Immunolocalization of aquaporin 5 during rat ovarian follicle development and expansion of the prevulatory cumulus oophorus

Agnieszka Starowicz a, Malgorzata Grzesiak a,*, Ali Mobasheri b, c, Maria Szoltys d

a Department of Endocrinology, Institute of Zoology, Jagiellonian University, Cronostojowa 9, 30–387 Krakow, Poland
b School of Veterinary Medicine and Science, Faculty of Medicine and Health Sciences, University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 9RD, United Kingdom
c King Abdulaziz University, Jeddah, Saudi Arabia

ARTICLE INFO

Article history:
Received 8 August 2013
Received in revised form
19 September 2013
Accepted 30 September 2013

Keywords:
Aquaporin 5
Follicle
Cumulus-oocyte complex
Ovary
Rat

ABSTRACT

Immunofluorescent localization of aquaporin 5 (AQP5) was investigated in rat ovarian follicles during development and prevulatory cumulus oophorus expansion. Ampullary cumuli oophori complexes (COCs) were examined. Analysis revealed that AQP5 immunostaining appeared in preantral follicles and formed a characteristic ring encircling and touching the oolemma. The staining represented most likely AQP5 functioning at the ends of corona radiata cell projections, anchoring on the oocyte surface. However, several hours after the presumptive preovulatory LH surge, when the process of expansion of COCs started, the AQP5 staining appeared also on the cumulus granulosa cells and in the extracellular matrix.

In the postovulatory ampullary COCs the fluorescent ring was not observed, which may be the result of the well-established prevulatory withdrawal of projections from the zona pellucida. At that time-point immunofluorescent staining of AQP5 appeared in most oocytes and was also present in the apical membrane of epithelial cells of the oviduct ampulla. The latter observation suggests that after ovulation AQP5 is involved in the transcellular movement of water in the oviduct ampulla and oocytes in rats.

© 2013 Elsevier GmbH. All rights reserved.

Introduction

Aquaporins (AQPs) are a well-conserved family of small (~30 kDa) membrane channel proteins that facilitate rapid movement of fluids (Agre et al., 2002). To date, 13 AQP isoforms (AQP0–AQP12) have been identified in mammals. They participate in a wide array of physiological processes and are the primary determinants of membrane osmotic water permeability (Ishibashi et al., 2009). Their functions in male and female reproductive systems were summarized by Zhang et al. (2012).

Ovarian folliculogenesis is a process that involves the formation and expansion of the fluid-filled antrum requiring aqueous solution influx. The precise mechanisms involving large amounts of fluid passing into the antral cavity of the follicles remain unknown. Rodgers and Irving-Rodgers (2010) have proposed that granulosa cell derived extracellular matrix (ECM) proteins, hyaluronan and versican, generate an osmotic gradient that drives fluid flow from the thecal vasculature, while AQPs present in the granulosa cell membranes facilitate transcellular water transport. Studies by McConnell et al. (2002), West-Farrell et al. (2009) and Thoroddsen et al. (2011) support the presence of AQPs 1–4 and AQPs 7–9 in the human, rat and mouse granulosa cells, suggesting that the water permeability of antral follicles can occur through transcellular mechanisms.

Hyaluronan and versican are also predominant components of ECM formed by the expanding cumulus-oocyte complexes (COCs), a process that occurs after the prevulatory LH surge. As hypothesized by Rodgers and Irving-Rodgers (2010), it is plausible that some components/enzymes allow the ECM of the COCs to form a gel. However, there are suggestions that water is responsible for the hydration of ECM and the increase of intercellular space among cumulus cells (Salustri, 2000). Hunger et al. (2012) also reported that hyaluronan is strongly hydrated in a biological environment and its high viscosity and viscoelasticity seems to be unique to aqueous solutions of hyaluronan. Therefore, it seems plausible, that AQPs may be involved in the mechanism that provides water for hyaluronan hydration.

AQP5 is expressed in the apical membrane at several sites in mammals, including secretory cells in salivary glands, lacrimal glands, sweat glands, corneal epithelium, nasopharyngeal and bronchial epithelia and type I pneumocytes of the lung (Takata et al., 2004). In the female reproductive system, it has been found in the uterus and oviduct (Branes et al., 2005; Lindsay and Murphy, 2006; Aralla et al., 2009; Skowronski et al., 2009; Skowronski, 2010; Klein et al., 2013), while in the ovarian follicles AQP5 was demonstrated in pigs and mice (Skowronski et al., 2009; Zhang et al., 2013).

* Corresponding author.
E-mail address: malgorzata.durlej@uj.edu.pl (M. Grzesiak).

