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Impact of Targeting β3 Receptor on Male Sex Hormonal Balance

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Abstract

BACKGROUND: Sympathetic stimulation has a significant impact on the physiology and pathology of the male reproductive system. β3 receptor is suspected to play a role in the regulation of fertility status in men.

AIM: The aim of the study is to investigate the role of the β3 receptor in regulating the fertility parameters (testosterone, estrogen, progesterone, and histology of testis) in male rats.

MATERIALS AND METHODS: Male albino rats have been given either placebo (controls) or β3 agonist (Mirabegron). Testosterone, estrogen, and progesterone are measured before and after the treatment for all cases and controls. Histology of testis is investigated for all the rats as well.

RESULTS: β3 receptor activation caused a significant increase in testosterone plasma concentration and a significant reduction in estrogen plasma concentration. β3 agonist did not affect the progesterone plasma concentration. Histological sections showed that β3 activation resulted in degeneration of the spermatocytes and accumulation of edema between the seminiferous tubules in the testis.

CONCLUSION: β3 receptor has a potentially important role in the fertility status of male rats through regulating sex hormonal profile and altering the histology of the testis.

Introduction

The testis is a male sex gonad that acts as an endocrine gland and is responsible for the production of spermatozoa [1], [2]. Seminiferous tubules, which develop from mesonephric cells, are considered a factory of the sperm in the testis as they have the migrating primitive germ cells. In addition, two different important specialized cells are present in the testis, Sertoli cells, and Leydig cells. Sertoli cells are located inside the seminiferous tubules and secrete factors that regulate spermatogenesis and spermiogenesis such as estrogen [3], [4]. Leydig cells are located in between seminiferous tubules and are responsible for testosterone production [5], [6].

The main reproductive hormones estrogen, testosterone, and progesterone are instrumental in sexuality and fertility. They are responsible for puberty, and sex drive and play a key roles in spermatogenesis and sperm maturation [7], [8]. Testosterone is necessary for normal sperm development. In addition, it activates genes in Sertoli cells, which promote the differentiation of spermatogonia [9], [10]. Although it is thought that estrogen is a female hormone, small amounts are produced in the males from the peripheral conversion of testosterone. The testicles, adipose cells, and adrenal glands have aromatase enzymes and can produce estrogen which is important for the fertility status of males [11]. In men, progesterone hormone is produced by the testis and adrenal glands, to act as a precursor to the sex hormones, estrogen, and testosterone [12], [13].

The sympathetic nervous system is part of the autonomic nervous system which is responsible for various physiological processes. The endogenous catecholamines, epinephrine, and norepinephrine stimulate different signaling pathways through action on different adrenergic receptors. There are nine different receptors of the sympathetic nervous system which belong to the superfamily of G protein-coupled receptors (α1a, α1b, α1d, α2a, α2b, α2c, β1, β2, and β3) [14], [15]. There is increasing evidence that testicular innervation and/or peripheral catecholamines can regulate male reproductive functions. Several potential targets for catecholamines have been identified in both Sertoli cells and Leydig cells in the testis of rats, mice, and pigs [4], [16]. Although noradrenergic innervation has multiple sites of action in the control of testicular functions, β1 and β2 subtypes have shown to be predominantly expressed in Sertoli and Leydig cells [9], [17]. In addition, recent studies found that β-adrenergic agonists can stimulate steroidogenesis and cAMP formation in Leydig cells [18], [19].

β3 receptor is one of the β-adrenergic subtypes receptors that (Figure 1) is shown to be predominantly expressed in adipose tissue as well as other parts in the body including urinary system, central nervous system (CNS), and heart [21], [22], [23]. However, whether it has a significant relationship with fertility status in males is yet to be determined. The study aims to investigate the role of the β3 receptor in the modulation of the sex-hormonal profile and histology of the testis in males.

