J Membrane Biol (2015) 248:1097–1105
DOI 10.1007/s00232-015-9823-8

Testosterone Induces Increase in Aquaporin (AQP)-1, 5, and 7 Expressions in the Uteri of Ovariectomized Rats

Naguib Salleh[1] · Helmy Mohd Mokhtar[1] · Normadiah M. Kassim[2] · Nelli Giribabu[1]

Received: 29 April 2015 / Accepted: 14 July 2015 / Published online: 22 July 2015

Abstract Testosterone has been reported to cause a decrease in uterine fluid volume in which this could involve the aquaporins (AQPs). This study aimed to investigate effect of testosterone on uterine AQP-1, 5, and 7 expressions in order to explain the reported reduction in uterine fluid volume under testosterone influence. Ovariectomized adult female rats received peanut oil, testosterone (1 mg/kg/day), estrogen (0.2 μg/kg/day), or combined estrogen plus testosterone for three consecutive days. Other groups received 3 days estrogen followed by 2 days either peanut oil or testosterone with or without flutamide or finasteride. A day after last injection, uteri were harvested, and the levels of AQP-1, 5, and 7 messenger RNA (mRNA) in uterine tissue homogenates were analyzed by real-time PCR (qPCR). Distributions of AQP-1, 5, and 7 proteins in uterus were observed by immunofluorescence. Levels of AQP-1 mRNA were elevated in rats receiving either estrogen or testosterone-only treatment; however, levels of AQP-5 and 7 mRNAs were elevated in rats receiving testosterone-only treatment. In rats pre-treated with estrogen, testosterone treatment resulted in higher AQP-1, 5, and 7 mRNA levels compared to vehicle treatment. Testosterone effects were antagonized by flutamide but not finasteride. Immunofluorescence study showed that AQP-1 was highly distributed in uterine lumenal epithelium following estrogen or testosterone-only treatment. However, AQP-5 and 7 distributions were high in uterine lumenal epithelium following testosterone-only treatment. Testosterone-induced up-regulation of AQP-1, 5, and 7 expressions in uterus could explain the observed reduction in uterine fluid volume as reported under this condition.

Keywords Testosterone · Uterine fluid · AQP 1, 5, 7

✉ Naguib Salleh
naguib.salleh@yahoo.com.my

[1] Department of Physiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

[2] Department of Anatomy, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Introduction

Aquaporin (AQP), a small hydrophobic, intrinsic membrane protein with low molecular weight of between 26 and 34 kDa, facilitates rapid and passive movement of H_2O (Denker et al. 1988). To date, thirteen AQP isoforms have been identified. Expression of AQP isoforms has been reported in male (Wilson et al. 2013) and female (He et al. 2006) reproductive tissues in rats, mice, marmosets, and humans. In uterus, expression of several AQP isoforms including AQP-1, 5, and 7 was found to be influenced by sex hormones (Jablonski et al. 2003). AQP-1 is the most commonly expressed isoform (Denker et al. 1988) and its expression has been reported in kidneys, lungs, red blood cells, brain, and uterus. This subunit participates in H_2O reabsorption and secretion across the secretory epithelia (Sales et al. 2013). Uterine AQP-1 was found to be distributed in stromal vasculature and was up-regulated by estrogen (Li et al. 1997).

AQP-5, a classic AQP isoform was reported to be expressed in ovaries, oviducts, and uterus (Skowronski 2010). In pig uterus, AQP-5 expression has been found to be influenced by progesterone (Skowronski et al. 2009). Meanwhile in rats, redistribution of uterine AQP-5 was observed under progesterone influence (Lindsay and Murphy 2006). AQP-7, a non-selective H_2O channels which regulate the transport of H_2O, glycerol, urea, and other

small non-electrolytes, was reported to be expressed in the uterus. This isoform was also expressed in the granulosa cells and oocytes of ovaries (Zhang et al. 2012). Uterine AQP-7 participates in decidualization (Huang et al. 2006), while ovarian AQP-7 participates in the H_2O movement into antral follicle which assists in the antrum formation (Huang et al. 2006).

In uterus, H_2O transport through AQP is important for maintaining the fluid volume within the lumen (Lindsay and Murphy 2004), which was known to be affected by sex-steroids. Estrogen increases, while progesterone decreases the uterine fluid volume (Salleh et al. 2005). Deviation from the normal volume could adversely affect the uterine reproductive processes, therefore could affect fertility. Previously, we have shown that testosterone could induce a decrease in the volume of uterine fluid (Mohd Mokhtar et al. 2014). However, the mechanisms underlying testosterone effects are still widely un-elucidated. We hypothesized that testosterone effect on uterine fluid volume could involve AQP-1, 5, and 7. Therefore, in this study, expressions of AQP-1, 5, and 7 in uterus under testosterone influence were investigated in order to explain the observed reduction in uterine fluid volume as observed under this condition.

Materials and Methods

Animal Preparation and Hormones Treatment

Three-month-old adult female Sprague–Dawley (SD) rats, weighing 225 ± 25 g were caged under standard conditions (lights on 06:00–18:00 h; room temperature 24 °C; 4 animals per cage). Animals were fed with rat chow diet (Harlan, Germany) and tap water ad libitum. All experimental procedures were approved by University of Malaya institutional ethics committee. Estrogen, testosterone, flutamide, finasteride, and peanut oil were obtained from Sigma–Aldrich, St Louis, MO, USA. Bilateral ovariectomy was performed 14 days prior to drug treatment to eliminate the effect of endogenous sex-steroids as previously described (Salleh et al. 2005). Drugs were dissolved in peanut oil prior to subcutaneous administration behind the neck scruff. Rats were divided into the following groups which received the following treatment. Each group consists of 6 animals.

