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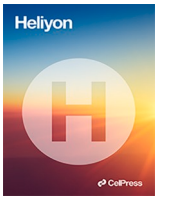

Proton pump inhibitors affect sperm parameters by regulating aquaporins

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ABSTRACT

Proton pump inhibitors (PPIs) were one of the most commonly used drugs in daily life. The adverse effects of long-term use of PPIs have aroused widespread controversy. It was of great significance to explore the molecular mechanism of sperm abnormality caused by PPIs. The PPI group was given omeprazole by gavage for 28 days. After the omeprazole intervention, the caudal epididymis was dissected to obtain sperms, and the sperm was counted through the microscope, as the acrosomal integrity was observed through PNA-FITC staining. The expression of aquaporins were detected by immunofluorescence and western blot in the testis, epididymis and spermatozoa. The liver cytochrome enzyme was evaluated by immunohistochemistry and western blot. We detected the serum estrogen level by ELISA, and the level of alanine transaminase (ALT) were detected through microplate method. The sperm count in PPI group was less than control group ($p < 0.05$), and the sperm acrosin integrity in PPI group was lower than control group ($p < 0.05$). In the testis, the expression of aquaporin 3 and aquaporin 8 in PPI group was higher than control group ($p < 0.05$), while the expression of aquaporin 7 was lower than control group ($p < 0.05$). In the epididymal and sperm, the expression of aquaporin 3 and aquaporin 7 in PPI group was higher than control group ($p < 0.05$), while the expression of aquaporin 8 in PPI group was lower than control group ($p < 0.05$). Meanwhile, the liver cytochrome enzyme in PPI group were lower than control group ($p < 0.05$), and estrogen and ALT in PPI group were higher than control group ($p < 0.05$). PPI may lead to the up-regulation of estrogen by inhibiting the activity of cytochrome enzyme, and then lead to the dysfunction of sperm parameters and acrosin integrity by affecting aquaporins function.

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1. Introduction

The prevalence of infertility was about 9%, ranging from 3.5 to 16.7% in developed countries and 6.9–9.3% in developing countries [1]. Among them, 20–30% of infertility were entirely caused by male sperm abnormality [2]. Male infertility was mainly related to sperm defects caused by genetic, infectious, and environmental. Over the past 60 years, sperm count had declined by 50% [3]. Asthenozoospermia (AZS), one of the main causes of male infertility, referred to the decrease or lack of sperm motility [4]. Large doses of Jinqing granules (gastric ulcer drug) were associated with decreased sperm count in seminiferous tubules [5]. Cimetidine (1200 mg/d for 9 weeks) resulted in an average 43% reduction in sperm count [6]. Sperm abnormalities were common in patients with chronic hepatitis C after antiviral therapy [7]. At present, there are few studies on the adverse effects of drugs on sperm count and semen quality. With the social reproductive pressure getting prominent, it is necessary to further explore the adverse effects of commonly used drugs on reproduction.

Proton pump inhibitors (PPIs) were one of the most commonly used stomach medicine in the world [8]. Short-term administration of PPIs was generally safe, while the adverse effects of long-term administration of PPIs had attracted much attention [9]. The long-term administration of PPIs may be associated with decreased sperm function and male infertility in humans. The nested case-control studies had found that the administration of PPI that inhibit gastric acid was associated with a decrease in total sperm motility and sperm concentration in male infertile patients [10]. The administration of PPIs between 6 and 12 months before semen analysis resulted in a reduction in TMSC in male patients by 3 times [11]. Recent studies had found that esomeprazole significantly reduced TMSC and forward motile sperm count by competitively inhibiting the activity of sperm choline acetyltransferase [12]. Similarly, in vitro studies have found that lansoprazole could also reduce the total motile sperm count and forward motile sperm count, which may be related to the calcium chelation and/or the inhibition of Na^+/K^+-ATPase activity in sperm of lansoprazole [13]. However, in a large retrospective study showed PPIs didn't seem to affect semen quality in male infertility patients [14]. Therefore, the effect of PPIs on sperm motility is still controversial, and the possible molecular mechanism of PPIs on sperm count still unknown.

Based on clinical work, we also found that some PPI drug users had low sperm motility. In order to further clarify the effect of PPIs on male sperm parameters, this study constructed the omeprazole intervention animal model to further explore correlation between PPIs and male sperm parameters and its molecular mechanism. Sperm parameters was evaluated by sperm count and PNA-FITC staining, and then aquaporin (AQP) expression levels in testis, epididymis and sperm were evaluated by Western blot (WB) immunofluorescence (IF) and qRT-PCR to detect differences in AQP in the PPI intervention group. Estrogen expression levels in serum samples were detected by ELISA. It was further proved that PPI affected the function of AQP in testis, epididymis and sperm through the enteric-testis axis. Finally, by improving the liver function test, we further explained that long-term consumption of PPI would lead to abnormal liver enzyme activity and liver function, and further speculated that the PPI affecting parameters of sperm through the enteric-liver-testis axis.

