animals or spermatozoa, N.D. = not described, ES = equatorial segment, AR = acrosomal region, N = nucleus; (2) Arkoun et al., 2014; (5) Chevalier et al., 2012; (6) Fietz et al., 2014; (7) Gautier et al., 2016; (11) Katleba et al., 2015; (13) Lucas et al., 2010; (14) Lucas et al., 2011; (15) Martínez-Traverso, Pearl, 2015; (18) Oliveira et al., 2014; (28) Rago et al., 2011; (29) Rago et al., 2014; (32) Siriani et al., 2008

in Sertoli cells, whereas germ cells were labelled very weakly. Oliveira et al. (2014) showed the distribution of GPER in the Leydig and Sertoli cell cytoplasm, but also in spermatogonia. In *rodent* testis, the presence of GPER has been detected in Leydig cells (Vaucher et al., 2014), Sertoli cells (Lucas et al., 2010, 2011), spermatocytes, and round spermatids of rats (Siriani et al., 2008; Chimento et al., 2010b; Lucas et al., 2010). The presence of GPER in germ cells of the testicles has been demonstrated in mice (Siriani et al., 2008).

In adult *rat* epididymis, the positivity for GPER was found mainly in the corpus and cauda parts, weaker in the caput epididymis (Martínez-Traverso, Pearl, 2015). Cao et al. (2016) detected the presence of GPER in tissue sections of cauda epididymis, especially in epithelial cells, in the juvenile rat by immunofluorescence. In *pigs* the findings of membrane-bound GPER expression in the tissues of male reproductive organs are limited to the epididymis in postnatal boars. Positive labelling was found in epithelial cells of epididymal tubules, and weaker in stroma and peritubular myoid cells (Katleba et al., 2015).

GPER in ejaculated spermatozoa. The presence of GPER in ejaculated sperm has so far been studied by only a limited number of researchers. Using antibody detection, Rago et al. (2014) found the membrane estrogen receptor GPER both in *human* and *boar* ejaculated sperm. This is probably the first report on the positive finding of GPER in the mature mammalian sperm. In ejaculated *human* sperm, the GPER receptor was located in the midpiece, but in the head and principle part of the flagellum the staining was negative (Rago et al., 2014). In *boar* sperm, GPER was localized in the acrosomal region and equatorial segment of the head, and in the mitochondrial part of the sperm cell with a permeabilized plasma membrane. No immunofluorescence was detected in the nucleus (Rago et al., 2014). In ejaculated sperm of *stallion*, GPER was found in the midpiece, neck, flagellum, and

head by the subcellular localization method (Gautier et al., 2016). Arkoun et al. (2014) detected this receptor in the connecting piece of the sperm.

CONCLUSION

The available literature suggests that the results on the detection and localization of estrogen receptors in the male reproductive organs and mammalian spermatozoa have not yet been verified and fully described. The ER distribution has not been determined across the different stages of development of spermatozoa by a uniform methodology, and particularly in the case of GPER, the findings may be considered incomplete.

In studies of mammalian infertility, and consequently of potential therapy, estrogen receptors have become one of the research objectives. The absence of estrogen receptors in reproductive tissues results in negative effects on spermatogenesis, hormone production, seminal sperm content, motility, and fertility.

REFERENCES

Acconcia F, Kumar R (2006): Signaling regulation of genomic and nongenomic functions of estrogen receptors. Cancer Letters, 238, 1–14. doi: 10.1016/j.canlet.2005.06.018.

Acconcia F, Bocedi A, Ascenzi P, Marino M (2003): Does palmitoylation target estrogen receptors to plasma membrane caveolae? Life, 55, 33–35. doi: 10.1080/1521654031000081256.

Aquila S, De Amicis F (2014): Steroid receptors and their ligands: Effect on male gamete functions. Experimental Cell Research, 328, 303–313. doi: 10.1016/j.yexcr.2014.07.015.

Aquila S, Sisci D, Gentile M, Middea E, Catalano S, Carpino A, Rago V, Ando S (2004): Estrogen receptor (ER) α and ERβ are both expressed in human ejaculated spermatozoa: evidence of their direct interaction with phosphatidylinositol-3-OH kinase/Akt pathway. The Journal of Clinical Endocrinology and Metabolism, 89, 1443–1451. doi: 10.1210/jc.2003-031681.

