The Water Permeability Channels Aquaporins 1–4 Are Differentially Expressed in Granulosa and Theca Cells of the Preovulatory Follicle during Precise Stages of Human Ovulation

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Context: Changes in vascular permeability and expansion of the fluid-filled antrum are major events in the LH-induced ovulatory process.

Objectives: Our objective was to investigate the presence and expression levels of aquaporins (AQPs) in the granulosa and theca cell compartments of the follicle during defined phases of human ovulation.

Design and Setting: We conducted a prospective experimental study at the Department of Obstetrics and Gynaecology at a university hospital.

Participants: Twenty-eight women underwent laparoscopic sterilization and at the same time follicle retrieval at four periovulatory phases.

Main Outcome Measures: mRNA levels of AQP1-4 were measured in separated granulosa and theca cells from preovulatory phase, early ovulatory (EO) phase, late ovulatory phase, and postovulatory phase. Immunohistochemistry was done for AQP1-4 in intact human follicles.

Results: All four AQPs were expressed in both the theca and granulosa cells during ovulation. In granulosa cells, AQP1 levels increased in the late ovulatory and postovulatory phases. Expression of AQP2-3 followed a similar pattern with a marked increase in the EO phase, whereas AQP4 levels decreased from preovulatory to the EO phase. The presence of AQP1-4 in the human follicle was verified by immunohistochemistry.

Conclusions: The results show for the first time the presence of AQP1-4 in human follicles during ovulation. The marked early rise in expression of AQP2 and AQP3 suggests a role during the process leading to follicular rupture, and the late rise of AQP1 suggests a role in corpus luteum formation. **(*J Clin Endocrinol Metab* 96: 1021–1028, 2011)**

Human ovulation is a highly synchronized process that extends over 36 h (1, 2) from the rise of the LH surge until follicular rupture with oocyte release. The LH surge induces biochemical pathways that lead to degradation of the extracellular matrix, changes in cellular morphology, and alterations of the follicular vasculature. During the preceding folliculogenesis, there is tremendous follicular growth with an estimated 19 doublings of the surface area of the follicle (3), and this growth is particularly great during later stages of the follicular phase and ovulation.

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Abbreviations: AQP, Aquaporin; Ct, cycle threshold; EO, early ovulatory; LO, late ovulatory; PO, preovulatory; PSO, postovulatory; rhCG, recombinant human chorionic gonadotropin.

The preovulatory (PO) follicle increases about 2–3 mm in diameter from the initiation of the LH surge until rupture (4). This accelerated growth is mostly due to expansion of the fluid-filled antrum (5), secondary to a massive fluid transport from the vasculature into the antrum (4, 6). The exact mechanisms for fluid transport from the vasculature of the theca layer into the avascular granulosa cell layer and antrum at ovulation are unclear. It is likely that not only passive diffusion from the capillaries of the theca layer into the antrum is responsible for the rapidly increased amount of fluid in the antrum at ovulation (7).

Several studies have shown that major alterations in ovarian blood vessels occur during the interval between the onset of the LH surge and follicular rupture. These changes include vasodilatation (8) and increased blood flow (9–11) as well as increased vascular permeability (12) of the microvasculature of the theca layer. The vascular changes during ovulation may be important in the mechanisms behind the constant (13) or possibly increased intrafollicular pressure (14) that is causing the rupture of the progressively weakened follicular wall.

The aquaporins (AQPs) are members of a family of small (25–34 kDa), hydrophobic, integral membrane channel proteins that facilitate rapid movement of water over cell membranes. The rate that water passes through cell membranes through the AQPs is around 100 times higher than the passage through diffusion (15). Thirteen AQPs (AQP0-12) have been identified in mammals (16). The so-called orthodox AQPs, including AQP0, -1, -2, and -4 to -6 are primarily water selective, whereas the aquaglyceroporins (AQP3, -7, -9, and -10) also transport glycerol, urea, and other small nonelectrolytes (15). Nine of the AQPs (AQP1–9) have been demonstrated in various compartments of the uterus-oviduct of the rat (17, 18), mouse (19, 20), pig (21), and human (22–24). The limited results of AQPs in the ovary suggested a role for AQPs in antrum expansion in the rat (25) and early folliculogenesis in the pig (21).

There are no studies concerning a possible role of AQPs in the cyclic changes of the human ovary. In the present study, we have explored whether some specific AQPs are expressed in the human ovary during ovulation and whether changes in the expression levels during different phases would indicate a role in human ovulation.

