Reduced Expression and Function of Aquaporin-3 in Mouse Metaphase-II Oocytes Induced by Controlled Ovarian Hyperstimulation were Associated with Subsequent Low Fertilization Rate

Qing-Xia Meng^{1*}, Hui-Juan Gao^{1*}, Chen-Ming Xu¹, Min-Yue Dong¹, Xia Sheng², Jian-Zhong Sheng³ and He-Feng Huang^{1,2}

¹Department of Reproductive Endocrinology, Women's Hospital, School of Medicine, Zhejiang University, ²Center of Tissue Engineering, School of Medicine, Zhejiang University, ³Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, *These authors made equal contribution to this work

Key Words

Aquaporin-3 • *In vitro* fertilization • Oocyte • Controlled ovarian hyperstimulation

Abstract

Background/Aims: Aquaporin-3 (AQP3), one isoform of water channel family, has been found to be expressed in mouse oocytes. The present study aimed to investigate whether functional AQP3 was expressed in oocytes induced by controlled ovarian hyperstimulation (COH), and whether altered oocyte AQP3 expression was associated with changes in fertilization rate. **Methods:** Sixty ICR female mice were divided into two groups: COH and control. AQP3 mRNA expression of mouse metaphase II (MII) oocytes was quantified by real-time RT-PCR. The water permeability of oocytes was assessed with cell swelling test. The fertilization profiles of oocytes were generated via *in vitro* fertilization. **Results:** AQP3 mRNA was expressed in both natural and COH-induced mouse oocytes. COH significantly reduced AQP3 mRNA expression. The volume of oocytes was significantly increased after exposure to hypotonic medium and pretreatment with HgCl₂ attenuated hypotonic medium-induced increase in oocyte volume

and water permeability coefficient (Pf). Furthermore, the expression of AQP3, Pf and the fertilization rate were significantly lower in COH oocytes than those in control. **Conclusion:** AQP3 might play an important role in controlling oocyte quality and a low *in vitro* fertilization rate of COH mice might, in part, result from reduced AQP3 expression and water permeability in mouse oocytes.

Copyright © 2008 S. Karger AG, Basel

Introduction

Water is a major component of cells and tissues. Its movement across cell membrane is a fundamental property of life. Water moves across cell membranes by diffusion through either the lipid bi-layer or protein water channels termed aquaporins (AQPs). The AQPs are a family of small (25~34 kDa), hydrophobic, integral membrane channel proteins that facilitate rapid passive movement of water. To date, thirteen isoforms of AQPs (AQP0-AQP12) have been identified in mammals [1-3] and eleven AQP isoforms (AQP0-AQP10) have been identified in male and female reproductive systems (3).

Fluid secretion and reabsorption are of central

importance to the metabolic processes of reproduction. Several studies have shown that AQPs play physiological and pathophysiological roles in mammalian reproductive systems by mediating fluid transport [3]. Our recent study demonstrated that AQP2 was expressed in human uterine endometrial cells and its expression level was menstrual cycle-dependent and reached the highest level at the mid-secretory phase during the time of embryo implantation [4]. AQP7, AQP8 and AQP9 were found in rat granulosa cells and the water movement into antral follicles might be mainly mediated by these AQPs [5]. AQPs were also detected in murine embryos from the one-cell stage up to the blastocyst stage [6, 7]. Several studies have been performed to examine expression of AQPs in oocytes. AQP3 and AQP7 were found in mouse oocytes [7] and AQP9 was found in rat oocytes [8]. Furthermore, induced expression of AQP3 in mouse oocytes was shown to improve water and glycerol permeability as well as oocyte survivability after cryopreservation [9].

