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Bisphenol A modifies human spermatozoa motility *in vitro*

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ABSTRACT

Introduction. The decrease of men's sperm quality was reported to be related to exposure to xenoestrogens. Bisphenol-A (BPA) is a synthetic xenoestrogen commonly present in our environment, for instance in food containers.
Aim. The aim of this study was to investigate the influence of BPA on human spermatozoa motility.
Materials and Methods. The effects on spermatozoa of BPA at final concentrations of 10^{-10}, 10^{-8} and 10^{-6} mol/L were studied regarding to the following phenomena: (1) evaluation of sperm motility using computer-aided sperm analysis system providing four parameters: velocity straight linear VSL, cross beat frequency CBF, lateral head displacement LHD and homogeneity of progressive movement velocity HPMV, (2) spermatozoa vitality (propidium iodide staining), (3) phosphatidylserine membrane translocation (staining with annexin V conjugated with fluorescein) and (4) kinetics of intracellular free calcium ions changes (using Fluo-3).
Results. BPA caused a transient, significant increase of VSL and HPMV at 15 minutes after stimulation. One hour incubation of spermatozoa with BPA did not alter cells vitality nor stimulated phosphatidylserine membrane translocation, for all three concentrations. BPA in the final concentration of 10^{-6} mol/L initiated a rapid (observed after a few seconds), and transient (resolving after a few minutes) increase of intracellular free calcium ions concentration.
Conclusions. Human spermatozoa can be considered target cells for BPA. BPA significantly modified spermatozoa motility. BPA affected spermatozoa involving free calcium ions as second messenger.

Keywords: bisphenol A; human spermatozoa; sperm cells motility.

Introduction

Male infertility contributes to 50% of all infertility cases. Risk factors for male infertility include: varicocele, aging, sexually transmitted diseases, lifestyle factors and long-term or intensive exposure to certain types of toxins, chemicals and medications [1]. Estrogen-like and endocrine disrupting (EDs) chemicals that alter the normal function of hormones, such as phthalates, organochlorines and bisphenol A are of particular potential concerns. When xenoestrogens enter the body they increase the total amount of estrogen resulting in a phenomenon called estrogen dominance. Xenoestrogens are not biodegradable therefore, they are stored in fat cells [2, 3].

Several studies have reported the association of exposure to phthalates and bisphenol A with impaired semen quality [4, 5]. Bisphenol A (BPA; 4,4'-(propane-2,2-diyl) diphenol) belongs to the group of diphenylmethane derivatives and bisphenols, with two hydroxyphenyl groups. BPA are non-persistent EDs mainly used as plasticizers, which are widely present in foods. BPA is used in food containers (bottles, microwave ovenware, and linings for canned foods and beverages) but also in non-food items, including epoxy-resin based paints,

PVC medical devices, thermal paper and parts of electronic devices [2, 6, 7].

Estrogenic effects are mediated by two types of intracellular receptors: estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2). BPA is mainly considered as an ESR1 and ESR2 agonist and in that manner has significant impact on human cells biology. Previous research revealed that it can also act as antagonist of the androgen receptor or as agonist of the aryl hydrocarbon receptor and other nuclear receptors [8, 9].

The review of available literature demonstrates that mature sperm cells are target cells for estrogen action. ESR1 and ESR2 presence was confirmed in spermatozoa [10, 11]. It is pointed out that estrogens may influence both the capacitation and acrosomal reaction [12, 13]. BPA is present in woman's reproductive tracts. It's concentration within the follicular fluid ranges from 1.0 ng/mL to 2.0 ng/mL [14]. It is assumed that BPA may affect the biology of mature sperm cells in woman's reproductive tract and thus influence the fertilization process [12].

Motility is the basic parameter that enables sperm cells to fertilize the egg cell. Motility parameters change throughout capacitation in process called hiperactivation and influence their biological quality [15]. The aim of the study was to investigate whether BPA influences sperm motility under *in vitro* conditions.

Materials and Methods

Preparation of human spermatozoa

Semen samples obtained from 20 normozoospermic men were analyzed according to WHO criteria 2010 [22]. 3–5 days period of sexual abstinence was required prior to obtaining the material. High motility sperm cells were isolated with the use of the swim-up technique [23]. Ham's F-10 medium served as sperm cell extender.

