Perfluorooctane sulfonate (PFOS) is associated with male reproductive disorders, but its targets and mechanisms are poorly understood. We used in vitro and in vivo models to explore the roles of Sertoli cells and the blood-testis barrier (BTB) in PFOS-induced male reproductive dysfunction. First, we used primary Sertoli cell to estimate PFOS-induced cytotoxicity, junction proteins expression, and the changes of barrier function. ICR mice were then administered PFOS (0.25–50 mg/kg/day) for 4 weeks. Sperm count, ultrastructure and permeability of the Sertoli cell-based BTB, and testicular PFOS were estimated. Furthermore, the expression and localization of proteins related to junctions between Sertoli cells and mitogen-activated protein kinase (MAPK) signaling pathway were evaluated. Apparent decreases in sperm count were found. PFOS significantly increased vacuolization in Sertoli cells in seminiferous tubules and BTB ultrastructural disassembly, which subsequently increased BTB permeability and testicular PFOS levels, which was confirmed by in vitro results that PFOS decreased transepithelial electrical resistance between Sertoli cells. Additionally, PFOS decreased the expression of junction proteins in Sertoli cells, which was further confirmed by in vivo results that PFOS decreased or dislocated junction proteins (i.e., ZO-1, occludin, claudin-11, and connexin-43) and increased proteins related to the MAPK signaling pathway (i.e., Erk and p38), whereas basal ectoplasmic specialization proteins did not change. The results were confirmed by SB203580, a p38 MAPK selective inhibitor. Sertoli cells appear to be a new cellular target for PFOS. Together with disruption of BTB integrity and function, these cells play an important role in PFOS-induced male reproductive toxicity.

Key Words: Sertoli cells; perfluorooctane sulfonate; blood-testis barrier; male reproductive dysfunction; MAPK signal pathway.

Perfluorooctane sulfonate (PFOS) is known as a persistent organic pollutant and has been used in a wide range of industrial and commercial products, including paints, toothpaste, drugs, surfactants, extinguishers, adhesives, and textiles (OECD, 2002). Since the United Nations Environment Programme (UNEP) listed PFOS under the Stockholm Convention on Persistent Organic Pollutants to control the PFOS emission in 2009, the production and the use of PFOS have been banned in Europe and North of America. However, in China, due to the lack of feasible substitutes, many kinds of the perfluorinated compounds have been still used, and the amount has increased quickly since 2005 (Lim et al., 2011; Zhang et al., 2012). Very high concentrations of PFOS were found in workshop dust, with concentrations as high as 4962 µg/g (Wang et al., 2010), and water samples, with concentrations as high as 99 ng/l (Bao et al., 2012). Furthermore, a human-based study showed that serum PFOS concentrations ranged from 5.55 to 8.07 ng/ml in Nanchang, a city in central China (Zhang et al., 2010, 2012).

PFOS has been reported to have adverse effects on several systems in mammals (OECD, 2002). Much data from prenatal and postnatal exposure studies in rodents showed that PFOS significantly reduced litter size and postnatal survival, reduced fetal weight, produced a cleft palate, delayed eye opening, and induced hormonal imbalance (OECD, 2002; Rosen et al., 2009; Thibodeaux et al., 2003). Additionally, recent epidemiological data showed that high levels of perfluoroalkyl acids, including PFOS, were associated with fewer normal sperm in humans (Joensen et al., 2009), and the infertility as well (La Rocca et al., 2012). Recently, ICR mice orally administered 10 mg/kg PFOS exhibited a dose-dependent decrease in serum testosterone levels and sperm count (Wan et al., 2011). However, the underlying targets and mechanisms of PFOS-induced male reproductive toxicity are still unclear.

During spermatogenesis, Sertoli cells provide structural and nutritional support to germ cells, induce phagocytosis of residual bodies, release spermatids, and maintain the spermatogenic microenvironment (Johnson et al., 2008). Furthermore,
an important and unique physiological function of Sertoli cells is that they are the main components of the blood-testis barrier (BTB), which protects germ cells from internal antigens and restricts the paracellular diffusion of various endogenous and xenobiotic toxic chemicals (Johnson et al., 2008). Notably, junction proteins in Sertoli cells have been regarded as early targets for different classes of reproductive toxicants and may contribute to their toxic reproductive effects (Fiorini et al., 2004). Dysfunction of the BTB between Sertoli cells has been considered an important mechanism involved in xenobiotic-induced reproductive toxicity (Cheng et al., 2011). However, no sufficient data have been generated to address the PFOS-induced disruption of Sertoli cells and their role in reproductive toxicity.