Assisted reproductive technology (ART) has been performed for decades, but in vitro fertilization rate, embryo implantation rate and pregnant rate of in-vitro fertilization and embryo transfer (IVF-ET) are still not satisfactory. Controlled ovarian hyperstimulation (COH) is used in ART to induce the development of numerous follicles. Previous studies demonstrated that the in vitro fertilization rate was reduced after superovulation with high-dose ovarian stimulation [10, 11]. Up to now, the mechanisms underlying low fertilization, embryo implantation and pregnant rates in IVF-ET are still not elucidated.

The findings of the expression of AQP3 in mouse oocyte and the improvement of water and glycerol permeability and oocyte survivability in cryopreservation by induced expression of AQP3 [9] suggest that the change in AQP3 expression and/or function in oocyte may influence oocyte quality. In order to examine possible roles of AQPs in oocyte quality and whether COH would affect the expression and function of AQP3 in oocyte, we compared, in the present study, the expression of AQP3 in metaphase-II (MII) oocytes between mice treated with COH and control using real-time PCR analysis. We also compared the water permeability of MII oocytes between mice treated with COH and control using cell swelling test. Furthermore, we compared the fertilization ability between natural and COH-induced oocytes. Our results suggested that COH could influence AQP3 expression and function in mouse oocyte, which might be one of factors inducing low quality of oocytes in IVF.

Materials and Methods

Experimental Animals

Animal care and use were in accordance with the institutional guidelines established by the Animal Care and Use Committee (ACUC) of School of Medicine, Zhejiang University. Female ICR mice (6-7 weeks old) were housed in 12/12 hrs light/dark cycle at 25 ± 0.5 °C and 50-60% humidity. The mice were fed *ad libitum* with a standard pellet diet and water. Estrus was examined by daily vaginal smear. Mice that exhibited estrus for more than two consecutive periods of regular 4-day cycles were included in the present study. Sixty mice were randomly allocated into two groups, COH and control. In COH group, mice were superovulated at the estrus phase as described previously [12]. Briefly, mice received intraperitoneal (i.p.) injection of 7.5 IU PMSG (pregnant mare serum gonadotropin; Pregnyl, Organon, the Netherlands) and followed by an injection (i.p.) of 5 IU human chorionic gonadotropin (hCG; Pregnyl, Organon, the Netherlands) 46-48 hrs after administration of PMSG. In the control mice, same volume of saline was injected at the same time as in COH group. Mice were killed by cervical dislocation 12-14 hrs after hCG (COH group) or saline (control) injection and the oviducts were excised. Cumulus masses were recovered from the dilated ampullae under a dissecting microscope. The collected cumulus masses were either placed in a drop of HTF (human tubal fluid solution, a medium for IVF) for in vitro fertilization or digested with hyaluronidase to remove granulosa cells for oocyte swelling assay or mRNA extraction. After digested with hyaluronidase, the naked oocytes were washed three times with Phosphate-buffered saline (PBS).

RT-PCR analysis

Total RNA was extracted from 80-100 oocytes of each group with TRIzol reagent (Invitrogen, Carlsbad, USA). One microgram of total RNA was reverse-transcribed using M-MLV (Sangon, Shanghai, China) and random primers. Ten microliters of reverse-transcription product were amplified by PCR with primers specific for AQP3 (sense primer: 5'-TGGTGGCTTCCTCACCATCAA-3' and antisense primer: 5'-CGA GCC CAA AAA CAA TCC CAG C-3') and β -actin. (sense primer: 5'-CGT ACC ACT GGC ATT GTG AT-3' and antisense primer: 5'-CATT GCC GAT AGT GAT GAC C-3'). cDNA corresponding to 5 μ g total RNA obtained from the mouse kidney were used as a positive control of AQP3 [13, 14]. The PCR was carried out in 25 μ l reaction mixture containing 2.5 μ l 10xPCR buffer (without Mg^{++}), 2 μ l 4xdNTP (each 2.5 mM), 1 μ l sense and antisense primers (10 μ M), 1.5 mM Mg^{++} , 0.18 μ l TaKaRa Taq (TaKaRa, Japan) and 10 μ l cDNA sample. The PCR was conducted in the following profile: 94 °C for 30 sec, 58 °C for 30 sec, and 72 °C for 30 sec for 42 cycles. For the positive control, the condition for PCR reaction was: 94 °C for 30 sec, 58 °C for 30 sec, and 72 °C for 30 s for 40 cycles. The PCR products were analyzed by 2% agarose gels electrophoresis and stained with ethidium bromide. RT-PCR was repeated at least three times.