Isolated cells were incubated with BFA in final concentrations of 10^{-10}, 10^{-8} and 10^{-6} M. Spermatozoa motility was noted at 5, 10, 15, 30 and 60 minutes after exposure to BPA. Spermatozoa incubated in Ham's F-10 medium were used as controls. Spermatozoa vitality and phosphatidylserine membrane translocation were assessed at 60 minutes after exposure to BPA.

Assessment of sperm cells motility

Sperm cells motility was analyzed in human spermatozoa (1×10^5 sperm cells/mL) suspended in Hams F-10 medium using a computer-assisted spermatozoa motility analysis system. Ten microliters of spermatozoa suspension was spotted onto a Cell Vision chamber slide, producing a specimen with uniform thickness. Images were collected at a frequency of 60 frames per second using a Pixel-Link camera. Motility in the microscopic specimen was assessed in a minimum of 10 different fields for each case, yielding a minimum of 700 analyzed sperm cells. Acquisition time was 2.08 s; the analyzed area was 640 × 470 μm; and the resolving power was 0.86 points. All measurements were made at a constant, controlled temperature of 24°C.

The following spermatozoa motility parameters were analyzed: (1) velocity straight linear (VSL), (2) cross-beat frequency (CBF), (3) lateral head displacement (LHD) and (4) homogeneity of progressive movement velocity (HPMV). These parameters were calculated as follows:

1. VSL was calculated matching the sperm path with two sectors (the method involves n sectors where n-1 is the period of observation in seconds). The sectors were obtained minimizing total sum, evaluated for all sectors, of squared distances from the sector to sperm mass center. The idea was to avoid erratic evaluation of VSL for colliding sperms, often found for higher sperm concentrations.
2. CBF was evaluated using Fourier series calculated on basis of distances from sperm mass centers to corresponding sectors (see VSL calculation).
3. LHD was calculated as standard deviation of distances from sperm mass center to corresponding sectors (see VSL calculation).
4. HPMV was evaluated as standard deviation of distances from predicted sperms orthogonal cast on corresponding (see VSL calculation) and sperm mass center cast. This parameter describes vibration of sperm observed at velocity VSL along sperm pathway.

Assessment of sperm cells vitality and phosphatidylserine membrane translocation

To determine phosphatidylserine membrane translocation (PST) from the inner to the outer layer of the plasma membrane, the annexin-V labeled with fluorescein (AnV-FLUOS) (Molecular Diagnostics, Darmstadt, Germany) was used. Simultaneously, to distinguish between viable and dead spermatozoa the propidium iodide (PI) staining was used, in the final concentration 0.125 μg/L (Sigma-Aldrich, St. Louis, MO). Double staining was conducted according to manufacturer's recommendations.

Flow cytometry

The fluorescence signals of labeled spermatozoa were analyzed by flow cytometer FACSCalibur (Becton–

Dickinson, USA). 10 000 cells were examined for each experiment. The fluorescence of An-V-FLUOS and PI was excited by argon laser (488 nm) and emission of An-V-FLUOS was measured in the FL1 channel (515–545 nm), while the red fluorescence of PI was detected in the FL3 channel (650 nm). All data was collected and analyzed using CellQuest Pro software (v.5.2.1) (Becton–Dickinson).

Changes in intracellular free calcium ions level

Fluo-3 (Molecular Probes; Ex/Em = 488/526 nm) was used to study changes in free calcium ions level in human sperm cells. Spermatozoa (1 x 10^6 cells/mL) were incubated with 4 μM Fluo-3 for 45 min at 37°C according to the manufacturer's protocol. For confocal microscopy, spermatozoa were immobilized in 1% (w/v) agarose and then treated with BPA. Microscopic images were used for gating single sperm cells in which fluorescence changes were recorded. Forty images were collected (every 10s) and used to study the kinetics of intracellular free calcium ions changes. Spermatozoa were observed using LSM 510 confocal microscope (Zeiss, Jena, Germany) equipped with a Plan Apochromat 63x/1.4 Oil DIC objective. Sperm cells incubated in Ham's F-10 medium were used as a control for fluorescence intensity changes. Changes of Ca^{2+} level were examined throughout 400 seconds after exposure to BPA, every 10 seconds. Sperm cells vitality was checked with saturated potassium chloride solution.