Patients and Methods

Patients and tissue sampling

The study, carried out at the Division of Gynaecology and Reproductive Medicine, was approved by the Human Ethics Committee of the Sahlgrenska Academy, University of Gothenburg, and all participants had given their informed written consent before participating. Twenty-eight healthy women (age 30–38 yr), with previously proven fertility (at least para 1) and regular menstrual cycles (cycle length 26–35 d) and who planned to undergo laparoscopic sterilization were included in the study. The participants had not been on any hormonal contraceptive during at least 3 months before the operation.

All women were monitored by repeated transvaginal ultrasound during one to three menstrual cycles before surgery to ascertain cycle regularity and normal increase in size of the dominant follicle during the follicular phase. During the actual cycle of the laparoscopy, transvaginal ultrasound was performed every 1–2 d from cycle d 9 until surgery to ascertain that the dominant follicle followed the normal increase in follicle diameter of about 2 mm/d (2). By these examinations, the surgery could be scheduled at a cycle stage when the dominant follicle had a diameter of more than14 mm, which is considered as a size of full LH responsiveness (1).

The surgery was planned to enable retrieval of follicles at four specific periovulatory intervals (Table 1) to cover the period from before the LH surge until after follicular rupture. The PO phase was defined as a stage with a size of the dominant follicle at surgery of more than 14 mm and no more than 17.5 mm and before the spontaneous LH surge. The majority of the women (n = 21) received 250 μg recombinant human chorionic gonadotropin (rhCG) (Ovitrelle; Merck Serono S.A., Geneva, Switzerland) sc for ovulation induction when the dominant follicle had a diameter of 14–20 mm. They were then operated at specific time intervals after rhCG injection to further differentiate the ovulatory stages. The early ovulatory (EO) phase was defined as a phase when surgery was performed 12 h to no more than 18 h after rhCG, late ovulatory (LO) phase when surgery was performed at least 18 h to no more than 24 h after rhCG, and postovulatory (PSO) phase when surgery was performed 44–77 h after rhCG injection. To confirm that the follicle to be used for expression analysis (see below) followed a normal hormonal

TABLE 1. Characteristics of the study population (n = 20) used for real-time qPCR

	PO phase	EO phase	LO phase	PSO phase
Age (yr)	35.4 ± 1.2 (31–38)	33.8 ± 1.4 (30–38)	37.4 ± 0.4 (36–38)	34.2 ± 0.5 (33–36)
Cycle length (d)	29.6 ± 0.6 (28–35)	27.2 ± 0.5 (26–28)	29.4 ± 0.9 (28–32)	28.4 ± 0.5 (27–30)
Cycle days of surgery	14 ± 0.3 (13–15)	12.6 ± 0.5 (11–14)	13 ± 0.6 (11–15)	13.1 ± 0.6 (11–15)
Time of surgery after rhCG (h)		14.0 ± 0.9 (12–18)	21.9 ± 0.8 (19–24)	49.1 ± 0.6 (44–70)
Follicle size (mm)	15.9 ± 0.7 (14–17)	17.1 ± 0.8 (15–19)	17.2 ± 0.8 (15–20)	16.7 ± 0.5 (15–18)
Progesterone (nmol/liter)	0.4 ± 0.04 (0.3–0.5)	1.2 ± 0.22 (0.5–1.9)	2.1 ± 0.3 (1.5–3.0)	3.1 ± 1.5 (1.3–9.5)
Estradiol (nmol/liter)	0.5 ± 0.1 (0.2–1.0)	0.6 ± 0.1 (0.4–0.9)	0.7 ± 0.1 (0.6–0.9)	0.3 ± 0.1 (0.21–0.6)

The clinical and laparotory parameters at surgery are shown with all data given as means ± SEM and ranges in *parentheses*; n = 5 samples in each group.

pattern, blood samples were taken at surgery for measurements of serum progesterone and estradiol (Table 1).

During surgery, the follicle was isolated by use of laparoscopic scissors that cut through the stroma of the follicular periphery. Diathermy was not used at this stage to avoid thermal injury. The whole intact follicle was removed inside a laparoscopic sac (ENDO CATCH; Covidien, Boston, MA) via a small suprapubic incision and then placed on ice and brought to the laboratory for dissection (see below). After follicle retrieval, sterilization was conducted.

The majority of follicles (n = 20) were used to obtain separated granulosa and theca cells for expression analysis. To isolate the granulosa cells, the follicle was opened with scissors to release any loosely attached granulosa cells. The mural granulosa cells were then gently scraped off from the interior of the follicle by a small tissue forceps. The follicular fluid and cell suspension were combined and was centrifuged at 500 × *g* to pellet the granulosa cells. After removal of the granulosa cells, the theca interna was mechanically separated from the underlying theca externa cell layer by pulling with watchmakers forceps. The granulosa cells and theca layer were frozen at −70 C within 30 min after follicle retrieval and stored until further analysis (see below). Some follicles (n = 8) were used for immunohistochemistry (see below).