2. Materials and methods

2.1. Animals and groups

Male SD rats weighing 180–220 g were obtained from Wu Animal Center (Fuzhou, China), and the animal certificate number was SCXK (Zhe)2019–0002. The animals were kept in SPF environment of experimental animal center of Fujian Medical University. The temperature was 20°C–25 °C and the humidity was 40%–70%. The animals were fed and watered freely. Omeprazole (Shanghai Maclin Biochemical Technology Co., LTD., Cas:73590-58-6) was administered by gavage (20 mg/kg/day) once a day for 28 days in the PPI intervention group, while the control group was treated by 0.9% normal saline (10 rats/group) [15]. The rats were sacrificed by gas anesthesia, and epididymis was cut up. The sperm was placed in the sperm rinse medium at 37 °C for 10 min to allow the sperm cells to fully escape and the sperm were collected for relevant detection. Testicular tissue was obtained at the same time. A part of the tissue was soaked in 10% neutral formalin, and the other part of the testicular tissue was stored in −80 °C refrigerator. All animal studies complied with the National Institutes of Health guidelines for the care and use of laboratory animals. The protocol was approved by the ethics committee the Fujian Medical University.

2.2. Sperm counting

The left epididymis of each rat was collected immediately after sacrifice and transferred to a test tube containing 1 mL normal saline (37 °C). 10 μL sperm suspension was transferred to a slide, fixed in formaldehyde, and sperm count was performed through a light lens. We evaluated whether there were differences in sperm count between the two groups of animal models mainly through the area of sperm cells in the visual field.

2.3. Quantitative-real-time-PCR

Tissues were isolated with the RNA isolate Total RNA Extraction Reagent (Vazyme Biotech Co., Ltd.). The concentration of total RNA in the samples was determined by spectrophotometer (TGem Pro, Thermo). cDNA was synthesized from 1000 ng total RNA by the SweScript RT I First Strand cDNA Synthesis Kit (Vazyme Biotech Co., Ltd). The cDNA was used for quantitative PCR (qPCR) by the 2 × SYBR Green qPCR Master Mix (Servicebio, China) and the threshold cycle (CT) was determined by the ABI QuantStudio 5 (Thermofisher, the United States). Relative gene expression levels of β-Actin were calculated (Primer sequences: Supplementary material 1).

This experiment was used to evaluate the mRNA expression of AQPs.

2.3.1. Western blot

Total proteins were separated by SDS-PAGE and transferred to PVDF membranes. Non-fat milk was used to block, and the PVDF membranes were incubated with targeted primary antibodies and secondary antibodies. At last, the protein bands were visualized with an ECL detection solution (Thermo). The following primary antibodies were used according to the manufacturer's instructions: rabbit anti-AQP 3 (1:1000, Yaji Biotechnology Co, LTD), rabbit anti-AQP 7 (1:1000, Yaji Biotechnology Co, LTD), rabbit anti-aquaporin 8 (1:1000, ABclonal), rabbit anti-aquaporin 11 (1:1000, affinity), rabbit anti-CYP3A4 (1:1000, ABclonal), rabbit anti-CYP2C11 (1:1000, Bioss)and rabbit anti-GADPH (1:10000, ABclonal), HRP Goat Anti-Rabbit (1:5000, ABclonal). All blotted bands was analyzed by ImageJ 1.48 software (Image Processing and Analysis in Java, Bethesda, MD, USA), and the intensity values were normalized to the bands of GADPH. The purpose of this experiment was to evaluate the expression of aquaporins and CYP family proteins.

2.4. Sperm PNA-FITC satining

The sperm of control and the PPI intervention group were centrifuged at 1000×*g*, resuspended in PBS, layered on adhesive slides (Servicebio, G6058-20), and fixed. Peanut PNA-FITC (GENMED SCIENTIFICS INC. U.S.A, GMS14015.1.1v.A) was incubated for 2 h at room temperature, washed with cleaning solution. And then it was incubated for 10 min with DAPI at room temperature, dropped anti-fluorescence quench and sealed with cover glass. Imaging was made using a positive immunofluorescence microscope (Olympus BX43, Japan). The experiment was used to evaluate the integrity of acrosomal enzymes in sperm.

2.5. Immunofluorescence and immunohistochemistry

Tissues from were fixed by 10% formalin at room temperature and sectioned. The sections were dewaxed with xylene and gradient ethanol, and antigen repair according to the instructions. Non-specific binding was blocked with goat serum. Primary monoclonal antibodies were incubated with the samples overnight at 4 °C. FITC-labeled goat anti-Rabbit IgG were applied for 2 h at room temperature, and the nucleus were stained with DAPI. Imaging was made using a positive immunofluorescence microscope (Olympus BX43, Japan).

The previous steps refer to the immunofluorescence section, HRP-labeled goat anti-Rabbit IgG were applied at room temperature, and the DAB staining and hematoxylin staining. Imaging was made using a positive light microscope (Olympus BX43, Japan). Primary antibodies: aquaporin 3 (1:400, Yaji Biotechnology Co., LTD), aquaporin 7 (1:400, Yaji Biotechnology Co., LTD), aquaporin 8 (1:400, ABclonal), aquaporin 11 (1:400, affinity), CYP3A4 (1:200, ABclonal), CYP2C11 (1:200, Bioss). This experiment was used to evaluate the expression of aquaporins and liver enzyme CYP family proteins.

3. ELISA

Serum samples were performed according to the manufacturer's protocol and the concentration of estrogrn was calculated using the appropriate standard curve. Absorbance were detected at 450 nm using a Sunrise Enzyme Standard Instrument (Tecan, Austria). Enzyme-linked immunoassay of human Estrogen protein (Jiangsu Enzyme industry Co., LTD, China, Lot:MM-055R1). The purpose of this study was to evaluate the effect of PPI intervention on serum estrogen.

