Effect of estrogen receptor-subtype-specific ligands on fertility in adult male rats

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Abstract

Maintenance of normal male fertility relies on the process of spermatogenesis which is under complex endocrine control by mechanisms involving gonadotropin and steroid hormones. Although testosterone is the primary sex steroid in males, estrogen is locally produced in the testis and plays a very crucial role in male fertility. This is evident from presence of both the estrogen receptors alpha (ERα) and beta (ERβ) in the testis and their absence, as in the case of knockout mice models, leads to sterility. The present study was undertaken to understand individual roles of the two ERs in spermatogenesis and their direct contribution towards the maintenance of male fertility using receptor-subtype-specific ligands. Administration of ERα and β agonists to adult male rats for 60 days results in a significant decrease in fertility, mainly due to an increase in pre- and post-implantation loss and a concomitant decrease in litter size and sperm counts. Our results indicate that ERα is mainly involved in negative feedback regulation of gonadotropin hormones, whereas both ERs are involved in regulation of prolactin and testosterone production. Histological examinations of the testis reveal that ERβ could be involved in the process of spermiation since many failed spermatids were observed in stages IX–XI following ERβ agonist treatment. Our results indicate that overactivation of estrogen signaling through either of its receptors can have detrimental effects on the fertility parameters and that the two ERs have both overlapping and distinct roles in maintenance of male fertility.

Key Words

- estradiol
- estrogen receptor
- male fertility
- selective ligands

Introduction

It is well established that maintenance of normal male fertility depends on spermatogenesis, the process of proliferation and differentiation of germ cells into mature spermatids, and is under hormonal control. Although testosterone and the gonadotropin hormones follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are key players in spermatogenesis, estradiol (E2) is now recognised to play an important role in testicular physiology and spermatogenesis (Carreau et al. 2007). The importance of E2 is highlighted by the fact that E2 is produced locally in the adult testis by most of the germ cells, Sertoli cells and Leydig cells through the conversion of testosterone to E2 by the enzyme aromatase cytochrome P450 (Carreau et al. 1999).

The cellular effects of E2 are mediated through its receptors; estrogen receptor α (ERα) and β (ERβ) which belong to the steroid hormone superfamily of nuclear receptors and upon binding to their ligands act as...
transcription factors and alter the rates of transcription of E2-responsive genes (Hall et al. 2001). Although both ERα and β bind to E2 with equal affinity, they have different biological functions as is evident from their distinct expression patterns and tissue distribution. In adult rat testes, ERα is expressed in Leydig cells and in pachytene spermatocytes and round spermatids in the seminiferous epithelium (Bois et al. 2010). On the other hand, ERβ is present in most cell types in the testis like the somatic Leydig cells and Sertoli cells; and germ cells like spermatogonia, pachytene spermatocytes and round spermatids (Pelt et al. 1999). In the efferent ductules, small tubules transporting sperm from the rete testis to the caput epididymis, ERα is the predominant receptor subtype expressed (Hess et al. 1997). In other reproductive organs of rats, like the epididymis, both the ERs are detectable, whereas in prostate ERβ is the predominant form expressed (Sar & Welsch 2000).

The importance of ERs in male fertility was further highlighted by the phenotypes of the knockout mice generated. Complete estrogen receptor α knock out (ERαKO) and estrogen receptor β knock out (ERβKO) male mice generated using the Cre-loxP system have been found to be infertile. Also, ERαKO testis shows atropic and degenerating seminiferous tubules and the epididymis was found to be hypospermic, whereas no histopathological defects in the testis and epididymis were observed in ERβKO male mice despite the sterility (Antal et al. 2008, Chen et al. 2009a). Another cause of the increased attention to the role of estrogens in male reproduction can be attributed to several reports that exposure to environmental estrogens may have a detrimental effect on the reproductive health of humans and wildlife (Toppari et al. 1996). Declining semen quality, sperm counts and other reproductive tract disorders in men in the last few decades have also been suggested to be associated with exposure to environmental estrogens and endocrine-disrupting compounds (Sharpe 1993, Daston et al. 1997, Marques-Pinto & Carvalho 2013). The effect of E2 and xenoestrogen exposure during the fetal and neonatal stages has been extensively studied (reviewed by Delbes et al. (2006)) yet relatively few studies have been done to investigate the role of E2 in maintenance of male fertility in adulthood. Administration of exogenous E2 to adult male rats causes a marked decrease in fertility and a concomitant suppression of the hypothalamus–pituitary–testis (HPT) axis (Rao & Chinoy 1983, Gill-Sharma et al. 2001). However, since treatment with E2 could affect both the ERs, the reduction in fertility observed after these treatments would be the cumulative effect brought about by both the receptors. Also, many environmental xeno-estrogens have different binding affinities to the two ERs and can thus act on them differently (Kuiper et al. 1997). Hence, there is little information on the individual roles of the two ERs in spermatogenesis and their direct contribution towards the maintenance of male fertility.