Group 1	3 days peanut oil (vehicle-C).
Group 2	3 days 1 mg/kg/day testosterone (T).
Group 3	3 days 0.2 μg/kg/day estrogen (E).
Group 4	3 days combined 0.2 μg/kg/day estrogen plus 1 mg/kg/day testosterone (ET).
Group 5	3 days 0.2 μg/kg/day estrogen followed by 2 days 1 mg/day testosterone (E + T).
Group 6	3 days 0.2 μg/kg/day estrogen followed by 2 days 1 mg/kg/day testosterone plus 2.5 mg/kg/day finasteride (5α-reductase inhibitor) [E + (T + FIN)].
Group 7	3 days 0.2 μg/kg/day estrogen followed by 2 days 1 mg/kg/day testosterone plus 5 mg/kg/day flutamide (androgen-receptor (AR) blocker) [E + (T + FLU)].
Group 8	3 days 0.2 μg/kg/day estrogen followed by 2 days peanut oil (E + PO).

Group 2 and 3 were designed to investigate the effect of individual sex-steroids. Group 1 acted as control for group 2 and 3. Group 4 was designed to investigate the combined effect of testosterone and estrogen. Group 5 was designed to investigate the effect of testosterone on uteri exposed to estrogen, where the later causing fluid to accumulate within the uterine lumen (Chinigarzadeh et al. 2014). Group 6 was designed to investigate dihydrotestosterone (DHT) involvement in mediating testosterone effect and group 7 was designed to investigate androgen-receptor involvement in mediating testosterone effect. Group 8 acted as a control for group 5–7.

Detection of AQP-1, 5, and 7 Protein Distributions in Uterus by Immunofluorescence

Uteri were cut into 5 μm sections, deparaffinized in xylene, and rehydrated in reducing concentrations of ethanol. Antigen retrieval was performed by incubating the sections in 0.01 M citrate buffer, pH 6.0 for 10 min. Subsequently, 3 % H_2O_2 in phosphate-buffered saline (PBS) was used to neutralize the endogenous peroxidase. Uterine sections were blocked in 10 % normal rabbit serum (sc-2338) (Santa Cruz, CA, USA) prior to incubation with goat polyclonal IgG AQP-1 primary antibody (sc-9878), goat polyclonal AQP-5 primary antibody (sc-9890), and rabbit polyclonal IgG AQP-7 primary antibody (sc-28625) (Santa Cruz,CA,USA). The antibodies were diluted at 1:100 in PBS with 1.5 % normal blocking serum at room temperature for 1 h. After three times rinsing with PBS, sections were incubated with rabbit anti-goat IgG–fluorochrome-conjugated secondary antibody (sc-2777) (Santa Cruz, CA, USA) at a dilution of 1:250 in PBS with 1.5 % normal blocking serum at room temperature for 45 min. The slides were rinsed three times with PBS and were mounted with Ultracruz mounting medium (Santa Cruz, CA, USA). Counterstaining was done to visualize the nuclei. All images were viewed under Nikon Eclipse 80i camera that was attached to a light microscope. Negative controls for immunofluorescence were performed by both omitting the

primary antibodies specific to AQP-1, 5, and 7 and by using non-immune IgG. In these experiments, no non-specific fluorescence signals were observed (data not shown).

Quantification *Aqp*-1, 5, and 7 mRNAs by Real-Time PCR (qPCR)

Whole uterine tissues were kept in RNALater solution (Ambion, Carlsbad, CA, USA) prior to RNA extraction. RNA was extracted by using RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) and its concentration was assessed by 260/280 UV absorption ratios (Gene Quant 1300, Cambridge, UK). Gene expression was evaluated by using two steps real-time PCR. TaqMan1 RNA-to-CT 1-Step Kit (Ambion, Carlsbad, CA, USA) was used. cDNA was reversely transcribed to RNA by using high-capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA). Amplifications on samples with no reverse transcriptase (-RT) acted as control. The amplified region of cDNA was probed with TaqMan fluorescence-labeled probe. TaqMan probe has a sensitivity of 100 % and specificity of 96.67 % (Tsai et al. 2012) and is capable of detecting as few as 50 copies of RNA/ml (Leutenegger et al. 2001) and as low as 5–10 molecules (Tandon et al. 2005).

The assay used (TaqMan®-catalog number: Rn005628 34-m1 for *Aqp-1*, Rn00562837 for *Aqp-5*, and Rn00569 727_m1 for *Aqp-7*—Applied Biosystems, Foster City, CA, USA) amplifies 60, 69, and 82 bp segments for *Aqp-1*, *Aqp-5,* and *Aqp-7,* respectively, from the whole messenger RNA (mRNA) length of 2623 bp (*Aqp-1*), 1426 bp (*Aqp-5*), and 1267 bp (*Aqp-7*). In this study, *Gapdh* (Rn99999916_s1) and *Hprt1* (Rn01527840-m1) (Applied Biosystems, Foster City, CA, USA) were used as reference or house-keeping genes as their expression were the most stable throughout the estrus cycle and in early pregnancy (Lin et al. 2013). The specificity of primer and probe ensures that the expression of target DNA was specifically evaluated. Validation was performed in silico by using whole rat genome and in vitro by using whole rat cDNA (Applied Biosystems, Foster, CA, USA) to ensure that specific sequences were detected. Therefore, additional sequencing was not required.

