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# Bisphenol A accelerates capacitation-associated protein tyrosine phosphorylation of rat sperm by activating protein kinase A

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## Abstract

Bisphenol A (BPA) is a synthetic estrogen-mimic chemical. It has been shown to affect many reproductive endpoints. However, the effect of BPA on the mature sperm and the mechanism of its action are not clear yet. Here, our *in vitro* studies indicated that BPA could accelerate sperm capacitation-associated protein tyrosine phosphorylation in time- and dose-dependent manners. *In vivo*, the adult male rats exposed to a high dose of BPA could result in a significant increase in sperm activity. Further investigation demonstrated that BPA could accelerate capacitation-associated protein tyrosine phosphorylation even if sperm were incubated in medium devoid of BSA, $HCO_3^-$, and $Ca^{2+}$. However, this action of BPA stimulation could be blocked by H89, a highly selective blocker of protein kinase A (PKA), but not by KH7, a specific inhibitor of adenylyl cyclase. These data suggest that BPA may activate PKA to affect sperm functions and male fertility.

**Key words:** bisphenol A, sperm, tyrosine phosphorylation, protein kinase A

## Introduction

Bisphenol A (BPA) is a small estrogenic monomer, and it is an important industrial material that is polymerized to produce polycarbonate plastics and epoxy resins. BPA is widely used in products for food and beverage packaging, baby bottles, dental sealants and fillings, adhesives, protective coatings, flame retardants, water supply pipes, compact discs, and so on. Humans are exposed to BPA through their diet, inhalation of household dust, and dermal exposure [1]. Numerous studies have shown that BPA is detectable in the urine of almost all tested adults and children [2,3], as well as in the serum of pregnant women and breast milk [4]. So BPA has been considered as a main endocrine disruptor and received a tremendous amount of attention due to its high potential for human exposure.

Overwhelming evidence has shown that BPA has profound adverse effects on human health, including reproductive and developmental effects, metabolic disease, and other health effects [5]. As for reproduction, BPA has been shown to affect many endpoints of fertility, such as spermatogenesis, fertilizing capacity, sexual function, ovarian response, and embryo development [5]. A large body of literature has demonstrated that prenatal or early environment

exposure to BPA leads to adverse adult outcome [6]. Fetus exposure to BPA in utero results in permanent alterations of morphology, histoarchitecture, and cell proliferation control in androgen- or estrogen-target tissues in adult life [7]. They were found to produce postnatal estrogenic effects, such as increased prostate gland weight, reduced daily sperm production in males, and accelerated growth and puberty in females [8]. In addition, some reports also demonstrated that BPA has an influence on the reproductive capacity of animal exposed to BPA. Female mice mated with male ones that had ingested BPA showed a significant reduction in pregnancy rates [9]. Male animals exposed to BPA showed an evident decrease in testicular and epididymal sperm counts [9,10]. Administration of BPA could cause a reduction in rat epididymal sperm count and motility in a dose-dependent manner [11]. In humans, higher urinary BPA was found to be significantly correlated with a lower sperm quality [12] and a poorer ovarian response [13]. However, there are also other reports indicating that BPA has no toxic effect on the reproductive system [7].

BPA has been found to bind to estrogen receptors (ERs) and exert its estrogenic or anti-estrogenic effects by competing with endogenous 17-β estradiol. BPA can also bind to androgen and thyroid receptors, and have agonistic or antagonistic effects on the function of corresponding target organs [5]. However, studies of molecular mechanism have revealed a variety of pathways through which BPA can stimulate cellular responses in addition to effects initiated by binding of BPA to the classical nuclear genomic receptors [14]. A few studies have revealed that endocrine-disrupting chemicals including BPA can exert their effects through hormone-independent mechanisms or through a nongenomic activation of membrane-initiated signaling pathways via membrane forms of ERs [15]. Exploring the molecular mechanism that mediates the effects of BPA on adverse aspects of health is the goal of many researchers.

Although BPA has been extensively studied for its reproductive toxicity in experimental animals, whether the mature sperms are affected by BPA, and the precise mechanism of action of BPA have not been elucidated [16,17]. To gain further insight into the effect of BPA on the male reproduction, the present study was conducted *in vitro* and *in vivo* to investigate the influence of BPA on the mature sperm and to explore the mechanism of its action.

## Material and Methods

### Animals

Healthy Sprague-Dawley (SD) rats were purchased from the Animal Center of the Chinese Academy of Sciences (Shanghai, China). Animals were housed under standard laboratory conditions. Experiments were conducted according to a protocol approved by the Institute Animal Care Committee of Shanghai Institute of Biochemistry and Cell Biology. The protocol conformed to internationally accepted guidelines for the humane care and use of laboratory animals.

### Chemicals and medium

All chemicals were purchased from Sigma-Aldrich Co. (St Louis, USA) with the following exceptions: human tubal fluid (HTF) for *in vitro* fertilization (IVF) medium (SAGE, Trumbull, USA); mouse monoclonal anti-phosphotyrosine antibody (clone 4G10, 1 mg/ml; Millipore, Boston, USA); and anti-phospho-PKA substrates antibody (clone 100G7E; Cell Signaling, Danvers, USA). Dimethyl sulphoxide (DMSO) was used as solvent. All compounds were used at a 1/1000 dilution to give desired final concentrations containing 0.1% DMSO. DMSO has no detectable effect on sperm function at very low final concentration (0.1%, 0.25%, and 0.5%) used in this study.