Arkoun B, Gautier G, Delalande C, Barrier-Battut I, Guenon I, Goux D, Bouraima-Lelong H (2014): Stallion spermatozoa: putative target of estrogens; presence of the estrogen receptors ESR1, ESR2 and identification of the estrogen-membrane receptor GPER. General and Comparative Endocrinology, 200, 35–43. doi: 10.1016/j.ygcen.2014.02.016.

Beato M, Chavez S, Truss M (1996): Transcriptional regulation by steroid hormones. Steroids, 61, 240–251. doi: 10.1016/0039-128X(96)00030-X.

Cao X, Huang J, Zhang G, Zuo W, Chongfeng L, Sun Q, Yang D, Gao D, Cheng CHK, Zhou WL (2016): Functional expression of G protein-coupled receptor 30 in immature rat epididymal epithelium. Cell Biology International, 41, 134–146. doi: 10.1002/cbin.10709.

Carpino A, Bilinska B, Siciliano L, Maggiolini M, Rago V (2004a): Immunolocalization of estrogen receptor beta in the epididymis of mature and immature pigs. Folia Histochemica et Cytobiologica, 42, 13–17.

Carpino A, Romeo F, Rago V (2004b): Aromatase immunolocalisation in human ductuli efferentes and proximal ductus epididymis. Journal of Anatomy, 204, 217–220.

Carreau S, Hess RA (2010): Oestrogens and spermatogenesis. Philosophical Transactions of the Royal Society B, 365, 1517–1535. doi: 10.1098/rstb.2009.0235.

Carreau S, Bouraima-Lelong H, Delalande C (2011): Estrogens in male germ cells. Spermatogenesis, 1, 90–94. doi: 10.4161/spmg.1.2.16766.

Chevalier N, Vega A, Bouskine A, Siddeek B, Michiels JF, Chevallier D, Fenichel P (2012): GPR30, the non-classical membrane G protein related estrogen receptor, is overexpressed in human seminoma and promotes seminoma cell proliferation. PLoS ONE, 7, e34672. doi: 10.1371/journal.pone.0034672.

Chimento A, Sirianni R, Delalande C, Silandre D, Bois C, Ando S, Maggiolini M, Carreau S, Pezzi V (2010a): 17 beta-estradiol activates rapid signaling pathways involved in rat pachytene spermatocytes apoptosis through GPR30 and ERα. Molecular and Cellular Endocrinology, 320, 136–144. doi: 10.1016/j.mce.2010.01.035.

Chimento A, Sirianni R, Zolea F, Bois C, Delalande C, Ando S, Maggiolini M, Aquila S, Carreau S, Pezzi V (2010b): GPER and ESRs are expressed in rat round spermatids and mediate oestrogen-dependent rapid pathways modulating expression of cyclin B1 and Bax. International Journal of Andrology, 34, 420–429. doi: 10.1111/j.1365-2605.2010.01100.x.

Dostalova P, Zatecka E, Dvorakova-Hortova K (2017): Of oestrogen and sperm: a review of the roles of oestrogens and oestrogen receptors in male reproduction. International Journal of Molecular Sciences, 18: 904. doi: 10.3390/ijms18050904.

Eddy E, Washburn T, Bunch D, Goulding E, Gladen B, Lubahn D, Korach K (1996): Targeted disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility. Endocrinology, 137, 4796–4805. doi: 10.1210/endo.137.11.8895349.

Fietz D, Ratzenbock C, Hartmann K, Raabe O, Kliesch S, Weidner W, Klug J, Bergmann M (2014): Expression pattern of estrogen receptors α and β and G-protein-coupled estrogen receptor 1 in the human testis. Histochemistry and Cell Biology, 142, 421–432. doi: 10.1007/s00418-014-1216-z.

Filardo EJ, Quinn J, Pang Y, Graeber C, Shaw S, Dong J, Thomas P (2007): Activation of the novel estrogen receptor G protein-coupled receptor 30 (GPR30) at the plasma membrane. Endocrinology, 148, 3236–3245. doi: 10.1210/en.2006-1605.

Franca LR, Avelar GF, Almeida FFL (2005):Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology, 63, 300–318. doi: 10.1016/j.theriogenology.2004.09.014.

Franco R, Boscia F, Gigantino V, Marra L, Esposito F, Ferrara D, Pariante P, Botti G, Caraglia M, Minucci S, Chieffi P (2011): GPR30 is overexpressed in post-pubertal testicular germ cell tumors. Cancer Biology and Therapy, 11, 609–613.