The ER knockout models are not appropriate for studying the involvement of E2 in spermatogenesis in adulthood as developmental defects due to absence of the receptors in the respective knockouts cannot be excluded. In studies by Oliveira et al. (2002) the complete ER antagonist ICI 182 780 was found to impair the functioning of efferent ductules, mediated by ERα, due to which there was failure of fluid reabsorption, resulting in accumulation of fluid in the testis and disruption of spermatogenesis. Thus, the use of pure anti-estrogens or ERα antagonists to study the role of E2 in spermatogenesis would not be effective due to the primary effect on efferent ductules. Therefore, in the present study, we investigated the effects of ER-subtype-specific ligands on spermatogenesis and fertility using an adult male rat model. The ligands used in our study were 4,4′,4″-(4-Propyl-[1H] pyrazole-1,3,5-triyl) (PPT), a 410-fold selective ERα agonist (Stauffer et al. 2000), 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN), a 70-fold selective ERβ agonist (Meyers et al. 2001) and 4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol (PHTPP), a 36-fold selective ERβ antagonist (Compton et al. 2004). Our results indicate that the two ERs have both overlapping and distinct roles in maintenance of spermatogenesis and male fertility.

Materials and methods

Animals

Randomly bred adult Holtzman strain male (75 days old weighing approximately 300 g) and female (90 days old weighing approximately 250 g) rats were used for the present study. The animals were maintained under controlled temperature (22 ± 1 °C) and humidity (55 ± 5%) conditions with a 14 h light:10 h darkness cycle. The animals were supplied with a diet of soy-free, in-house prepared rat pellets and water and allowed to feed and drink ad libitum. Prior approval for the use of animals was obtained from the Institutional Animal Ethics Committee.

PPT, DPN and PHTPP treatment

PPT (>99%, Axon Medchem, Groningen, The Netherlands), DPN and PHTPP (>99%, Tocris Biosciences,
Bristol, UK) were dissolved completely in vehicle DMSO (Sigma): saline (75:25). A total of 120 male and 240 female rats were used in the study. For each drug, male rats were divided into five groups, four treatment groups each receiving one dose of the drug and one control group which received the vehicle alone. Each group had eight male rats. For PPT and DPN doses of 0.05, 0.1, 0.2, 0.4 mg/kg per day and for PHTPP doses of 0.05, 0.1, 0.4, 0.8 mg/kg per day were used. The doses were administered subcutaneously every day between 10 and 12 h for 60 days. The duration of the study was chosen to cover the entire spermatogenic cycle of the rat (of 54 days). The doses were selected on the basis of previous studies reported in literature (Santollo & Eckel 2009, Campbell et al. 2010, Santollo et al. 2010, Umar et al. 2011).