3.1. Microplate method

Serum were collected from postcava and the levels of ALT, Aspartate aminotransferase (AST) and γ-Glutamyl transpeptidase (γ-GT) were measured according to the protocol of ALT, AST and γ-GT Assay Kit (Purchase from Nanjing Jiancheng Bioengineering Institute, catalogue number C009-2-1/C010-2-1/C017-2-1). This part of the experiment was used to evaluate the effect of PPI intervention on liver function.

3.2. Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0 (Graph Pad Prism Software Inc, San Diego, CA, USA). Continuous variable were presented as the mean ± standard error of mean (SEM). Student's unpaired *t*-test was utilized for comparisons between two groups. Frequencies of categorical variables were compared by the χ^2 test. $P < 0.05$ was considered significant (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

4. Results

4.1. PPI leads to decreased sperm count and acrosomal enzyme activity

Full lavage was performed on unilateral epididymal tissue, and the sperm count in the lavage solution was observed under the microscope, we conducted a semi-quantitative analysis of the visual field of sperm, and found that the ratio of sperm area to total area in the control group was 16.58% ± 2.029% (n = 6) and that in the PPI group was 3.392% ± 0.9887% (n = 6). There was a statistically

significant difference between the two group ($p < 0.05$) (Fig. 1A). PNA-FITC fluorescence staining showed that the acrosomal integrity of the PPI group was lower than that of the control group ($p < 0.05$) (Fig. 1B and D). The morphology of spermatogenic cells and sertoli cells in the control group was normal after HE staining. Compared with the control group, spermatogenic cells in the lumen were detached and shed, spermatogenic cells were reduced and arranged loosely, and dense sperm accumulation in lumen was reduced in the PPI group (Fig. 1C).

4.2. PPI results in the dysfunction of AQPs in testicular tissue

To further evaluate the expression of AQPs in testicular tissue of PPI group, the WB and qRT-PCR were performed on the testicular tissue. Our result showed that the expression of AQP3 and AQP11 in testicular tissue of PPI group were higher than control group ($p < 0.05$) (Fig. 2A,B,C and F). However, the expression of AQP7 in testicular tissue of PPI group was lower than control group ($p < 0.05$) (Fig. 2A and D). There was no significant difference in the expression of AQP8 in testicular tissue between the two groups (Fig. 2B and E). At the same time, qRT-PCR showed that the average relative expression level of AQP3 mRNA ($p = 0.195$) in testicular tissue of PPI group was higher than that in control group (Fig. 2G). The average relative expression of AQP7 mRNA ($p = 0.3526$), AQP8 mRNA ($p = 0.5938$) and AQP11 mRNA ($p = 0.1705$) were lower than those in control group, with no statistical difference (Fig. 2H, I and J).

4.3. PPI results in the dysfunction of AQPs in epididymal tissue

In order to further evaluate the expression level of AQP in epididymal tissues in PPI group, a series of molecular experiments were performed on epididymal tissues. The result showed that the expression of AQP3 and AQP7 in epididymis tissues of PPI group were higher than those in control group ($p < 0.05$) (Fig. 3A,C and D), and the expression of AQP8 in epididymis tissues of PPI group was lower than that in control group ($p < 0.05$) (Fig. 3B and E). There was no significant difference in the expression of AQP11 in epididymal tissues between the two groups (Fig. 3B and F). At the same time, qRT-PCR results showed the average relative expression levels of AQP3 mRNA ($p < 0.05$), AQP7 mRNA ($p = 0.3004$) and AQP11 mRNA ($p = 0.5427$) in epididymal tissue of PPI group were higher than control group, as the AQP7 and AQP11 were no statistical difference (Fig. 3G, H and J). However, the average relative expression of AQP8 mRNA in epididymal tissue of PPI group was lower than that in control group ($p < 0.05$) (Fig. 3I).

4.4. Abnormal function of sperm AQPs induced by PPI

To further evaluate the expression level of AQPs of spermatozoa in PPI group, the molecular experiments was performed. The result