Gautier C, Barrier-Battut I, Guenon I, Goux D, Delalande C, Bouraima-Lelong H (2016): Implication of the estrogen receptors GPER, ESR1, ESR2 in post-testicular maturations of equine spermatozoa. General and Comparative Endocrinology, 233, 100–108. doi: 10.1016/j.ygcen.2016.05.022.

Grober OMV, Mutarelli M, Giurato G, Ravo M, Cicatiello L, De Filippo MR, Ferrato L, Nassa G, Papa MF, Paris O, Tarallo R, Luo S, Schroth GP, Benes V, Weisz A (2011): Global analysis of estrogen receptor beta binding to breast cancer cell genome reveals an extensive interplay with estrogen receptor alpha for target gene regulation. BMC Genomics, 12: 36. doi: 10.1186/1471-2164-12-36.

Gruber CJ, Tschugguel W, Schneeberger C, Huber JC (2002): Production and actions of estrogens. The New England Journal of Medicine, 346, 340–352. doi: 10.1056/NEJMra000471.

Guido C, Perrotta I, Panza S, Middea E, Avena P, Santoro M, Marsico S, Imbrogno P, Ando S, Aquila S (2011): Human sperm physiology: estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) influence sperm metabolism and may be involved in the pathophysiology of varicocele-associated male infertility. Journal of Cellular Physiology, 226, 3403–3412. doi: 10.1002/jcp.22703.

Gunawan A, Kaewmala K, Uddin MJ, Cinar MU, Tesfaye D, Phatsara C, Tholen E, Looft C, Schellander K (2011): Association study and expression analysis of porcine ESR1 as a candidate gene for boar fertility and sperm quality. Animal Reproduction Science, 128, 11–21. doi: 10.1016/j.anireprosci.2011.08.008.

Gunawan A, Cinar MU, Uddin MJ, Kaewmala K, Tesfaye D, Phatsara C, Tholen E, Looft C, Schellander K (2012): Investigation on association and expression of ESR2 as a candidate gene for boar sperm quality and fertility. Reproduction in Domestic Animals, 47, 782–790. doi: 10.1111/j.1439-0531.2011.01968.x.

Gur Y, Breitbart H (2015): Mammalian sperm translate nuclear-encoded proteins by mitochondrial-type ribosomes. Genes and Development, 20, 411–416. doi: 10.1101/gad.367606.

Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Strom A, Treuter E, Warner M, Gustafsson JA (2007): Estrogen receptors: how do they signal and what are their targets. Physiological Reviews, 87, 905–931. doi: 10.1152/physrev.00026.2006.

Hess RA (2003): Estrogen in the adult male reproductive tract: a review. Reproductive Biology and Endocrinology, 1: 52. doi: 10.1186/1477-7827-1-52.

Hess RA, Carnes K (2004): The role of estrogen in testis and the male reproductive tract: a review and species comparison. Animal Reproduction, 1, 5–30.

Hess RA, Bunick D, Bahr J (2001): Oestrogen, its receptors and function in the male reproductive tract – a review. Molecular and Cellular Endocrinology, 178, 29–38. doi: 10.1016/S0303-7207(01)00412-9.

Katleba KD, Legacki EL, Conley AJ, Berger T (2015): Steroid regulation of early postnatal development in the corpus epididymidis of pigs. Journal of Endocrinology, 225, 125–134. doi: 10.1530/JOE-15-0001.

Kotula-Balak M, Hejmej A, Lydka M, Cierpich A, Bilinska B (2012): Detection of aromatase, androgen, and estrogen receptors in bank vole spermatozoa. Theriogenology, 78, 385–392. doi: 10.1016/j.theriogenology.2012.02.018.

Kulkoyluoglu E, Madak-Erdogan Z (2016): Nuclear and extranuclear-initiated estrogen receptor signaling crosstalk and endocrine resistance in breast cancer. A review. Steroids, 114, 41–47. doi: 10.1016/j.steroids.2016.06.007.

Kumar P, Wu Q, Chambliss KL, Yuhanna IS, Mumby SM, Mineo C, Tall GG, Shaul PW (2007): Direct interactions with Gαi and Gβγ mediate nongenomic signaling by estrogen receptor α. Molecular Endocrinology, 21, 1370–1380. doi: 10.1210/me.2006-0360.