In this study, we investigated the role of Sertoli cells in PFOS-induced male reproductive dysfunction in adult mice. The mechanisms of PFOS-induced dysfunction of the BTB between Sertoli cells and male reproductive toxicity were also evaluated. The present results will help reveal the toxicological and physiological importance of PFOS-induced male reproductive disruption.

MATERIALS AND METHODS

Isolation of Sertoli cells and analysis of PFOS-induced cytotoxicity. Sertoli cells were isolated from 14-day-old ICR mouse testes as described previously (Mnuk and Cheng, 2011) and seeded on Marigel (BD Biosciences, Bedford, MA) coated 96-well plates at high density (0.5 × 10^6 cells/cm²). After 24h, the cells were treated with 20mM Tris (pH 7.4) at 22°C to remove residual germ cells (Su et al., 2010). A series of concentrations of PFOS (5–60 μg/mL) or p38 mitogen-activated protein kinase (MAPK) signal pathway specific inhibitor SB203580 (10 μM) were added into wells. The control group was treated with 1:1 mixture of Ham’S F12 and Dulbecco’s Modified Eagle Medium contains 0.1% dimethyl sulfoxide (DMSO), 15mM HEPES (Invitrogen, Carlsbad, CA), 10 μg/mL insulin, 5 μg/mL human transferrin, 5 μg/mL bacitracin, and 2.5 mg/mL epidermal growth factor (Sigma-Aldrich, St Louis, MO). After 24-h treatment, the supernatant was replaced with 200 μl medium containing 20 μl cell counting kit-8 (CCK-8, Dojindo, Tokyo, Japan) for additional 2-h incubation. The plate was then read at 450 nm by TECAN SUNRISE (Tecan Group Ltd, Switzerland).

Assessment of BTB permeability in vitro. As described previously, Sertoli cell barrier function in vitro was monitored by measuring transperithelial electrical resistance (TER) with a Minicell ERS system (Millipore Corp., Bedford, MA) (Su et al., 2010). Briefly, Sertoli cells (1 × 10^6 cells/cm²) were maintained as monolayer and seeded on Marigel-coated micrilical bicameral units (Millipore Corp.). After 24h, the TER was detected in each unit at four different areas (12, 3, 6, and 9 o’clock positions), which were averaged into a single value and presented as R_{sample}. The blank control was conducted in the unit without Sertoli cell and presented as R_{blank}. The true value of TER was obtained using a corrected formula: TER_{corrected} (Ω cm²) = (R_{sample} − R_{blank}) × Effective Membrane Area (cm²) (the membrane area of Millipore bicameral unit). Once a functional barrier was assembled (approximate 3 days after plated), a series of concentrations of PFOS (10–30 μM) or SB203580 (10 μM) were added into unit. Treatment of vehicle was used as control.

Animals and treatment. One hundred male ICR mice (8 weeks old, 35–40g) were randomly divided into five groups, housed in a room with controlled temperature (25±1°C) and a 12-h/12-h light/dark cycle, and given free access to rat chow. The care and use of the animals followed the guidelines of the Animal Care and Welfare Committee of Nanjing Medical University. The mice were orally administered PFOS (Sigma-Aldrich) at doses of 0.25, 2.5, 25, and 50 mg/kg/day by gavage for 28 days. The control group was given the same volume of corn oil. After the last treatment, the mice were sacrificed, and the blood, testes, and epididymis were isolated. The blood was prepared by centrifugation and then stored at −20°C until analysis.

Sperm count. The fresh epididymis was weighted and immersed in 1 ml of 0.9% sodium chloride solution. Subsequently, six deep cuts were made in each cauda using iris scissors, and sperm were released into the media for 10 min. The suspension was filtered through 80 μm pore size nylon mesh to remove the debris. For sperm count analysis, the filtrate was diluted with 0.9% sodium chloride containing 0.5% formalin and 0.25% eosin Y. The numbers of sperm were counted using a Neubaur’s counting chamber and expressed as a total sperm count/epididymis (Wang et al., 2012).