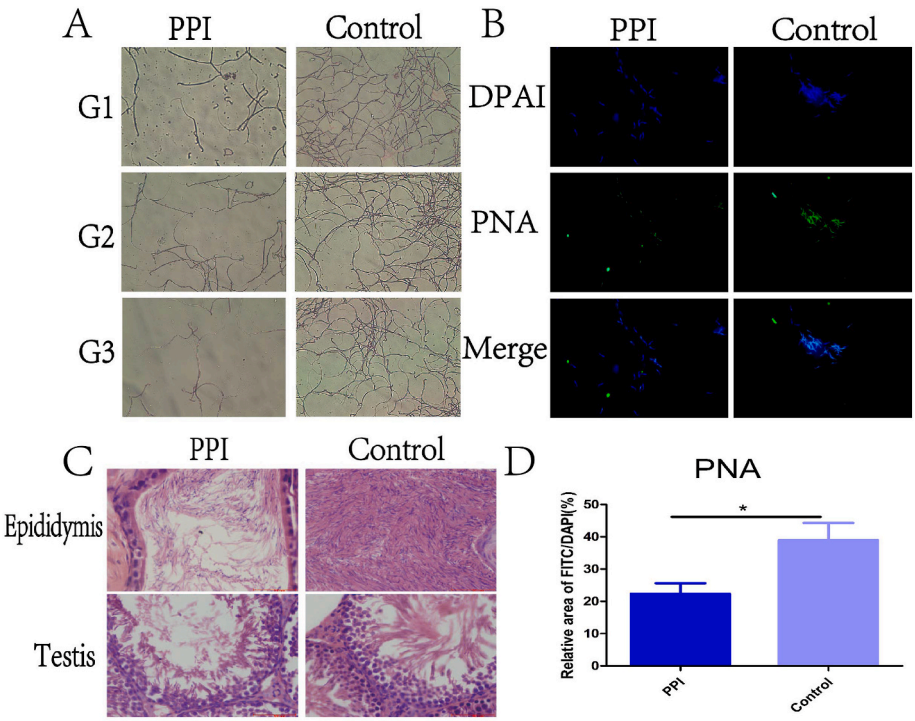

Fig. 1. Correlation between PPI and sperm count and acrosomal activity. (A) Sperm count in sperm rinse medium (400×); (B) HE staining of the rat testis; (C) HE staining of the rat epididymis; (D) PNA-FITC of the rat epididymis; (E)The semi-quantification analysis of PNA-FITC in the epididymis ($n > 3$).

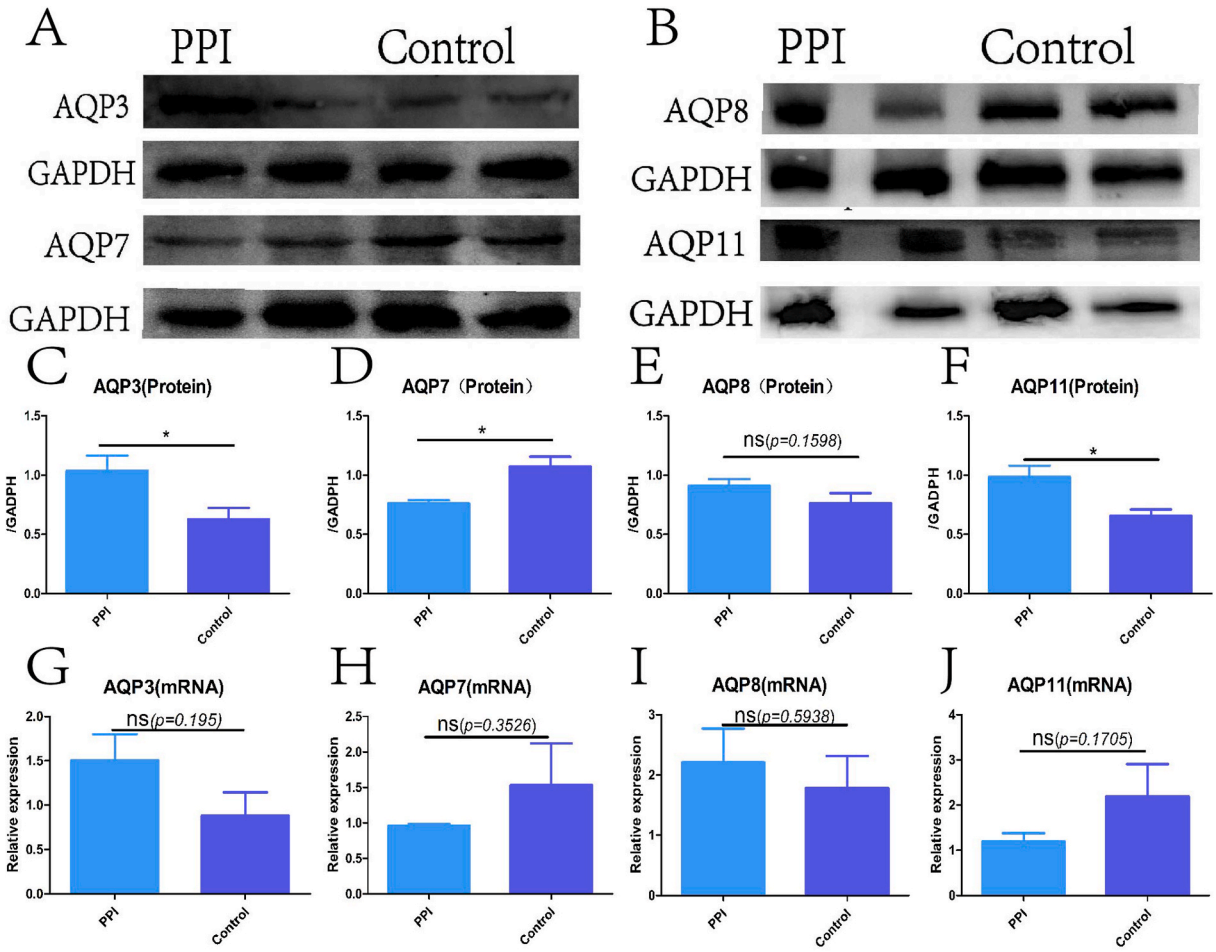

Fig. 2. Correlation between PPI and testicular AQPs. (A) (B)Western blot of AQP 3, AQP 7, AQP 8 and AQP 11 in the testis; (C) (D) (E) (F) The semi-quantification analysis of WB of AQP 3, AQP 7, AQP 8 and AQP 11 in the testis (n > 3); (G) (H) (I) (J) The mRNA expression level analysis of AQP 3, AQP 7, AQP 8 and AQP 11 in the testis (n > 3).

showed that the AQP3 expression of spermatozoa in PPI group was higher than that in control group ($p < 0.05$) (Fig. 4A and G), while the AQP8 and AQP11 expression of spermatozoa in PPI group were lower than those in control group ($p < 0.05$) (Fig. 4B, I and J). And there was no significant difference in the AQP7 expression of spermatozoa between two groups (Fig. 4A and H). Immunofluorescence showed that the AQP3 expression of spermatozoa in PPI group was higher than that in control group ($p < 0.05$) (Fig. 4C and K), while the AQP8 expression of spermatozoa in PPI group was lower than that in control group ($p < 0.05$) (Fig. 4E and M). There was no significant difference in the expression of AQP7 ($p = 0.3004$) and AQP11 ($p = 0.7835$) in spermatozoa between two groups (Fig. 4D, L, F and N).