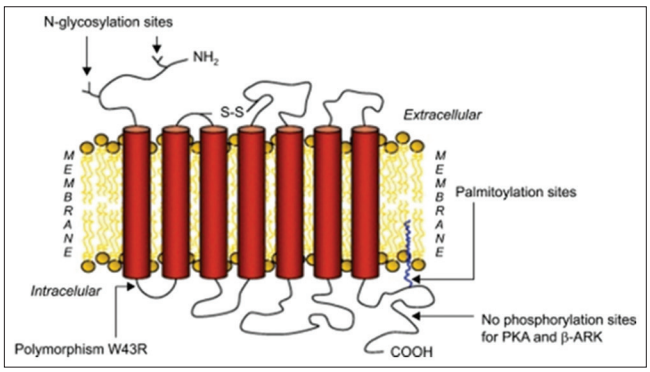

Figure 1: The primary structure of β3-adrenoceptor [20]

Materials and Methods

Ten randomly selected male Albino rats of reproductive age (between 10 and 12 weeks) have been used in this study. The rats, which are weighing approximately 200–300 g, are kept under the standard laboratory condition including a controlled temperature of 23 ± 3°C and humidity (50 ± 10%) with free access to water and food provided. All the rats were kept strictly under the protocols of the animal ethics committee as well as all the experiments were approved by the institution. Rats were equally subdivided into two groups, a case group in which β receptors are activated by β agonist (mirabegron 5 mg/kg/day, orally) and a control group which received distilled water as a placebo. The duration of the study (administration of β agonist or placebo) was 3 months with 12 h light and 12 h dark cycles.

The levels of testosterone, estrogen, and progesterone were measured using TOSOH AIA 360 automated immunoassay analyzer and following the manufacturer's protocols (ST AIA-pack testosterone 0025204, ST AIA-pack iE2 0025224, and ST AIA-pack PROGII 0025239, respectively).

The laboratory animals were killed for histological studying. Then, the testes were dissected and fixed using a 10% buffered formalin solution for 24 h before treating with different amounts of alcohol and ultimately with xylene. Tissue samples were mounted in paraffin to make blocks for microtomy. Tissues were sectioned to a thickness of 4 μm with a microtome and stained with hematoxylin and eosin (Sigma). Then, examination and photographing of the slides were performed using a Model BM-2101 light microscope (Olympus, Yuyao, China).

Data are expressed as mean ± standard error of the mean where n = the number of different animals. One-way ANOVA in conjugation with Sidak's *post hoc* test was used to assess the differences between the groups. $p < 0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad Prism.

Results

The effect of β3 receptor activation on sex hormones levels

To understand the role of the β3 receptor in the regulation of male fertility status, the effect of activation of the β3 receptor is investigated on the male sex hormone profile. Mirabegron (β3 agonist) could significantly increase the level of testosterone plasma concentration compared with controls (Control: 225 ± 27.47 vs. 285.4 ± 21.11; Mirabegron: 273.7 ± 31.97 vs. 444.6 ± 18.67) (Figure 2).

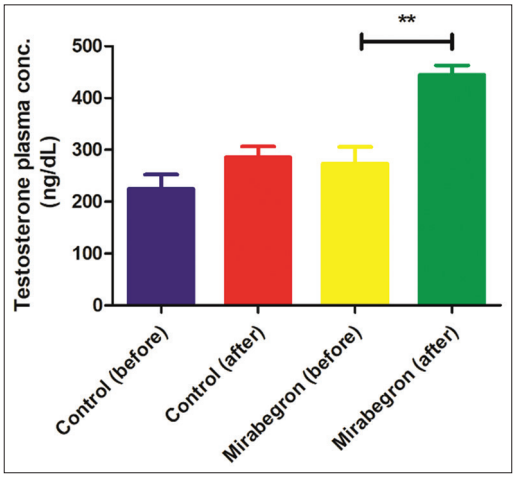

Figure 2: *Effect of β3 agonist on testosterone plasma concentration. Plasma concentration of testosterone before and after the treatment with (5 mg/kg/day) mirabegron or placebo (controls). Data are expressed as mean ± standard error of the mean of five different rats. ** indicates p < 0.01, one-way ANOVA v control followed by a Tukey's post hoc test versus control*

In contrast, β3 receptor activation caused significantly reduction of estrogen plasma concentration compared with controls (Control: 16.75 ± 2.629 vs. 15.16 ± 1.544; Mirabegron: 16.92 ± 2.479 vs. 5.988 ± 1.106) (Figure 3).