Detection of PFOS in serum and testicular samples. According to Yeung et al. (2006), the fresh testes were washed with Milli-Q water thrice to remove adhesive blood stains. Testicular homogenate (250 μl; 200 mg testes) or serum was mixed with 27.8 μl sodium perchlorate-1-[1,2,3,4-13C4] octanesulfonate (500 ng/ml; MPFO, Wellington, Guelph, Ontario, Canada), 1 ml of 0.25M sodium carbonate buffer solution, 0.5M tetra-n-butylammonium hydrogen sulfate (pH 10; J&K Chemical, Beijing, China), and 2.5 ml methyl tertiary-butyl ether for extraction. Instrumental analysis was performed using ultraperformance liquid chromatography-tandem mass spectrometry (Waters, Milford, MA) with electrospray ionization. The extracts (5 μl injection volume) were loaded onto a BEH Shield RP18 column (2.1×50 mm, 1.7 μm; Waters) maintained at 35°C. The limits of detection for the testes and serum analyses were 4.35 ng/ml and 1.42 ng/mL, respectively. The recoveries and relative standard deviations were 88.37–104.97% and 5.75–12.82%, respectively.

Immunoblot and immunohistochemistry analysis. The immunoblot and immunohistochemistry analysis was performed according to a previous report (Xiao et al., 2011). The fresh dissected testes and PFOS-treated Sertoli cells were homogenized and lysed for 30 min in lysis buffer on ice. After sonication and centrifugation, the lysates (50 μg) were subjected to SDS-PAGE and transferred to PVDF membranes (Millipore). Immune complexes were detected by enhanced chemiluminescence using specific antibodies including ZO-1, claudin-11, occludin, and connexin-43, p-connexin-43, α-catenin, β-catenin, N-cadherin, Erk, pErk, p38, p-p38, and glyceraldehyde-3-phosphate dehydrogenase. (The details related to antibody dilution and vendor, see Supplementary table S1.) For the densitometric analysis, the protein bands on the blots were measured using ImageJ software.

For the immunohistochemistry analysis, the fresh testes were fixed with 10% formalin for 10% formalin, followed by routine dehydration, paraffin embedding, slicing, and microwave-based antigen retrieval. After blocking, suitable concentrations of primary antibodies were added to each slide and incubated at 4°C. The following immunostaining procedure was conducted according to the Streptavidin–Biotin Complex immunohistochemistry protocol and DAB enzyme substrate color development kit (SA1022, P0203; BOSTER, Wuhan, China).

Immunofluorescence analysis. The Sertoli cells were seeded at 2 × 10^4 cm² in Matrigel-coated 35-mm glass confocal dish and incubated for 24h. Then, the cells were treated with 0, 20, or 30 μM concentration of PFOS or 10μM SB203580 for 24h. The control was treated with 0.1% DMSO. The tight junction (TJ) protein claudin-11 and gap junction (GJ) protein connexin-43 were selected. Immunofluorescence analysis was preformed according to a previous report (Su et al., 2011).

Light and electron microscopy. The testicular light and electron microscopy analyses were conducted according to a previous report (Morrow et al., 2009). Briefly, the freshly isolated testes were immersed in 10% formalin or 2.5% glutaraldehyde for 12h. For light microscopy, the testes were routinely dehydrated, embedded in paraffin, sliced, and subjected to hematoxylin and eosin staining. For electron microscopy, the testes were postfixed in 1% osmium tetroxide for 1h. After dehydration and embedding, the ultrathin sections were prepared and mounted on copper grids, stained with uranyl acetate and citrate, and analyzed using a transmission electron microscope (JOEL, Tokyo, Japan).

Assessment of BTB permeability in vivo. The permeability of the BTB was analyzed according to Morrow et al. (2009). Briefly, 50 μl of...
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biodin tracer (EZ-Link Sulfo-NHS-LC-Biotin, Pierce, Rockford, IL) that was freshly dissolved in sterile PBS that contained 1mM CaCl₂, was directly administered to the right testis of the mice via an intratesticular injection. After 30 min, the testes were removed and embedded in Tissue-Tek OCT (Sakura Finetek Japan, Tokyo, Japan) for cryosection preparation. The sections (5 μm thick) were fixed in a mixture of methanol and acetone (1:1) for 10 min at 20°C and blocked in 0.1% Triton X-100, 15% horse serum, and 1% bovine serum albumin prior to incubation with Qdot565 streptavidin conjugates (Invitrogen) for 1 h at room temperature. After mounting with mounting medium, the sections were analyzed by fluorescence microscopy.