4.5. PPI inhibits the activity of liver enzymes, leading to the upregulation of estrogen concentration in serum

Omeprazole may affect sperm parameters by regulating the expression of AQPs in the testicular and epididymal tissue. The abnormal expression of CYP3A4 was likely to be an important bridge between omeprazole and the abnormal expression of AQPs in the testicular and epididymal tissue. Our result showed that the expression levels of CYP3A4 and CYP2C11 in liver tissues of PPI group were lower than those in control group ($p < 0.05$) (Fig. 5A–D). Immunohistochemistry showed that the expression of CYP3A4 and CYP2C11 in liver tissues of PPI intervention group were lower than those in control group ($p < 0.05$) (Fig. 5E). In addition, ELISA showed the serum estrogen level of PPI group was higher than that in control group ($p < 0.05$) (Fig. 5F). At the same time, the liver function was tested by microplate method. It was found that there were no significant differences in the serum AST ($p = 0.9490$) and γ-GT ($p = 0.4158$) between two groups (Fig. 5G and I). However, the concentration of serum ALT in PPI group was higher than that in control group ($p < 0.05$) (Fig. 5H).

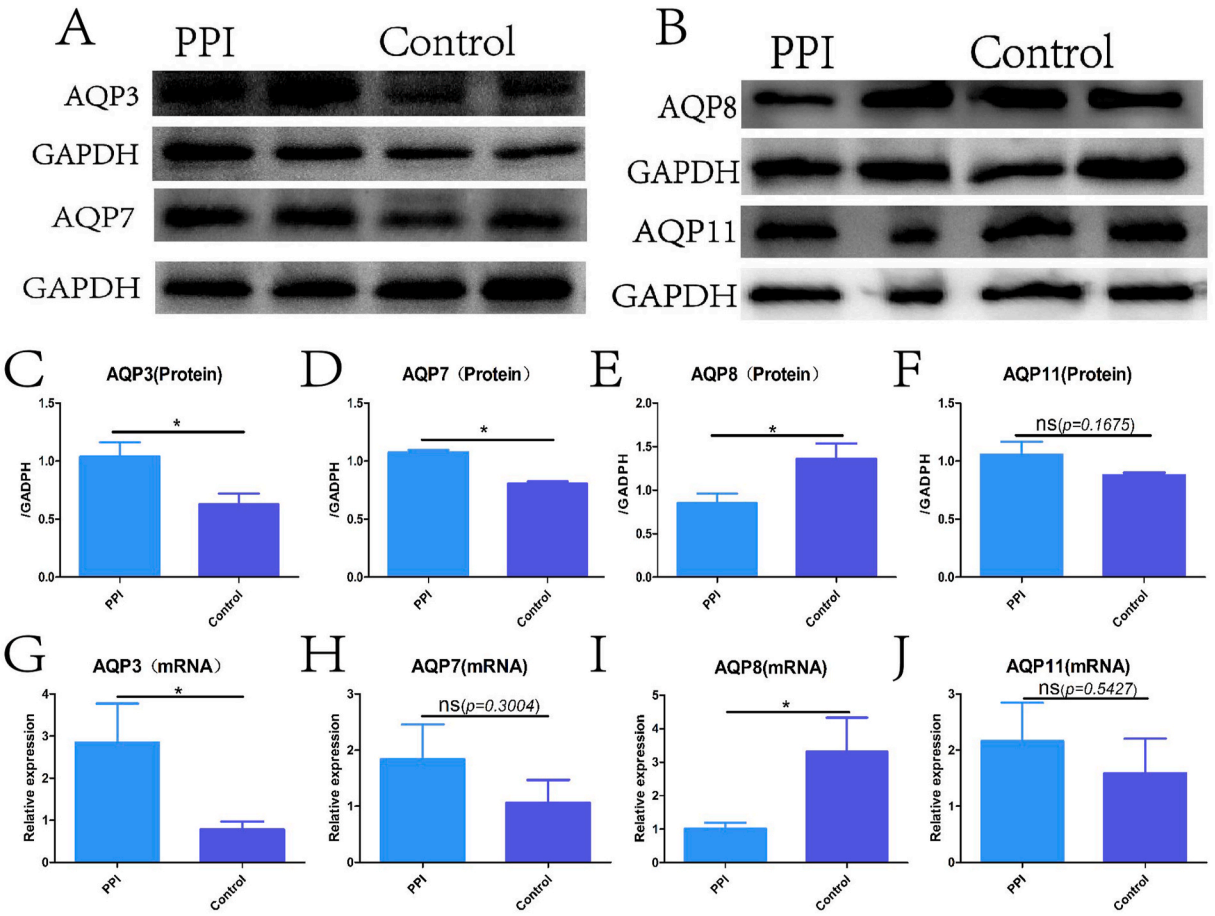

Fig. 3. Correlation between PPI and epididymal AQPs. (A) (B)Western blot of AQP 3, AQP 7, AQP 8 and AQP 11 in the epididymis; (C) (D) (E) (F) The semi-quantification analysis of WB of AQP 3, AQP 7, AQP 8 and AQP 11 in the epididymis ($n > 3$); (G) (H) (I) (J) The mRNA expression level analysis of AQP 3, AQP 7, AQP 8 and AQP 11 in the epididymis ($n > 3$).