0065-1281/5 – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.acthis.2013.10.001
However, its expression has never been investigated in the pre-ovulat‌ory follicles after the LH surge. Therefore, the main objective of the present study was to investigate the expression of AQPS in preovulat‌ory rat ovarian follicles, with special reference to expanding COCs, including postovulatory ampullary COCs. The choice of AQPS was supported by the fact that its gene includes androgen-respondence element (Moehren et al., 2008), while the rat cumulus granulosa cells express androgen receptors (AR) during their whole lifespan (Szołtys and Słomczynska, 2000; Szołtys et al., 2010). To date, recent findings by Grzesiak et al. (2013) demonstrated increased swelling of porcine granulosa cells following testosterone treatment.

Materials and methods

Antibodies

Polyclonal antibody raised against a unique epitope of rat AQPS (peptide sequence NH2-CWEDHREERKKTIEL--COOH) conjugated to keyhole limpet hemocyanin (KLH) was developed in partnership with Sigma-Genosys (Poole, Dorset, UK). The peptide sequence chosen was checked on the protein blast database to ensure that it corresponded to AQPS (http://www.ncbi.nlm.nih.gov/BLAST/). A 77-day immunization protocol was used in New Zealand white rabbits that consisted of pre-immune serum collection, injection with 200 μg of peptide conjugated to KLH in complete Freund’s adjuvant on day one. This was followed by 5 × 100 μg booster injections in incomplete Freund’s adjuvant on days 14, 28, 42, 56 and 70 (Mobasher et al., 2011).

A mouse monoclonal antibody against β-actin was obtained from Sigma–Aldrich (St. Louis, MO, USA). Horseradish peroxidase-conjugated anti-mouse IgG and anti-rabbit IgG were from Vector Laboratories (Burlingame, CA, USA). Alexa Fluor 633 goat anti-rabbit antibody was purchased from Invitrogen (Carlsbad, CA, USA).

Ethics experimentation

The study was conducted in accordance with the Polish legal requirements, under the license given by Local Ethics Committee at the Jagiellonian University in Krakow, Poland.

Animals and tissue preparation

Female Wistar rats 2–3 months of age were kept in a 12 h light:12 h darkness schedule (lights from 08.00 to 20.00 h) and an ordinary laboratory diet was available ad libitum. Estrous cycle was followed by the condition of vaginal smears and only those animals exhibiting a regular 4-day estrous cycle were included in this study. Our laboratory rats generally ovulate at early estrus between 04.00 and 06.00 h. The females were killed in groups (n = 3–5 per group) with an overdose of Aerrane (Isoluranum, Baxter, Poland) on the subsequent days of estrous cycle (estrus, metestrus, diestrus, proestrus) at 11.00 h. Additionally, on the day of proestrus a group of animals was placed with males. These animals were then subsequently killed at estrus: (1) before ovulation at 23.00 h, 01.30 h and 03.00 h, and (2) at 11.00 h, that is several hours after ovulation, on day 1 of pregnancy, previously confirmed by the presence of spermatozoa in vaginal smears. Excised ovaries and postovulatory oviducts of pregnant and non-pregnant females were fixed in 10% neutral buffered formalin overnight and routinely embedded in Paraplast (Monoject Scientific Division of Sherwood Medical, St. Louis, MO, USA).

For the Western blot analysis, the oviduct ampulla and COCs were isolated from both oviducts on day 1 of pregnancy (confirmed by the presence of spermatozoa in vaginal smears). Oviductal COCs were then incubated in a drop of hyaluronidase solution 50IU/ml in phosphate-buffered saline (PBS). After incubation, oocytes (approximately 80) and granulosa cells (both collected from all COCs) were carried out separately with a mouth pipette, centrifuged, placed in Tris/EDTA buffer (50 mM Tris, 1 mM EDTA, pH 7.5) and sonicated. The homogenates of the oviduct ampulla, COCs and granulosa cells were stored at –80 °C until further analysis.