### Preparation of sperms

Caudal epididymal sperms were released into the capacitating medium containing 94.6 mM NaCl, 25 mM KCl, 1.19 mM $KH_2PO_4$, 1.19 mM $MgSO_4$, 1.71 mM $CaCl_2$, 25 mM $NaHCO_3$, 10.76 mM sodium lactate, 0.5 mM sodium pyruvate, 5.56 mM glucose, 0.0002% phenol red, 4 mg/ml bovine serum albumin (BSA), 50 μg/ml streptomycin sulfate, and 75 μg/ml potassium penicillin, with the pH adjusted to 7.3. The final concentration of sperm was adjusted to 5–10 × $10^6$ sperms/ml in the medium.

In some experiments, non-capacitating medium, such as $Ca^{2+}$-free medium (capacitating medium without $CaCl_2$), BSA-free medium (capacitating medium without BSA), and $HCO_3^-$-free medium (capacitating medium in which 25 mM $NaHCO_3$ was replaced by 25 mM NaCl), were also used, with the pH adjusted to 7.3.

### Sperm motility analysis

Sperm motility was analyzed using an HTM-TOX IVOS sperm motility analyzer (Rat Head Toxicology, version 12.3A; Hamilton-Thorn Research, Beverly, USA) as in our previous method [18]. The instrument settings used during the analysis were: temperature, 37°C; minimum cell size, 5 pixels; minimum contrast, 50; minimum static contrast, 25; low VAP cut-off, 20.0; low VSL cut-off, 30.0; threshold straightness, 50%; static head size, 0.3–1.95; static head intensity, 0.5–1.3; and magnification, 0.89. Thirty frames were acquired at a frame rate of 60 Hz. The playback feature was used during analysis to check the accuracy of the method. At least 10 microscopy fields were analyzed in each test.

### SDS-PAGE and immunoblotting

The protein tyrosine phosphorylation was detected as described in our previous studies [18,19] with some modifications on the treatment of spermatozoa. Briefly, the spermatozoa from the caudal epididymis were released into the capacitating medium, adjusted to a concentration of 5–10 × $10^6$ sperms/ml. After incubation for various periods of time with different concentrations of BPA, the sperms were collected and proteins were extracted as previously described [20]. Total sperm protein was separated by 12% SDS-PAGE. Tyrosine-phosphorylated proteins of sperms were probed with mouse monoclonal anti-phosphotyrosine antibody (clone 4G10, 1:10,000). PKA substrate phosphorylation was measured using anti-phospho-PKA substrates antibody (clone 100G7E, 1:5000). To confirm equal protein loading, the blots were stripped and reprobed with anti-α-tubulin monoclonal antibody (clone B515, 1:50,000; Sigma-Aldrich Co.).

### *In vitro* fertilization assay

IVF was carried out following a standard protocol [21] with some modifications on the processing of spermatozoa. Briefly, the spermatozoa from the caudal epididymis were released into the capacitating medium. After incubation with different concentrations of BPA for 3 h, capacitated sperms were washed by capacitation medium to remove BPA, and then added into the medium containing oocytes. After 6 h of insemination, the eggs were removed and transferred serially through several drops of fresh fertilization medium to remove the free sperms. The fertilized eggs were incubated in HTF medium. The percentage of 2-cell-stage embryos was recorded after 48 h of incubation.

### BPA exposure *in vivo* and male fertility assay

Healthy male SD rats, body weight (bw) > 450 g, were randomly assigned into three groups ($n$ = 10 in each group): group I (0 mg/kg

bw/day BSA, corn oil vehicle), group II (0.0005 mg/kg bw/day BSA), and group III (200 mg/kg bw/day BSA). The concentration of 0.0005 mg/kg bw/day BSA was based on the acceptable amount of BPA for human daily intake from food [22]. The dose of 200 mg/kg bw/day BSA was designed and selected according to our unpublished data. BPA was orally administrated with a micropipette for consecutive 8 weeks. At the end of 4 and 8 weeks, five male rats of each group were subject to mate with normal female SD rats for 7 days. Then the pups and fetuses from pregnant females were recorded respectively. All male rats were killed at the end of 8 weeks. The motility of sperms from these rats was analyzed as described above.

### Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison post hoc test and was calculated using the GraphPad Prism 5. $P < 0.05$ was considered as statistically significant.

## Results

### BPA can accelerate sperm capacitation-associated protein tyrosine phosphorylation

To test the effect of BPA on sperm maturation *in vitro*, caudal epididymal sperms were incubated in the capacitating medium containing different concentrations of BPA. The results showed that BPA could markedly accelerate sperm capacitation-associated protein tyrosine phosphorylation in dose- and time-dependent manners (**Fig. 1A,B**). In contrast, BPA was able to induce a decrease of the percentage of sperm motility in dose- and time-dependent manners, except that at certain dose range and at 10 min of sperm incubation, BPA could