Real-time RT-PCR

Three microliters of reverse transcription products were

Fig. 1. Expression of AQP3 mRNA in mouse MII oocytes. Messenger RNA encoding AQP3 was analyzed by RT-PCR in mouse MII oocytes. Bands of 209 bp representing AQP3 mRNA and bands of 319 bp representing β -actin were detected in mouse kidney (positive control, panel A) and mouse oocytes (panel B). K: kidney; O: oocyte; M: marker.

amplified by real-time PCR with SYBR-Green I (SG; Sangon, Shanghai, China) using β -actin as an internal control. Real-time PCR was carried out in a 25 μ l reaction system containing 2.5 μ l 10xPCR buffer (without Mg^{++}), 2 μ l 4xdNTP (each 2.5mM), 1 μ l sense and anti-sense primers (10 μ M), 1.5 mM Mg^{++} , 0.18 μ l TaKaRa Taq (TaKaRa, Japan), 0.5 μ l SG and 3 μ l cDNA sample. The thermal cycling conditions were: 94 $^{\circ}$ C for 30 sec, 58 $^{\circ}$ C for 30 sec, and 72 $^{\circ}$ C for 30 sec, followed by 42 cycles. Data were analyzed by the comparative threshold cycle (CT) method and the following formula [15]:

$$\text{Expression} = 2^{-\Delta\Delta CT}$$

Where, $\Delta\Delta CT = \Delta CT (\text{COH group}) - \Delta CT (\text{control group})$, and $\Delta CT = CT (\text{sample}) - CT (\text{internal control})$. The threshold cycle for each sample was determined in triplicate. The values of real-time PCR were presented as relative expression levels of AQP3 mRNA.

Oocyte Swelling Assay

Naked MII oocytes were incubated in HTF (osmotic pressure: 280 mosm) and initial photographs (x80) were taken. Oocytes were incubated for 15 min in the presence (+ $HgCl_2$) or absence (- $HgCl_2$) of 50 μ M $HgCl_2$, washed three times with PBS and exposed to hypotonic medium (70% H_2O /30% HTF, osmotic pressure: 120 mosm) for 45 seconds. Photographs (x80) were taken again from 0 sec to 45 sec at an interval of 5 sec. The diameters were measured and volumes calculated based on the assumption that the oocytes approximate a complete sphere [5, 16]. The osmotic water permeability coefficient (P_f , cm/sec) was calculated from [17]:

$$P_f = V_0 d(V/V_0)/dt / [S V_w (\text{osm}_{out} - \text{osm}_{in})]$$

Where, V_0 is initial mouse oocyte volume, V is mouse oocyte volume 45 s after switching to hypotonic media, and S is initial surface area of the mouse oocyte immediately before the change in media, osm_{in} is 280 mosm and osm_{out} is 120 mosm.

In vitro fertilization

Male ICR mice (8-10 weeks) were killed and the epididymis dissected. The vas deferens close to epididymis was cut and placed in a drop of 500 μ l HTF under oil. The HTF was incubated for 12 hrs at 37 $^{\circ}$ C in 5% CO_2 prior to use. The sperm were gently taken out using watchmaker's forceps and incubated for 1.5 hrs at 37 $^{\circ}$ C with 5% CO_2 to be capacitated. The collected cumulus masses were placed in a drop of 1000 μ l HTF under oil and incubated for 1 hr at 37 $^{\circ}$ C with 5% CO_2 . 100 μ l of sperms were then added into the drop containing oocytes. The sperm concentration was in a range of 1×10^6 to 2×10^6 /ml.