Immunofluorescent assay

Seven μm-thick sections were cut and mounted on slides coated with 3-amino-isopropyl-triethoxysilane (APES; Sigma–Aldrich). After routine deparaffinization and dehydration, sections were immersed in 0.01 M citrate buffer (pH 6.0) and heated in a microwave oven (3 × 4 min, 750 W) to retrieve antigenicity. Endogenous peroxidase activity was prevented by incubation with 0.3% H2O2 in TBS (Tris-buffered saline, pH 7.4) and non-specific binding sites were blocked by using 10% normal goat serum (Sigma–Aldrich) for 40 min at room temperature (RT). For visualizing AQPS, sections were first incubated overnight at 4 °C in a humidified chamber with rabbit polyclonal anti-AQPS antibody (diluted 1:400). After being rinsed in TBS with 0.1% Tween 20, the sections were incubated with far-red fluorescent Alexa Fluor 633 goat anti-rabbit antibody (diluted 1:100) for 1.5 h in the dark. Finally, the slides were mounted in Vectashield mounting medium with DAPI (4,6-diamidino-2-phenylindole, Vector Laboratories) and viewed under a Zeiss confocal laser-scanning microscope LSM510 (GmbH, Jena, Germany). Negative controls were performed by substituting the primary antibody with non-immune rabbit IgG. The sections of rat lung were used as a positive control of AQPS immunostaining.

Western blot analysis

The protein concentration for each sample was estimated using Bradford dye-binding with bovine serum albumin as a standard (Bradford, 1976). Supernatants containing 30 μg of protein were solubilized in a sample buffer consisting of 62.5 mM Tris–HCl pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue, 5% β-mercaptoethanol and heated at 99.9 °C for 3 min. After denaturation, samples were electrophoretically resolved using 15% SDS-polyacrylamide gels under reducing conditions according to Laemmli (1970). Separated proteins were transferred onto nitrocellulose membranes using a wet blotter in a Transfer Buffer (20 mM Tris, 150 mM glycine in 20% methanol, pH 8.4) for 90 min at a constant voltage of 135 V. After overnight blocking with 5% non-fat milk in TBS with 0.1% Tween 20 at 4 °C with gentle shaking, the membranes were probed with rabbit polyclonal anti-AQPS antibody, diluted 1:1000 at RT for 1.5 h. Membranes were washed and incubated with a secondary horseradish peroxidase-conjugated anti-rabbit IgG (diluted 1:3000) for 1 h at RT. Next, the membranes were stripped and reprobed with mouse monoclonal anti-β-actin antibody (dilution 1:3000) and with horseradish peroxidase-conjugated anti-mouse IgG (dilution 1:3000) to control the variable amounts of protein. Immunoreactive proteins were detected by chemiluminescence using the Luminol Reagent (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and visualized using ChemiDoc XR+ System (Bio-Rad Laboratories, GmbH, Munich, Germany). To obtain the relative amounts of AQPS protein (arbitrary units), the bands representing each examined sample were densitometrically scanned using Image Lab 2.0 software (Bio-Rad Laboratories) and normalized against its corresponding β-actin data point. Western blot analysis was performed for three separately repeated experiments. A sample of rat lung was used as a positive control for AQPS expression.
**Results**

**Immunofluorescent localization of AQP5 in various ovarian follicles**

In primordial, primary, small and large secondary ovarian follicles present at different days of the estrous cycle, the AQP5 immunostaining was almost negative (Fig. 1A). The AQP5-associated fluorescence appeared in the preantral follicles and formed a characteristic ring encircling the oocytes (Fig. 1B). A similar staining pattern with a stronger immunostaining was observed in the antral follicles continuing growth and differentiating during estrus, metestrus, diestrus and proestrus (Fig. 1C–E). The oocyte membrane associated with the positive ring of immunoreactivity was situated very close to the oolemma and was in close contact with it in many areas (Fig. 1C–E). Several hours after the presumptive preovulatory LH surge (estrus 23.00 h), when the process of cumulus expansion had started and the outer cumulus granulosa cells had dispersed, the characteristic AQP5-positive ring was still present. However, at that time staining appeared also on the...
Fig. 2. Representative micrographs of aquaporin 5 (AQP5) localization in the preovulatory ovarian follicles of the rat during cumulus-oocyte complexes (COCs) expansion (A–F) and in the postovulatory COCs (G–I) as well as the oviduct ampulla (H inset). Immunoreactive proteins were visualized using an Alexa Fluor 633 detection system (red). Nuclei were counterstained with DAPI (blue). (A) A fragment of preovulatory follicle (estrus, 1.30 h), in which the AQP5 immunostaining pattern resembles that expressed in follicles found in the ovary two hours earlier (Fig. 1F). Also partial granulosa cells lying close to the COC and extracellular matrix (ECM) between them feature a positive reaction for AQP5 (bold arrows). (B and C) Different magnifications of a fragment of another preovulatory follicle (estrus, 1.30 h). It features a very strong fluorescent reaction in ECM (bold arrows), richly gathered between the cumulus cells. Arrowheads point to the oocytes, while open arrow in C indicates a blue-stained oolemma. (D–F) Different magnifications of preovulatory follicle (estrus, 3.00 h). The majority of the corona radiata cells are disintegrated and lie far away from the oocyte. Some fragments of a former ring are still visible (arrowheads) marking the surfaces of groups of not disintegrated yet corona radiata cells. Large amounts of ECM feature a strong AQP5 fluorescent staining (bold arrows). (G–I) Different magnifications of postovulatory COCs and ampullary wall (estrus, day 1 of pregnancy, 11.00 h). Less abundant ECM lying between disintegrated cumulus cells still features immunofluorescent staining (bold arrows). Note, most of oocytes show a positive staining. In the oviduct ampulla, AQP5 immunostaining is observed in the apical membrane of epithelial cells (H inset, arrowhead). The replacing of primary antibody with non-immune rabbit IgG (G inset) shows lack of AQP5 staining in the oocytes and oviduct ampulla. O – oocyte. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Positive immunostaining was also observed around the parietal granulosa cells lying close to the COC, the cells thought to be responsible for the production of hyaluronan (Fig. 2C). At estrus at 3.00 h also two various patterns of immunofluorescent localization of AQP5 of preovulatory COCs were apparent. Firstly, in COCs which corona radiata cells still formed a compact layer situated close to the oocyte, immunofluorescent ring was complete (not shown) and similar to that observed two hours earlier. Secondly, in COCs