Statistical analysis

Each variable was tested using the Shapiro-Wilk W-test for normality. Homogeneity of variance was assessed with Levene's test. Since the distribution of the variables was normal and the values were homogeneous in variance, all statistical analyses were performed using parametric tests. The analysis was made using Statistica 10 software (StatSoft Inc., Tulsa, OK, USA). Data were presented as mean ± SD and considered statistically significant at $P < 0.05$.

Ethics Statement

The study protocol was approved by the Institutional Review Board of the Poznan University of Medical Sciences (No 119/09). All the involved patients provided written informed consent.

Results

Control group motility

Marked heterogeneity of human sperm cells motility parameters was observed (**Table 1**). No significant parameters change ($P > 0.05$) was observed during 60 minutes observation.

Table 1. Control group motility parameters

	Mean	Minimum	Maximum	SD
VSL [μm/s]	13.6	2.6	56.7	11.7
CBF [Hz]	7.6	0.5	30.0	7.5
LHD [μm]	0.6	0.2	3.5	0.5
HPMV [μm/s]	1.1	0.2	8.2	0.7

VSL – velocity straight linear, CBF – cross-beat frequency LHD – lateral head displacement, HPMV – homogeneity of progressive movement velocity, SD – standard deviations

Impact of bisfenol A on spermatozoa motility

Velocity Straight Linear

Spermatozoa stimulated with BPA in final concentration of 10^{-10} mol/L and 10^{-8} mol/L revealed transient, statistically significant increase of VSL at 15 minutes after stimulation. The VSL values at 30 and 60 minutes after stimulation did not significantly differ from controls. Spermatozoa stimulated with BPA in final concentration of 10^{-6} mol/L revealed statistically significant ($P < 0.05$) increase of VSL both at 15 minutes and at 30 minutes after stimulation (**Figure 1A**).

Cross-Beat Frequency

No significant CBF changes were observed for all BPA concentrations or times after BPA stimulation (**Figure 1B**).

Lateral Head Displacement

No significant LHD changes were observed for all BPA concentrations or times after BPA stimulation (**Figure 1C**).

Homogeneity of Progressive Movement Velocity

Spermatozoa stimulated with BPA in final concentration of 10^{-10} mol/L and 10^{-8} mol/L revealed transient, statistically significant increase of HPMV at 15 minutes after stimulation. The VSL values at 30 and 60 minutes after stimulation did not significantly differ from controls ($P > 0.05$).

Spermatozoa stimulated with BPA in final concentration of 10^{-6} mol/L revealed statistically significant ($P < 0.05$) increase of VSL both at 15 minutes and at 30 minutes after stimulation (**Figure 1D**).

Effects of bisphenol-A on sperm vitality and phosphatidylserine membrane translocation

Flow cytometry analyses identified four fractions of spermatozoa: (1) An-V^-/PI^- viable sperm without PST, (2) An-V^+/PI^- viable sperm with PST, (3) An-V^-/PI^+ dead sperm without PST and (4) An-V^+/PI^+ dead sperm with PST (Figure 2). The sperm cells percentage of each frac-

