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# Reproductive Toxicity of Bisphenol A (BPA) in Albino Rats

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**ABSTRACT**

This study evaluates the effect of oral administration of BPA on male reproductive parameters and female reproductive hormones. Male and female rats were evenly distributed to get 6 groups (5 rats per group) the first three groups were all male rats while the remaining three groups were female rats. BPA doses of 50 and 250mg/kg body weight were administered weekly to groups II, III, V and VI respectively while groups I and IV served as vehicle control. After 6 weeks of exposure, epididymis and blood samples were collected for semen and biochemical analysis. The parameters analysed include; actively motile sperm cells, dead cells, total sperm count, sluggishly motile cells, abnormal cells, viable cells, estradiol, prolactin and progesterone. The reproductive studies on male rats revealed that there was a significant reduction ($p<0.05$) in the percentage of actively motile sperm cells (93.33 -72.33), total sperm count 600.00% - 327.67%, while viable cells counts ranged from 82.67% to 67.33%. Dead sperm cells increased significantly from the control group (9.00%) to group 3 (19.67%), abnormal cells increased from the control at (22.00%) to group 3 (35.33%) while sluggishly motile cells increased in the same sequence (5.67% to 19.00%). Results on the female reproductive hormones showed that there was a steady reduction sequence from the control group to BPA-treated groups (group 3) at ($p<0.05$) in all parameters; with estradiol (84.00 to 68.00), prolactin (5.84 to 0.34) and progesterone (43.85 to 23.11). Results from this study indicate that exposure to BPA resulted in insidious alteration in female reproductive hormones and can also induce impotency in males.

## 1 Introduction

Bisphenol A (BPA), also known as 2, 2-bis (4-hydroxyphenyl propane) is a synthetic organic compound that is commonly used all over the world. It is categorised within the group of diphenylmethane derivatives and bisphenols. The significance of BPA became obvious in the 1950s, in the manufacture of plastics, food packaging materials, industrial materials, dental sealants and personal hygiene products (Huo *et al*., 2015). The general human population is vulnerable to BPA through the oral, inhalatory and dermal routes of exposure (Witorsch, 2002; Le *et al*., 2008; Vandenberg *et al.*, 2009; Shuo *et al*., 2011; Matuszczak *et al*., 2019). It is highly dissolvable in ether, benzene, acetone and other organic solvents but slowly dissolves in water. Over the years, BPA has become an important component for the manufacture of epoxy resins and polycarbonate (PC) plastics. These materials can be found in water bottles, food cans and containers, compact discs, flooring, lenses, sports safety equipment, and paints (Biedermann *et al*., 2010). BPA is known to have deleterious effects on numerous systems especially on the reproductive system as it possesses estrogenic property (Tinwell *et al*., 2002; Sugiura-Ogasawara *et al*., 2005). Apart from the reproductive effects, BPA is also associated with endocrine abnormalities (Zoeller *et al*., 2005), carcinogenesis (Ho *et al*., 2006), neural and behavioural alterations (Patisaul *et al*., 2007), cardiac and hepatic abnormalities (Lang *et al*., 2008), signifying the long term effects of BPA. Recent studies regarding the environmental distribution

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and presence of BPA in organisms have produced persistent scientific, regulatory, and public interest in evaluating the possible health effects linked to BPA exposure. Endocrine disruptors cause deleterious effects in wildlife species and humans with subsequent alterations in endocrine function. BPA is classified as an endocrine disruptor which is associated with its estrogenic properties and toxic effects on germinal cells, leading to an alteration in hormone production. From thermal paper, which many people handle as receipts regularly, to food containers, to cans, to plastic objects around the household and beyond, manmade sources of BPA are numerous and virtually everyone, young or old, can be vulnerable to this chemical. This study has been undertaken to determine the impact of oral administration of BPA in adult female and male albino rat by examining associated alterations in male reproductive framework and female reproductive hormones.

# 2 Materials and Method

## 2.1 Test Material

Bisphenol A (purity 99.9%, CAS no. 80-05-7) was procured from Geochem Ventures Limited, Choba, Rivers State. BPA solution was prepared by dissolving it in olive oil on a weekly basis to obtain the doses which were orally administered to each of the groups.