Mating studies

Control and treated rats were cohabited with normal cycling females, at a ratio of one male:two females, a week before completion of 30 and 60 days of treatment. Daily vaginal smears were taken to check for the presence of sperm. The occurrence of mating was confirmed by the presence of copulatory plug/spermatozoa in the vaginal smear/persistent diestrus (>11 days). The day when spermatozoa were seen in vaginal smears was designated as day 0 of gestation. In cases of persistent diestrus, the last estrus before the appearance of persistence diestrus was considered as day ‘0’ of gestation. The gravid females were killed between gestation days 17 and 19 and the uterus and ovaries were exposed. The numbers of live and resorbed fetuses, implantation sites (IS) and corpora lutea (CL) were noted. The following fertility parameters were calculated (Gill-Sharma et al. 1993, Balasinor et al. 2001):

\[
\% \text{ Pre-implantation loss (PIL)} = \frac{\text{No. of CL} - \text{No. of IS}}{\text{No. of CL}} \times 100
\]

\[
\% \text{ Post-implantation loss (POL)} = \frac{\text{No. of IS} - \text{No. of live fetuses}}{\text{No. of IS}} \times 100
\]

Litter size: the average number of live fetuses in one litter sired by male.

Potency: the ability of male rats to inseminate the females. It is expressed as:

\[
\text{Potency} = \frac{\text{No. of female rats inseminated}}{\text{No. of female rats exposed for mating}} \times 100
\]

Sample collection

After the mating experiments, the control and treated rats in all groups were weighed and killed by decapitation, in order to reduce stress- and anaesthesia-induced changes in the hormonal profile, and trunk blood was collected. The blood was allowed to coagulate at room temperature and serum was separated and stored at −80 °C for hormone assays. Reproductive organs like testes, epididymes, prostate, seminal vesicles, accessory glands and pituitary gland were dissected out and wet weights were recorded. Paired weights were taken for testes, epididymes, seminal vesicles and accessory glands.

Epididymal sperm counts and motility

Epididymal sperm counts and motility assessment were performed for all the control and treated male rats. Briefly, both the cauda epididymides were excised and dissected in 10 ml of DMEM (Sigma) pH 7.4, preincubated at 37 °C with 5% CO₂, in a petri dish to disperse sperm from the tubules. The petri dish was incubated at 37 °C for 10 min and sperm concentration was measured using Neubauer’s hemocytometer after appropriately diluting the sample. The sperm count for each group was expressed as millions per cauda epididymidis. Sperm motility was analyzed by computer-assisted semen analysis using an HTM-IVOS motility analyser (Hamilton Thorne Research, Beverly, MA, USA). All the procedures were performed at 37 °C, and all equipment and reagents that came into contact with the sperm were prewarmed to and maintained at 37 °C.

Hormone assays

Steroid hormones like testosterone and E₂ were estimated from the serum using commercial ELISA kits from Diagnostic Biochem Canada, Inc. (London, Ontario, Canada) following the manufacturer’s instructions. Serum FSH, LH and prolactin were estimated on a Luminex 200IS platform using a Milliplex Map Rat Pituitary Magnetic Bead Panel Kit (Millipore Corporation, Billerica, MA, USA) as per the manufacturer’s instructions. Serum samples were diluted 1:3 with serum matrix and all samples were measured in duplicate.

Histological examination of the testis

The testes were dissected out and fixed in Bouin’s fixative for 24 h. After primary fixation, each testis was cut into
three pieces (3–5 mm thick) and refixed in fresh fixative for 24 h. The tissues were then dehydrated through grades of alcohol, cleared in xylene and embedded in paraffin wax. The paraffin-embedded tissue blocks were sectioned at a thickness of 5 μm, mounted on glass slides and stained with Hematoxylin and Eosin then observed under a Zieiss Axioskop photomicroscope at 100× magnification. The staging of seminiferous tubules was carried out according to the standard published criteria (Hess 1990). For evaluation of spermiation failure, the numbers of stage IX–XI tubules showing failed spermatid(s) were counted in five cross sections for each animal (five animals per group). The percentage of tubules showing spermiation failure was calculated as a ratio of number of stage IX–XI tubules showing failed spermatids to the total number of stage IX–XI tubules counted $\times 100$.

Statistical analysis

Data are presented as mean±S.E.M. and were analysed using GraphPad Prism Software version 5.0 (San Diego, CA, USA). The percentage pre- and post-implantation loss (PIL and POL) calculated for each of the two females mated with each male, was averaged and a total average of (PIL and POL) calculated for each of the two females mated with males treated with PPT and DPN at doses of 0.05–0.2 mg/kg per day after both 30 and 60 days of PPT treatment and 60 days of DPN treatment (Fig. 1B and E). There was no significant change in post-implantation embryo loss in females mated with PHTPP-treated males at any of the doses after 30 and 60 days (Fig. 1H).