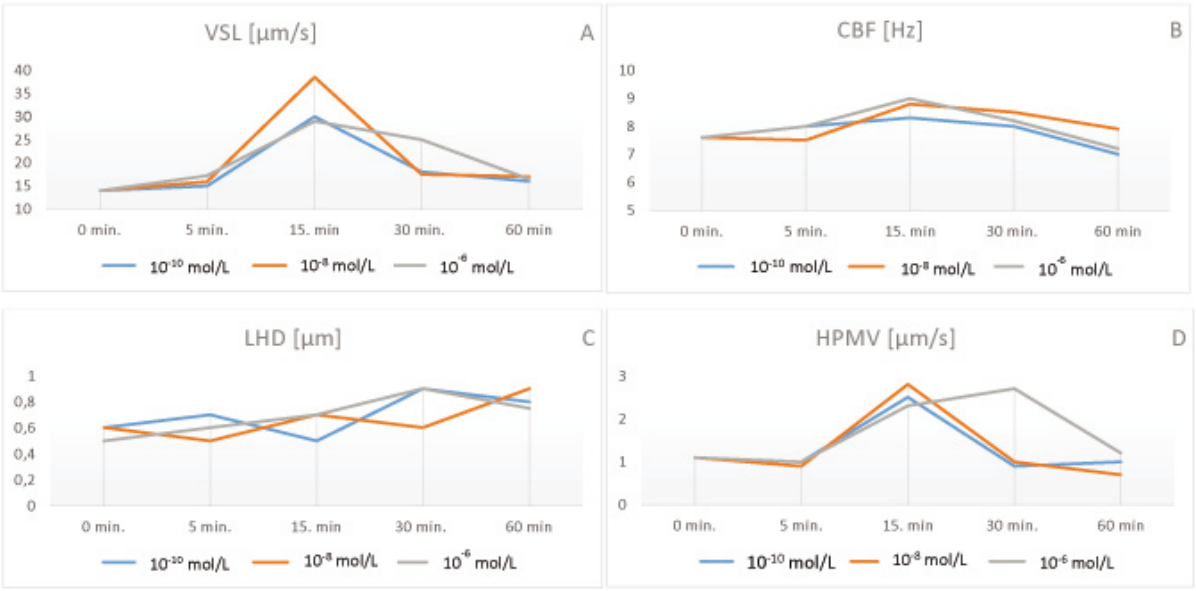

Figure 1. Changes of spermatozoa motility parameters after BPA stimulation. (A) VSL – velocity straight linear, (B) CBF – cross-beat frequency, (C) LHD – lateral head displacement, (D) HPMV – homogeneity of progressive movement velocity

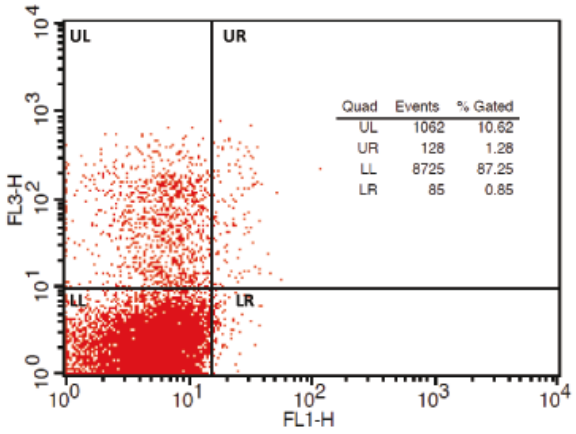

Figure 2. Flow cytometry analysis of spermatozoa vitality and phosphatidylserine membrane translocation in swim-up selective cells – representative graph. UL (upper left quadrant) – positive propidium iodide cells, UR (upper right quadrant) – positive propidium iodide and annexinV cells, LL (lower left quadrant) – non stained cells; LR (lower right quadrant) – cells positive only to annexin V. FL1-H – fluorescence channel 515 – 545 nm, FL3-H – fluorescence channel > 650 nm

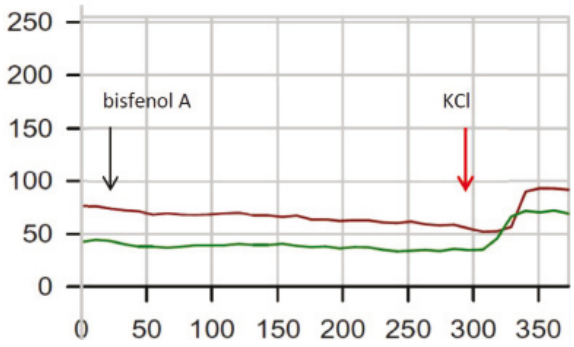

Figure 3. Kinetics of free intracellular calcium ions change in human spermatozoa stimulated with BPA at 10^{-10} mol/L final concentration. Arrows indicate administration of BPA and KCl