## 2.2 Test Animals

A total of thirty adult male (15) and female (15) albino rats, weighing 180-280 grams were used to carry out this study. The rats were bred in the animal house with a 12-hour light and 12-hour dark photoperiod, in the Department of Animal and Environmental Biology, University of Port Harcourt. Before the administration of this chemical, the test animals were allowed to acclimatize to the laboratory environment for a period of fourteen days. They were kept in cage made of polycarbonate base with wire mesh tops and given a standard feed and water throughout the study.

## 2.3 Experimental Design

The animals were divided into six groups with each group containing five animals which were approximately equal to their average body weight. The experimental groups were designated as follows:

For males;

Group I: Control (vehicle-treated)

Group II: Oral administration of 50mg BPA/kg body-weight/week for 6weeks

Group III: Oral administration of 250mg BPA/kg body-weight/week for 6weeks

For females;

Group IV: Control (vehicle-treated)

Group V: Oral administration of 50mg BPA/kg body-weight/week for 6weeks

Group VI: Oral administration of 250mg BPA/kg body-weight/week for 6weeks

At the end of the experiment, the test animals were sedated by placing them in a sealed jar containing cotton soaked with chloroform anaesthesia. The testicles of male rats were removed to collect epididymis for semen analysis. Blood samples were obtained by cardiac prick and 5ml of blood was placed in an eppendorf tube for measurement of biochemical parameters.

## 2.4 Semen Analysis

### 2.4.1 Sperm Count

This was done using the method described by Amann (1986). The epididymis was crushed and dissolved in 10 ml of normal saline, and 0.1 ml of the dissolved sperm suspension was further dissolved in 0.9 ml of white blood cell-diluting fluid in a test tube. A drop of the diluted sperm was used to charge the Neubauer chamber and viewed under the microscope. The number of sperm cells was counted on the four corner squares and estimated in Neubauer chamber. These sperm cells were counted in the volume of the Neubauer chamber which is $2.5\times10^{-4}$ ml; hence, the number of sperm cells was multiplied by 10 to get the total number of sperm cells in 10 ml of normal saline.

## 2.5 Sperm Motility

Epididymal sperm motility was carried out as per the procedure suggested by Saalu *et al*. (2010). Sperm motility was also made by transferring a small amount of the diluted suspension on a pre-warmed slide and then applying a cover slip. Sperm motility was recorded in percentage.

## 2.6 Sperm Morphology

Sperm head morphology evaluated by the process adopted by Luke *et al*., (2014). Briefly, 1 ml of sperm suspension was transferred to a marked test tube. Then 5-6 drops of 1% Eosin yellow was added to it and gently mixed it by simple finger tapping. 45 minutes incubation was done in room temperature to facilitate the staining. Then the suspension was agitated gently by pipette. Smear was prepared by simple push technique. The smears were air-dried and preserved by mounting with cover slips on the following day using DPX mount. About 100 sperms cells/ rat from each experimental group was examined at high power.

## 2.7 Live and Dead Sperm Count

The microscopic identification of live and dead spermatozoa was carried out as per the method suggested by Correia *et al*., (2013). A drop of the epididymal sperm suspension was placed on one edge of the clean glass slide and mixed with a drop of 3% eosin using a glass rod. Immediately this mixture was again mixed with a drop of 10% nigrosin using the same glass rod. A small quantity of this was smeared on another slide and air-dried. 200 sperms of each rat were counted at high power and live and dead spermatozoa counts were recorded. The nigrosin stain produces a dark background on which the sperms stand out as lightly coloured objects. Normal live sperms excluded the eosin stain and appeared whitish, whereas dead sperms lost membrane integrity, taken up eosin, and appeared pinkish in colour.

## 2.8 Female Hormonal Assay

Blood samples were centrifuged for 600 seconds using 40 revolutions per minute. After centrifugation, serums were isolated with the aid of a micropipette. Serum estradiol, progesterone and prolactin were ascertained using Elecsys auto-analyzer (Model 1010, Roche, Mannheim, Germany).

## 2.9 Statistical Analysis

The results were presented as Mean ± Standard Deviation and subjected to analysis by using one-way analysis of variance (ANOVA). Post hoc test was used to determine the significant difference among means of different groups. The SPSS (Statistical Package for Social Sciences) software (version 20) was used for the analysis of data and the level of significance was set at $p \leq 0.05$. The graphs were plotted using Microsoft Excel.