PCR amplification program included 2 min at 50 °C Uracil N-glycosylase (UNG), 20 s, 95 °C activation of ampliTaq gold DNA polymerase and 1 min denaturation at 95 °C, 20 s and annealing/extension at 60 °C for 1 min. Denaturation and annealing were performed for 40 cycles. Negative controls were performed which include omission of RT or omission of cDNA. GenEx software (MultiD, Odingatan, Sweden) was used to normalized all measurements followed by Data Assist v3 (Applied Biosystems, Foster City, CA, USA) that was used to calculate RNA fold changes. Data were analyzed according to comparative CT ($2^{\Delta\Delta C_t}$) method. The relative quantity of target in each sample was determined by comparing the normalized target quantity in each sample to average normalized target quantity of references.

Statistical Analysis

Student's t test and one-way analysis of variance (ANOVA) were used for statistical analyses. A probability level of less than 0.05 ($p < 0.05$) was considered significant. Post-hoc statistical power analysis was performed and all values were > 0.8 which indicate adequate sample size.

Results

Distribution of AQP-1, 5, and 7 Proteins in Uterus

Aqp-1

Figure 1 shows AQP-1 distribution in uterus. High level of AQP-1 was seen in epithelium lining the uterine lumen of rats receiving estrogen (E)-only and testosterone (T)-only treatment. In E-only treated rats, high distribution could also be seen in stroma and myometrium. However, in rats receiving T-only treatment, only mild stromal distribution could be seen. In rats receiving concomitant estrogen plus testosterone (ET) treatment, moderate epithelial distribution could be seen, with no stromal distribution. In rats receiving estrogen followed by testosterone (E + T) treatment, moderate epithelial and stromal distribution could be seen. Administration of estrogen followed by combined testosterone and finasteride [E + (T + FIN)] resulted in lower stromal distribution compared to E + T treatment; however, no differences in epithelial distribution were observed. In rats receiving estrogen followed by combined testosterone and flutamide treatment [E + (T + FLU)], very low distribution could be seen in both stroma and lumenal epithelium.

Aqp-5

Figure 2 shows AQP-5 distribution in uterus. High levels of AQP-5 could be seen at apical and basolateral membranes of uterine lumenal epithelium and stroma of T-only and E + T treated rats. Additionally, in these rats, myometrial distribution could also be seen. In rats receiving E + (T + FIN) treatment, high stromal and moderate epithelial distribution could be seen. In rats receiving E + (T + FLU) treatment, moderate stromal and epithelial distribution could be seen. In E and E + V treated rats, very low distribution could be seen in lumenal epithelium,

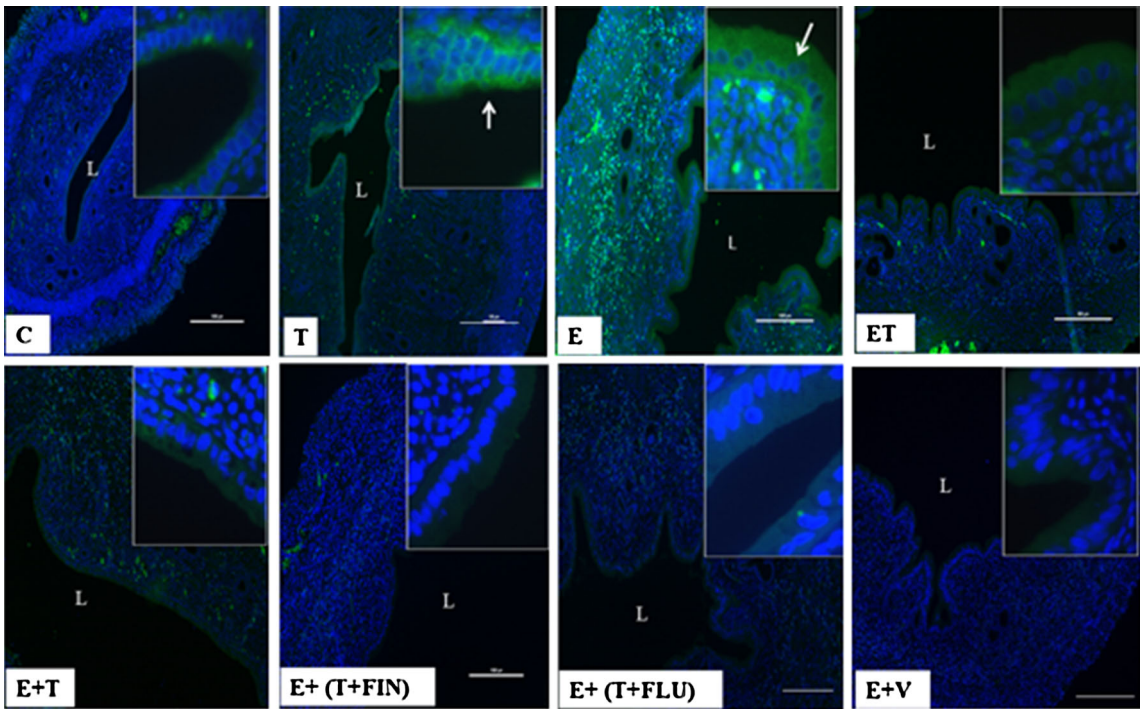

Fig. 1 Distribution of AQP 1 protein in uterus. Under estrogen influence, AQP1 could be seen to be distributed in the stroma, myometrium and lumenal epithelium. Under testosterone influence, the distribution is mainly in the lumenal epithelium *Arrows* pointing toward AQP 1. *L* lumen, *scale bar* 50 μm. *C* control, *T* testosterone, *E* estrogen, *FLU* flutamide, *FIN* finasteride, *V* peanut oil (vehicle)