surface of some cumulus granulosa cells and in the ECM (Fig. 1F and G). Since the process of COC expansion began in the outside areas and gradually proceeded toward the oocyte, the outer regions showed more prominent AQP5 immunoreactivity than the corona radiata layer (Fig. 1F). Two hours later (estrus 1.30 h) some COCs resembled a pattern of staining observed at estrus at 23.00 (Fig. 2A), while in others immunostaining was much stronger and included also areas around corona radiata cells (Fig. 2A and B).
Expression of AQP5 protein after ovulation using Western blot analysis

Western blot analysis revealed the expression of AQP5 as a single band of approximately 30 kDa. AQP5 was detected in granulosa cells, ampullary COCs, and oviduct ampulla. Rat lung homogenate was used as a positive control (Fig. 3A). Quantitative analysis of relative AQP5 protein expression was assessed densitometrically and normalized against its corresponding β-actin (Fig. 3B).

Discussion

The most conspicuous feature of the rat ovarian follicles subjected to AQP5 staining was the presence of a fluorescent ring encircling the oocyte membrane, which was in close contact with it in many places. This ring appeared in the preantral follicles and persisted during their further antral growth at estrus, metestrus and preovulatory differentiation at proestrus, including the expansion of COCs in the late preovulatory follicles at estrus. This ring of immunoreactivity most likely reflects AQP5 proteins present at the ends of projections of corona radiata cells, which are known to penetrate the zona pellucida and anchor on the oocyte surface (Sotello and Porter, 1959; Dekel et al., 1978; Motta et al., 1999). It is known that gap junctions form at the ends of these processes, allowing the transfer of small molecules between cumulus cells and oocyte that are necessary for oocyte growth and development (Gilchrist et al., 2004). Our results suggest that the ends of corona radiata cells are also equipped with AQP5, which is likely to be functionally involved in water transport (Fig. 4).