# 3 RESULTS

## 3.1 Effects of BPA on Sperm Cell Parameters of Male Albino Rats

The result in Table 1 is the summary of effect of BPA on sperm cell parameters. The data revealed that there was a significant reduction ($p < 0.05$) in actively motile cells, total sperm count and viable cells whereas there was a significant increase ($p < 0.05$) in the number of dead cells, sluggishly motile cells and abnormal cells in BPA-treated groups in comparison with the control. The highest value for actively motile cells was 93.33 ± 4.73 while the lowest value was 72.33 ± 2.52, the highest value for dead cells was 19.67 ± 1.53 while the lowest value was 9.00 ± 1.73, the highest value for total sperm count was 600.00 ± 50.00 while the lowest value was 327.67s ± 16.62, the highest value for sluggishly motile cells was 19.00 ± 3.61 while the lowest value was 5.67 ± 1.15, the highest value for abnormal cells was 35.33 ± 1.53 while the lowest value was 22.00 ± 2.65, and the highest value for viable cells was 82.67 ± 4.62 while the lowest value was 67.33 ± 3.79.

**Table 1: Effect of oral administration of BPA on the sperm cell parameters of male albino rats**

| Parameters | Control | 50mg/kg | 250mg/kg |
|---|---|---|---|
| Actively Motile Cells (%) | $93.33 \pm 4.73^{b}$ | $85.00 \pm 4.36^{b}$ | $72.33 \pm 2.52^{a}$ |
| Dead Cells (%) | $9.00 \pm 1.73^{a}$ | $12.67 \pm 3.06^{a}$ | $19.67 \pm 1.53^{b}$ |
| Total Sperm Count (%) | $600.00 \pm 50.00^{c}$ | $446.67 \pm 15.28^{b}$ | $327.67 \pm 16.62^{a}$ |
| Sluggishly Motile Cells (%) | $5.67 \pm 1.15^{a}$ | $10.00 \pm 0.00^{a}$ | $19.00 \pm 3.61^{b}$ |
| Abnormal Cells (%) | $22.00 \pm 2.65^{a}$ | $28.67 \pm 1.53^{b}$ | $35.33 \pm 1.53^{c}$ |
| Viable Cells (%) | $82.67 \pm 4.62^{b}$ | $75.67 \pm 1.15^{ba}$ | $67.33 \pm 3.79^{a}$ |

Data are presented as Mean ± SD. Values with different superscript characters indicate a significant difference ($p < 0.05$).

## 3.2 Effects of BPA on some Hormones of Female Albino Rats

The result in Table 2 shows the summary of effect of BPA on some female hormones evaluated. The mean values of estradiol, prolactin and progesterone revealed that there was a significant reduction ($p < 0.05$) in the groups that received BPA-treatment in comparison with the control. Estradiol had mean values of 84.00 ± 4.58, 77.67 ± 1.15 and 68.00 ± 2.65 for the control, 50mg/kg and 250mg/kg groups respectively. The mean values for prolactin in the control, 50mg/kg and 250mg/kg groups were 5.84 ± 0.48, 3.64 ± 0.47 and 0.34 ± 0.04 respectively. The mean progesterone values for BPA-treated groups (50mg/kg and 250mg/kg) were 27.88 ± 0.64 and 23.11 ± 0.46 respectively, while the control group had a mean value of 43.85 ± 0.43.

**Table 2: Effect of oral administration of BPA on the hormones of female albino rats**

| Parameters | Control | 50mg/kg | 250mg/kg |
|---|---|---|---|
| Estradiol | 84.00 ± 4.58$^{b}$ | 77.67 ± 1.15$^{b}$ | 68.00 ± 2.65$^{a}$ |
| Prolactin | 5.84 ± 0.48$^{c}$ | 3.64 ± 0.47$^{b}$ | 0.34 ± 0.04$^{a}$ |
| Progesterone | 43.85 ± 0.43$^{c}$ | 27.88 ± 0.64$^{b}$ | 23.11 ± 0.46$^{a}$ |

Data are presented as Mean ± SD. Values with different superscript characters indicate a significant difference ($p < 0.05$).