Litter size A significant decrease in litter size was observed in females mated with males treated with PPT and DPN at doses 0.05–0.2 mg/kg per day after both 30 and 60 days of treatment (Fig. 1C and F). No significant change in litter size was observed in females mated with males treated with PHTPP after 30 and 60 days treatment (Fig 1I).

Potency A significant decrease in potency upon PPT treatment with a complete loss of potency at the highest dose of 0.4 mg/kg per day was observed (Fig. 2A). There was a significant decrease in potency at 0.1 mg/kg per day DPN and at 0.8 mg/kg per day PHTPP group. The potency was unaffected by all the other doses of DPN and PHTPP (Fig. 2B and C).

Effect on sperm counts and motility

Caudal sperm counts were significantly reduced after all doses of PPT treatment, whereas motility was not affected (Fig. 2D and G). Sperm counts and motility were significantly decreased only at 0.05 and 0.1 mg/kg per day DPN treatment, whereas in the groups treated with higher dosages of DPN there was no effect (Fig. 2E and H). No difference in sperm counts and motility was observed after 60 days of PHTPP treatment at any of the doses as compared with the vehicle control group (Fig. 2F and I).

Effect on body weight and reproductive tissue weights

The terminal body weights and reproductive organ weights after PPT, DPN and PHTPP treatment are summarized in Table 1. A decrease in terminal body weights were observed with higher doses of PPT, whereas they were not affected by DPN or PHTPP treatment. Testis weights were significantly decreased with the highest dose of PPT, with no effect due to DPN and PHTPP treatment. The weights of epididymis were decreased after treatment with 0.2 and 0.4 mg/kg per day PPT and 0.05 and 0.1 mg/kg per day DPN. The weights of the prostate were significantly reduced in response to treatment with the highest dose of 0.4 mg/kg per day PPT, and 0.8 mg/kg per day PHTPP. The weights of seminal vesicles and coagulating glands were decreased after 0.4 mg/kg per day of PPT treatment only. There is a dose-dependent increase in
weights of pituitary after treatment with both the agonist treatment of agonists, PPT and DPN, whereas no change was observed upon PHTPP treatment.

Effect on hormonal profile

There was a significant decrease in serum FSH for all doses of PPT treatment whereas FSH levels were not affected by either DPN or PHTPP treatment (Fig. 3A, B, and C). Similarly, serum LH was deceased by treatment with higher doses of 0.2 and 0.4 mg/kg per day PPT and LH levels were unchanged after DPN and PHTPP treatment (Fig. 3D, E, and F). Serum prolactin levels were increased by all doses of PPT treatment and by the treatment with the highest dose of DPN and contrastingly they were reduced by treatment with the highest dose of PHTPP (Fig. 3G, H, and I). Serum testosterone was decreased with increasing doses of PPT treatment. However, there was a significant decrease in testosterone levels only in the 0.1 mg/kg per day DPN and 0.8 mg/kg per day PHTPP treatment groups (Fig. 3J, K, and L). Serum E2 levels were unaffected by any of the treatments except for treatment with the highest dose of PPT, 0.4 mg/kg per day, where a significant increase was observed (Fig. 3M, N, and O).

Effect on testicular histology

No gross morphological changes were observed in testicular sections from animals treated with PPT and PHTPP as compared with vehicle control at any of the stages of spermatogenesis. All the germ cell types with normal stage-specific cellular associations were observed for all the doses of PPT and PHTPP treatments (data not shown). The somatic cells like Sertoli and Leydig cells also appeared normal. In the case of DPN treatment, many hook-shaped failed step 19 spermatids were observed towards the basal membrane of the tubules in stages IX–XI (Fig. 4C); although no other histological differences were noted in the other stages. There was a significant increase in the percentage of stage IX–XI tubules showing spermiation failure in the 0.05 and 0.1 mg/kg per day DPN groups as compared with the control group (Fig. 4E).
Significantly different from control (*P<0.05, †P<0.01 and ‡P<0.001).
**Figure 3**