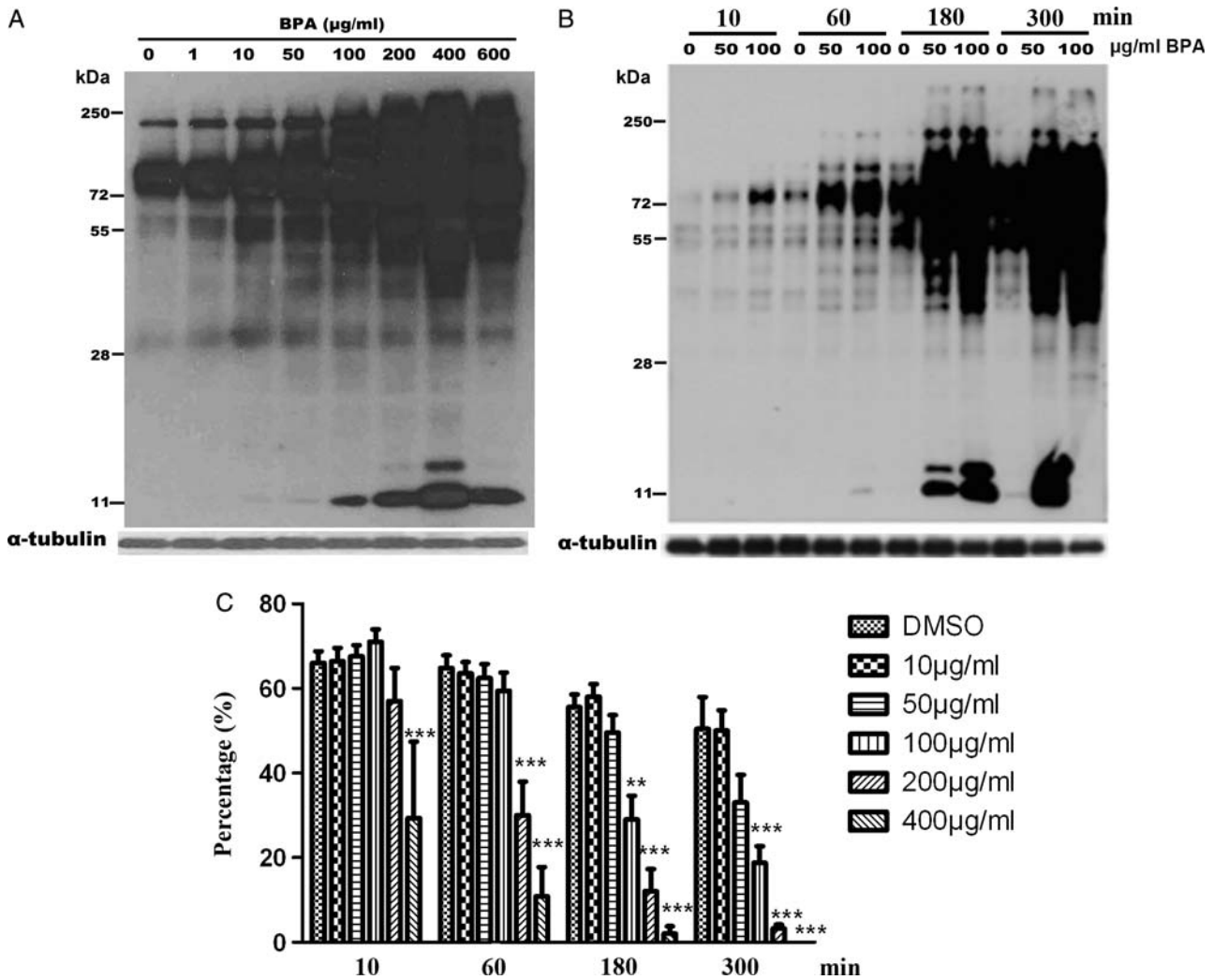


**Figure 1. Effect of BPA on capacitation-associated protein tyrosine phosphorylation and motility of sperms** (A) Dose-dependent effect of BPA on sperm protein tyrosine phosphorylation. Sperms were incubated in capacitation medium with different dosages of BPA (0, 1, 10, 50, 100, 200, 400, and 600 μg/ml) for 3 h. Protein tyrosine phosphorylation was then assessed by western blot analysis. α-tubulin was used as the loading control. The western blot is a representative of five independent experiments. (B) Time-dependent effect of BPA on sperm protein tyrosine phosphorylation. Sperms were incubated in capacitation medium with BPA (0, 50, and 100 μg/ml) and collected at 10, 60, 180, and 300 min after incubation. Protein tyrosine phosphorylation was then assessed by western blot analysis. α-tubulin was used as the loading control. The western blot is a representative of five independent experiments. (C) Dose- and time-dependent effects of BPA on percentage of sperm motility. Sperms were incubated in capacitation medium with different dosages of BPA (0, 10, 50, 100, 200, and 400 μg/ml). The percentage of sperm motility was examined using computer-assisted semen analysis (CASA) at 10, 60, 180, and 300 min after incubation. Data are expressed as the mean ± SEM ($n = 3$). $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the corresponding control.

gradually increase the percentage of motile sperms (**Fig. 1C**). As shown in **Fig. 1**, BPA at 50 μg/ml stimulated the increase of tyrosine phosphorylation, but it did not have a significant effect on sperm motility. BPA at 100 μg/ml induced significant changes of both sperm tyrosine phosphorylation and motility. Our data demonstrated that BPA could significantly affect the functions of mature sperms. So, unless otherwise noted, we used these two concentrations of BPA for the subsequent experiments in this study.