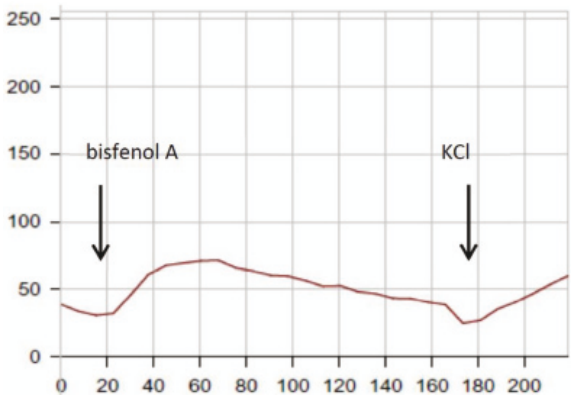

Figure 4. Kinetics of free intracellular calcium ions change in human spermatozoa stimulated with BPA at 10^{-6} mol/L final concentration. Arrows indicate administration of BPA and KCl

tion was 81.2 ± 6.1%, 0.4 ± 0.3%, 15.9 ± 5.5% and 2.4 ± 1.4%, respectively. It did not change after 60 minutes incubation with BPA, for all concentrations used.

Changes in intracellular free calcium ions level

In spermatozoa isolated with swim-up technique the highest concentration of intracellular free calcium ions was observed within the midpiece and distal part of head. Stimulation with 10^{-10} mol/L or 10^{-8} mol/L BPA did not change intracellular free calcium ions level (**Figure 3**). Stimulation with 10^{-6} mol/L caused a rapid,

transient increase of intracellular free calcium level. The reaction was observed at 10 seconds after stimulation and lasted a few minutes (**Figure 4**).

Discussion

Bisphenol A is the synthetic compound that exhibits activity similar to 17β-estradiol (E2). Due to its physicochemical properties it is extensively used in the manufacture of wide variety of common consumer goods. The safety of BPA is often disputed. Therefore, its usage in production process has to be regulated according to applicable law [6, 7].

Sperm cells are potential target cells for estrogens and xenoestrogens, which may alter their biological function. It is established that exogenous 17β-estradiol stimulates hamster's sperm cells motility. Jin et al. indicated that different concentrations of 17β-estradiol caused significant increase of all spermatozoa motility parameters, excluding the linear index in the group of animals treated with high doses of E2. The research also revealed that active immunization to E2 decreased significantly the sperm cells motility [16].

Available literature demonstrates that also xenoestrogens, including BPA, may influence semen quality and its parameters such as sperm motility and velocity [17, 18].

Rat sperm cells motility decreased in relation to the diet containing health hazardous xenoestrogens such as dicofol and mixture of dieldryn, endosulfan, dicofol, dichlorvos and permethrin. There were no changes in motility after administration of above mentioned compounds separately [19].

It was revealed that genistein and 4-tert-octylphenol affect capacitation and acrosome reaction of boar spermatozoa. It turned out that genistein acts similarly to estrogen while, in comparison to 4-tert-octylphenol, its stimulative effect on both above mentioned processes is stronger [20].

The correlation between increased level of BPA in urine and decreased semen quality was also proven in men exposed to BPA in work environment. BPA exposure resulted in decreased sperm cells count, concentration, motility and vitality [21].

Described research data is not consistent with results obtained in our study. For all the BPA doses we observed transient increase of both, velocity straight linear and homogeneity of progressive movement velocity parameters, which occurred 15 minutes after stimulation. We also observed increase of these parameters at 30 minutes after stimulation with the highest concentration of BPA (10^{-6} mol/L). The lateral head displacement and cross-beat frequency values did not change significantly with regard to either BPA dose or time of incubation. In presented research model sperm cells were incubated in estrogen free medium, thus the only compound able to mimic estrogen action was BPA. Therefore, it can be assumed that bisphenol-A stimulates spermatozoa motility in a transient way and the reaction is dose dependent.

Probably, BPA uses endogenous estrogen signaling pathway and similarly to 17β-estradiol modulates sperm motility. This may be related to its effects on sperm mitochondrial potential, and thus, the production of ATP. It seems probable because of the proven midpiece localization of estrogen receptors in human sperm. This region of sperm cell is also characteristic for exclusive mitochondrial occurrence and mitochondria are supposedly target organelles for estrogen action [10, 22, 23].

There are few studies examining the effects of estrogens and xenoestrogens on vitality and apoptosis of mature sperm. In the conducted studies we analyzed whether bisphenol-A affects the vitality and the process of phosphatidylserine membrane translocation, which is the marker of apoptosis. There were no significant changes in viable sperm cells percentage and the percentage of spermatozoa presenting phosphatidylserine membrane translocation, irrespectively to the applied doses [24–26].