Hormonal profile of serum FSH (A, B, and C), LH (D, E, and F), prolactin (G, H, and I), testosterone (J, K, and L), estrogen (M, N, and O) after 60 days of PPT (A, D, G, J, and M), DPN (B, E, H, K, and N) and PHTPP (C, F, I, L, and O) treatment. Values are mean ± S.E.M. n = 8. Asterisks indicate significant difference over the control (*P < 0.05 and **P < 0.01).
Discussion

The effects of E$_2$ can be mediated by the ERs present in the germ cells in a direct autocrine manner or indirectly by affecting Sertoli and Leydig cell function (Shaha 2008). On the basis of their localisation in the different cell types in the testis, the two ERs can have distinct physiological roles and can mediate different responses. In the present study, we have attempted to understand the individual roles of the ERs in various aspects contributing towards male fertility. In our study, we report that treatment of adult male rats with ER$_a$- and ER$_b$-selective agonists and ER$_b$ antagonist decreases fertility. An increase in PIL and POL and a consequent decrease in litter size were observed upon treatments with both the agonists. However, upon ER$_b$ antagonist treatment, an increase in only PIL was observed with no significant decrease in litter size, which could be because the increase in PIL was very subtle. PIL was the most sensitive fertility parameter since an increase in its incidence was observed for all treatments after both 30 and 60 days of treatment. This indicates that post-meiotic early spermatids were likely to be affected and attenuation of E$_2$ signaling though either of the ERs could lead to PIL. The POL was observed after 60 days of PPT and DPN treatment (Fig. 1B and E), indicating that germ cells at earlier stages of spermatogenesis, like spermatogonia and spermatocytes, could be affected by these treatments (Dixon & Hall 1982). An increase in POL was also observed after 30 days of PPT treatment. This indicates that POL could be brought about by the two ERs through different mechanisms. The results of studies investigating the effects of E$_2$ administration on fertility have indicated total loss of potency to be the main reason for the infertility observed (Rao & Chinoy 1983, Gill-Sharma et al. 2001). Hence, the effects of such treatments on the pre- and post-implantation embryo loss have not been investigated. Administration of tamoxifen, a selective ER modulator (SERM), which can affect both the ERs, to adult male rats also causes an increase in preimplantation (approximately 60%) and post-implantation (approximately 10%) loss with a decrease in litter size and fertility (Gill-Sharma et al. 1993, Balasinor et al. 2001). Taken together these results indicate that although normal estrogen signaling through its receptors is important for maintenance of spermatogenesis and male fertility, over-activation, as in the case of agonists treatment, can be detrimental.

In several studies have interesting hypotheses have been proposed to explain the decrease in male fertility parameters upon estrogen exposure. For example, decreases in steroid receptor expression and sperm nuclear condensation, on exposure to E$_2$ and tamoxifen respectively, have been associated with disturbed spermatogenesis and decreased fertility (Aleem et al. 2005, Kaushik et al. 2010). Also, aberrant sperm DNA methylation pattern of
imprinted genes have been reported on treatment with SERMs, tamoxifen and bisphenol A (BPA) (Pathak et al. 2009, 2010, Doshi et al. 2013) and have been correlated with the sub-fertility observed after these treatments (Gill-Sharma et al. 1993, Balasinor et al. 2001, Salian et al. 2009). The precise causes contributing to the decrease in fertility upon administration of the two agonists in our study are currently being pursued.

In rodents, male sexual behaviour and ability to mate is under the influence of the testosterone, yet E2 is also thought to play an important role (Freeman & Rissman 1996, McCarthy & Albrecht 1996). It is evident that there was a decrease in potency in the treatment groups where 1996, McCarthy & Albrecht 1996). It is evident that there was a decrease in potency in the treatment groups where there was a decrease in serum testosterone levels (Fig. 2A, B, and C). Interestingly, there was a complete lack of potency after treatment with the highest concentration of 0.4 mg/kg per day PPT. Although the serum testosterone levels were not significantly different at the highest dose from those for the other PPT treatment groups, there was a significant increase in serum E2 levels at this dose. This finding indicates that the increase in E2 could have a suppressive effect on sexual behaviour in male rats.