## Acceleration of tyrosine phosphorylation induced by BPA is independent on BSA, $Ca^{2+}$, and $HCO_3^-$ in the medium

It is well known that sperm capacitation-associated protein tyrosine phosphorylation is regulated by the soluble adenylyl cyclase (sAC)/cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway. The presence of BSA, $Ca^{2+}$, and $HCO_3^-$ in the medium is essential for the activation of this pathway [20]. To test whether the effect of BPA on tyrosine phosphorylation was dependent on BSA, $Ca^{2+}$, and $HCO_3^-$ in the medium, non-capacitating medium without BSA, $Ca^{2+}$, and $HCO_3^-$ was also used. It was found that BPA could still accelerate sperm capacitation-associated tyrosine phosphorylation when sperms were incubated in non-capacitating media which devoid of BSA (**Fig. 2A**), $Ca^{2+}$ (**Fig. 2B**), and $HCO_3^-$ (**Fig. 2C**), respectively. These data demonstrated that the effect of BPA on tyrosine phosphorylation is independent of the presence of BSA, $Ca^{2+}$, and $HCO_3^-$ in the medium.

## BPA accelerates sperm capacitation-associated protein tyrosine phosphorylation by activating PKA activity

sAC and PKA play vital roles in the sAC/cAMP/PKA pathway. The entry of bicarbonate and calcium from the medium into the sperm cell activates sAC, which results in elevated cAMP levels, subsequent PKA activation, and the increase of protein tyrosine phosphorylation [23]. To test whether the effect of BPA on tyrosine phosphorylation