Real-time quantitative PCR of mRNA for AQP1-4

Total RNA was extracted from separated granulosa and theca cells using Trizol (Invitrogen, Carlsbad, CA) and the RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction but with the modification that the samples precipitated on ice instead of at room temperature. The RNeasy kit was used as a clean-up step after extraction with Trizol. cDNA was synthesized from 1 μg total RNA using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Applera Corp., Foster City, CA). Real-Time RT-PCR was performed with the ABI Prism 7000 Sequence Detector (Applied Biosystems). TaqMan MGB probes targeting AQP1 (Hs00166067_m1), AQP2 (Hs00166640_m1), AQP3 (Hs 00185020_m1), AQP4 (Hs00242342_ml), and RPLPO (large ribosomal protein) were purchased as TaqMan gene expression assays (Applied Biosystems). Each amplification reaction consisted of 20 ng cDNA, 1× probe mix, and 1× TaqMan Universal PCR mix (Applied Biosystems) to a final volume of 25 μl. The PCR parameters were according to the manufacturer's protocol. After performing a validation experiment to demonstrate that efficiencies of target and endogenous control (large ribosomal protein) were approximately equal, relative expression levels were presented with the comparative cycle threshold (ΔΔCt) method (26). The expression of target gene was normalized to the endogenous control by subtracting the Ct value of the target gene with the Ct value of the endogenous control. To compare levels relative to a calibrator (mean ΔCt for the PO group), ΔCt of PO were subtracted from the ΔCt of each samples. Relative expression is given by $2^{-\Delta\Delta Ct}$. All reactions were performed in duplicate for both target gene and endogenous control, and means of these values were used as a single observation points in the statistical analysis and presentation of data.

Immunohistochemistry

Follicles from eight individuals (PO, n = 1; EO, n = 4; LO, n = 3) were used for immunohistochemistry. The characteristics of this study population were comparable to their respective groups according to Table 1. After fixation in 4% formaldehyde, the follicle was embedded in paraffin. Sections (approximately 4 μm thick) were placed on glass slides, deparaffinized in xylene, rinsed in ethanol, and brought to water through a series of decreasing concentrations of ethanol. Antigens were retrieved by boiling with an antigen unmasking solution (Vector Laboratories, Burlingame, CA) for 20 min at 120 C and then cooled in water for 5 min. Nonspecific binding was blocked with background sniper (Biocare Medical, Concord, CA) for 10 min at room temperature. The sections were incubated in a humidified chamber at 4 C overnight, with primary antibodies against AQP1 (ab9566, mouse monoclonal, dilution 1:50; Abcam, Cambridge, UK), AQP2 (ab15081, rabbit polyclonal, dilution 1:200; Abcam), AQP3 (SC-20811, rabbit polyclonal, dilution 1:200; Santa Cruz Biotechnology, Santa Cruz, CA), and AQP4 (ab11026, mouse monoclonal, dilution 1:100; Abcam). The MACH 3 mouse/rabbit alkaline phosphatase and mouse horseradish-peroxidase polymer kits (Biocare Medical) were used as detection systems, and the immunostaining was visualized using Vulcan Fast Red (AQP1-3)/diaminobenzidine (AQP4) Chromagen kits (Biocare Medical) according to the manufacturer's protocol. Sections were counterstained with hematoxylin, dehydrated through a series of increasing ethanol concentrations of ethanol to xylene, and then coverslipped. Negative control slides were prepared in an identical manner and processed with a ready-to-use mouse or rabbit IgG negative control (Biocare Medical) instead of primary antibody. Slides were viewed on a Nikon EFD-3 (Nikon, Tokyo, Japan) microscope under bright-field optics by two independent viewers and photographed with a Digital Sight DS-U1 camera (Nikon).

Statistics

The nonparametric Kruskal-Wallis test was used to test whether significant differences were present between the four groups. Subsequent analysis was performed with Mann-Whitney test. A P value <0.05 was considered significant.

Results

Aquaporin 1

The relative levels of AQP1 mRNA during the PO phase were 45-fold higher in the theca cells compared with the granulosa cells. No significant changes could be seen in the AQP1 mRNA levels in theca cells during ovulation, but in granulosa cells, a significant increase was seen during the PSO phase in comparison with PO and EO phases (Fig. 1). Immunohistochemistry demonstrated abundant AQP1 protein in the large rounded cells of the theca layer, representing the theca interna cells (Fig. 2A). In the granulosa layer, the staining intensity appeared more pronounced in cells toward the luminal surface compared with those of the basal layers. The blood vessels of the theca layer and the theca externa exhibited only minor staining. The stroma surrounding the follicle did not exhibit any staining. No staining was observed in the negative control (Fig. 2B).