# 4 Discussion

The findings of this study are in agreement with the observations of Kourouma *et al*. (2014). They administered BPA via intraperitoneal injection and observed that there was a decrease in sperm quality and total sperm count. An increment in BPA dosage will lead to reduced sperm mobility, number of sperm count and abnormal sperm cells (Meeker *et al*., 2010; Li *et al*., 2011; Manfo *et al*., 2014). The reduction in the number of sperm cells was also observed in some workers who were exposed to BPA at low dosage (Salian *et al*., 2009; Salian *et al*., 2011; Jin *et al*., 2013; Qiu *et al.*, 2013; Isling *et al*., 2014). The main feature associated with the transportation of sperm cell throughout the male and female reproductive tract is the sperm motility. Data of the current research revealed that there was a significant reduction in the percentage of actively motile cells of BPA-treated groups when compared to the control. This result suggests that BPA may have altered the mitochondrion of the cell which in turn results in a delay in motility and in most cases leads to sperm mortality thereby resulting in the reduction of total sperm count (Hulak *et al*., 2013; Vilela *et al*., 2014). These reasons are likely causes for an increase in the number of dead sperm cells and a decrease in the percentage of sperm count in the group that received BPA-treatment in comparison with the control. The observed abnormalities of sperm cell show that BPA may result in genotoxic injury in sex cells (Karnam *et al*., 2015). High doses of BPA increases the percentage of abnormal sperm cells and therefore, shows a dose-dependent response (Karman *et al*., 2015).

It has been reported that BPA has the ability to disrupt the process of spermatogenesis (Zhou et al. 2016; Lassen et al. 2014). At concentrations that is high, BPA is known binds to and acts as an androgen receptor (AR) antagonist (Hejmej et al. 2011). Furthermore, aside receptor binding, the compound has been reported to affect the process of Leydig cell steroidogenesis, including affecting the expression of 17$\alpha$-hydroxylase/17,20 lyase and aromatase, and interfering with LH receptor-ligand binding (Hejmej et al. 2011). An exposure to BPA is related to a reduction in the antioxidant system activity, leading to oxidative stress, which has been implicated among the general causes of sperm damage (Wang et al. 2014; O'Connell et al. 2002; Matuszczak *et al*., 2019)

Sperm cells also known as spermatozoa are highly vulnerable to oxidative stress as this is due to the cells losing a great majority of their cytoplasm during the terminal stage of the process of spermatogenesis (De Lamirande and Gagnon 1995). The cellular damage caused by oxidative stress is achieved by increasing the levels of oxygen and oxygen-derived oxidants, generally known as reactive oxygen species (ROS). Despite that ROS are essential for the normal functions of the sperm at low levels, excessive high levels of ROS exert a detrimental and negative effects on the acrosome reaction of spermatozoa, the motility and capacitation (Rahman et al., 2014; Agarwal et al., 2006). In addition, high levels of ROS can result in damages to lipids, proteins, and DNA in spermatozoa (Aitken et al., 1995). It is important to note that the level of oxygen in the testis are lower when compared to the level in other cells/tissues due to the functional weakness of the testicular artery. Consequently, in other to maintain the very high levels of cell division that accompanies germ cell production/ differentiation, testicular cells are in serious competition for oxygen (Asadi et al., 2017). As a result,

the cells of the testis and the process of spermatogenesis are significantly vulnerable to oxidative stress, which may result in abnormal production of sperm cells and the generation of morphologically abnormal spermatozoa. Increased oxidative stress as indicated earlier directly affects DNA, lipids, and antioxidant enzymes in the germ cells, thereby causing apoptosis and resulting in a quality decline in the overall semen parameters (Agarwal et al., 2014; Rahman and Pang 2019).

The present study also investigated the association between exposure to BPA and some female reproductive hormones. The data obtained from this investigation revealed that there was a significant decrease in the serum levels of estradiol, prolactin and progesterone in BPA-treated groups when compared to the control. The female sex hormones, estradiol and progesterone are both produced by the ovaries. Estradiol and progesterone are vital hormones that aid normal reproductive development and fecundity in females and these hormones are also responsible for the growth of follicles.