However, β3 receptor activation has no significant effect on the progesterone plasma concentration (Figure 4).

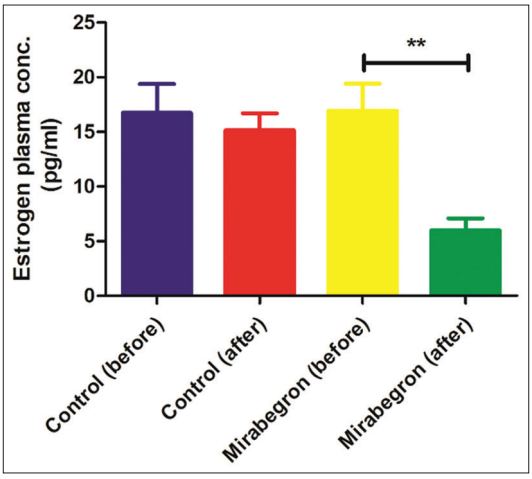

Figure 3: *Effect of β3 agonist on estrogen plasma concentration. Plasma concentration of estrogen before and after the treatment with (5 mg/kg/day) mirabegron or placebo (controls). Data are expressed as mean ± standard error of the mean of five different rats. **indicates p < 0.01, one-way ANOVA v control followed by a Tukey's post hoc test versus control*

The effect of β3 receptor activation on histology of the testis

Next, a histological examination was performed to assess the effect of β3 receptor activation on the normal morphological appearance of the testis (Figure 5A and B). Histopathological sections of testicular tissue showed that mirabegron caused accumulation of fluids (edema) between the seminiferous tubules as well as degeneration of spermatocytes inside the tubules (Figure 6A and B).

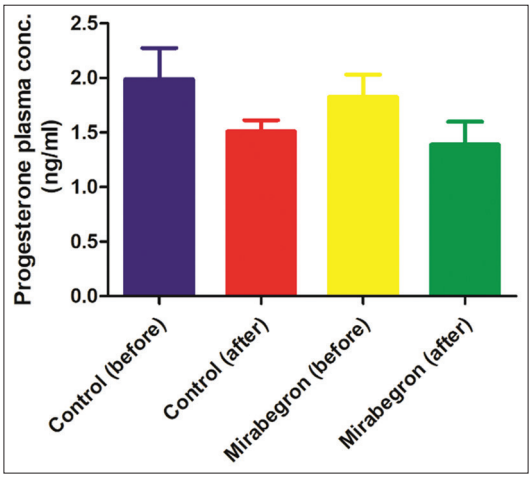

Figure 4: *Effect of β3 agonist on progesterone plasma concentration. Plasma concentration of progesterone before and after the treatment with (5 mg/kg/day) mirabegron or placebo (controls). Data are expressed as mean ± standard error of the mean of five different rats*

Discussion

β3-adrenergic receptor (β3-AR) is a seven-transmembrane domain G-protein coupled receptor

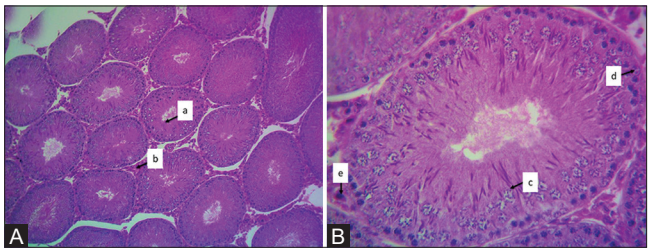

Figure 5: Photomicrographs of rat testis of the control group show the normal architecture of seminiferous tubules with spermatogenesis (a and c) and interstitial tissue with Leydig cells (b and e) and Sertoli cells (d). (A: H and E, ×100 and B: H and E, ×400)

and its stimulation increases the production of cAMP [21], [24], [25]. β3-AR is expressed in many tissues including the reproductive system, urinary system, CNS, and adipose tissue [26], [27]. A β3-AR agonist can improve urinary characteristics and the attributed erectile dysfunction in patients with lower urinary tract symptoms/benign prostatic obstruction [28]. Although sex difference generally has a significant impact on the nervous system [29], [30], [31], there is not too much information about the relationship between the plasma level of sexual hormones and the activation of β-adrenoceptors, especially β3-AR.