Exogenous administration of E2 (Gill-Sharma et al. 2001) and exposure to the synthetic estrogen diethylstilbestrol (DES) (Goyal et al. 2001) causes a decrease in gonadotropin hormones. However, the treatments with selective ligands affected the HPT axis differently. Serum FSH and LH levels were significantly decreased upon PPT treatment, whereas there was no change as a result of treatment with DPN and PHTPP (Fig. 3A, B, C, D, E, and F). These results concur with results published in earlier reports indicating that the negative feedback regulation of gonadotropins by E2 could be mediated through ERα (Lindzey et al. 1998). Interestingly, ERα is also the predominant ER in the gonadotrope cells in the pituitary (Mitchner et al. 1998). E2 is known to be a major regulator of prolactin gene expression and release (Dannies 1985), and E2 treatment increases serum prolactin levels (Gill-Sharma et al. 2001). An increase in serum prolactin was observed with both the agonists and a corresponding decrease was found with the antagonist PHTPP (Fig 3G, H, and I), indicating that the effects of E2 on prolactin levels are brought about by both the ERs in a ligand-dependent manner. Results of recent in vitro studies also support this observation that prolactin gene expression can be promoted by both the ERs through their respective agonists (Chen et al. 2009b).

Steroidogenic enzymes responsible for production of androgens are known to be regulated by E2 (Sakaue et al. 2002) and a decrease in testosterone levels is commonly observed with E2 and DES administration (Gill-Sharma et al. 2001; Goyal et al. 2001). The serum testosterone levels at higher doses of PPT were reduced to 25% of the control values (Fig. 3J); this could be due to the combined effect of the decrease in serum LH and local effects of ERα on Leydig cell steroidogenesis (Akingbemi et al. 2003). However, regulation by ERβ seems to be dose-dependent as only one of the lower doses of 0.1 mg/kg per day DPN and the highest dose of 0.8 mg/kg per day PHTPP (Fig. 3K and L) have caused a 50% decrease in serum testosterone levels. This could be due to the direct effect on Leydig cells as the serum LH levels are not changed. The levels of serum E2 itself were not affected in any of the dosage groups with the exception of the highest dose of 0.4 mg/kg per day PPT where there is an increase in the E2 levels (Fig. 3M, N, and O). This could be due to changes in aromatase, since exogenous administration of E2 to adult rats, increases intratesticular levels of aromatase (Bharti et al. 2013) and activation of E2 signaling specifically through ERα is known to upregulate aromatase activity in other tissues (Villablanca et al. 2013). Taken together, these results indicate that activation of E2 signaling through ERα suppresses the levels of gonadotropins and testosterone, thus the observed decrease in fertility could be a cumulative effect of HPT axis disruption and local steroidogenic defects on spermatogenesis. However, activation of E2 signaling via ERβ did not affect the gonadotropin levels thus the decreased fertility could be due to localised effects on the reproductive tract.

Reports of several studies have described a sharp decline in caudal sperm counts and motility on E2 administration (Rao & Chinoy 1983, Gill-Sharma et al. 2001) and exposure to synthetic estrogen DES (Goyal et al. 2001) and BPA (Salian et al. 2009). Caudal sperm counts were affected by all doses of PPT treatment and lower doses of DPN treatment (Fig. 2D and E) which could be due to reduced sperm production in the testis, apoptosis of germ cells or retention of spermatids in the seminiferous epithelium, all of which are typical effects of E2 exposure (D’Souza et al. 2005). The exact reasons for the low counts are currently being investigated. In the present study, sperm motility was affected by lower doses of DPN (Fig. 2H) which could be due to testicular defects and/or defects in maturation of the sperm in the epididymis. Although sperm motility was not affected by PPT under the experimental conditions examined, the involvement of ERα cannot be ruled out.