Estradiol is an estrogen steroid hormone that is derived from the molecules of cholesterol, another form of estrogen is estriol. BPA is reported to be an estrogenic endocrine distruptor, and can interfere with the activity of nuclear receptors, noncanonical steroid hormone receptors and other receptors such as aryl hydrocarbon receptor, AhR (Wilcox et al., 1988; Norwitz et al., 2001). The decrease in estradiol and progesterone might be as a result of BPA induced low secretion of GnRH which is initiated in the hypothalamus; thereby leading to a low production of FSH (estradiol) and LH (progesterone) released from the anterior pituitary gland (Sembulingam and Sembulingam, 2012). It may also be as a result of BPA destroyed ovaries resulting in the low levels of estradiol and progesterone. The decreased levels of estradiol may result in sterility or irregular cycles in female rats (Fernandez *et al*., 2009; Monje *et al*., 2010; Lee *et al*., 2013).

## 5 Conclusion

It can be concluded that oral administration of BPA for 6 weeks in adult male and female rats resulted in insidious alteration in female reproductive hormones and may also result to impotency in males.

### REFERENCES

[1] Agarwal A, Sharma RK, Nallella KP, Thomas AJ Jr, Alvarez JG, Sikka SC. (2006). Reactive oxygen species as an independent marker of male factor infertility. *Fertil Steril* 86:878-85.

[2] Agarwal A, Virk G, Ong C, du Plessis SS (2014). Effect of oxidative stress on male reproduction. *World J Mens Health* 32:1-17.

[3] Aitken RJ, Paterson M, Fisher H, Buckingham DW, van Duin M. (1995). Redox regulation of tyrosine phosphorylation in human spermatozoa and its role in the control of human sperm function. *J Cell Sci* 108 (5) 2017-2025

[4] Amann, R. P. (1986). Detection of alteration in testicular and epididymal function in laboratory animals. *Environmental Health Perspective*, 70, 119-158.

[5] Asadi N, Bahmani M, Kheradmand A, Rafieian-Kopaei M. (2017). The impact of oxidative stress on testicular function and the role of antioxidants in improving it: a review. *J Clin Diagn Res* 11: 01- 5

[6] Biedermann, S., Tschudin, P. and Grob, K. (2010). Transfer of bisphenol A from thermal printer paper to the skin. *Analytical and Bioanalytical Chemistry*, 398(1), 571-576.

[7] Correia, S., Oliveira, P. F., Guerreiro, P. M., Lopes, G., Alves, M. G., Canário, A. V., Cavaco, J. E. and Socorro, S. (2013). Sperm parameters and epididymis function in transgenic rats overexpressing the Ca2+-binding protein regucalcin: A hidden role for $Ca^{2+}$ in sperm maturation? *Mol. Hum. Reprod*., 19, 581-589.

[8] De Lamirande E, and Gagnon C. (1995). Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects. *Hum Reprod* 10 (1)15-21

[9] Fernandez, M., Bianchi, M., Lux-Santos, V. and Libertun, C. (2009). Neonatal exposure to bisphenol A alters reproductive parameters and gonadotropin releasing hormone signaling in female rats. *Environmental Health Perspectives*, 117(5), 757-762.

[10] Hejmej A., M. Kotula-Balak, and B. Bilinsk, (2011). "Antiandrogenic and estrogenic compounds: effect on development and function of male reproductive system,"in *Steroids – Clinical Aspect*.

[11] Ho, S. M., Tang, W. Y., Belmonte, J. and Prins, G. S. (2006). Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Resources*, 66, 5624.

[12] Hulak, M., Gazo, I., Shaliutina, A., Linhartova, P. (2013). In vitro effects of bisphenol A on the quality parameters, oxidative stress, DNA integrity and adenosine triphosphate content in sterlet (Acipenser ruthenus) spermatozoa. *Comparative Biochemistry and Physiology C: Toxicology and Pharmacol*ogy, 158 (2), 64–71.

[13] Huo, X., Chen, D., He, Y., Zhu, W., Zhou, W. and Zhang, J. (2015). Bisphenol-A and female infertility: a possible role of gene environment interactions. *International Journal of Environmental Research and Public Health*, 12(9), 11101–11116.