Kumar R, Zakharov MN, Khan SH, Miki R, Jang H, Toraldo G, Singh R, Bhasin S, Jasuja R (2011): The dynamic structure of the estrogen receptor. Journal of Amino Acids, 2011, 1–7. doi: 10.4061/2011/812540.

Lambard S, Galeraud-Denis I, Saunders PTK, Carreau S (2004): Human immature germ cells and ejaculated spermatozoa contain aromatase and oestrogen receptors. Journal of Molecular Endocrinology, 32, 279–289. doi: 10.1677/jme.0.0320279.

Levin ER (2009): Plasma membrane estrogen receptors. A review. Trends in Endocrinology and Metabolism, 20, 477–482. doi: 10.1016/j.tem.2009.06.009.

Lombardi G, Zarrilli S, Colao A, Paesano L, Di Somma C, Rossi F, De Rosa M (2001): Estrogens and health in males. Molecular and Cellular Endocrinology, 178, 51–55. doi: 10.1016/S0303-7207(01)00420-8.

Lucas TFG, Siu ER, Esteves CA, Monteiro HP, Oliveira CA, Porto CS, Lazari MFM (2008): 17beta-estradiol induces the translocation of the estrogen receptors ESR1 and ESR2 to the cell membrane, MAPK3/1 phosphorylation and proliferation of cultured immature rat Sertoli cells. Biology of Reproduction, 78, 101–114. doi: 10.1095/biolreprod.107.063909.

Lucas TF, Royer C, Siu ER, Lazari MFM, Porto CS (2010): Expression and signaling of G protein-coupled estrogen receptor 1 (GPER) in rat Sertoli cells. Biology of Reproduction, 83, 307–317. doi: 10.1095/biolreprod.110.084160.

Lucas TF, Pimenta MT, Pisolato R, Lazari MF, Porto CS (2011): 17beta-estradiol signaling and regulation of Sertoli cell function. Spermatogenesis, 1, 318–324. doi: 10.4161/spmg.1.4.18903.

Luconi M, Bonaccorsi L, Forti G, Baldi E (2001): Effects of estrogenic compounds on human spermatozoa: evidence for interaction with a nongenomic receptor for estrogen on human sperm membrane. Molecular and Cellular Endocrinology, 178, 39–45. doi: 10.1016/S0303-7207(01)00416-6.

Luconi M, Forti G, Baldi E (2002): Genomic and nongenomic effects of estrogens: molecular mechanisms of action and clinical implications for male reproduction. A review. Journal of Steroid Biochemistry and Molecular Biology, 80, 369–381. doi: 10.1016/S0960-0760(02)00041-9.

Lukoseviciute K, Zilinskas H, Januskauskas A (2005): The effect of oestradiol, progesterone and heparin on bovine spermatozoa function after thawing. Reproduction of Domestic Animals, 40, 100–107. doi: 10.1111/j.1439-0531.2004.00559.x.

Makinen S, Makela S, Weihua Z, Warner M, Rosenlund B, Salmi S, Hovatta O, Gustafsson JA (2001): Localization of oestrogen receptors alpha and beta in human testis. Molecular Human Reproduction, 7, 497–503. doi: 10.1093/molehr/7.6.497.

Martínez-Traverso GB, Pearl CA (2015): Immunolocalization of G protein-coupled estrogen receptor in the rat epididymis. Reproductive Biology and Endocrinology, 13: 48. doi: 10.1186/s12958-015-0042-z.

Mutembei HM, 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. Reproduction in Domestic Animals, 40, 228–236. doi: 10.1111/j.1439-0531.2005.00586.x.

Ogawa S, Inoue S, Watanabe T, Hiroi H, Orimo A, Hosoi T, Ouchi Y, Muramatsu M (1998): The complete primary structure of human estrogen receptor beta (hER beta) and its heterodimerization with ER alpha *in vivo* and *in vitro*. Biochemical and Biophysical Research Communications, 243, 122–126. doi: 10.1006/bbrc.1997.7893.

Oliveira PF, Alves MG, Martins AD, Correia S, Bernardino RL, Silva J, Barros A, Sousa M, Cavaco JE, Socorro S (2014): Expression pattern of G protein-coupled receptor 30 in human seminiferous tubular cells. General and Comparative Endocrinology, 201, 16–20. doi: 10.1016/j.ygcen.2014.02.022.