Fig. 2. Real-time PCR analysis showed that expression levels of AQP3 mRNA in mouse oocytes at MII. A, typical tracings in a real-time PCR experiment. B, Summary data showed that the relative expression levels of AQP3

The oocytes were incubated with sperms for 4 hrs at 37 $^{\circ}$ C with 5% CO_2 . Fertilization was judged if the oocyte showed extrusion of second polar body 3-6 hrs after incubation with sperms.

Statistical Analysis

Unpaired Student t test was used to compare the expression of AQP3 mRNA in real-time PCR analysis and P_f in cell swelling assay between COH group and control. One-way analysis of variance (ANOVA) was used to analyze the difference in cell volume changes among groups in cell swelling assay. The in vitro fertilization rates were compared with χ^2 test. SPSS 10.0 software was used to analyze data and $p < 0.05$ was considered statistically significant.

Fig. 3. Functional expression of AQP in mouse MII oocytes in control and COH groups. A: photographs ($\times 80$) of typical oocytes pre-incubated with (+HgCl₂) or without (-HgCl₂) of 50 μ M HgCl₂ for 15 min before (a and b) and 45 sec (c and d) after exposure to hypotonic medium. B, Summary data of the changes in the cell volume in control and COH oocytes pretreated without or with 50 μ M HgCl₂ after exposure to hypotonic medium. C, Summary data of the changes in P_f in control and COH oocytes pretreated without or with 50 μ M HgCl₂ after exposure to hypotonic medium. **, $P < 0.01$ compared with oocytes pretreated without HgCl₂. ##, $p < 0.01$ compared with corresponding control. Data were presented in mean $\pm$ SD.

Results

The expression of AQP3 mRNA in mouse MII oocytes

Figure 1 shows the results of RT-PCR of AQP3 mRNA in mouse MII oocytes. Bands of 209 bp, which represented AQP3 mRNA, were detected in mouse kidney (positive control, figure 1 A) and mouse MII oocytes (figure 1 B) using specific AQP3 primers. The RT-PCR data confirmed the expression of mRNA encoding AQP3 in mouse MII oocytes.

The expression levels of AQP3 mRNA in mouse oocytes from the COH group and the control group

Figure 2A shows typical tracings in a real-time PCR experiment. The difference between CT(AQP3) and CT(actin) in the oocytes of control was larger than that in COH. Analysis of the data with the formula of $2^{-\Delta\Delta CT}$ showed that the relative expression levels of AQP3 mRNA in the control oocytes and in the COH oocytes were 1 (0.97 – 1.04) and 0.28 (0.25 – 0.32), respectively (figure 2B). The expression level of AQP3 mRNA in the COH oocytes was significantly lower than that in the control oocytes ($p < 0.01$).

The analysis of function of AQPs in control and COH mouse MII oocytes

Figure 3A shows the recordings of oocytes pretreated without or with 50 μ M HgCl₂ before and after exposure to hypotonic medium in an experiment. Exposure of oocytes to hypotonic medium induced a significant increase in the cell volume. Pretreatment of oocytes with 50 μ M HgCl₂ significantly attenuated the increase in the cell volume induced by hypotonic medium. The results indicated that AQP(s) expressed in mouse oocytes was functional and mercury-sensitive. Figure 3B shows the