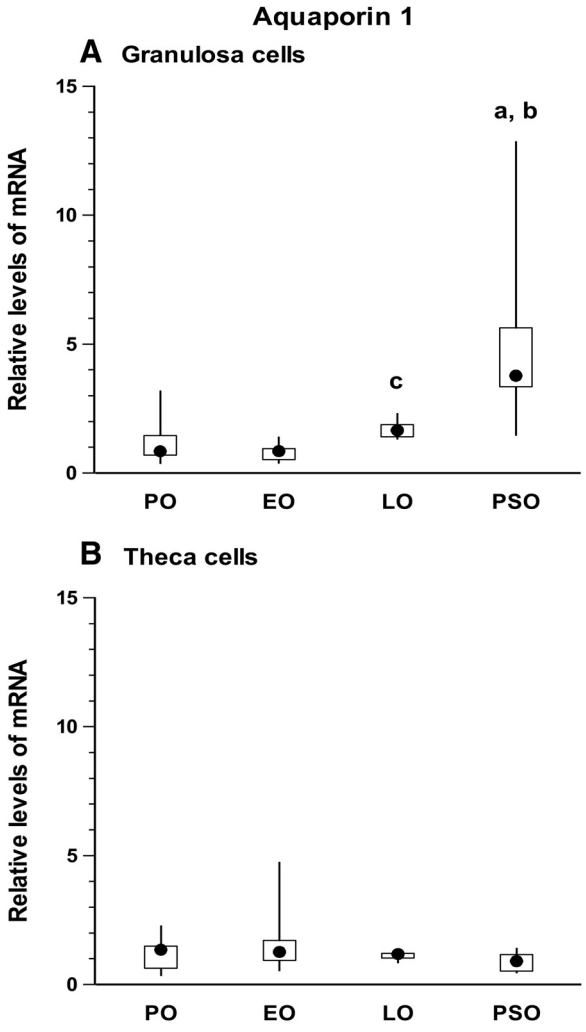

FIG. 1. Expression levels of AQP1 in human granulosa cells (A) and theca cells (B) during different periovulatory phases. The mRNA levels in the PO, EO, LO, and PSO phases were calculated by the ΔΔCt method. The values shown are the levels of mRNA for each phase relative to the mRNA level for the PO phase; n = 5 samples in each ovulatory phase. Medians are indicated by *dots*, 25–75th percentiles by *boxes*, and 10–90th percentiles by *vertical lines*. a, Significantly ($P < 0.05$) higher than PO; b and c, significantly ($P < 0.01$ and $P < 0.05$ respectively) higher than EO.

Aquaporin 2

The mRNA levels of AQP2 of the PO phase were similar in granulosa and theca cells. mRNA levels in theca cells showed a modest but significant increase during the PSO phase compared with the PO phase (Fig. 3), with 6-fold higher median levels. In granulosa cells, a marked increase in AQP2 mRNA levels was seen already during the EO phase (median levels 35-fold higher in EO compared with PO phase). The mRNA levels stayed elevated in the granulosa cells throughout the ovulation period with 8- and 19-fold increases in median levels of LO and PSO phases, respectively, compared with the PO phase. In immunohistochemical analysis of the granulosa layer, increased staining was seen during later ovulatory phases with the staining evenly distributed among the cells (Fig. 2C). The staining of the theca layer was most evident in the large rounded cells of the theca interna area. Negative control sections showed only staining of blood cells within large blood vessels (Fig. 2D).

Aquaporin 3

AQP3 mRNA expression levels were 5-fold higher in theca cells compared with the granulosa cells in the PO phase. The expression patterns of AQP3 followed a similar time course in theca and granulosa cells. The mRNA levels were low in both cell types in the PO phase, and a large increase was seen already during the EO phase (Fig. 4), with 43- and 16-fold increase in granulosa and theca cells, respectively, when compared with the PO phase. The mRNA levels remained elevated during the LO and PSO phases. A strong immunostaining was seen in both granulosa and theca cell compartments (Fig. 2E). In the theca cell layer, the staining was concentrated in the larger theca cells. A patchy staining was seen in the stroma.