5. Discussion

Our study indicated that oral administration of omeprazole for 28 days (20 mg/kg/day) caused a decrease in sperm count and dysfunction in sperm acrosomal state. This conclusion was consistent with recent reports by Hujgen [13] and Banihani [14] et al., which suggested that long-term use of PPIs resulted in poor sperm quality and motility. Hujgen et al. found that patients who used PPI between 6 and 12 months before semen analysis suffered increased risk of reduced TMSC by 2–3 times [11]. The Banihani et al. showed that lansoprazole decreased the TMSC and the forward motile sperm count, but did not reduce the viability of human sperm [14]. The results of this study are similar to the above research. PPI administration can affect sperm count and sperm acrosomal state, which may be positively correlated with the asthenospermia.

However, some studies have shown that PPIs do not affect sperm motility, and it was irrelevant to male infertility. Omeprazole at multiple concentrations does not change motility, viability and DNA integrity of human sperm in vitro [16]. A large patient-based retrospective study by Keihani et al. also suggested that PPIs did not affect semen quality in infertile men [14]. Omeprazole was irrelevant to sperm function, which may be related to the omeprazole does not affect the basal secretion level of pituitary-gonadal axis hormones, and it can counteract the destructive effect of reactive oxygen species [17]. In addition, the omeprazole and its metabolites were genotoxic [18]. The adverse reactions of PPIs may vary greatly among individuals, so, The spermatogenic effect of PPI needs further study in the future.

The current studies assumed a series of possible molecular pathways, which still cannot definitively explain the PPI-related decrease in sperm motility. Pantoprazole may reduce sperm motility by significantly affecting capacitation-induced membrane potential hyperpolarization and capacitation-related protein phosphorylation [19]. Esomeprazole significantly reduced the total number and motility of sperm by inhibiting the activity of choline acetyltransferase [12]. The causes of male reproductive toxicity induced by PPIs in Wistar rats were related to the changes of testosterone, FSH, LH and other hormones [20]. The decrease in sperm motility may be due to the calcium chelation of lansoprazole and a decrease in Na^+/K^+ -ATPase activity [16]. The use of PPIs during the 6–12 months before semen analysis was associated with an increased risk of TMSC reduction by 3 times, which suggested that long-term use