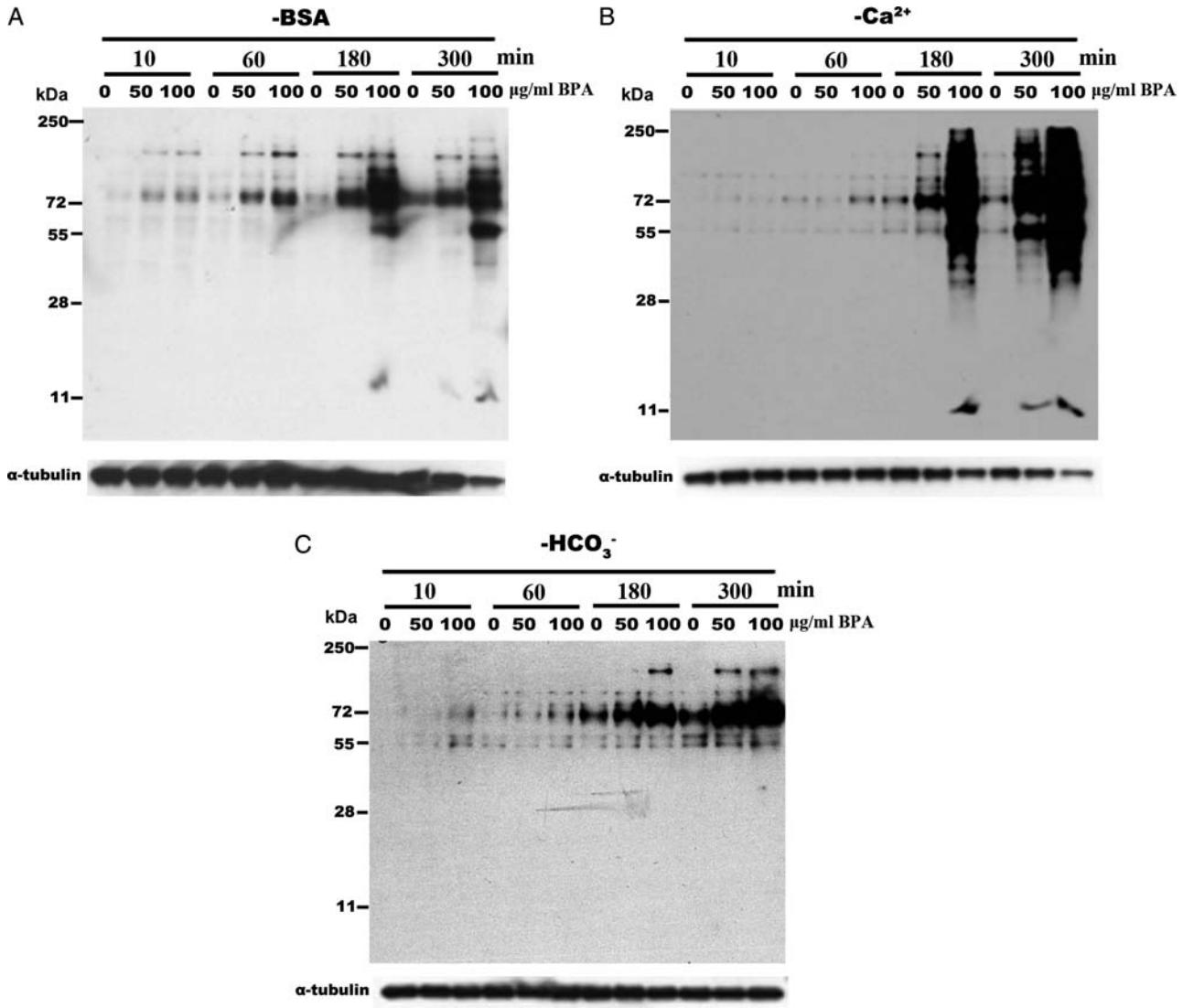


**Figure 2. The acceleration of BPA-induced sperm protein tyrosine phosphorylation is independent on BSA, $Ca^{2+}$, and $HCO_3^-$ in the medium** Sperms were treated with BPA (50 and 100 μg/ml) and collected at 10, 60, 180, and 300 min in the absence of BSA (A), $Ca^{2+}$ (B), and $HCO_3^-$ (C). Protein tyrosine phosphorylation was then assessed by western blot analysis. α-tubulin was used as the loading control. The western blot is a representative of five independent experiments.

depends on the sAC or/and PKA activity, a specific inhibitor (KH7) of sAC and a highly selective blocker (H89) of PKA were used. The results indicated that KH7 could not block the acceleration of BPA-induced tyrosine phosphorylation in sperms incubated in the medium for 3 or 5 h (**Fig. 3A,B**). However, H89 was able to prevent the increase of BPA-induced tyrosine phosphorylation (**Fig. 3C**). PKA activity can be analyzed by using anti-phospho-PKA substrates antibodies [24], therefore the changes of PKA substrates were further determined. The results showed that BPA could accelerate the phosphorylation of PKA substrates in a dose-dependent manner, and this acceleration could be suppressed by H89 (**Fig. 3C**). These data suggested that BPA can promote sperm capacitation-associated protein tyrosine phosphorylation by inducing PKA activity.

## Effect of BPA-treated sperms on *in vitro* fertilization

Mammalian sperms must undergo capacitation process after maturation in the epididymis before they can competently interact with oocytes. The protein tyrosine phosphorylation is closely