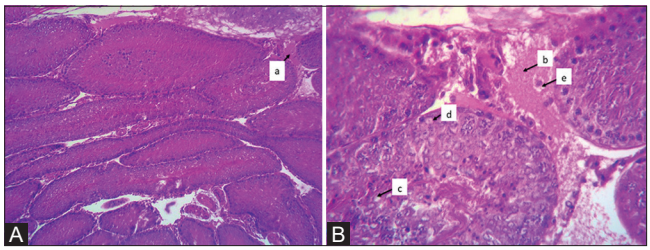

Figure 6: Photomicrographs of rat testis of mirabegron group show the presence of edema between seminiferous tubules (a and b), degeneration of spermatocytes (c), Sertoli cells (d), and Leydig cells (e). (A: H and E, ×100 and B: H and E, ×400)

The complex interaction of germ cells, Sertoli cells, estrogen, and androgen is responsible for the regulation of the spermatogenesis process within the seminiferous tubules [32], [33], [34]. A study showed that spermiogenesis and spermatogenesis, which is the genomic function of Sertoli cells, are regulated by adrenergic receptor signaling [35]. The authors have found that there is a significant decrease in androgen levels in mice lacking adrenergic receptors. Regarding α-adrenoceptors, it is known that these receptors have a significant impact on the control of sexual behavior [36], [37], [38]. A recent review highlighted the important link between androgens and the adrenergic system in both health and disease states [39].

The present study found that activation of β3-AR by Mirabegron 5 mg/kg/day for 3 months caused a significant increase in plasma testosterone concentration as well as a significant reduction in plasma estrogen concentration. However, there is no effect of β3-AR stimulation on progesterone concentration. In addition, histological sections showed degeneration of the spermatocytes and accumulation of edema between the seminiferous

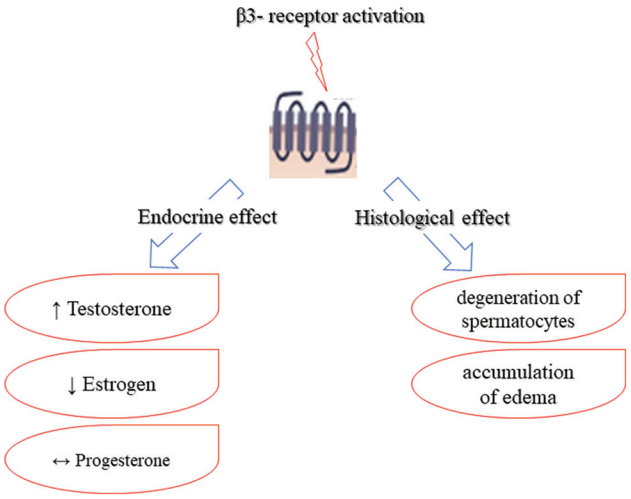

Figure 7: Summary of the endocrine and histological effects of adrenergic receptor stimulation

tubules in the testis after activation of β3-AR for 3 months (Figure 7). However, these results were inconsistent with those of Yuno *et al*., (2014) who found that administration of β3-AR agonist (Mirabegron) at doses of 10, 30, and 100 mg/kg/day once daily for only 2 weeks showed no changes in the serum levels of gonadotropic or sex steroidal hormones (luteinizing hormone, follicle-stimulating hormone, testosterone, or dihydrotestosterone). In addition, the authors did not see histopathological changes in the pituitary gland, adrenal glands, liver, testes, epididymis, prostate, and seminal vesicle within the given doses and period [40]. The possible explanation for the differences in the results between this present study and Yuno *et al*. study could be the difference in the duration of administration of Mirabegron as only 2 weeks were not sufficient to result in these detectable biochemical and histological effects in comparison with the 3 months used in this study. Moreover, a study by Kallner *et al*., (2016) has found that treatment of women suffering from an overactive bladder for 2 months with Mirabegron 50 mg once daily could reduce estrogen levels and elevate testosterone levels, but not significantly, in comparison with patients who did not take the drug [41]. Again, the duration of treatment possibly could be the key behind the varied responses to β3-AR stimulation. Of note, stimulation of β3-adrenoceptors can cause slow onset enhancing, secreting, and elevating serum levels of steroid hormone. This effect of β3-adrenoceptors activation could be through induction of lipolysis and thermogenesis within the adipose tissue which needs a long time to affect hormonal levels [20]. Thus, further studies with same duration of treatment by Mirabegron, or even longer, are required to investigate and confirm the mechanism of action of β3-AR stimulation and its impact on hormonal profile and fertility statues in different species including human.