The level of calcium ions, which is one of important factors controlling the process of capacitation and acrosome reaction, was also assessed in the present study. Unlike somatic cells, spermatozoa have much less buffering capacity of Ca^{2+} in its organelles. That is why the proper calcium homeostasis is maintained through the system of calcium channels and pumps. Calcium ions storage takes place mainly in mitochondria, acrosome and posterior part of the head [27, 28]. Stimulation with 17β-estradiol causes transient increase of intracellular free calcium ions level in sperm cells [29]. Our research revealed that stimulation with bisphenol-A at final concentrations 10^{-10} mol/L and 10^{-8} mol/L induced the increase of calcium ion levels only in single cells while the concentration 10^{-6} mol/L caused rapid, transient, significant increase of intracellular free Ca^{2+} level in the whole population of observed cells.

In conclusion, our study along with literature findings have shown that human spermatozoa are target cells for BPA. BPA significantly modified spermatozoa motility. BPA affected spermatozoa involving free calcium ions as second messenger.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

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References

1. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson AM, Eisenberg ML, Kold Jensen T, et al. Male reproductive disorders and fertility trends: Influences of environment and genetic susceptibility. Physiol Rev. 2016;96:55–97.
2. La Rocca C, Tait S, Guerranti C, Busani L, Ciardo F,Bergamasco B, Perra G, et al. Exposure to endocrine disruptors and nuclear receptors gene expression in infertile and fertile men from italian areas with different environmental features. Int J Environ Res Public Health. 2015;12:12426–45.
3. Watson CS, Jeng YJ, Guptarak J. Endocrine disruption via estrogen receptors that participate in nongenomic signaling pathways. J Steroid Biochem Mol Biol. 2011;127:44–50.
4. Rozati R, Reddy PP, Reddanna P, Mujtaba R. Role of environmental estrogens in the deterioration of male factor fertility. Fertil Steril. 2002;78:1187–94.
5. Rozati R, Reddy PP, Reddanna P, Mujtaba R. Xenoesterogens and male infertility: myth or reality? Asian J Androl. 2000;2:263–9.
6. World Health Organization, Food and Agriculture Organization of Unated Nations: Bisphenol A (BPA) Current state of knowledge and future actions by WHO and FAO. International Food Safety Authorities Network (INFOSAN), 2009 [dostęp: 26 września. 2013], dostępne:http://www.who.int/foodsafety/publications/fs_management/No_05_Bisphenol_A_Nov09_en.pdf.
7. EFSA. Scientific Opinion on the Risks to Public Health Related to the Presence of Bisphenol A (BPA) in Foodstuffs—Part I: Exposure Assessment. Available online: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3978part1.pdf (accessed on 6 August. 2015).
8. Sui Y, Ai N, Park SH, Rios-Pilier J, Perkins JT, Welsh WJ, et al. Bisphenol A and its analogues activate human pregnane X receptor. Environ Health Perspect. 2012;120:399–405.
9. Tait S, Tassinari R, Maranghi F, Mantovani A. Bisphenol A affects placental layers morphology and angiogenesis during early pregnancy phase in mice. J Appl Toxicol. 2015;35:1278–91.
10. Aquila S, Sisci D, Gentile M, Middea E, Catalano S, Carpino A, Rago V. Estrogen receptor (ER) alpha and ER beta are both expressed in human ejaculated spermatozoa: evidence of their direct interaction with phosphatidylinositol-3-OH kinase/Akt pathway. J Clin Endocrinol Metab. 2004;89:1443–51.
11. Rago V, Siciliano L, Aquila S, Carpino A. Detection of estrogen receptors ER-alpha and ER-beta in human ejaculated immature spermatozoa with excess residual cytoplasm. Reprod Biol Endocrinol. 2006;4:1–6.
12. Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Hum Reprod. 2002;17:2839–41.
13. Ded L, Dostalova P, Dorosh A, Dvorakova-Hortova K, Peknicova J. Effect of estrogens on boar sperm capacitation in vitro. Reprod Biol Endocrinol. 2010;8:87.
14. Filannino A, Stout TAE, Gadella BM, Sostaric E, Pizzi F, Colenbrander B, et al. Dose-response effect of estrogenic mycotoxins (zearalenone, alpha- and beta-zearalenol) on motility, hyperactivation and the acrosome reaction of stallion sperm. Reprod Biol Endocrinol. 2011;9:134.
15. Fujinoki M, Takei GL, Kon HJ. Non-genomic regulation and disruption of spermatozoal in vitro hyperactivation by oviductal hormones. J Physiol Sci. 2015 Nov;1–6.
16. Jin W, Arai KY, Watanabe G, Suzuki AK, Takahashi S, Taya K. The stimulatory role of estrogen on sperm motility in the male golden hamster (Mesocricetus auratus). J Androl. 2005;26:478–84.
17. Hatef A, Alavi SM, Abdulfatah A, Fontaine P, Rodina M, Linhart O. Adverse effects of bisphenol A on reproductive physiology in male goldfish at environmentally relevant concentrations. Ecotoxicol Environ Saf. 2012;76:56–62.
18. De Flora S, Micale RT, a Maestra S, Izzotti A, D'Agostini F, Camoirano A, Davoli SA, et al. Upregulation of clusterin in prostate and DNA damage in spermatozoa from bisphenol A-treated rats and formation of DNA adducts in cultured human prostatic cells. Toxicol Sci. 2011;122:45–51.
19. Perobelli JE, Martinez MF, da Silva Franchi CA, Fernandez CD, de Camargo JL, Kempinas WG. Decreased sperm motility in rats orally exposed to single or mixed pesticide. J Toxicol Environ Heath. 2010;73:991–1002.
20. Mohamed EA, Park Y, Song W, Shin D, You Y, Ryu B, Pang M. Xenoestrogenic compounds promote capacitation and an acrosome reaction in porcine sperm. Theriogenology. 2011;75:1161–9.
21. Li DK, Zhou Z, Miao M, He Y, Wang J, Ferber J, Herrinton LJ, Gao E, Yuan W. Urine bisphenol-A (BPA) level in relation to semen quality. Fertil Steril. 2011;95:625–30.
22. Durkee TJ, Mueller M, Zinaman M. Identification of estrogen receptor protein and messengerribonucleic acid in human spermatozoa. Am J Obstet Gynecol. 1998;178:1288–97.
23. Solakidi S, Psarra AM, Nikolaropoulos S, Sekeris CE. Estrogen receptors alpha and beta (ERalpha and ERbeta) and androgen receptor (AR) in human sperm: localization of ERbeta and AR in mitochondria of the midpiece. Hum Reprod. 2005;20:3481–7.
24. Kotwicka M. Błonowa translokacja fosfatydyloseryny jako wykładnik biologicznych procesów zachodzących w plemnikach ludzkich. Wydaw. Nauk. Uniw. Med. im. K. Marcinkowskiego, Poznań. 2008.
25. Kotwicka M, Jendraszak M, Skibińska I, Jędrzejczak P, Pawelczyk L. Decreased motility of human spermatozoa presenting phosphatidylserine membrane translocation--cells selection with the swim-up technique. Hum Cell. 2013;26:28–34.
26. Kotwicka M, Filipiak K, Jedrzejczak P, Warchol JB. Caspase-3 activation and phosphatidylserine membrane translocation in human spermatozoa: is there a relationship? Reprod Biomed Online. 2008;16:657–63.

27. Baldi E, Luconi M, Muratori M, Marchiani S, Tamburrino L, Forti G. Nongenomic activation of spermatozoa by steroid hormones: facts and fictions. Mol Cell Endocrinol. 2009;308:39–46.
28. Espinosa F, López-González I, Muñoz-Garay C, Felix R, De la Vega-Beltrán JL, Kopf GS,Visconti PE, Darszon A. Dual regulation of the T-type Ca2+ current by serum albumin and betaestradiol in mammalian spermatogenic cells. FEBS Lett. 2000;475:251–6.
29. Luconi M, Bonaccorsi L, Forti G, Baldi E. Effects of estrogenic compounds on human spermatozoa: evidence for interaction with a nongenomic receptor for estrogen on humansperm membrane. Mol Cell Endocrinol. 2001;178:39–45.

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