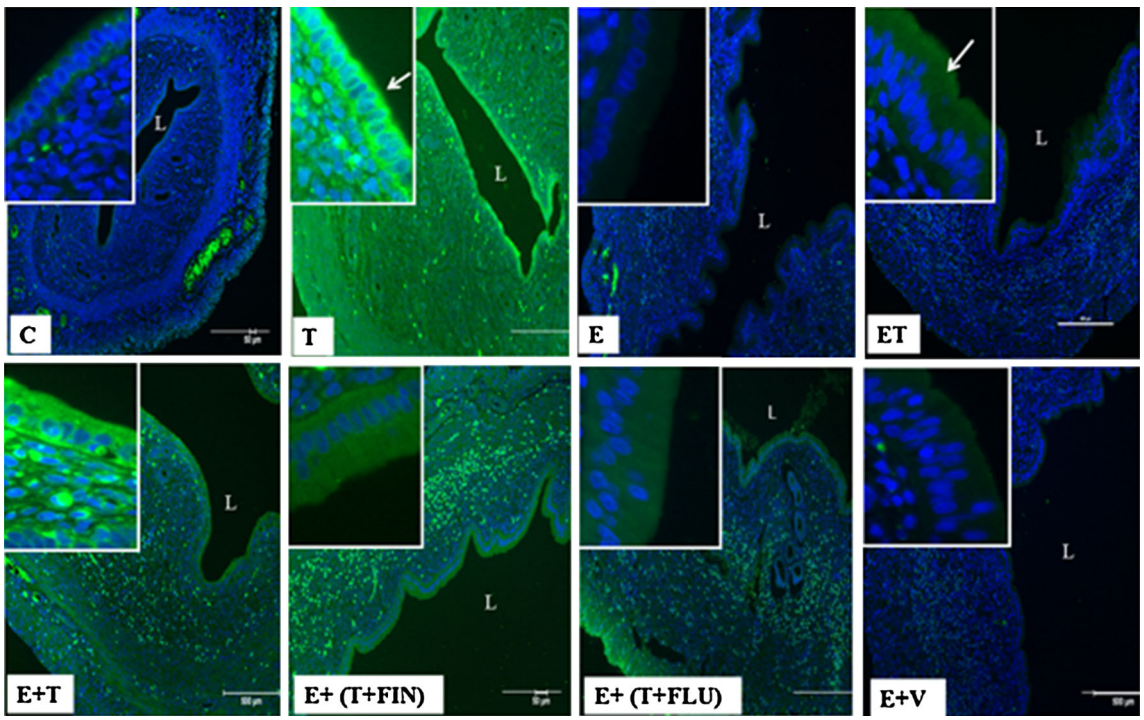

Fig. 2 Distribution of AQP5 protein in uterus. Under testosterone influence, high distribution of AQP5 could be seen at apical and basolateral membranes of uterine lumenal epithelium. Very low distribution could be seen under estrogen influence. *L* lumen, *scale bar* 50 μm. *C* control, *T* testosterone, *E* estrogen, *FLU* flutamide, *FIN* finasteride, *V* peanut oil (vehicle)

stroma, and myometrium. Meanwhile, moderate distribution could be seen in lumenal epithelium following concomitant ET treatment with the levels lower than T-only treatment.

Aqp-7

Figure 3 shows distribution of AQP-7 in uterus. AQP-7 was highly distributed in lumenal epithelium and stroma of rats receiving T-only treatment. In rats receiving E-only treatment, very low epithelial and stromal distribution could be seen. Low distribution could also be seen in lumenal epithelium of rats receiving combined ET treatment. In E + T treated rats, mild to moderate lumenal epithelial distribution could be seen. In rats receiving E + (T + FLU) treatment, very low lumenal epithelial distribution could be seen, while in rats receiving E + (T + FIN) treatment, lower lumenal epithelial distribution could be seen compared to E + T treatment.

Levels of *Aqp*-1, 5, and 7 mRNAs in Uterine Tissue Homogenates

Aqp-1 mRNA

Figure 4 shows *Aqp-1* mRNA levels in uterus of rats receiving different treatments. The levels of *Aqp-1* mRNA were 1.95- and 1.75-fold higher following E-only and T-only treatment, respectively, compared to control. Concomitant ET treatment resulted in lower *Aqp-1* mRNA level compared to E-only and T-only treatment. In rats receiving E + T treatment, levels of *Aqp-1* mRNA were threefold higher compared to E + V treatment. In these rats, treatment with E + (T + FLU) resulted in approximately twofold lower *Aqp-1* mRNA level compared to E + T treatment. A slight but no significant changes in the levels of *Aqp-1* mRNA were noted in rats receiving E + (T + FIN) compared to E + T treatment.

Aqp-5 mRNA

Figure 5 shows levels of *Aqp-5* mRNA in uterus of rats receiving different treatments. *Aqp-5* mRNA was the highest in rats receiving T-only treatment which was approximately threefold higher compared to control. In rats receiving E-only treatment, *Aqp-5* mRNA levels were not significantly different compared to control. Concomitant ET treatment resulted in the level of *Aqp-5* mRNA to be markedly lower than T-only treatment; however, slightly higher than E-only treatment. In rats receiving E + T treatment, levels of *Aqp-5* mRNA were higher compared to E + V treatment ($p < 0.05$). In rats receiving E + (T + FIN) treatment, no significant difference in *Aqp-5* mRNA levels was noted compared to E + T treatment.