Conclusion

The finding of the present study showed that β3-adrenoceptor stimulation could affect the sexual hormones and the normal histology of the testis which supports the suggestion about the possible role of adrenergic receptors, especially β3 activation, in controlling the general fertility status of males. Duration of β3-AR activation plays a key role in this neuroendocrine effect. As there is a deficiency in the information about the relationship between activation of β3-AR and secretion of sex hormones, more studies are required to clarify the exact molecular mechanism behind this relationship.

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References

1. Victor E, Osarugue I, Mega Obukohwo O, Eze Kingsley N, Alexander Obidike N. Endocrine functions of the testes. In: Male Reproductive Anatomy. London: IntechOpen; 2022. https://doi.org/10.5772/intechopen.101170
2. Mäkelä JA, Koskenniemi JJ, Virtanen HE, Toppari J. Testis development. Endocr Rev. 2019;40(4):857-905. https://doi.org/10.1210/er.2018-00140
   PMid:30590466
3. O'Donnell L, Smith LB, Rebourcet D. Sertoli cells as key drivers of testis function. Semin Cell Dev Biol. 2022;121:2-9. https://doi.org/10.1016/j.semcdb.2021.06.016
   PMid:34229950
4. França LR, Hess RA, Dufour JM, Hofmann MC, Griswold MD. The Sertoli cell: One hundred fifty years of beauty and plasticity. Andrology. 2016;4(2):189-212. https://doi.org/10.1111/andr.12165
   PMid:26846984
5. Zirkin BR, Papadopoulos V. Leydig cells: Formation, function, and regulation. Biol Reprod. 2018;99(1):101-11. https://doi.org/10.1093/biolre/ioy059
   PMid:29566165
6. De Miguel MP, Gonzalez-Peramato P, Nistal M. Morphological bases of human leydig cell dysfunction. In: Advances in Testosterone Action. London: IntechOpen; 2018. https://doi.org/10.5772/intechopen.79201
7. Patel DP, Chandrapal JC, Hotaling JM. Hormone-based treatments in subfertile males. Curr Urol Rep. 2016;17(8):56. https://doi.org/10.1007/s11934-016-0612-4
   PMid:27292256
8. O'Hara L, Smith LB. Androgen receptor roles in spermatogenesis and infertility. Best Pract Res Clin Endocrinol

Metab. 2015;29(5):595-605. https://doi.org/10.1016/j.beem.2015.04.006
PMid:26303086

9. Smith LB, Walker WH. The regulation of spermatogenesis by androgens. Semin Cell Dev Biol. 2014;30:2-13. https://doi.org/10.1016/j.semcdb.2014.02.012
PMid:24598768

10. Di Guardo F, Vloeberghs V, Bardhi E, Blockeel C, Verheyen G, Tournaye H, *et al*. Low testosterone and semen parameters in male partners of infertile couples undergoing ivf with a total sperm count greater than 5 million. J Clin Med. 2020;9(12):3824. https://doi.org/10.3390/jcm9123824
PMid:33255908

11. Hamilton KJ, Hewitt SC, Arao Y, Korach KS. Estrogen hormone biology. Curr Top Dev Biol. 2017;125:109-46. https://doi.org/10.1016/bs.ctdb.2016.12.005
PMid:28527569

12. Filicori M. Clinical roles and applications of progesterone in reproductive medicine: An overview. Acta Obstet Gynecol Scand. 2015;94 Suppl 161:3-7. https://doi.org/10.1111/aogs.12791
PMid:26443945