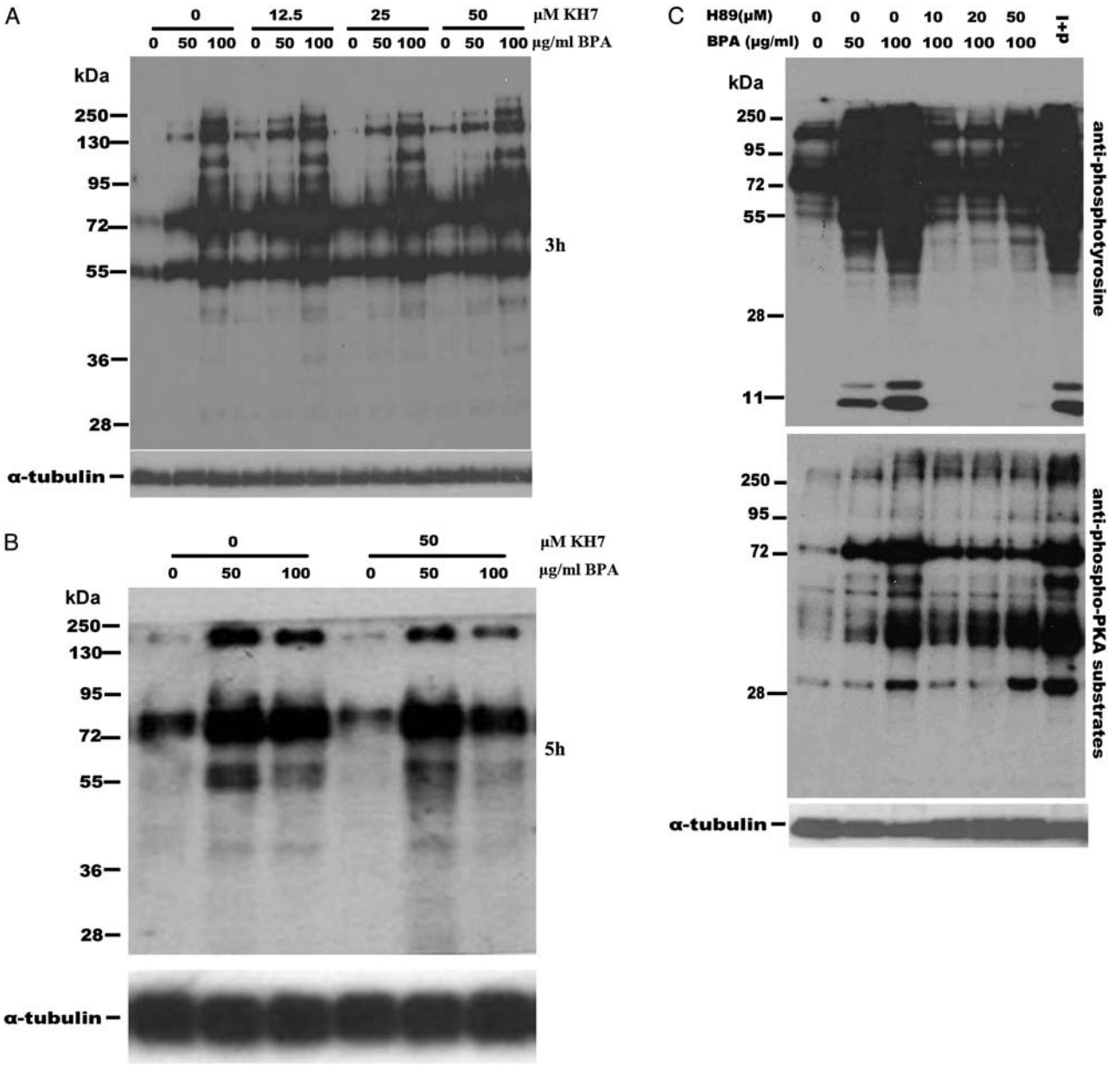


**Figure 3. BPA stimulates protein tyrosine phosphorylation by activating PKA activity** (A) Spermatozoa were treated by BPA (50 and 100 μg/ml) for 180 min in the presence of 12.5, 25, and 50 μM KH7. Protein tyrosine phosphorylation was then assessed by western blot analysis. α-tubulin was used as the loading control. The western blot is a representative of five independent experiments. (B) Spermatozoa were treated by BPA (50 and 100 μg/ml) for 300 min in the presence of 50 μM KH7. Protein tyrosine phosphorylation was then assessed by western blot analysis. α-tubulin was used as the loading control. The western blot is a representative of three independent experiments. (C) Spermatozoa were treated by BPA (50 and 100 μg/ml) for 180 min in the presence of 10, 20, and 50 μM H89 respectively. d + I (positive control): sperms were incubated in capacitating medium containing 1 mM dibutyryl cAMP (db-cAMP, a cyclic AMP analogue) and 100 μM 3-isobutyl-1-methylxanthine (IBMX). Protein tyrosine phosphorylation was then assessed by western blot analysis. α-tubulin was used as the loading control. The western blot is a representative of five independent experiments.

correlated with sperm capacitation. To explore whether the BPA-induced change of protein tyrosine phosphorylation affects the fertilizing capacity of sperms, the experiment of *in vitro* fertilization was conducted by using the BPA-treated sperms. The result showed that BPA at 100 μg/ml could significantly increase the percentage of oocyte cleavage (**Fig. 4**). This result further indicated that BPA could affect the fertilizing capacity of mature sperms.

### Effect of BPA exposure on fertilizing ability of male rats

To investigate the effect of BPA exposure on fertilizing ability of male rats, both low and high dose of BPA were used for exposure to male rats. The results illustrated that BPA exposure at low dose did not have any obvious effect on the sperm motility (**Fig. 5**). However, BPA exposure at high dose resulted in significant increase in percentage of progressive motility (**Fig. 5B**), straightness (STR) (**Fig. 5C**), and beat cross frequency (BCF) (**Fig. 5D**). As for fertilizing capacity, there is a decreasing trend in the numbers of pups and fetuses in the litters from receptive female rats that mated with BPA-treated male rats (fetuses: 14.45 ± 0.67 versus 11.73 ± 1.18, 11.86 ± 1.87, and pups: 12.56 ± 0.94 versus 12.20 ± 0.57, 12.17 ± 1.97) (**Fig. 6**). These *in vivo* results suggested that BPA could affect sperm maturation.

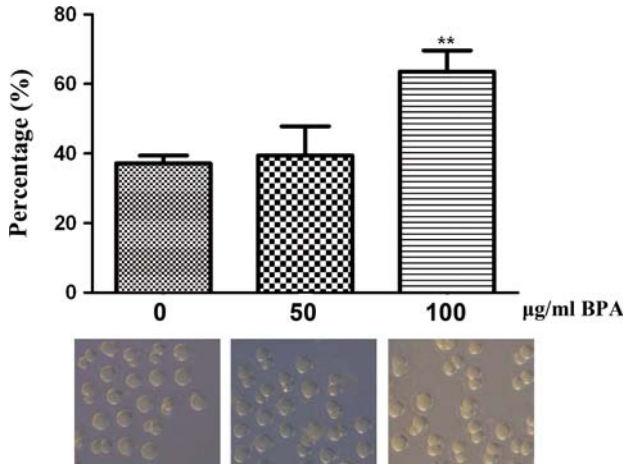


**Figure 4. Effects of BPA-treated sperms on IVF** Sperms were treated with BPA (50 and 100 μg/ml) for 3 h in capacitation medium. Sperm-egg fertilization was performed as described in methods. The percentage of cleaved oocytes at 48 h was calculated. More than 350 oocytes were evaluated in each group. Results were expressed as the mean ± SEM ($n = 3$). $^{**}P < 0.01$ compared with the corresponding control.

## Discussion

Previous data from several studies have indicated that BPA-treated male animals showed deficiency in reproduction, such as the decrease in the weight of testis and epididymis, the reduction of sperm motility and count, decrease in pregnancy rates, and so on. The objective of the present study was to assess whether BPA exposure also affected the process of sperm maturation. We found that male rats fed with 200 mg/kg/day BPA showed a significant increase in the epididymal sperm motility. Moreover, we found that BPA exposure *in vitro* could accelerate capacitation-associated protein tyrosine phosphorylation of rat sperm by activating PKA. These data suggested that the BPA exposure may cause significant functional changes of mature sperm *in vitro* and *in vivo*. This action of BPA broadens our understanding of the effect of BPA on reproductive health.