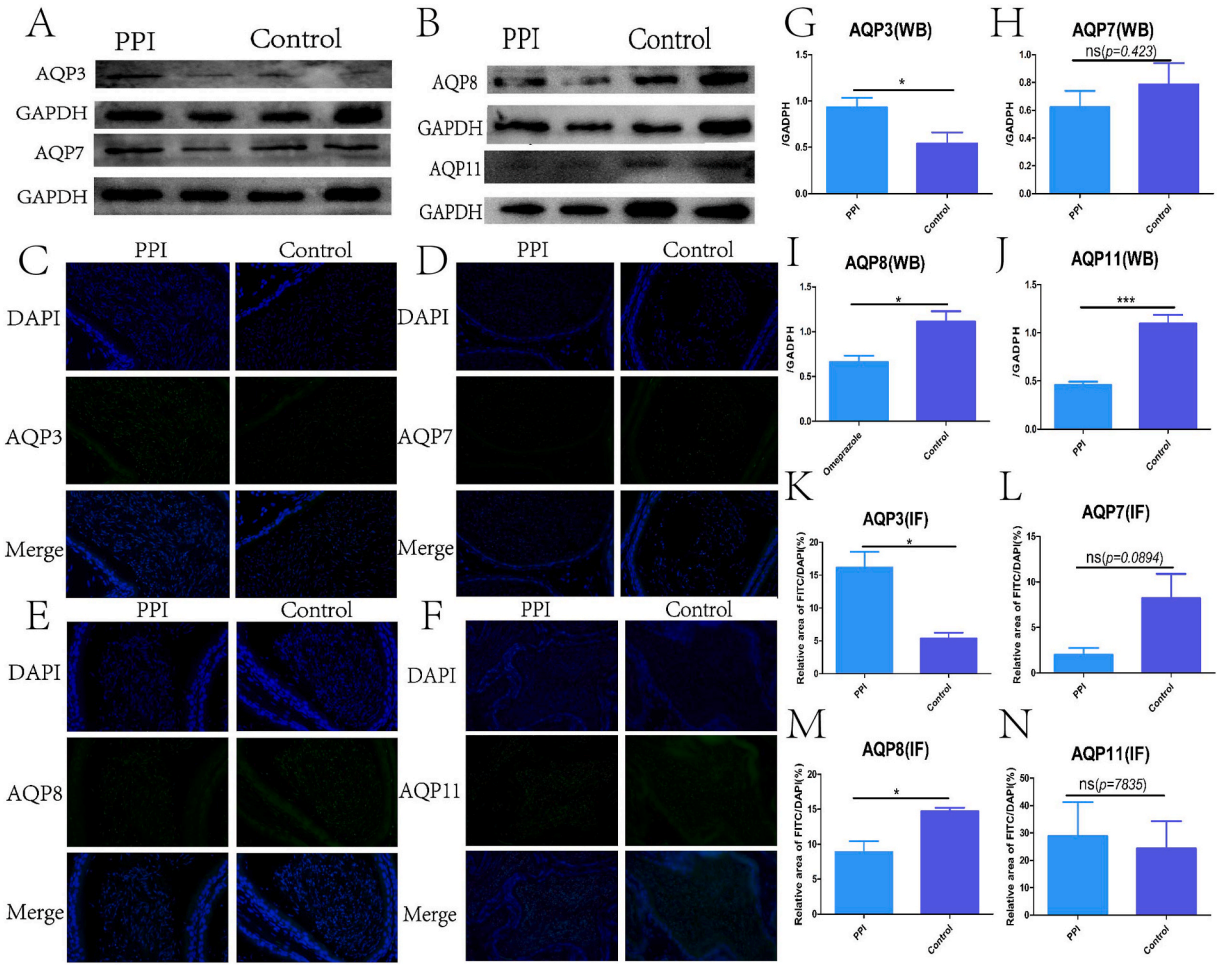

Fig. 4. Correlation between PPI and AQPs in sperm. (A) (B) (C) (D) Immunofluorescence of AQP 3, AQP 7, AQP 8 and AQP 11 in the epididymis (400×); (E) (F)Western blot of AQP 3, AQP 7, AQP 8 and AQP 11 in the sperm; (G) (H) (I) (J)Semi-quantitative analysis of AQP 3, AQP 7, AQP 8 and AQP 11(IF) (n > 3); (K) (L) (M) (N)Semi-quantitative analysis of AQP 3, AQP 7, AQP 8 and AQP 11(WB) (n > 3).

of PPIs may lead to decreased sperm quality [12]. The effect of PPIs was due to increased gastric pH leading to depletion of vitamin B12 and other micronutrients, which supported sperm function [11]. PPI-related asthenospermia may be related to sex hormone levels, ATPase activity and so on.

In patients with liver cirrhosis, omeprazole, esomeprazole and lansoprazole have inhibitory effects on liver enzyme microsomal activity [21]. In the presence of omeprazole, the N-demethylase activity of CYP3A4 measured by formaldehyde generation method to erythromycin obvious decreased [22]. The formation of eprazole sulfone, the main metabolite of eprazole was mainly catalyzed by CYP3A4 [23]. At the same time, PPIs may have harmful effects by inhibiting the metabolism of active metabolites of clopidogrel, mainly CYP2C19 [24]. Lansoprazole and pantoprazole are the most effective in vitro inhibitors of CYP2C19 and CYP2C9, respectively. Compared with omeprazole and its R-enantiomer, esomeprazole has a weaker inhibitory effect [25]. High-dose esomeprazole has a strong inhibitory effect on CYP2C19, a weak inhibitory effect on CYP3A4 and a small induction effect on CYP1A2 [26]. The use of omeprazole and other PPI may inhibit the activity of liver enzymes like CYP3A4, resulting in a series of adverse reactions, including changes in the concentration of sex hormones in the body and decreased sperm motility.

The first step in themetabolic response of estrogens was mainly regulated by cytochrome P450 enzymes [27]. A major metabolic reaction of estradiol, was mainly catalyzed by CYP1A2 and CYP3A4 in liver [27]. The CYP2C11 catalyzed the hydroxylations of estradiol at the 2 and 16 alpha positions [28]. The protein expression of aquaporins 3 and aquaporins 11 was significantly lower in the ovariectomy group than in the control [29]. Estrogen directly upregulates aquaporins 3 and aquaporins 7 by activating estrogen response elements in the promoter of the aquaporins gene [30,31]. Aquaporins 8 was related to oestrogen mediated buffalo follicular development by regulating cell cycle progression in granulosa cells [32]. Previous literature has adequately demonstrated the relevance of P450 enzymes (particularly CYP3A4, CYP2C11) to estrogen and the promoting effects of high expression of estrogen on aquaporins.

Alterations in the aquaporins (aquaporins 3, aquaporins 7, aquaporins 8 and aquaporin 11) of the testis-epididymis-sperm axis may