Statistical analysis. The statistical analysis was conducted using SPSS 17.0 software (Chicago, IL). The results are expressed as mean ± SD. Where appropriate, the data were analyzed using ANOVA followed by Duncan’s post hoc test. Correlations were determined using the Pearson correlation test and Stata 9.2 software (StataCorp, LP, College Station, TX). Values of \( p < 0.05 \) were considered statistically significant.

RESULTS

PFOS-Induced Sertoli Cells Junction Barrier Function Disassembly In Vitro

As shown in Figure 1, PFOS dose dependently decreased the Sertoli cell permeability barrier, and there was a significant difference between the 20 or 30μM PFOS treatment and that of vehicle control (\( p < 0.05 \) or \( p < 0.01 \)). Besides, PFOS significantly decreased the expression of TJ proteins (ZO-1, claudin-11, and occludin, \( p < 0.01 \) for 20 and 30μM) and GJ proteins (connexin-43 and p-connexin-43, \( p < 0.01 \) for 20 and 30μM), whereas basal ectoplasmic specialization proteins (N-cadherin, α-catenin, and β-catenin) did not change (Fig. 2). Moreover, PFOS significantly decreased the location of claudin-11 and connexin-43 at interface between the Sertoli cells, especially in the treatment group with 30μM PFOS (Fig. 3). The results indicated that PFOS induced the functional disassemble of Sertoli cells junction barrier; thus, Sertoli cells and its BTB integrity might be sensitive targets that contribute to the adverse effects of PFOS.

PFOS-Induced Male Reproductive Damage

As shown in Figure 4, significant dose-dependent decreases in sperm count were observed in PFOS-treated mice (\( p < 0.05 \) or \( p < 0.01 \)). This result indicates that PFOS disrupted spermatogenesis. Moreover, to confirm it, the testicular morphology was also evaluated. In the control group, the structure and morphology of seminiferous tubules, Sertoli cells, various stages of germ cells, and spermatozoa in the seminiferous epithelium were normal (Figs. 5A, a). However, a significant increase in Sertoli cell vacuolization and derangement of the cell layers

**FIG. 1.** Effects of PFOS on primary Sertoli cell junction barrier permeability. (A) Cytotoxicity. Primary Sertoli cells were maintained as monolayer and seeded on Marigel-coated 96-well plates. After 24h, a series of concentrations of PFOS (5–60μM) were added into wells. 0.1% DMSO was used as vehicle control. (B) Junction barrier permeability. Primary Sertoli cells (1 × 10⁶ cells/cm²) were treated with PFOS (10, 20, and 30 μM) on day 4 (a functional barrier is assembled), and then the TER was monitored using a Millicell ERS system. The data are expressed as the mean ± SD of three independent experiments with triplicate samples. *\( p < 0.05 \), **\( p < 0.01 \), compared with the vehicle control.
were observed with PFOS treatment at doses of 2.5 mg/kg/day and higher (Figs. 5C–E, c–e, black arrow). Dislocated immature germ cells were found in the lumens of seminiferous tubules in the highest dose group (Fig. 5e), whereas the changes were slight in the 0.25 mg/kg/day group (Figs. 5B, b). To confirm these results, the ultrastructure of seminiferous epithelia was analyzed by electron microscopy. The control group exhibited a normal ultrastructure, with a Sertoli cell nucleus and two germ cell nuclei adjacent to the basement membrane; the cellular layer was clear, and adjacent cells were compact and intact (Figs. 5F, f). However, a significant increase in the vacuolization of Sertoli cells was found in the groups treated with PFOS at doses of 2.5 mg/kg/day and higher (Figs. 5H–J, h–j, black asterisk), whereas the changes were slight in the 0.25 mg/kg/day group (Figs. 5G, g). These results indicate that PFOS damaged seminiferous tubules and specifically disrupted Sertoli cells.

**PFOS-Induced Structural Changes in the BTB Between Sertoli Cells**

The normal and clear ultrastructure of the BTB between two adjacent Sertoli cells (Fig. 6A, opposing white arrowheads)
showed a classic basal ectoplasmic specialization structure, actin filament bundles (black arrowheads) sandwiched between the endoplasmic reticulum membrane and opposing plasma membranes of two adjacent Sertoli cells, and a TJ (white arrow) in the BTB (Fig. 6a). However, significant changes, such as the absence of actin filament bundles (black arrow) and disassembly of the TJ and vacuoles (black asterisk), were observed in the groups treated with PFOS at doses of 2.5 mg/kg/day and higher (Figs. 6C–E, c–e). These results confirmed the disruption of BTB integrity and function via damage to the ultrastructure of the BTB between Sertoli cells.