13. Oettel M, Mukhopadhyay AK. Progesterone: The forgotten hormone in men? Aging Male. 2004;7(3):236-57. https://doi.org/10.1080/13685530400004199
PMid:15669543

14. Brodde OE. β1- and β2-Adrenoceptor polymorphisms and cardiovascular diseases. Fundam Clin Pharmacol. 2008;22(2):107-25. https://doi.org/10.1111/j.1472-8206.2007.00557.x
PMid:18353108

15. Xanthopoulos A, Daskalopoulou I, Frountzi S, Papadimitriou E. A systematic review on the role of adrenergic receptors in angiogenesis regulation in health and disease. Int J Transl Med. 2021;1:353-65. https://doi.org/10.3390/ijtm1030021

16. Su L, Kopera-Sobota IA, Bilinska B, Cheng CY, Mruk DD. Germ cells contribute to the function of the Sertoli cell barrier. Spermatogenesis. 2013;3:e26460. https://doi.org/10.4161/spmg.26460

17. Troispoux C, Reiter E, Combarnous Y, Guillou F. β2 adrenergic receptors mediate cAMP, tissue-type p]asminogen activator and transferrin production in rat Sertoli cells. Mol Cell Endocrinol. 1998;142(1-2):75-86. https://doi.org/10.1016/s0303-7207(98)00115-4
PMid:9783905

18. Pointis G, Latreille MT. β-Adrenergic stimulation of androgen production by fetal mouse Leydig cells in primary culture. Experientia. 1986;42(6):617-9. https://doi.org/10.1007/bf01955560
PMid:2424782

19. Moger WH, Murphy PR. β-Adrenergic agonist induced androgen production during primary culture of mouse leydig cells. Arch Androl. 1983;10(2):135-42. https://doi.org/10.3109/01485018308987554
PMid:6134500

20. Ursino MG, Vasina V, Raschi E, Crema F, De Ponti F. The β3-adrenoceptor as a therapeutic target: Current perspectives. Pharmacol Res. 2009;59(4):221-34. https://doi.org/10.1016/j.phrs.2009.01.002
PMid:19429463

21. Michel LY, Farah C, Balligand JL. The beta3 adrenergic receptor in healthy and pathological cardiovascular tissues. Cells. 2020;9(12):2584. https://doi.org/10.3390/cells9122584
PMid:33276630

22. Sripad AA, Raker CA, Sung VW. Overactive bladder medication: Anticholinergics versus mirabegron by specialty. Urol J. 2022. https://doi.org/10.1177/03915603221076949
PMid:35195050

23. Cannavo A, Koch WJ. Targeting β3-adrenergic receptors in the heart: Selective agonism and β-blockade. J Cardiovasc Pharmacol. 2017;69(2):71-8. https://doi.org/10.1097/fjc.0000000000000444
PMid:28170359

24. Perrone MG, Scilimati A. β 3 -Adrenoceptor agonists and (Antagonists as) inverse agonists. History, perspective, constitutive activity, and stereospecific binding. Methods Enzymol. 2010;484:197-230. https://doi.org/10.1016/b978-0-12-381298-8.00011-3
PMid:21036234

25. Valentine JM, Ahmadian M, Keinan O, Abu-Odeh M, Zhao P, Zhou X, *et al*. β3-Adrenergic receptor downregulation leads to adipocyte catecholamine resistance in obesity. J Clin Invest. 2022;132(2):e153357. https://doi.org/10.1172/jci153357
PMid:34847077

26. Schena G, Caplan MJ. Everything You Always Wanted to Know about β3-AR * (* But Were Afraid to Ask). Cells. 2019;8:357. https://doi.org/10.3390/cells8040357
PMid:30995798

27. Michel MC, Ochodnicky P, Summers RJ. Tissue functions mediated by β 3-adrenoceptors - Findings and challenges. Naunyn Schmiedebergs Arch Pharmacol. 2010;382(2):103-8. https://doi.org/10.1007/s00210-010-0529-2
PMid:20517594