[14] Isling, L. K., Boberg, J., Jacobsen, P. R., Mandrup, K. R., Axelstad, M., Christiansen, S., Vinggaard, A. M., Taxvig, C., Kortenkamp, A. and Hass, U. (2014). Late life effects on rat reproductive system after development exposure to mixture of endocrine disruptors. *Reproduction*, 147(4), 465-476.

[15] Jin, P., Wang, X., Chang, F., Bai, Y., Li, Y., Zhou, R. and Chen, L. (2013). Low dose bisphenol A impairs spermatogenesis by suppressing reproductive hormone production and promoting germ cell apoptosis in adult rats. *The Journal of Biomedical Research*, 27(2), 135-144.

[16] Lassen T. H., H. Frederiksen, T. K. Jensen (2014). "Urinary bisphenol a levels in young men: Association with reproductive hormones and semen quality," *Environmental Health Perspectives*, 122(5) 478–484.

[17] Karnam, S. S., Ghosh, R. C., Mondal, S. and Mondal, M. (2015). Evaluation of subacute bisphenol -A toxicity on male reproductive system. *Veterinary World*, 8, 2231-2916.

[18] Kourouma, A., Peng, D., Chao, Q., Changjiang, L., Chengmin, W., Wenjuan, F., Suqin, Q., Tingting, Y. and Kedi, Y. (2014). Bisphenol A induced reactive oxygen species (ROS) in the liver and affect epididymal semen quality in adults Sprague-Dawley rats. *Journal of Toxicology and Environmental Health Science*, 6, 103–112.

[19] Lang, I. A., Galloway, T. S., Scarlett, A., Henley, W. E., Depledge, M. and Wallace, R. B. (2008). Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. The *Journal of the American Medical Association*, 300, 1303.

[20] Le, H. H., Carlson, E. M., Chua, J. P. and Belcher, S.M. (2008). Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. *Toxicology Letters*, 176, 149-156.

[21] Lee, S. G., Kim, J. Y. and Chung, J. Y. (2013). Bisphenol a exposure during adulthood causes augmentation of follicular atresia and luteal regression by decreasing 17$\beta$-estradiol synthesis via down regulation of aromatase in rat ovary. *Environmental Health Perspectives*, 121(6), 663–669.

[22] Li, D. K., Zhou, Z., Miao, M., He, Y., Wang, J., Ferber, J., Herrinton, L. J., Gao, E. and Yuan, W. (2011). Urine bisphenol A (BPA) level in relation to semen quality. *International Journal of Fertility and Sterility*, 95(2), 625-630.

[23] Luke, L., Vicens, A., Tourmente, M. and Roldan, E. R. (2014). Evolution of protamine genes and changes in sperm head phenotype in rodents. *Biology of Reprod*uction, 90, 67.

[24] Matuszczak, E., Komarowska, D. M., Debek, W. and Hermanowicz, A. (2019). The impact of bisphenol a on fertility, reproductive system, and development: a review of the literature. *International Journal of Endocrinology*, 8.

[25] Manfo, F. P., Jubendradass, R., Nantia, E. A., Moundipa, P. F. and Mathur, P. P. (2014). Adverse effects of bisphenol A on male reproductive function. *Reviews of Environmental Contamination and Toxicology*, 228: 57-82.

[26] Meeker, J. D., Ehrlich, S., Toth, T. L., Wright, D. L., Calafat, A. M., Trisini, A. T. and Hauser, R. (2010). Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. *Reproductive Toxicology*, 30, 532–539.

[27] Monje, L. Varayoud, J. Munoz-de-Toro, M. Luque, E. H. and Ramos, J. G. (2010). Exposure of neonatal female rats to bisphenol A disrupts hypothalamic LHRH pre-mRNA processing and estrogen receptor alpha expression in nuclei control lingestrous cyclicity. *Reproductive Toxicology*, 30(4), 625–634.

[28] Norwitz ER, Schust DJ, Fisher S. J. (2001). Implantation and the survival of early pregnancy. *N Engl J Med*. 345:1400–8

[29] O'Connell M., N. McClure, and S. E. M. Lewis, (2002). "The effects of cryopreservation on sperm morphology, motility and mitochondrial function," *Human Reproduction*, 17(3)704–709.