**PFOS-Induced Changes in BTB Function**

To further verify BTB integrity and function, the permeability of the BTB was evaluated using a biotin tracer. The results showed the biotin tracer located in the interstitial spaces and basal compartment but excluded from the adluminal compartment of seminiferous tubules in the control group (Fig. 7i, a). However, it passed though the BTB and entered the adluminal compartment of seminiferous tubules in the groups treated with PFOS at doses of 2.5 mg/kg/day and higher (Figs. 7i, C–E, c–e, white arrow). These results indicate significant disruption of the integrity and function of the BTB after PFOS exposure.

Furthermore, correlations between external PFOS exposure and serum/testes PFOS levels were analyzed. As shown in Figure 7ii, significant dose-dependent increases in PFOS levels in serum and testes were found after PFOS treatment. The correlation coefficients were 0.9628 for serum (Pearson, \( p < 0.0001 \)) and 0.9557 for testes (Pearson, \( p < 0.0001 \)). Additionally, a positive correlation was found between serum and testes PFOS levels, with a correlation coefficient of 0.9676 (Pearson, \( p < 0.0001 \)). These results indicate that PFOS was

![FIG. 4](image.png)

**FIG. 4.** Effects of PFOS on sperm count in mice. The data are expressed as the mean ± SD of 20 mice in each group. \( *p < 0.05 \), \( **p < 0.01 \), compared with control group.

![FIG. 5](image.png)

**FIG. 5.** Effects of PFOS on seminiferous tubules under light and electron microscopy. (A–E) Light microscopy (scale bar = 200 μm). (a–e) Magnification of boxed areas from A–E. The black arrow indicates Sertoli cell vacuolization (scale bar = 100 μm). (F–J) Electron microscopy (scale bar = 5 μm). (f–j) Magnification of boxed areas from F–J (scale bar = 2 μm). SC, Sertoli cell; GC, germ cell; BM, basement membrane; LD, lipid droplet. The black asterisk indicates Sertoli cell vacuolization and vacuoles.
absorbed, passed through the BTB, and was transferred to the testes via disruption of BTB integrity between Sertoli cells.

**PFOS-Induced Changes in the Expression and Localization of Testicular TJ and GJ Proteins**

Similar to the *in vitro* changes (Fig. 2), PFOS dose dependently decreased the expression of testicular TJ proteins (ZO-1, claudin-11, and occludin) and the GJ protein (connexin-43 and phosphorylated connexin-43) at doses of 2.5 mg/kg/day and higher (*p* < 0.05 or *p* < 0.01). However, the expression of basal ectoplasmic specialization proteins (N-cadherin, α-catenin, and β-catenin) did not change (Fig. 8). To further determine the mechanism of action, proteins related to the MAPK signaling pathway were evaluated. As shown in Figure 8, the expression of phosphorylated Erk (pErk) and p-p38 significantly increased after PFOS exposure (25 mg/kg/day and higher for pErk and 2.5 mg/kg/day and higher for p-p38) although total Erk and p38 levels did not change (Fig. 8).

To confirm the above results, the localization of BTB functional junction proteins was evaluated. Although TJ proteins (ZO-1,
occludin, and claudin-11) were still consistently and clearly present at the BTB adjacent to the basal membrane in seminiferous tubules after treatment with 0.25 mg/kg/day PFOS (Figs. 9B, b, G, g, L, l, black arrow), they decreased (Figs. 9C–E, H–J, M–O) and significantly dissociated from the BTB after treatment with PFOS at doses of 2.5 mg/kg/day and higher (Figs. 9d–e, h–j, m–o, black arrow). Additionally, the GJ protein connexin-43 was found at the BTB and apical region of seminiferous tubules in the control and 2.5 mg/kg/day PFOS groups (Figs. 9P and Q, p and q, black arrow). However, a significant decrease in connexin-43 localization was found after treatment with PFOS at doses of 2.5 mg/kg/day and higher (Figs. 9R–T, r–t, black arrow). Notably, connexin-43 disappeared at the apical region in seminiferous epithelia after treatment with PFOS at doses of 25 and 50 mg/kg/day (Figs. 9s and t). These results suggest that PFOS disrupted BTB integrity and function, possibly by downregulating the expression and dislocating BTB-related TJ and GJ proteins, with possible involvement of the MAPK signaling pathway.