Otto C, Rohde-Schulz B, Schwarz G, Fuchs I, Klewer M, Brittain D, Langer G, Bader B, Prelle K, Nubbemeyer R, Fritzemeier KH (2008): G protein-coupled receptor 30 localizes to the endoplasmic reticulum and is not activated by estradiol. Endocrinology, 149, 4846–4856. doi: 10.1210/en.2008-0269.

Parlevliet JM, Pearl CA, Hess MF, Famula TR, Roser JF (2006): Immunolocalization of estrogen and androgen receptors and steroid concentrations in the stallion epididymis. Theriogenology, 66, 755–765. doi: 10.1016/j.theriogenology.2005.12.013.

Pearl CA, Berger T, Roser JF (2007a): Estrogen and androgen receptor expression in relation to steroid concentrations in the adult boar epididymis. Domestic Animal Endocrinology, 33, 451–459. doi: 10.1016/j.domaniend.2006.09.003.

Pearl CA, At-Taras E, Berger T, Roser JF (2007b): Reduced endogenous estrogen delays epididymal development but has no effect on efferent duct morphology in boars. Reproduction, 134, 593–604. doi: 10.1530/REP-06-0239.

Pearl CA, Mason H, Roser JF (2011): Immunolocalization of estrogen receptor alpha, estrogen receptor beta and androgen receptor in the pre-, peri- and post-pubertal stallion testis. Animal Reproduction Science, 125, 103–111. doi: 10.1016/j.anireprosci.2011.03.007.

Pedram A, Razandi M, Sainson RCA, Kim JK, Hughes CC, Levin ER (2007): A conserved mechanism for steroid receptor translocation to the plasma membrane. The Journal of Biological Chemistry, 282, 22278–22288. doi: 10.1074/jbc.M611877200.

Pedram A, Razandi M, Deschenes RJ, Levin ER (2012): DHHC-7 and -21 are palmitoylacyltransferases for sex steroid receptors. Molecular Biology of the Cell, 23, 188–199. doi: 10.1091/mbc.E11-07-0638.

Pelletier G, El-Alfy M (2000): Immunocytochemical localization of estrogen receptors alpha and beta in the human reproductive organs. The Journal of Clinical Endocrinology and Metabolism, 85, 4835–4840. doi: 10.1210/jcem.85.12.7029.

Pelletier G, Labrie C, Labrie F (2000): Localization of oestrogen receptor alpha, oestrogen receptor beta and androgen receptors in the rat reproductive organs. Journal of Endocrinology, 165, 359–370. doi: 10.1677/joe.0.1650359.

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*. The Journal of Clinical Endocrinology and Metabolism, 85, 2057–2067. doi: 10.1210/jc.85.5.2057.

Prelusky DB (1994): Residues in food products of animal origin. In: Miller JD, Trenholm HL (eds): Mycotoxins in grain: compounds other than aflatoxins. Eagen Press, St. Paul, 405–419.

Prossnitz ER, Arterburn JB, Sklar LA (2007): GPR30: a G protein-coupled receptor for estrogen. Molecular and Cellular Endocrinology, 265–266, 138–142. doi: 10.1016/j.mce.2006.12.010.

Prossnitz ER, Oprea TI, Sklar LA, Arterburn JB (2008): The ins and outs of GPR30: a transmembrane estrogen receptor. Journal of Steroid Biochemistry and Molecular Biology, 109, 350–353. doi: 10.1016/j.jsbmb.2008.03.006.

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. The Anatomical Record Part A, 281A, 1234–1239. doi: 10.1002/ar.a.20131.

Rago V, Siciliano L, Aquila S, Carpino A (2006): Detection of estrogen receptors ER-alpha and ER-beta in human ejaculated immature spermatozoa with excess residual cytoplasm. Reproductive Biology and Endocrinology, 4: 36. doi: 10.1186/1477-7827-4-36.

Rago V, Aquila S, Panza R, Carpino A (2007): Cytochrome P450arom, androgen and estrogen receptors in pig sperm. Reproductive Biology and Endocrinology, 5, 23–29. doi: 10.1186/1477-7827-5-23.

Rago V, Romeo F, Giordano F, Maggiolini M, Carpino A (2011): Identification of the estrogen receptor GPER in neoplastic and non-neoplastic human testes. Reproductive Biology and Endocrinology, 9: 135. doi: 10.1186/1477-7827-9-135.

Rago V, Giordano F, Brunelli E, Zito D, Aquila S, Carpino A (2014): Identification of G protein-coupled estrogen receptor in human and pig spermatozoa. Journal of Anatomy, 224, 732–736. doi: 10.1111/joa.12183.