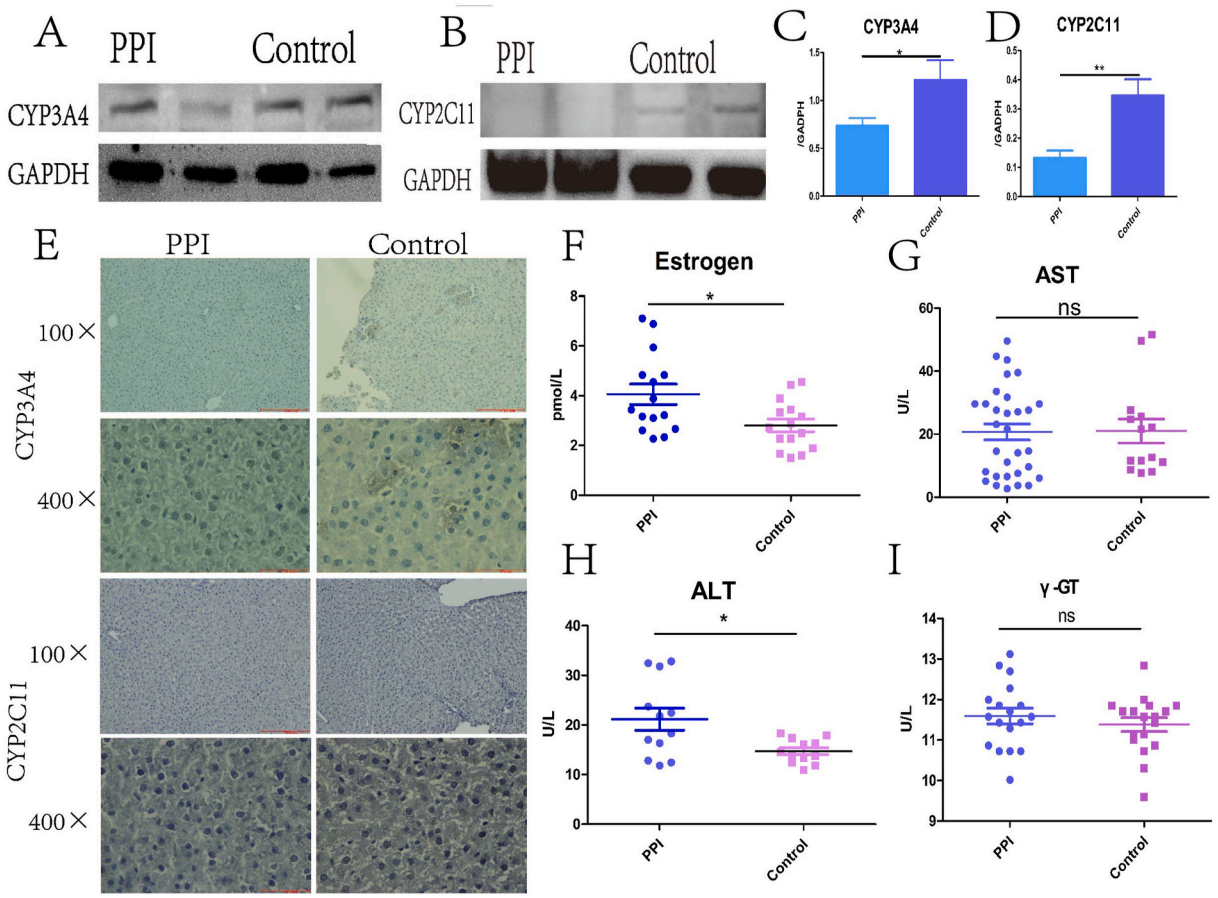

Fig. 5. Correlation between PPI and liver enzyme-estrogen axis. (A) (B) Western blot of CYP3A4 and CYP2C11 in liver tissue; (C) (D) Semi-quantitative analysis of CYP3A4 and CYP2C11 in liver tissue (WB) ($n > 3$); (E) Immunohistochemistry of CYP3A4 and CYP2C11 in liver tissues; (F) Serum estrogen levels; (G) Serum AST level; (H) Serum ALT level; (I) Serum γ-GT level.

affect sperm number and motility. Aquaporin 11 expression is related to sperm motility and membrane integrity, while aquaporin 7 expression is not [33]. Aquaporin 3, aquaporin 7, and aquaporin 11 appear to be involved in the regulation of sperm volume. Aquaporin 8 seems to play a role in regulating the elimination of hydrogen peroxide (the most abundant ROS) [34]. The expression of sperm aquaporin 7 is related to forward motility, and the expression of sperm aquaporin 8 is negatively correlated with the degree of sperm frizziness [35]. Aquaporin 3, aquaporin 7 and aquaporin 11 are involved in the response of spermatozoa to osmotic pressure changes and freeze-thaw processes [36]. At the same time, aquaporins are involved in volume regulation and ROS elimination, affecting sperm number and sperm motility [37]. In conclusion, aquaporins family plays an important role in sperm number and motility.

Our study still has some deficiencies, which required more effort and work to complete this series of studies. First, our team collected semen of some patients with long-term PPI oral administration for molecular detection of aquaporins to further clarify the inhibitory effect of PPI inhibitors on aquaporins, and conducted correlation analysis between aquaporins expression levels and sperm motility. In addition, we can further demonstrate the reliability of the PPI-liver enzyme-estrogen-aquaporins-sperm axis through the expression intervention of liver enzymes, estrogen supplementation and functional antagonism of aquaporins in the future. Finally, We need to directly test rat sperm motility through sperm function test or other related test in the future.

6. Conclusion

Omeprazole may lead to upregulation of estrogen level in serum by inhibiting the activity of liver enzymes such as CYP3A4 in rats, and then affect the function of aquaporins in testis, epididymis and sperm, thus affecting the number of sperm cells and acrosomal enzyme level. This study only confirmed the effect of omeprazole on sperm count and function in animal. In the future, a lot of work is still needed to further confirm the effect of PPI on clinical patients, and to explore the molecular changes in this process, so as to further balance between PPI medication and infertility.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declarations

Ethics approval and consent to participate

All experimental procedures in animal work were approved by the Ethical Committee of Fujian Medical University. This study was carried out in accordance with the recommendations of Medical ethics committee of the First Affiliated Hospital of Fujian Medical University. The protocol was approved by the Medical ethics committee of the First Affiliated Hospital of Fujian Medical University [2022] 551.

Consent for publication

No consent was involved in this publication.

Availability of data and materials

All data and materials generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Conceived and designed the experiments: Q.-C.L., and J.-M.W.
Performed the experiments: Q.-C. L., F.-D.D., and Y.-X.L.
Analyzed and interpreted the data: J.-M.W., and Q.-C.L.
Contributed reagents, materials, analysis tools or data: X.-X.G., Y.-X.L., Y.-Z.L., J.-T.C., and Q.-C.L.
Performed immunhistochemistr: F.-D.D., and Y.-X.L.
Wrote the paper: Q.-C.L., and F.-D.D.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgements

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17911.

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