p38 MAPK Signal Pathway Contributes to PFOS-Induced BTB Disassembly

To investigate the mechanism of PFOS-induced BTB disassembly and breakdown of the barrier integrity, p38 MAPK signal pathway was conducted in the study. As shown in Figure 10, treatment of PFOS resulted in a significant activation of p38 MAPK and the decrease in the expression of TJ and GJ proteins. Similarly, the changes of location of claudin-11 and connexin-43 between Sertoli cells were observed (Fig. 11). Furthermore, a significant loss in TER was also observed in PFOS-treated group. Accordingly, treatment of SB203580 (a selective inhibitor of p38 MAPK) significantly suppressed the decrease or loss of junction proteins and TER (Fig. 12). These results suggested that PFOS might break down the Sertoli-based BTB integrity through the activation of p38 MAPK signal pathway.

DISCUSSION

Epidemiological data indicate that PFOS exposure is associated with fewer normal sperm and poor semen quality in humans (Joensen et al., 2009; Toft et al., 2012). This has been supported by recent in vivo and in vitro studies that showed that PFOS exposure significantly decreased epididymal sperm count (Wan et al., 2011; Zhang et al., 2011). In this study, PFOS dose-dependently decreased sperm count in ICR mice, suggesting that PFOS compromised male reproductive function and the animal model...
was established successfully. Endocrine-disruptive effects, decreases in the testicular signaling of gonadotropin, growth hormone, insulin-like growth factor, and inhibins/activins, and increases in reactive oxygen species induced by PFOS might be related to its reproductive toxicity (Jensen and Leffers, 2008; Wan et al., 2011; Zhang et al., 2011). However, the mechanisms of PFOS-induced male reproductive dysfunction still need to be addressed by evaluating testicular target cells, such as Sertoli cells, and their involvement in BTB integrity and function.

Sertoli cells play a key role in spermatogenesis by providing structural and nutritional support and an immune and permeation barrier for germ cells. An important function of Sertoli cells is to protect germ cells from endogenous and exogenous toxicants that are transmitted by the blood and lymph system. However, Sertoli cells are sometimes the primary target of some reproductive toxicants (Fiorini et al., 2004). We first found that PFOS decreased the expression and localization of TJ and GJ proteins in primary Sertoli cells and subsequently disrupted the junction barrier function, indicating that Sertoli cells might be a target for PFOS and play an important role in PFOS-induced reproductive toxicity by disrupting BTB integrity and function.

To confirm the above hypothesis, a further in vivo study was conducted in adult ICR mice. A significant increase in the vacuolization of Sertoli cells and large lipid droplets in the cytoplasm were found, suggesting that Sertoli cells were destroyed by PFOS. Typically, vacuolization is a common abnormal characteristic of xenobiotic-induced Sertoli cell destruction (Eid et al., 2012; Rajendar et al., 2011). Moreover, PFOS induced ultrastructural damage of the BTB between adjacent Sertoli cells, further indicating that Sertoli cell–related BTB function might be disrupted, which was supported by other xenobiotic...
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studies (Li et al., 2009; Wong et al., 2005). To confirm this functional hypothesis, biotin was used as a tracer to evaluate BTB permeability. Biotin passed through the BTB between adjacent Sertoli cells and entered the adluminal compartment of seminiferous tubules after PFOS exposure, suggestion that PFOS disrupted Sertoli cell–related BTB function. These results are consistent with the results of other studies of endogenous reproductive toxicants, such as bisphenol A and cadmium (Cheng et al., 2011). Once BTB function between Sertoli cells is disrupted, PFOS could enter the testes and subsequently trigger direct toxic effects on germ cells, indicating that PFOS destroys Sertoli cells and related BTB integrity. Thus, PFOS passes though the BTB and enters the testes to exert direct reproductive toxicity.