28. Elbaz R, El-Assmy A, Zahran MH, Hashem A, Shokeir AA. Mirabegron for treatment of erectile dysfunction concomitant with lower urinary tract symptoms in patients with benign prostatic obstruction: A randomized controlled trial. Int J Urol. 2022;29(5):390-6. https://doi.org/10.1111/iju.14792
PMid:35043484

29. Kurth F, Gaser C, Luders E. Development of sex differences in the human brain. Cogn Neurosci. 2021;12(3-4):155-62. https://doi.org/10.1080/17588928.2020.1800617
PMid:32902364

30. Shansky RM. Sex Differences in the Central Nervous System.1st Edition: Elsevier; 2015;1-414.

31. Giatti S, Diviccaro S, Serafini MM, Caruso D, Garcia-Segura LM, Viviani B, *et al*. Sex differences in steroid levels and steroidogenesis in the nervous system: Physiopathological role. Front Neuroendocrinol. 2020;56:100804. https://doi.org/10.1016/j.yfrne.2019.100804
PMid:31689419

32. Wolgemuth DJ, Laurion E, Lele KM. Regulation of the mitotic and meiotic cell cycles in the male germ line. Recent Prog Horm Res. 2002;57:75-101. https://doi.org/10.1210/rp.57.1.75
PMid:12017557

33. Sofikitis N, Giotitsas N, Tsounapi P, Baltogiannis D, Giannakis D, Pardalidis N. Hormonal regulation of spermatogenesis and spermiogenesis. J Steroid Biochem Mol Biol. 2008;109(3-5):323-30. https://doi.org/10.1016/j.jsbmb.2008.03.004
PMid:18400489

34. Hasbi H, Gustina S. Androgen regulation in spermatogenesis to increase male fertility. Indonesian Bull Animal Vet Sci. 2018;28:13. https://doi.org/10.14334/wartazoa.v28i1.1643

35. Mhaouty-Kodja S, Lozach A, Habert R, Tanneux M, Guigon C, Brailly-Tabard S, *et al*. Fertility and spermatogenesis are altered in α1b-adrenergic receptor knockout male mice. J Endocrinol. 2007;195(2):281-92. https://doi.org/10.1677/joe-07-0071
PMid:17951539

36. Chen Y, Li H, Dong Q. Alpha1-adrenoceptor antagonists and ejaculation dysfunction. Zhonghua Nan Ke Xue. 2008;14(4):364-7.
PMid:18481434

37. Lehtimäki J, Ventura-Aquino E, Chu X, Paredes RG, Ågmo A. Sexual incentive motivation and copulatory behavior in male rats treated with the adrenergic α2-adrenoceptor agonists tasipimidine and fadolmidine: Implications for treatment of premature ejaculation. J Sex Med. 2021;18(10):1677-89. https://doi.org/10.1016/j.jsxm.2021.07.010
PMid:34493465

38. Sanbe A, Tanaka Y, Fujiwara Y, Tsumura H, Yamauchi J, Cotecchia S, *et al*. α 1-Adrenoceptors are required for normal male sexual function. Br J Pharmacol. 2007;152(3):332-40. https://doi.org/10.1038/sj.bjp.0707366
PMid:17603545

39. Carbajal-García A, Reyes-García J, Montaño LM. Androgen effects on the adrenergic system of the vascular, airway, and cardiac myocytes and their relevance in pathological processes. Int J Endocrinol. 2020;2020:8849641. https://doi.org/10.1155/2020/8849641
PMid:33273918

40. Yuno K, Onishi Y, Arima A, Zaizen K, Aoki Y, Nakagawa S, *et al*. Effect of mirabegron on plasma gonadotropic and steroidal hormone levels in rats after two weeks of oral administration. J Toxicol Sci. 2014;39(3):507-14. https://doi.org/10.2131/jts.39.507
PMid:24849685

41. Kallner HK, Elmer C, Andersson KE, Altman D. Hormonal influence on the effect of mirabegron treatment for overactive bladder. Menopause. 2016;23(12):1303-6. https://doi.org/10.1097/gme.0000000000000708
PMid:27529461