Aquaporin 4

The AQP4 mRNA expression levels were generally low and about 3-fold higher in theca cells than in granulosa cells of the PO phase. A temporary decrease in mRNA levels was seen in EO phase in granulosa cells (Fig. 5). A similar pattern was seen in the theca cells, although the decrease was not significant. The general staining intensity of AQP4 protein was low, but the staining was stronger in the granulosa cell layer compared with the theca cell layer (Fig. 2F).

Discussion

The largest volume of the human PO follicle, which has a size of about 20 mm in diameter, is taken up by the fluid-filled antrum, which further expands during ovulation (4) that extends over 1.5 d. Furthermore, there is a leakage of follicular fluid from the progressively degraded follicular apex for several hours before rupture (27). The great increase in follicular antrum volume and the fact that the ovulatory follicle is expanded, despite fluid leakage through the apex, suggest that there is a rapid and massive transport of water from the microvasculature of the follicle into the antral cavity. Any movement of water could be provided by either slow passive diffusion/pericellular transport or rapid active transport through the AQPs (15).

The major finding of the present study is that AQP1-4 show high expression in the ovulating human follicle and that prominent alterations in expression patterns occur during ovulation. The expression pattern of AQP1 exhibited an increase after follicular rupture, suggesting that it may be important in processes associated with the tran-

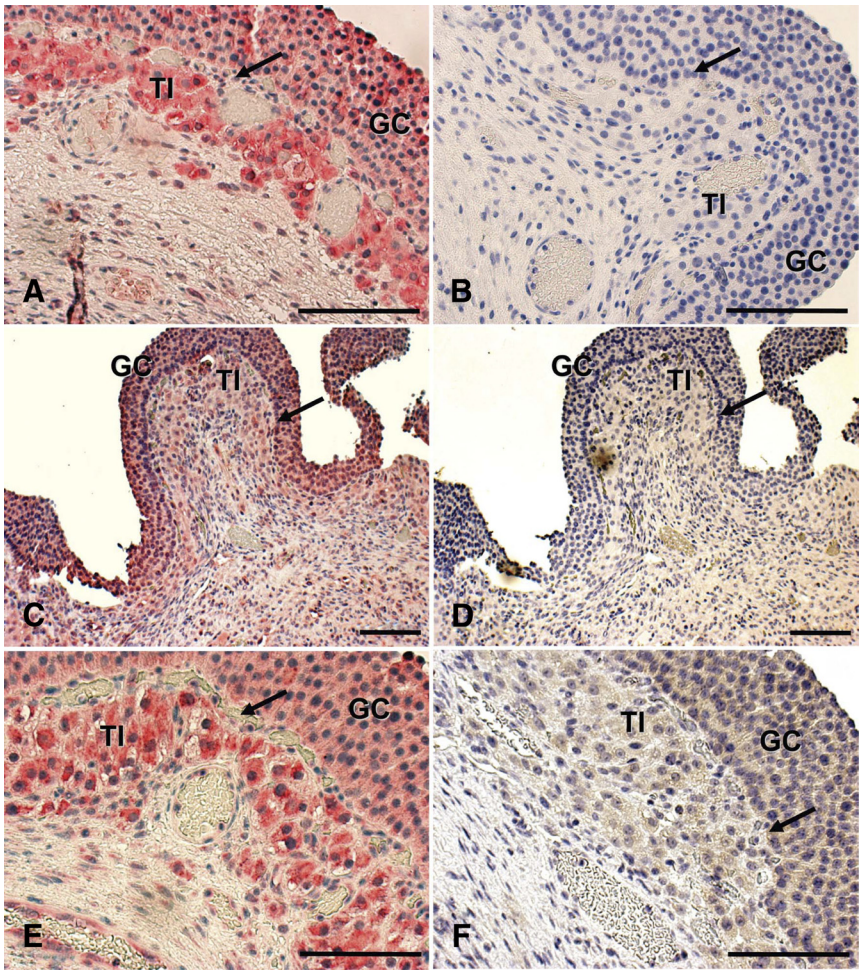

FIG. 2. Immunohistochemical localization of AQP1-4 in ovarian tissue. Sections of whole follicles are shown from EO phase with staining of AQP1 (A), AQP2 (C), AQP3 (E), and AQP4 (F) in the theca interna (TI) and the granulosa cells (GC). Basal lamina (BL) is indicated by an *arrow*. No staining was observed in the absence of the primary antibody. Negative controls are shown only for AQP1 (B) and AQP2 (D). *Scale bar*, 100 μm.

sition of the follicle into a corpus luteum. The time-related changes of AQP2 and AQP3, with distinct elevations from the PO to the EO stage, suggest that these AQPs are operative in the events leading to follicular rupture. All the AQPs studied were localized in both the granulosa and theca cells by immunohistochemistry. Interestingly, the mRNA expression levels during the PO phase were generally higher in theca cells than in the granulosa cells, which was also apparent by immunohistochemistry.