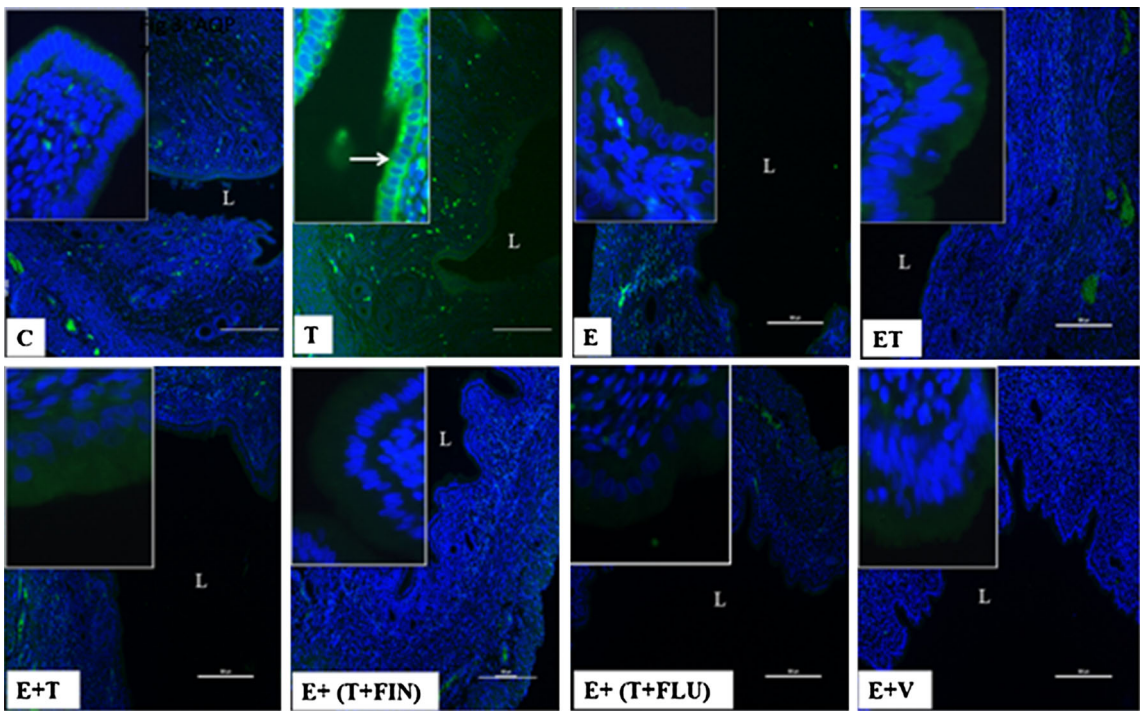

Fig. 3 Distribution of AQP7 protein in uterus. Under testosterone influence, high levels of AQP7 could be seen in uterine lumenal epithelium and stroma. Following estrogen-only treatment, low AQP7 expression was observed. *L* lumen, *scale bar* 50 μm. *C* control, *T* testosterone, *E* estrogen, *FLU* flutamide, *FIN* finasteride, *V* peanut oil (vehicle)

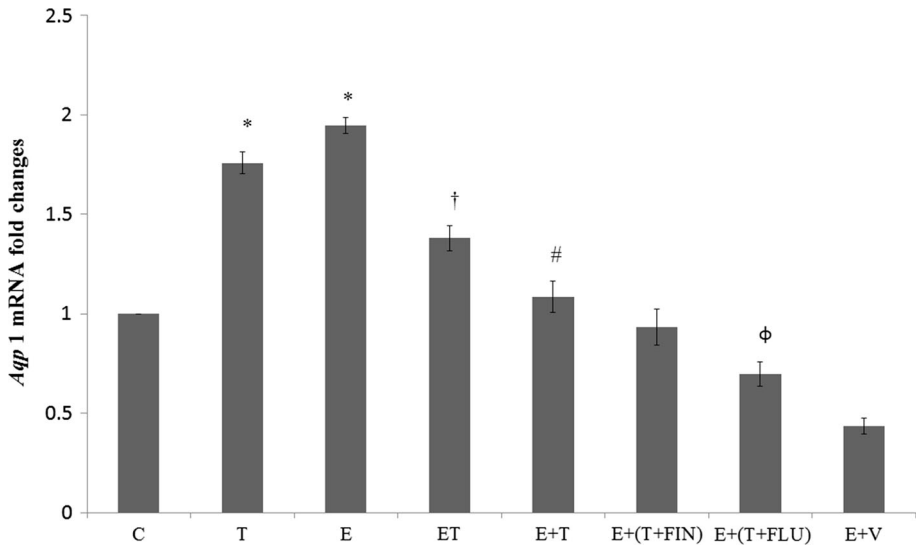

Fig. 4 Levels of expression of *Aqp*-1 mRNA in uterus. The highest mRNA levels were noted in rats receiving estrogen followed by testosterone treatment. In rats receiving E + T treatment, *Aqp*-1 mRNA levels were higher than E + V treatment. $^{*}p < 0.05$ as compared to control, $^{\dagger}p < 0.05$ as compared to E, $^{\#}p < 0.05$ as compared to E + V, $^{\varphi}p < 0.05$ as compared to E + T