FIG. 11. Expression and location of claudin-11 and connexin-43 in Sertoli cells treated with PFOS or SB203580. The primary Sertoli cells (2×10⁴/cm²) were seeded in Matrigel-coated 35-mm glass confocal dish and incubated for 24 h and then treated with 30μM PFOS, 10μM SB203580, 30μM PFOS + 10μM SB203580, or 0.1% DMSO (control) for 24 h. The protein expression was conducted using immunofluorescence analysis. Each treatment was conducted from triplicate parallel dishes. Claudin-11 and connexin-43 (green); Sertoli cell nuclear (blue); Scale bar = 20 μm.
More importantly, the BTB between Sertoli cells is created by the coexistence of several proteins, including TJ, GJ, desmosome-like, and basal ectoplasmic specialization proteins. Decreases in TJ or GJ protein expression and their redistribution have been thought to be an important molecular mechanism for the xenobiotic-induced disruption of the BTB between Sertoli cells (Siu et al., 2009). TJ proteins, such as ZO-1, claudin-11, and occludin, are important structural components and essential to BTB function (i.e., the regulation of BTB dynamics; Su et al., 2011). Claudin-11 is a key TJ protein that contributes to the maintenance of BTB permeability. A lack of TJ in Sertoli cells was found in claudin-11 knockout mice (Nah et al., 2011). These results suggest that TJ protein expression and localization are involved in the regulation of BTB function. Additionally, connexin-43, a predominant GJ protein in the testes, is thought to be an early molecular target for the adverse effects of environmental toxicants on male reproductive function. It has also been found to be associated with the regulation of TJ proteins (Pointis et al., 2011). Decreased connexin-43 resulted in hindrance of the reassembly of TJ proteins and increased TJ permeability (Yao et al., 2010). Interestingly, an in vitro study found that PFOS compromised TJ function in brain endothelial cells by decreasing TJ protein expression, indicating that the blood-brain barrier might also be a target for PFOS (Wang et al., 2011). Similarly, our data showed that PFOS decreased the expression and localization of the junction proteins ZO-1, claudin-11, occludin, and connexin-43 at the BTB in mouse testes, further indicating that Sertoli cells–related BTB is a target for PFOS. Additionally, two members of the MAPK signaling pathway family, Erk and p38, were significantly activated by PFOS. Activation of the MAPK signaling pathway, especially Erk, JNK, and p38 proteins, in Sertoli cells was reported to contribute to environmental toxicant-induced BTB disruption (Lui et al., 2003b; Wong and Cheng, 2011). Activation of the Erk and p38 signaling pathway plays an important role in the regulation of TJ and GJ proteins and promotes the redistribution of junction proteins in Sertoli cells.

FIG. 12. The changes of the junction barrier permeability of Sertoli cells treated with PFOS or SB203580. (A) Cytotoxicity. The primary Sertoli cells were treated with 30μM PFOS, 10μM SB203580, 30μM PFOS + 10μM SB203580, or 0.1% DMSO (control) for 24 h. (B) Junction barrier permeability. Primary Sertoli cells (1 × 10^6 cells/cm^2) were maintained as monolayer, seeded on Marigel-coated millicell bicameral units, and treated with 30μM PFOS, 10μM SB203580, 30μM PFOS + 10μM SB203580, or 0.1% DMSO on day 4 (a functional barrier is assembled). The TER was monitored using a Millicell ERS system. The data are expressed as the mean ± SD of three independent experiments with triplicate samples. *p < 0.05, **p < 0.01, compared with the vehicle control. *p < 0.05, **p < 0.01, compared with PFOS-treated group.
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(Lui et al., 2003a; Wong and Cheng, 2011). Our study showed that SB203580, a selective inhibitor of p38 MAPK, significantly rescued the PFOS-induced decrease/loss of junction protein and the deficiency of Sertoli cell junction barrier integrity, suggesting that PFOS-induced activation of p38 MAPK signaling pathway might be involved in the disruption of BTB function by decreasing TJ and GJ protein expression. These results indicate that Sertoli cells in the BTB might play an important role in PFOS-induced male reproductive dysfunction.

In summary, this study confirmed that PFOS destroyed Sertoli cells and BTB integrity, allowing PFOS to enter the testes and directly cause male reproductive dysfunction. The observed sensitivity of Sertoli cells indicates that they may play an important role in PFOS-induced reproductive toxicity. A decrease and dislocation of TJ and GJ proteins at the BTB between Sertoli cells and activation of the p38 MAPK signaling pathway might be mechanisms of PFOS-induced disruption of BTB integrity and function. The BTB, therefore, may be a sensitive target for, rather than a protective barrier to, xenobiotic-induced male reproductive toxicity.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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