The terminal body weight were unaltered by DPN and PHTPP treatment and decreased only with PPT. E2 administration has been known to affect weight gain in...
males (Robaire et al. 1987), and results from some recent studies have indicated that the anorexigenic effect of E2 in females is modulated specifically through ERα and not ERβ (Santollo & Eckel 2009, Santollo et al. 2010). The weights of most of the reproductive organs were more or less unaffected by the treatments except in the 0.4 mg/kg per day PPT dose group where the weights of prostate, seminal vesicles and coagulating glands are significantly reduced (Table 1). This could be due to both increased E2 and decreased testosterone levels. There was a decrease in the epididymal weights with the higher doses of PPT and lower doses of DPN which could be due to the decreased sperm counts observed after these treatments. Interestingly, a significant decrease in the weight of prostate gland was observed upon treatment with 0.8 mg/kg per day PHTPP. Since the prostate gland shows the highest expression of ERβ in the male reproductive tract in rats (Kuiper et al. 1997), disruption of its function by the antagonist treatment could lead to the observed reduction in the weight of the prostate gland. There was also a dose-dependent increase in weights of pituitary upon treatment with both the agonists. Similar effects on pituitary have also been observed with exogenous E2 administration and this supports the well-established observation that mitogenic activity of E2 causes a significant increase in lactotrophs and, due to their prevalence, a commensurate increase in pituitary weight (Dannies 1985).

Although no visible effect on testicular morphology was observed at the light microscopy level as a result of PPT treatment, changes in different testicular cell populations cannot be ruled out. An increase in the number of stage IX–XI tubules showing failed spermatids towards the basal membrane was observed after treatment with 0.05 and 0.1 mg/kg per day DPN (Fig. 4E). This could be one of the reasons for the observed low sperm counts upon DPN treatment. Surprisingly, this effect was not seen with PPT treatment, despite the reduced testosterone and FSH levels. This indicates that E2 through ERβ, along with testosterone, plays an important role in the process of spermatogenesis and activation of E2 signaling through ERβ alone can cause retention of mature spermatids. The results of parallel investigations in our lab have also indicated that cytoskeletal genes regulated by ERβ are involved in spermatiation and their downregulation leads to failure of spermatiation (Kumar et al. 2015).

To summarise, normal E2 levels are essential for maintenance of normal spermatogenesis and male fertility. Results of studies have revealed a correlation between decreasing E2 levels in the testis and decreasing sperm production during ageing and indicated that E2 can attenuate the age-related decline in spermatogenesis (Hamden et al. 2008, Clarke & Pearl 2014). On the other hand, overexposure to E2, synthetic estrogens, like DES, and SERMs, like tamoxifen and BPA, cause deleterious effects on the male reproductive tract and fertility. Thus, a balance of estrogen action is essential and either deficit or excess would be detrimental to male fertility.

**Conclusion**

The results of this study indicate that overactivation of E2 signaling through both the ERs can affect male fertility but presumably through different mechanisms. The effects observed after administration of an ERα agonist, are mediated mainly through HPT axis, whereas treatment with an ERβ agonist mainly affects the male reproductive tract directly. The most interesting finding from the present study is that overstimulation of E2 signaling through ERβ alone, as in the case of 0.05 DPN treatment, is sufficient to cause a decrease in most of the fertility parameters like litter size, sperm counts and motility, without affecting the levels of any other hormones, or sexual behaviour. The results of this study indicate that treatments with ERα and β selective agonists, which cause a decrease in fertility, can be used as models to further delineate the individual roles of the two receptor subtypes and the molecular mechanisms involved therein.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Funding**

This study (RA/179/08-2014) has been funded by the National Institute for Research in Reproductive Health (NIRRH) core budget.

**Acknowledgements**

The authors gratefully acknowledge Indian Council of Medical Research (ICMR) for providing financial support and fellowship to Ms K Dumasia. The authors appreciate the technical expertise and assistance of Mr S M Mandavkar in animal experiments, dissections and histology work. The authors are grateful for the technical assistance provided by Ms G Suryavanshi, Ms S Deshpande and Mr D Shelar during the course of this work.

**References**


http://joe.endocrinology-journals.org  DOI: 10.1530/JOE-15-0045 © 2015 Society for Endocrinology  Published in Great Britain  Printed in Great Britain