change curves of the cell volume of the control oocytes and COH oocytes pretreated without or with 50 μ M HgCl₂ in the cell swelling assay. Hypotonic medium induced a time-dependent and mercury-sensitive increase in the cell volume of mouse MII oocytes. The effect of hypotonic shock on the cell volume was significantly attenuated in COH oocytes ($p < 0.01$), suggesting that COH might influence the function of AQP(s) in mouse oocyte. Data analysis also showed that water permeability coefficient (P_f) in cell swelling assay in control oocytes pretreated without HgCl₂ were 0.0078 ± 0.0003 cm/s, significantly larger than that (0.0040 ± 0.0003 cm/s, $p < 0.01$) in control oocytes pretreated with 50 μ M HgCl₂ (figure 3C). Furthermore, P_f in COH oocytes pretreated without HgCl₂ was 0.0052 ± 0.0006 cm/s, significantly smaller than that in control ($p < 0.01$, figure 3C). On the other hand, there was no significant difference in the cell volume and P_f between COH and control oocytes pretreated with 50 μ M HgCl₂ (figure 3B and 3C).

Table 1. Fertilization rates in vitro of COH group and control.

	Control	COH group	P
Number of MII oocytes	53	142	-
Number of fertilized oocytes	41	97	-
Fertilization rate (%)	77.36	68.31	<0.05

Comparison of fertilization rates in vitro of mouse MII oocytes in COH and control groups

One hundred and forty two MII oocytes of COH group and 53 of control were used for *in vitro* fertilization. The fertilization rate in COH group was 68.31%, significantly lower than that in control (77.36%, $P < 0.05$, Table 1), indicating that COH might reduce the fertilization rate *in vitro* of mouse oocytes.

Discussion

In consistent with the previous study [7], we demonstrated expression of AQP3 mRNA in mouse MII oocytes in the present research. Compared with control, COH was shown to down-regulate AQP3 expression, attenuate the mercury-sensitive water permeability and reduce fertilization rate in mouse oocytes. These results suggest that COH might influence the quality of oocytes by altering AQP expression and function in oocytes.

Up to date, 11 isoforms of AQPs have been found in mammalian reproductive systems and play physiological and pathophysiological roles in reproductive procedures [3]. AQP3 and other three isoforms (AQP7, AQP9 and AQP10) are classified into a group called aquaglyceroporins [3]. AQP isoforms in aquaglyceroporin group are non-selective water channels which are permeable to glycerol, urea and other small nonelectrolytes as well as to water [1, 3, 18]. In mammal reproductive system, expression of AQP3 was demonstrated in uterus [19], cervix [20], placenta [21], fetal membrane [22], epididymis [23] and oocyte [7]. Edashige et al. [7] first reported that AQP3 and AQP7, but not other AQPs, were expressed in mouse MII oocytes. It was subsequently found that induced expression of AQP3 in mouse oocytes improved water and glycerol permeability as well as oocyte survival after cryopreservation and retained their fertilization ability [9]. These findings revealed a possibility that AQP3 might play an important role in the survival and fertilization of oocytes.

Although both AQP3 and AQP7 expression have been located in mouse MII oocytes, the previous studies showed that AQP3, but not AQP7, was mercury-sensitive [24]. Up to date, mercury is still an effective inhibitor of

most AQP isoforms and widely used to evaluate functions of mercury-sensitive AQPs [5, 15]. Dramatic swelling of mouse MII oocytes induced by hypotonic medium could be inhibited by the pretreatment of oocytes with HgCl_2 , indicates that the contribution of AQP3 to water transport in mouse oocytes is physiologically substantial.

In the present study, we found for the first time that COH not only reduced the expression of AQP3 mRNA in mouse M II oocytes, but also attenuated its function, i.e. water permeability, and subsequently decreased fertilization ability of oocytes. COH is a main process in ART to stimulate the development of follicles and obtain numerous oocytes for IVF-ET purpose. Although a lot of oocytes could be obtained by COH, the quality of oocytes might not be satisfactory in IVF-ET practice. Several studies demonstrated that the *in vitro* fertilization rate was reduced after superovulation with high-dose ovarian stimulation [10, 11]. The mechanisms underlying low fertilization rate, low embryo implantation rate and low pregnancy rate in IVF-ET remain unclear. Based on the results from the present and other studies that AQP3 expression and its function were attenuated in COH-induced mouse MII oocytes and that AQP3 was important for mouse oocyte survival and fertilization [9], it is reasonable to speculate that AQP3 may be among the factors leading to low fertilization rate of oocytes in IVF-ET. The mechanism underlying attenuation of expression levels of AQP3 in mouse oocyte by COH is unclear and needed to be clarified in further study.