A major strength of the present study is that it was performed on human ovarian tissue material obtained from well-defined stages of ovulation. Previous studies by us (28) and others (29, 30) of changes in the human follicle during ovulation have mostly relied on tissue obtained at planned surgery with dating into a specific stage based on menstrual cycle data and hormonal levels. Most studies on the molecular mediators of ovulation have been performed in the immature equine CG/hCG-primed rat or mouse models (31), with ovarian tissue obtained at several well-defined time points during the ovulatory interval. The present study was designed to acquire tissue from similarly well-defined phases of ovulation. To ascertain high quality of the patient material, only women with proven fertility, regular menstrual cycles, and without any hormonal medications were included. The women were monitored with repeated ultrasound to enable planning of surgery at a stage when the dominant follicle could be considered to be PO. Because detection of the PO rise in LH levels during the endogenous gonadotropin surge requires repeated measurements of LH levels in blood, we chose to give exogenous hCG to mimic the LH surge to enable retrieval of follicles at defined stages. The use of hCG as a substitute for the endogenous LH surge is a well-established method to induce ovulation in animal models (32, 33) and in human *in vitro* fertilization.

We were able to obtain follicles from only two phases during the interval between hCG and follicular rupture (EO and LO) because of practical problems in scheduling surgery more frequently in relation to hCG administration. Some studies on ovarian expression of ovulatory mediators in rodents have been able to obtain ovarian tissue more frequently during the ovulatory interval of only 12–15 h (32). However, these studies have analyzed the expression levels in the entire ovaries, with the disadvantage of a heterogeneous cell population where major changes in expression levels in one cell type may be masked by an inverse expression profile in another ovarian cell type. The great size of the human ovulatory follicle enabled us to separate granulosa and theca cells to examine the expression levels in these separate compartments.

The present study represents the first one on AQPs in the normal human ovary of the menstrual cycle, although there are reports of presence of AQP9 in *in vitro* fertilization granulosa cells of women with polycystic ovary syndrome (34) and other AQPs in ovaries of the mouse (35), rat (25), and pig (21). AQP1, a 28-kDa protein, was initially isolated from red blood cells (36) and later found to be a potent water channel protein. Since then, it has been shown to be present in an increasing number of tissues, with especially high expression in kid-

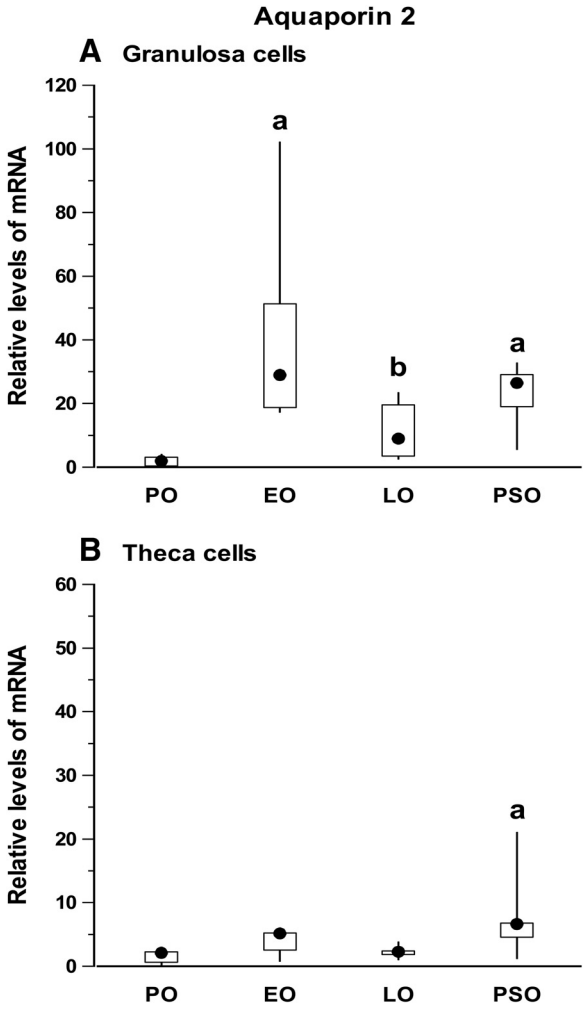

FIG. 3. Expression levels of AQP2 in human granulosa cells (A) and theca cells (B) during different periovulatory phases. The mRNA levels in the PO, EO, LO, and PSO phases were calculated by the ΔΔCt method. The values shown are the levels of mRNA for each phase relative to the mRNA level for the PO phase; n = 5 samples in each ovulatory phase. Medians are indicated by *dots*, 25–75th percentiles by *boxes*, and 10–90th percentiles by *vertical lines*. a, Significantly ($P <$ 0.01) higher than PO; b and c, significantly ($P < 0.05$) higher than PO.