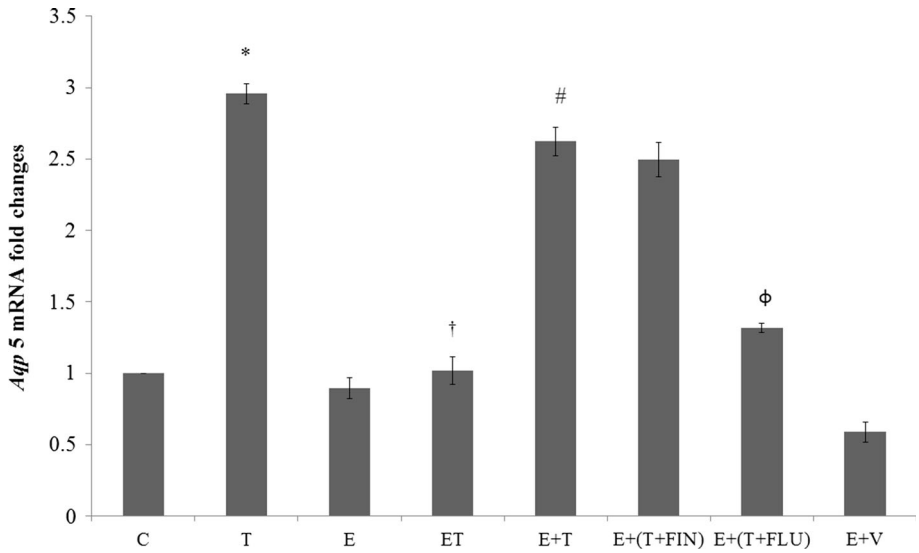

Fig. 5 Levels of expression of *Aqp*-5 mRNA in uterus. The highest expression levels were noted in testosterone-only treated rats. Treatment with estrogen followed by testosterone (E + T) resulted in higher *Aqp*-5 mRNA levels compared to E + V treatment. $^{*}p < 0.05$ as compared to control, $^{\dagger}p < 0.05$ as compared to E, $^{\#}p < 0.05$ as compared to E + V, $^{\varphi}p < 0.05$ as compared to E + T

However, markedly reduced *Aqp-5* mRNA levels were noted in rats receiving E + (T + FLU) compared to E + T treatment.

Aqp-7 mRNA

Figure 6 shows levels of expression of *Aqp-7* mRNA in uterus of rats receiving different treatments. *Aqp-7* mRNA was the highest in rats receiving T-only treatment (nearly fourfold higher compared to control). In rats receiving E-only treatment, levels of *Aqp-7* mRNA were not significantly different from control. Concomitant ET treatment resulted in markedly reduced *Aqp-7* mRNA levels compared to T-only treatment. In rats receiving E + T treatment, levels of *Aqp-7* mRNA were higher compared to E + V treatment ($p < 0.05$). In E + (T + FIN) treated rats, *Aqp-7* mRNA levels were not significantly different compared to E + T treatment. However, in rats receiving E + (T + FLU) treatment, levels of *Aqp-7* mRNA were significantly lower compared to E + T treatment.

Discussion

In this study, we have shown that expressions of AQP-1, 5. and 7 mRNA and proteins in uterus were markedly increased under testosterone influence (compared to control). However, under the influence of estrogen, expression of AQP-1 but not AQP-5 or 7 was markedly elevated in the inner stroma and myometrium. The stromal and myometrial AQPs might indicate distribution of this protein in the blood vessels. Our findings were supported by the previous

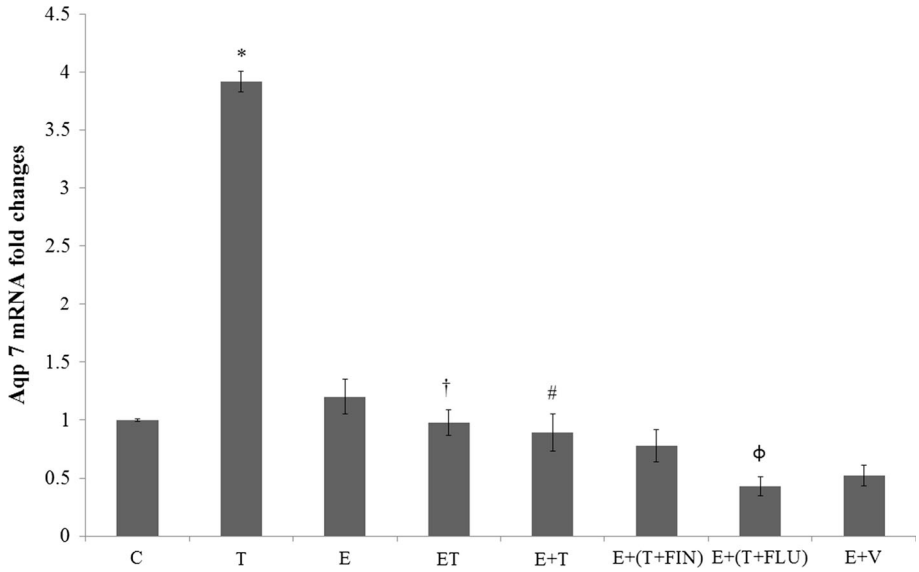

Fig. 6 Levels of expression of *Aqp-7* mRNA in uterus. The highest mRNA levels were noted in testosterone-only treated rats. In E + T treated rats, *Aqp-7* mRNA levels were higher than E + V treated rats. $^{*}p < 0.05$ as compared to C, $^{\pm}p<0.05$ as compared to T, $^{\#}p < 0.05$ as compared to E + V, $^{\varphi}p < 0.05$ *as compared* to E + T

studies in mice which showed that under estrogen influence, AQP-1 was highly expressed in the myometrial (Jablonski et al. 2003) and stromal blood vessels (Richard et al. 2003). Further report indicates that estrogen induced shifting of AQP-1 to the blood vessels in the stroma (Richard et al. 2003). In addition to the stroma and myometrium, we have found that estrogen also causes high expression of AQP-1 in the epithelium lining the uterine lumen. High epithelial AQP-1 expression indicated that this channel might be involved in mediating the H_2O imbibition process into the uterine lumen.