The present study demonstrated that the mean expression level of AQP3 mRNA in COH oocytes was around 28% of that in control. However, the mean water permeability coefficient in COH oocytes was around 67% of that in control. A very strong down-regulation of AQP3 expression in COH oocytes did not correspond to a parallel decrease in the water permeability. This result suggested that other pathways, such as simple diffusion or/and other AQP isoform(s), may also facilitate water transport in mouse oocytes.

Unfortunately, the resource and ethic considerations restrict studies on human oocytes and thus no reports of study on AQPs in human oocytes were available. Therefore the results of the present study could not be directly applied to explain the mechanisms underlying low fertilization rate in IVF-ET. However, our data provide a

clue to understand the mechanisms underlying low fertilization rate in IVF-ET practice and a potential way to improve fertilization rate in IVF-ET through improving AQP expression in oocyte [25, 26].

In conclusion, the present study provides further evidence that AQP3 is expressed in mouse MII oocytes. The down-regulation of AQP3 expression associated with attenuation of the water permeability and *in vitro* fertilization rate in oocytes of the mice treated with mimic COH in IVF-ET suggest that a low *in vitro* fertilization rate might, in part, result from reduced expression AQP3 and relatively altered water permeability in mouse oocytes.

Acknowledgements

This work was carried out in Key Laboratory of Women's Reproductive health of Zhejiang Province and financially supported by National Natural Science Foundation of China (30670789), National Basic Research Program of China (2006CB944006, 2006CB504004), National Natural Science Foundation Chinese-Canadian Institutes of Joint Health Research Project (30711120577) and Research Grants from Key Research Program of Education of Zhejiang Province (20061382).