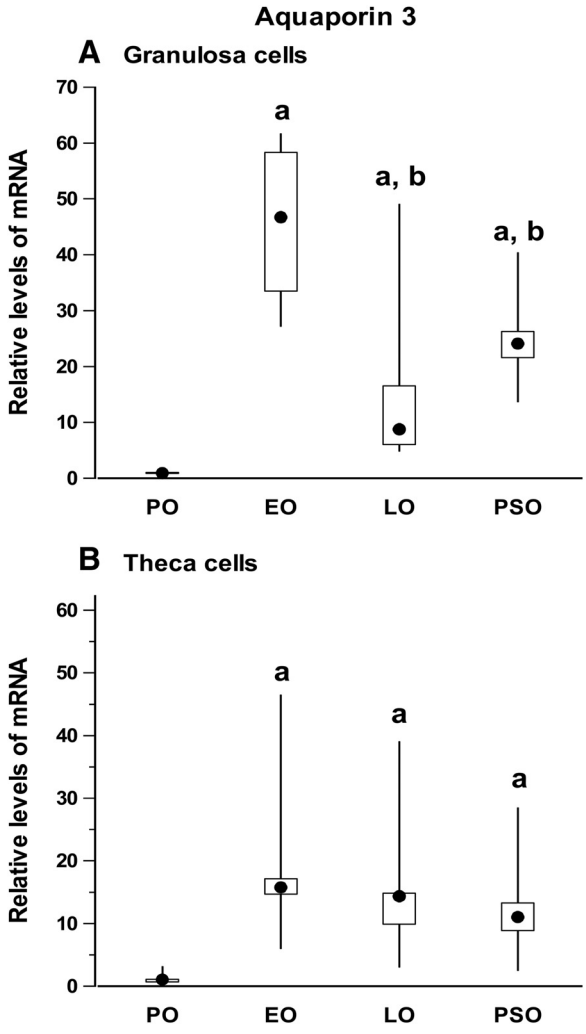

FIG. 4. Expression levels of AQP3 in human granulosa cells (A) and theca cells (B) during different periovulatory phases. The mRNA levels in the PO, EO, LO, and PSO phases were calculated by the ΔΔCt method. The values shown are the levels of mRNA for each phase relative to the mRNA level for the PO phase; n = 5 samples in each ovulatory phase. Medians are indicated by *dots*, 25–75th percentiles by *boxes*, and 10–90th percentiles by *vertical lines*. a, Significantly ($P <$ 0.01) higher than PO; b, significantly ($P < 0.05$) higher than EO.

neys. A previous study on pig ovaries demonstrated AQP1 immunoreactivity in the endothelium of capillaries surrounding the follicle (21), and a study of granulosa cells obtained from PO rat follicles detected low or absent expression of AQP1 (25). In the present study, mRNA levels in granulosa cells were slightly elevated in the LO phase and increased more after rupture in the PSO phase. The finding of low levels before and during ovulation corresponds to the finding of low expression in rat PO granulosa cells (25). In that study, the AQP1 expression was evaluated only at the PO stage, and the presence of PSO rise was not assessed. The presence and regulation of AQP1 in the female reproductive tract have been well studied concerning the uterus, where AQP1 has been found in rats (37), mice (19, 20), and humans (24, 38). Expression of AQP1 in the uterus of both rats and humans is up-regulated by steroid hormones (37, 38). In the present study, the low APQ1 expression in granulosa cells during the PO phase, followed by a small increase in LO phase and then a sharp rise in the PSO phase, may be regulated by progesterone, which rises during the later stages of the ovulatory process and especially after ovulation. This pattern of expression was seen only in AQP1 and may indicate a role in corpus luteum formation.