There is still controversy about the effect of BPA on male fertilizing capacity. In mice, male animals exposed to BPA at the level of 25 and 100 ng/kg body weight showed a significant reduction in testicular and epididymal sperm counts [9]. *In vitro* exposure data on mouse sperms showed that 100 μM BPA resulted in the increase of the capacitation-associated protein tyrosine phosphorylation and the decrease of motility, and induced a precocious acrosome reaction [25].

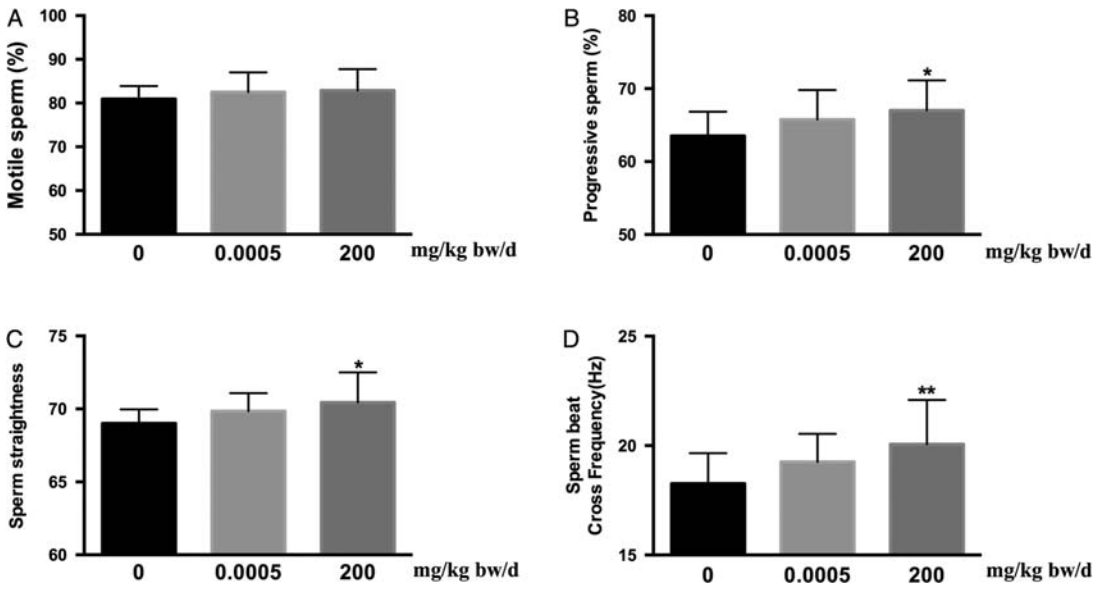


**Figure 5. Effects of BPA exposure on sperm motility of rats** Healthy male SD rats were orally administrated with BPA (0, 0.0005 and 200 mg/kg bw/day) for consecutive 8 weeks, and then sperm motility was analyzed by CASA. (A) Percentage of motile sperms. (B) Percentage of progressive motile sperms. (C) Sperm straightness (STR) of progressive sperms. (D) Sperm beat cross frequency (BCF). Results were expressed as the mean ± SEM ($n = 10$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the corresponding control.

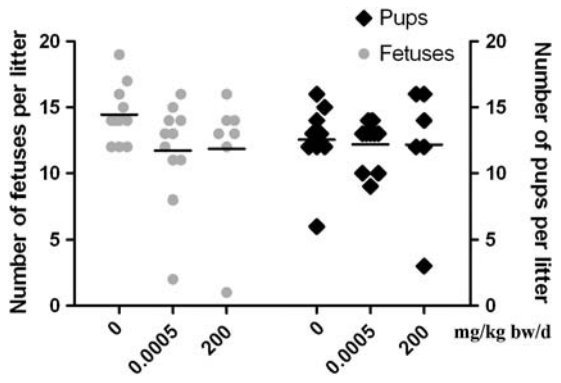


**Figure 6. Effect of BPA exposure on the fertility of male rats** Healthy male SD rats were orally administrated with BPA (0.0005 and 200 mg/kg bw/day), and then mated with normal female rats at the end of 4 or 8 weeks. The number of pups and fetuses per litter were recorded. Horizontal lines indicate the mean from each group, $n \geq 10$ in BPA at 0 and 0.0005 mg/kg bw/day group, and $n \geq 6$ in 200 mg/kg bw/day BPA group.

The exposure of human sperms to 1 μM BPA for 2 h did not affect the acrosome reaction [26]. For bovine sperm *in vitro*, BPA at the doses of 100 and 200 μg/ml significantly decreased motility at the time of 2, 4, 6, and 24 h [27]. However, the doses of 1 and 10 μg/ml BPA markedly increased motility after 24 h [27]. In rats, some reports have indicated that there is no effect of BPA on SD rats [7]. Whilst in male rat of Wistar strain, administration of BPA at the level of 2 and 20 μg/kg bw/day caused a decrease in the weight of testis and epididymis, as well as sperm count and motility [11]. In our *in vitro* work, we found that BPA, at the dosages of 1, 10, 50, 100, 200, 400, and 600 μg/ml, could markedly promote the capacitation-associated protein tyrosine phosphorylation of rat sperm in a dose-dependent manner. This *in vitro* data demonstrated that the effect of BPA on sperm function was similar in rat and mice. Male rats exposed to 0.0005 mg/kg bw/day BPA *in vivo* showed no significant changes in reproductive parameters. This result is not consistent with the previous study that showed that administration of BPA at a dose of 0.2 μg/kg (0.0002 mg/kg) had significant effect on reproduction function [11]. This discrepancy may be caused by the difference in rat strain, since BPA stimulates responses of Sprague-Dawley rats at doses much higher than those required to stimulate responses in other rat strains [14]. On the other hand, the difference in the manner of BPA administration, dosage of BPA, and time of BPA exposure might result in the different response. However, rats exposed to BPA at the high dose of 200 mg/kg showed a significant increase in sperm motility. This result is opposite to the results that exposure of BPA at doses of 0.2, 2, and 20 μg/kg all caused a reduction in the epididymal sperm motility [11]. This reflects the difference in the effect of BPA on reproductive function at the condition of low and high dosage. A previous finding has suggested that the effects of BPA, and perhaps other estrogenic compounds, at low doses are opposite to those observed at high doses [28].