References

- Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A, Nielsen S: Aquaporin water channels-from atomic structure to clinical medicine. *J Physiol* 2002;542(Pt 1):3-16.
- Agre P, Kozono D: Aquaporin water channels: molecular mechanisms for human diseases. *FEBS Lett* 2003;555(1):72-78.
- Huang HF, He RH, Sun CC, Zhang Y, Meng QX, Ma YY: Function of aquaporins in female and male reproductive systems. *Hum Reprod Update* 2006;12(6):785-795.
- He RH, Sheng JZ, Luo Q, Jin F, Wang B, Qian YL, Zhou CY, Sheng X, Huang HF: Aquaporin-2 expression in human endometrium correlates with serum ovarian steroid hormones. *Life Sci* 2006;79:423-429.
- McConnell NA, Yunus RS, Gross SA, Bost KL, Clemens MG, Hughes FM Jr: Water permeability of an ovarian antral follicle is predominantly transcellular and mediated by aquaporins. *Endocrinology* 2002;143(8):2905-2912.
- Offenberg H, Barcroft LC, Caveney A, Viuff D, Thomsen PD, Watson AJ: mRNAs encoding aquaporins are present during murine preimplantation development. *Mol Reprod Dev* 2000;57(4):323-330.
- Edashige K, Sakamoto M, Kasai M: Expression of mRNAs of the aquaporin family in mouse oocytes and embryos. *Cryobiology* 2000;40(2):171-175.
- Ford P, Merot J, Jawerbaum A, Gimeno MA, Capurro C, Parisi M: Water permeability in rat oocytes at different maturity stages: aquaporin-9 expression. *J Membr Biol* 2000;176(2):151-158.
- Edashige K, Yamaji Y, Kleinhans FW, Kasai M: Artificial expression of aquaporin-3 improves the survival of mouse oocytes after cryopreservation. *Biol Reprod* 2003;68(1):87-94.
- Fossum GT, Davidson A, Paulson RJ: Ovarian hyperstimulation inhibits embryo implantation in the mouse. *J In Vitro Fert Embryo Transf* 1989;6(1):7-10.
- Sato F, Marrs RP: The effect of pregnant mare serum gonadotropin on mouse embryos fertilized *in vivo* or *in vitro*. *J In Vitro Fert Embryo Transf* 1986;3(6):353-357.
- Ruan HC, Zhu XM, Luo Q, Liu AX, Qian YL, Zhou CY, Jin F, Huang HF, Sheng JZ: Ovarian stimulation with GnRH agonist, but not GnRH antagonist, partially restores the expression of endometrial integrin beta3 and leukaemia-inhibitory factor and improves uterine receptivity in mice. *Hum Reprod* 2006;21(10):2521-2529.
- Echevarria M, Windhager EE, Tate SS, Frindt G: Cloning and expression of AQP3, a water channel from the medullary collecting duct of rat kidney. *Proc Natl Acad Sci U S A* 1994;91(23):10997-11001.
- Ecelbarger CA, Terris J, Frindt G, Echevarria M, Marples D, Nielsen S, Knepper MA: Aquaporin-3 water channel localization and regulation in rat kidney. *Am J Physiol* 1995;269(5 Pt 2):F663-F672.
- Scheffé JH, Lehmann KE, Buschmann IR, Unger T, Funke-Kaiser H: Quantitative real-time RT-PCR data analysis: current concepts and the novel "gene expression's CT difference" formula. *J Mol Med* 2006;84(11):901-910.
- Yasui M, Hazama A, Kwon TH, Nielsen S, Guggino WB, Agre P: Rapid gating and anion permeability of an intracellular aquaporin. *Nature* 1990;402(6758):184-187.
- Zhang RB, Logee KA, Verkman AS: Expression of mRNA coding for kidney and red cell water channels in *Xenopus* oocytes. *J Biol Chem* 1990;265(26):15375-15378.
- Borgnia M, Nielsen S, Engel A, Agre P: Cellular and molecular biology of the aquaporin water channels. *Annu Rev Biochem* 1999;68:425-458.
- Jablonski EM, McConnell NA, Hughes FM Jr, Huet-Hudson YM: Estrogen regulation of aquaporins in the mouse uterus: potential roles in uterine water movement. *Biol Reprod* 2003;69(5):1481-1487.
- Anderson J, Brown N, Mahendroo MS, Reese J: Utilization of different aquaporin water channels in the mouse cervix during pregnancy and parturition and in models of preterm and delayed cervical ripening. *Endocrinology* 2006;147(1):130-140.
- Damiano A, Zotta E, Goldstein J, Reisin I, Ibarra C: Water channel proteins AQP3 and AQP9 are present in syncytiotrophoblast of human term placenta. *Placenta* 2001;22(8-9):776-781.
- Mann SE, Rieke EA, Yang BA, Verkman AS, Taylor RN: Expression and localization of aquaporin 1 and 3 in human fetal membranes. *Am J Obstet Gynecol* 2002;187(4):902-907.
- Hermo L, Krzczunowicz D, Ruz R: Cell specificity of aquaporins 0, 3, and 10 expressed in the testis, efferent ducts, and epididymis of adult rats. *J Androl* 2004;25(4):494-505.
- Kuwahara M, Gu Y, Ishibashi K, Marumo F, Sasaki S: Mercury-sensitive residues and pore site in AQP3 water channel. *Biochemistry* 1997;36(46):13973-13978.
- King LS, Yasui M: Aquaporins and disease: lessons from mice to humans. *Trends Endocrinol Metab* 2002;13(8):355-360.
- Yasui M: Molecular mechanisms and drug development in aquaporin water channel diseases: structure and function of aquaporins. *J Pharmacol Sci* 2004;96(3):260-263.