The mRNA levels for AQP2 and AQP3 in granulosa cells showed expression patterns that mimicked each other with a sharp rise in the EO phase and then extended elevation in later periovulatory phases. This up-regulation in the EO phase was especially high for AQP3 with a 43-fold increase compared with PO levels. The same pattern was seen for AQP3 in the theca cells, although the changes

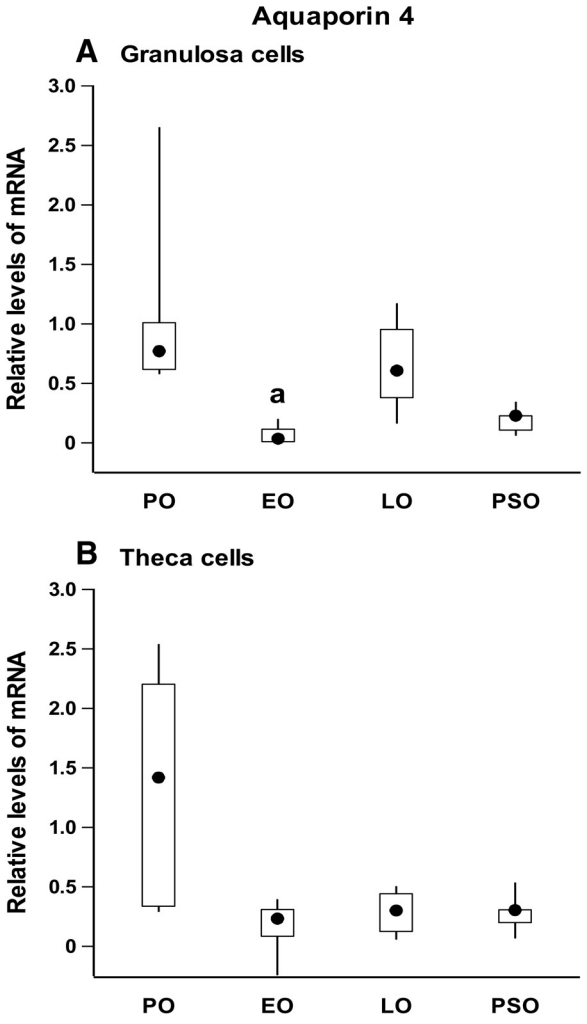

FIG. 5. Expression levels of AQP4 in human granulosa cells (A) and theca cells (B) during different periovulatory phases. The mRNA levels in the PO, EO, LO, and PSO phases were calculated by the ΔΔCt method. The values shown are the levels of mRNA for each phase relative to the mRNA level for the PO phase; n = 5 samples in each ovulatory phase. Medians are indicated by *dots*, 25–75th percentiles by *boxes*, and 10–90th percentiles by *vertical lines*. a, Significantly ($P < 0.05$) lower than PO.

were not as large. The early and marked rise in the expression of AQP2 and -3 during ovulation suggest a role for these water channels in the mechanisms leading to follicular rupture. These AQPs were abundant both in the granulosa and theca cell layer, as shown also by immunohistochemistry. It is quite possible that these AQPs are important for expansion of the follicular antrum before ovulation. The permeability of the follicular wall, determined by the blood follicular barrier, increases dramatically after the LH surge. There are reasons to believe that AQPs in granulosa cells could be important in this process. Thus, in large rat follicles, it was found that 70% of permeability is transcellular and not pericellular (25). Furthermore, it was demonstrated that water permeability of the antral follicles was markedly decreased by the addition of the AQP inhibitor $HgCl_2$, indicating the importance of AQPs in mediating water movement into the antral compartment.

Studies have shown that AQP2 expression levels in the human endometrium correlates with both estrogen and progesterone levels with highest expression levels in the midluteal phase (38, 39). The expression patterns of AQP2 and -3 in the present study, with increased mRNA levels after LH/hCG, suggest that the expression of these AQPs is regulated by progesterone, which is a main positive regulator of several ovulatory mediators (40).

Few studies exist on AQP4 in the reproductive system. The present study is the first that provides evidence of AQP4 expression in human ovaries, although both the mRNA and protein expression was low. A recent study on AQP4 knockout mice found that AQP4-deficient mice exhibited subfertility, evidenced by lower pregnancy rates (35). The AQP4 knockout mice showed fewer antral follicles and corpora lutea, indicating a dysfunction of ovulation. These data and our results, with a temporary decrease in AQP4 expression in EO phase in granulosa cells, indicate that AQP4 protein may be important in antrum formation during follicular development as recently suggested for the AQPs (7). However, the mechanisms involved in the regulation of AQP4 remain unclear.

In conclusion, there are reasons to believe that selective transport of water across cell membranes is crucial for ovulation to occur. By using a unique, well-defined material, we demonstrate for the first time expression of AQP1-4 in isolated human granulosa and theca cells. Different expression profiles of the AQPs in the diverse time phases of ovulation suggest a highly selective regulation of expression and indicate roles in ovulation. It could be speculated that high expression of AQPs in the theca interna would be of importance to transport fluid into the interstitium underlying the basal lamina and that water would pass through this membrane passively to be transported by granulosa cell AQPs into the antrum.

Acknowledgments

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