BPA possesses the estrogenic activity and has a lower affinity for ERs. There is growing evidence indicating that as a selective ER modulator and relative estradiol, BPA (i) interacts differently within the ligand-binding domain of ERs; (ii) shows a different binding affinity and regulation for ERα and ERβ in target cells; and (iii) interacts differently with transcriptional coregulators [14]. In addition, there is evidence showing that similar to estradiol, BPA can elicit its action in cells through nongenomic signaling systems. BPA has been shown to bind to both membrane ER (mER) and G protein-coupled receptor 30, and studies have demonstrated that these membrane-bound receptors are capable of nongenomic actions [29]. BPA can also signal through a nongenomic pathway in cultured mouse endothelial cells to increase nitric oxide production, although it was not specifically demonstrated that these effects were mediated via mER. In fact, most studies did not specify whether the nongenomic effects of BPA were due to actions via ER in the plasma membrane, the cytosol, or elsewhere [29]. Our study demonstrated that BPA could directly stimulate the PKA activity. This suggests that BPA may act via nongenomic (nonnuclear) receptor to activate cell-signaling pathways in sperm.

Sperm capacitation occurs *in vivo* in the female reproductive tract, however, it can be mimicked *in vitro* by incubating in a chemically defined medium supplemented with $Ca^{2+}$, $HCO_3^-$, serum albumin (usually BSA), and energy substrates such as glucose, lactate, and pyruvate [18,19]. Now it has widely been accepted that sperm tyrosine phosphorylation is regulated by the sAC/cAMP/PKA pathway [18,19,23]. Some components such as $Ca^{2+}$, $HCO_3^-$, and BSA in surrounding medium have been shown to be essential for sperm capacitation-associated tyrosine phosphorylation. BSA in the medium is used as a sink to promote cholesterol removal from the plasma membrane, and subsequently increase the sperm membrane fluidity; and then the entry of bicarbonate and calcium in the medium into the sperm cell activates sAC, which results in elevated cAMP levels and the subsequent activation of PKA. Finally phosphorylation of PKA substrates and protein tyrosine takes place. Here, we found that BPA could accelerate capacitation-associated protein tyrosine phosphorylation of sperm *in vitro*. Moreover, the stimulatory effect of BPA on tyrosine phosphorylation is independent of extracellular $Ca^{2+}$, BSA, and $HCO_3^-$ in the medium. Further investigation using the inhibitor of PKA (H89) indicated that H89 could completely block the increase of PKA substrates and tyrosine phosphorylation stimulated by BPA. In somatic cells, low dose of BPA could promote human seminoma cell proliferation by activating sAC/PKA pathway via a membrane G-protein coupled ER [15]. However, mammalian sperms are enriched in the atypical sAC, which is not regulated by G proteins but rather by $HCO_3^-$ [30,31]. So our results suggest that BPA accelerates capacitation-associated protein tyrosine phosphorylation of rat sperm by activating PKA. In addition, Sperm motility is known to be closely related to the activation of PKA [32]. In our *in vitro* study, it is only at 10 min of sperm incubation, that BPA, at the certain range of dose, could moderately and gradually increase the percentage of motile sperm. This suggests that the effect of BPA on sperm motility and capacitation is not synchronous. *In vivo*, BPA at high dose significantly increases sperm motility. However, as shown in **Fig. 6**, these changes of sperm motility did not result in the significant deficiency of fertility of BPA-treated rats. So it is more complex to explain the mechanism of BPA action *in vivo*. It has not been ruled out that BPA, by acting directly on the brain, hypothalamus, or anterior pituitary gland, may indirectly affect the sperm functions and fertilizing capacity [33].

This research has both practical and theoretical implications. BPA-induced acceleration of sperm capacitation results in the increase of fertilized egg cleavage *in vitro* and decrease of male fertility, indicating that an increased rate of capacitation by BPA stimulation would be detrimental for sperm and embryo development. Our previous data also indicated that the percentage of capacitated sperm was clearly increased in rats when the epididymis-specific secretory protein HongrES1 was down-regulated by RNAi *in vivo*. Subsequently, the increase of capacitated sperm *in vivo* will lead to

reduced fertility accompanied with deformed appearance of fetuses and pups [18]. Our present study on BPA-induced functional changes of mature sperm has enriched our knowledge of BPA on reproductive health, and shed new light on our understanding of the mechanism of BPA action.

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