[30] Patisaul, H. B., Fortino, A. E. and Polston, E. K. (2007). Differential disruption of nuclear volume and neuronal phenotype in the preoptic area by neonatal exposure to genistein and bisphenol A. *Neurotoxicology*, 28, 1.

[31] Qiu, L. L., Wang, X., Zhang, X. H., Zhang, Z., Gu, J., Liu, L., Wang, Y., Wang, X. and Wang, S. L. (2013). Decreased androgen receptor expression may contribute to spermatogenesis failure in rats exposed to low concentration of bisphenol A. *Toxicological Letters*, 219(2), 116-124.

[32] Rahman MS, Kwon WS, Lee JS, Kim J, Yoon SJ, Park YJ, (2014). Sodium nitroprusside suppresses male fertility in vitro. *Andrology* 2:899-909.

[33] Rahman, M. S. and Pang, M. G. (2019). Understanding the molecular mechanisms of bisphenol A action in spermatozoa. *Clin Exp Reprod Med* 46(3) 99-106

[34] Saalu, L. C., Kpela, T., Benebo, A. S., Oyewopo, A. O., Anifowope, E. O. and Oguntola, J. A. (2010). The dose-dependent testiculoprotective and testiculotoxic potentials of telfairia occidentalis hook f. Leaves extract in rat. *International Journal Applied Research in Natural Products*, 3, 27-38.

[35] Salian, S., Doshi, T. and Vanage, G. (2009) Neonatal exposure of male rats to bisphenol A impairs fertility and expression of sertoli cell junctional proteins in the testis. *Toxicology*, 265(1-2), 56-67.

[36] Salian, S., Doshi, T. and Vanage, G. (2011). Perinatal exposure of rats to bisphenol A affects fertility of male offspring an overview. *Reproductive Toxicology*, 31 359–362. (doi:10.1016/j.reprotox.2010.10.008).

[37] Sembulingam, K. and Sembulingam, P. (2012). *Essentials of Medical Physiology* (6th ed.). New Delhi: Jaypee Brothers Medical Publishers. 478-481.

[38] Shuo, X., Honglu, D., Mary, A. S., Xiao, S. and Xiaoqin, Y. (2011). Preimplantation exposure to bisphenol A (BPA) affects embryo transport, preimplantation embryo development and uterine receptivity in mice. *Reproductive Toxicology*, 32(4), 434-441.

[39] Sugiura-Ogasawara, M., Ozaki, Y., Sonta, S., Makino, T. and Suzumori, K. (2005). Exposure to bisphenol A is associated with recurrent miscarriage, *Human Reproduction*, 20, 2325.

[40] Tinwell, H., Haseman, J., Lefevre, P. A., Wallis, N. and Ashby, J. (2002). Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. *Toxicological Science*, 68, 339.

[41] Vandenberg, L. N., Maffini, M. V., Sonnenschein, C., Rubin, B. S. and Soto, A. M. (2009). Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. *Endocrine Reviews*, 30, 75-95.

[42] Vilela, J., Hartmann, A., Silva, E. F., Cardoso, T., Corcini, C. D., Varela-Junior, A. S., Martinez, P. E. and Colares, E. P. (2014). Sperm impairments in adult vesper mice (*Calomys laucha*) caused by in uteroexposure to bisphenol A. *Andrologia*, 46(9), 971-978.

[43] Wang, P., C. Luo, Q. Li, S. Chen, and Y. Hu, (2014). "Mitochondrion-mediated apoptosis is involved in reproductive damage caused by BPA in male rats," *Environmental Toxicology and Pharmacology*, 38(3) 1025–1033.

[44] Wilcox AJ, Weinberg CR, O'Connor JF, (1988). Incidence of early loss of pregnancy. *N Engl J Med*. 319:189–94.

[45] Witorsch, R. J. (2002). Endocrine disruptors: can biological effects and environmental risks be predicted? *Regulatory Toxicology and Pharmacology*, 36, 118-130.

[46] Zhou W, F. Fang, W. Zhu, Z. Chen, Y. Du, and J. Zhang,( 2016). "Bisphenol A and ovarian reserve among infertile women with polycystic ovarian syndrome," International Journal of Environmental Research and Public Health, 27 pp. 14.

[47] Zoeller, R. T., Bansal, R. and Parris, C. (2005). Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. *Endocrinology*, 146, 607.