Ramesh R, Pearl CA, At-Taras E, Roser JF, Berger T (2007): Ontogeny of androgen and estrogen receptor expression in porcine testis: effect of reducing testicular estrogen synthesis. Animal Reproduction Science, 102, 286–299. doi: 10.1016/j.anireprosci.2006.10.025.

Razandi M, Oh P, Pedram A, Schnitzer J, Levin ER (2002): ERs associate with and regulate the production of caveolin: implications for signaling and cellular actions. Molecular Endocrinology, 16, 100–115. doi: 10.1210/mend.16.1.0757.

Razandi M, Pedram A, Merchenthaler I, Greene GL, Levin ER (2004): Plasma membrane estrogen receptors exist and functions as dimers. Molecular Endocrinology, 18, 2854–2865. doi: 10.1210/me.2004-0115.

Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER (2005): A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science, 307, 1625–1630. doi: 10.1126/science.1106943.

Rochira V, Granata AR, Madeo B, Zirilli L, Rossi G, Carani C (2005): Estrogens in males: what have we learned in the last 10 years? Asian Journal of Andrology, 7, 3–20. doi: 10.1111/j.1745-7262.2005.00018.x.

Saunders PTK, Sharpe RM, Williams K, Macpherson S, Urquart H, Irvine DS, Millar R (2001): Differential expression of oestrogen receptor α and β proteins in the testes and male reproductive system of human and non-human primates. Molecular Human Reproduction, 7, 227–236. doi: 10.1093/molehr/7.3.227.

Shemes M, Shore LS (2012): Effects of environmental estrogens on reproductive parameters in domestic animals. Israel Journal of Veterinary Medicine, 67, 6–10.

Siriani R, Chimento A, Ruggiero C, De Luca A, Lappano R, Ando S, Maggiolini M, Pezzi V (2008): The novel estrogen receptor, G protein-coupled receptor 30, mediates the proliferative effects induced by 17beta-estradiol on mouse spermatogonial GC-1 cell line. Endocrinology, 149, 5043–5051. doi: 10.1210/en.2007-1593.

Smith LC, Ralston-Hooper KJ, Ferguson PL, Sabo-Attwood T (2016): The G protein-coupled estrogen receptor agonist G-1 inhibits nuclear estrogen receptor activity and stimulates novel phosphoproteomic signatures. Toxicological Sciences, 151, 434–446. doi: 10.1093/toxsci/kfw057.

Solakidi S, Psarra AMG, Nikolaropoulos S, Sekeris CE (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. Human Reproduction, 20, 3481–3487. doi: 10.1093/humrep/dei267.

Vaucher L, Funaro MG, Mehta A, Mielnik A, Bolyakov A, Prossnitz ER, Schlegel PN, Paduch DA (2014): Activation of GPER-1 estradiol receptor downregulates production of testosterone in isolated rat Leydig cells and adult human testis. PLoS ONE, 9, e92425. doi: 10.1371/journal.pone.0092425.

Wang C, Liu Y, Cao JM (2014): G protein-coupled receptors: extranuclear mediators for the non-genomic actions of steroids. International Journal of Molecular Sciences, 15, 15412–15425. doi: 10.3390/ijms150915412.

Whiting KP, Restall CJ, Brain PF (2000): Steroid hormone-induced effects on membrane fluidity and their potential roles in non-genomic mechanisms. Life Sciences, 67, 743–757.

Zaya R, Hennick C, Pearl CA (2012): *In vitro* expression of androgen and estrogen receptors in prepubertal and adult rat epididymis. General and Comparative Endocrinology, 178, 573–586. doi: 10.1016/j.ygcen.2012.07.004.

Zhao C, Dahlman-Wright K, Gustafsson JA (2008): Estrogen receptor β: an overview and update. Nuclear Receptor Signaling, 6: e003. doi: 10.1621/nrs.06003.

Zhou Q, Nie R, Prins GS, Saunders PTK, Katzenellenbogen BS, Hess RA (2002): Localization of androgen and estrogen receptors in adult male mouse reproductive tract. Journal of Andrology, 23, 870–881.

Corresponding Author:

RNDr. Pavla P o s t l e r o v á, Ph.D., Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Veterinary Sciences, Kamýcká 129, 165 00 Prague 6-Suchdol, Czech Republic, phone: +420 224 382 934, e-mail: postlerova@af.czu.cz