Besides estrogen, AQP-1 expression was also found to be enhanced by other sex hormones. In this study, we have shown that testosterone could also cause increased in AQP1 expression in uterus. Expression of uterine AQP-1 could also be enhanced by progesterone. Both estrogen and progesterone have been shown to influence uterine AQP-1 expression as this isoform expression in pig uterus was reported to increase throughout the oestrous cycle (Skowronski 2010). Meanwhile, studies in rats indicated that progesterone was able to induce redistribution of AQP-1 to the stromal endothelial cells and to the inner circular layer of the myometrium (Lindsay and Murphy 2006). While the progesterone effect was thought to be important in the development of stromal edema at the implantation site (Huang et al. 2006), effect of this hormone was also proposed to participate in the fluid loss from the uterine lumen (Lindsay and Murphy 2006).

In this study, levels of AQP-1 expression were moderately increased under testosterone influence. However, pattern of AQP-1 distribution in uterus was slightly different from that observed under estrogen. Under testosterone influence, AQP-1 was found to be distributed mostly in the epithelia with lesser distribution in stroma and myometrium. The different distribution pattern indicates different roles of AQP-1. For examples, under testosterone influence, AQP-1 might participate in uterine fluid reabsorption resulting in luminal fluid loss; however, under estrogen influence, high stromal distribution suggested that this channel might participate in the fluid imbibition process into the uterine lumen. Similar effects were observed in rats receiving estrogen followed by testosterone treatment. In these rats, levels of AQP-1 were found to be higher compared to rats receiving estrogen followed by vehicle treatment with this protein mostly distributed in lumenal epithelium.

We have further shown that in estrogen-sensitized uteri, testosterone effect on uterine AQP-1 was markedly lesser compared to testosterone effect in non-estrogen-treated uteri. When estrogen and testosterone were concomitantly given, levels of AQP-1 expression were markedly lower compared to estrogen-only treatment. The reasons being were unknown, however, there was a possibility that testosterone could increase redistribution of this channel to the epithelium, while decreases the expression levels of this channel in the stroma and myometrium. Testosterone effect was supported by the observation in post-menopausal uteri. Under this condition, high testosterone level was found to be associated with increased expression of androgen receptor in endometrium (Zang et al. 2008) and vagina (Traish et al. 2007). This could explain the increased uterine responsiveness toward androgen in the state of sex-steroid deficient. There was a possibility that pre-exposure to estrogen could lead to decreased in tissue sensitivity toward testosterone in view that estrogen was reported to down-regulate androgen-receptor expression (Stover et al. 1987).

Our findings indicated that expression of AQP-5 in uterus was enhanced by testosterone and was diminished

following estrogen treatment. Under testosterone influence, AQP-5 was localized to the apical and basolateral membranes of lumenal epithelium and stroma. These findings raised possibility that AQP-5 might participate in fluid reabsorption from the uterine lumen under testosterone influence. Previous studies showed that AQP-5 expression in uterus was enhanced by progesterone (Lindsay and Murphy 2006) and at the time of embryo implantation, when progesterone level was high (Richard et al. 2003). Enhanced expression of AQP-5 has been proposed to mediate progesterone-induced uterine fluid reabsorption (Salleh et al. 2005), where similar mechanism might occur in uterus under testosterone influence.

In this study, we have found that the levels of AQP-7 expression were elevated under testosterone influence, while estrogen had no effect on the expression of this protein channel. Our findings were supported by the report that uterine AQP-7 expression increases after ovulation, at the time when testosterone level is high and this increase might be essential for decidualization (Klein et al. 2013; Peng et al. 2011). The involvement of AQP-7 in testosterone-induced decidualization, therefore, warrants further investigation. Besides testosterone, progesterone was also reported to stimulate increased in AQP-7 expression. In non-pregnant mares, high expression of AQP-7 eight days after ovulation coincides with high level of circulating progesterone (Klein et al. 2013) indicating that this isoform might be involved in progesterone-induced uterine fluid reabsorption. We hypothesized that similar effects might occur under testosterone in which testosterone-induced up-regulation of AQP-7 in uterine lumenal epithelium could mediate the efflux of water from the uterine lumen.

We have shown that testosterone effect on uterine AQP expression involved the androgen-receptor-mediated pathway. However, this effect did not involve active testosterone metabolite, DHT. These findings were consistent with our previous observation which indicates that testosterone but not DHT mediates uterine lumenal fluid loss via downregulating the expression of cystic fibrosis transmembrane regulator (CFTR) protein in uterus in rats (Mohd Mokhtar et al. 2014). In conclusions, up-regulation of AQP-1, 5, and 7 expressions in uterus by testosterone could explain the observed reduction of uterine fluid volume as observed under this condition. These findings are of clinical importance since the presence of high levels of testosterone which occurs in diseases such as polycystic ovaries (PCO) (Nestler et al. 1998) could interfere with uterine fluid volume regulation. Under normal physiological condition, slightly elevated testosterone level following ovulation (Doi et al. 1980) could be involved in regulating the precise uterine fluid volume that is needed for embryo implantation.

Acknowledgments This study was funded by PPP Grant (PG007-2013B), University of Malaya, Kuala Lumpur, Malaysia.

Compliance with Ethical Standards

Conflict of interest No conflict of interest is reported.

References

Chinigarzadeh A, Kasim NF, Muniandy S, Kassim NM, Salleh N (2014) Genistein Induces increase in fluid pH, Na^+ and $HCO3^-$ concentration, SLC26A6 and SLC4A4 (NBCe1)-B expression in the uteri of ovariectomized rats. Int J Mol Sci 15:958–976

Denker BM, Smith BL, Kuhajda FP, Agre P (1988) Identification, purification, and partial characterization of a novel Mr 28,000 integral membrane protein from erythrocytes and renal tubules. J Biol Chem 263:15634–15642

Doi O, Takai T, Nakamura T, Tanabe Y (1980) Changes in the pituitary and plasma LH, plasma and follicular progesterone and estradiol, and plasma testosterone and estrone concentrations during the ovulatory cycle of the quail (Coturnix coturnix japonica). Gen Comp Endocrinol 41:156–163

He RH, Sheng JZ, Luo Q, Jin F, Wang B, Qian YL, Zhou CY, Sheng X, Huang HF (2006) Aquaporin-2 expression in human endometrium correlates with serum ovarian steroid hormones. Life Sci 79:423–429

Huang HF, He RH, Sun CC, Zhang Y, Meng QX, Ma YY (2006) Function of aquaporins in female and male reproductive systems. Hum Reprod Update 12:785–795

Jablonski EM, McConnell NA, Hughes FM, Huet-Hudson YM (2003) Estrogen regulation of aquaporins in the mouse uterus: potential roles in uterine water movement. Biol Reprod 69:1481–1487

Klein C, Troedsson MH, Rutllant J (2013) Expression of aquaporin water channels in equine endometrium is differentially regulated during the oestrous cycle and early pregnancy. Reprod Domest Anim 48:529–537

Leutenegger CM, Higgins J, Matthews TB, Tarantal AF, Luciw PA, Pedersen NC, North TW (2001) Real-time TaqMan PCR as a specific and more sensitive alternative to the branched-chain DNA assay for quantitation of simian immunodeficiency virus RNA. AIDS Res Hum Retroviruses 17:243–251

Li X, Yu H, Koide S (1997) Regulation of water channel gene (AQP-CHIP) expression by estradiol and anordiol in rat uterus. Yao Xue Xue Bao 32:586–592

Lin P, Lan X, Chen F, Yang Y, Jin Y, Wang A (2013) Reference gene selection for real-time quantitative PCR analysis of the mouse uterus in the peri-implantation period. PLoS One 8:e62462

Lindsay LA, Murphy CR (2004) Aquaporin-1 increases in the rat myometrium during early pregnancy. J Mol Histol 35:75–79

Lindsay LA, Murphy CR (2006) Redistribution of aquaporins 1 and 5 in the rat uterus is dependent on progesterone: a study with light and electron microscopy. Reproduction 131:369–378

Mohd Mokhtar H, Giribabu N, Kassim N, Muniandy S, Salleh N (2014) Testosterone decreases fluid and chloride secretions in the uterus of adult female rats via down-regulating cystic fibrosis transmembrane regulator (CFTR) expression and functional activity. J Steroid Biochem Mol Biol 144:361–372

Nestler JE, Jakubowicz DJ, Falcon de Vargas A, Brik C, Quintero N, Medina F (1998) Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system 1. J Clin Endocrinol Metab 83:2001–2005

Peng H, Zhang Y, LeiL Chen Q, Yue J, Tan Y, Duan E (2011) Aquaporin 7 expression in postimplantation mouse uteri: a potential role for glycerol transport in uterine decidualization. Fertil Steril 95:1514–1517

Richard C, Gao J, Brown N, Reese J (2003) Aquaporin water channel genes are differentially expressed and regulated by ovarian steroids during the periimplantation period in the mouse. Endocrinology 144:1533–1541

Sales AD, Lobo CH, Carvalho AA, Moura AA, Rodrigues AP (2013) Structure, function, and localization of aquaporins: their possible implications on gamete cryopreservation. Genet Mol Res 12:6718–6732

Salleh N, Baines DL, Naftalin RJ, Milligan SR (2005) The hormonal control of uterine lumenal fluid secretion and absorption. J Membr Biol 206:17–28

Skowronski MT (2010) Distribution and quantitative changes in amounts of aquaporin 1, 5 and 9 in the pig uterus during the estrous cycle and early pregnancy. Reprod Biol Endocrinol 8:109

Skowronski MT, Kwon TH, Nielsen S (2009) Immunolocalization of aquaporin 1, 5, and 9 in the female pig reproductive system. J Histochem Cytochem 57:61–67

Stover EP, Krishnan AV, Feldman D (1987) Estrogen down-regulation of androgen receptors in cultured human mammary cancer cells (MCF-7). Endocrinology 120:2597–2603

Tandon R, Cattori V, Gomes-Keller MA, Meli ML, Golder MC, Lutz H, Hofmann-Lehmann R (2005) Quantitation of feline leukaemia virus viral and proviral loads by TaqMan real-time polymerase chain reaction. J Virol Methods 130:124–132

Traish AM, Kim SW, Stankovic M, Goldstein I, Kim NN (2007) Testosterone increases blood flow and expression of androgen and estrogen receptors in the rat vagina. J Sex Med 4:609–619

Tsai YL, Wang HTT, Chang HFG, Tsai CF, Lin CK, Teng PH, Su C, Jeng CC, Lee PY (2012) Development of TaqMan probe-based insulated isothermal PCR (iiPCR) for sensitive and specific on-site pathogen detection. PLoS One 7:e45278

Wilson JL, Miranda CA, Knepper MA (2013) Vasopressin and the regulation of aquaporin-2. Clin Exp Nephrol 17:751–764

Zang H, Sahlin L, Masironi B, Hirschberg AL (2008) Effects of testosterone and estrogen treatment on the distribution of sex hormone receptors in the endometrium of postmenopausal women. Menopause 15:233–239

Zhang D, Tan YJ, Qu F, Sheng JZ, Huang HF (2012) Functions of water channels in male and female reproductive systems. Mol Aspects Med 33:676–690