Although the zona pellucida is not a significant barrier for the movement of water molecules, projections possessing AQP5 can considerably facilitate the transfer of water to the oocyte. However, the characteristic fluorescent ring started to disappear in
Fig. 4. Hypothetical sequence of events explaining the changes of aquaporin 5 (AQP5) expression in the rat cumulus–oocyte complexes (COCs). (A) In the preovulatory follicles (proestrus 11.00 h), cumulus granulosa cells tightly surround the oocyte. Most likely, AQP5 (red dots) is present at the ends of granulosa cells’ projections, which penetrate zona pellucida and anchor on the oocyte surface, since visualization of the oocyte membrane allowed to establish that AQP5 was situated very close to oolemma and was getting in contact with it in many regions forming a ring (reflecting micrographs in Fig. 1D–E). (B) In the preovulatory follicles at estrus (1.30 h), AQP5 expression pattern around the oocyte resembles that expressed in proestrus follicles. Interestingly, the process of COC expansion is accompanied by appearance of AQP5 (red dots) located in and between the cumulus granulosa cells that, most likely are connected by projections, which possibly express AQP5. At this stage of follicle development, perivitelline space begins to form (reflecting micrographs in Fig. 2A–C). (C) In the late estrous follicles (3.00 h) the majority of corona radiata cells are disintegrated and their projections are retracted. However, some granulosa cells still possess projections expressing AQP5 at the ends, therefore the AQP5 immunoreaction visible as fragments of a fluorescent ring is still observed. They are distant from oolemma due to perivitelline space enlargement (reflecting micrographs in Fig. 2D–F). (D) After ovulation (estrus, day 1 of pregnancy), cumulus granulosa cells are disintegrated and outlying the oocytes. Due to complete withdrawal of their projections from zona pellucida, the fluorescent ring or its fragments are absent. Postovulatory cumulus granulosa cells are covered with numerous protruding blebs (black arrowheads) and projections (yellow arrowheads), which express AQP5 and might be the source of extracellular immunofluorescence (reflecting micrographs in Fig. 2G–I). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Some COCs at estrus at 3.00 h and was absent in all investigated postovulatory COCs. This disappearance can easily be explained by a well-established preovulatory withdrawal of projections from zona pellucida, a process that is completed in the oviduct, immediately after ovulation (Sotello and Porter, 1959; Dekel et al., 1978; Motta et al., 1999). This implies that thereafter AQP5 at the ends of the projections no longer participate in the transfer of water. However, after ovulation relatively strong
immunofluorescence appeared in the ampullary oocytes suggesting the possibility of AQPS5-facilitated water transport through the oolemma, which is the source of the extracellular immunofluorescence signal. Further studies are needed to clarify the presence of AQPS5 in the rat oocytes. Previously, expression of AQPS5, AQPS3, and AQPS7 but not that of AQPS5 was reported in the rat or mouse preovulatory and ampullary oocytes by Ford et al. (2000), Edashige et al. (2000) and Meng et al. (2008). Interestingly, the process of cumulus expansion was accompanied by the appearance of AQPS5 immunoreactivity on the surface of cumulus granulosa cells and in the ECM, which is composed predominantly of hyaluronan. It is known that in various species, including rats, late preovulatory and/or postovulatory cumulus cells form numerous protruding blebs and numerous membrane extensions (Dekel et al., 1978; Motta et al., 1999; Familiar, 1998; Kawashima et al., 2012). These surface structures of cumulus cells richly penetrate ECM and may contain AQPS5, being the source of the extracellular immunofluorescence signal. To date, hyaluronan is known as the main component of this ECM and this molecule has been reported to be strongly hydrated in a biological environment (Hunger et al., 2012). Therefore, AQPS5 could participate in the supply of the water that is present in follicular fluid, and oviduct fluid. However, in COCs present in the postovulatory ampullae, less abundant fluorescent ECM than in the late preovulatory follicles was observed. This finding can be explained by the process of ECM disassembly and degradation, which may be occurring at that time (Camandini et al., 1997). As reported by D’Alessandris et al. (2001) in the mouse COCs degradation of ECM occurs within a few hours of ovulation and plasminogen activator seems to be involved in this process. Brown et al. (2010) have underlined the role of ADAMTS1, which mediates neomorphogenesis of the ovulating follicle wall and COC matrix necessary for successful ovulation and fertilization. A subsequent catabolism of versican is required for degradation of COC matrix after fertilization.

There are no previous reports of the presence of AQPS5 in the cumulus granulosa cells. The only demonstration of AQPS5 expression in ovarian follicles concerns flattened cells surrounding oocytes in the porcine primordial follicles and the granulosa cells of developing follicles (Skowronska et al., 2009) and the granulosa cells of antral follicles of mice (Zhang et al., 2013). These results contradict our findings, since the AQPS5 immunostaining was almost negative in the rat mural granulosa cells of antral follicles. Also in earlier studies concerning rodents only AQPS7, 8, and 9 have been detected in the granulosa cells (McConnell et al., 2002; Branes et al., 2005; West-Farrell et al., 2009). In the oviduct and uterus the expression of AQPS5 was observed in many mammalian species, including rats (Branes et al., 2005; Huang et al., 2006; Lindsay and Murphy, 2006; Aralla et al., 2009; Skowronska et al., 2009; Skowronska, 2010; Klein et al., 2013). A more detailed investigation of immunoeexpression of AQPS5 in the rat ampulla and isthmus was performed by Branes et al. (2005). The authors have found cytoplasmic immunoeexpression of AQPS5 in the epithelial cells of isthmus during the entire estrous cycle, while in the epithelial cells of ampulla exclusively during diestrus 1 and 2. The absence of AQPS5 in the rat ampulla during estrus and proestrus contradicts our results, since we found a very strong apical AQPS5 immunostaining in the epithelial ampullary cells during preovulatory stages, which precede and represent the maximal water contents in the ampullary fluid. In the reported studies by Branes et al. (2005) the main rat oviductal aquaporin seemed to be AQPS9, identified in ampulla and isthmus segments throughout the entire estrous cycle. Its expression was regulated by estradiol and progesterone, while the expression of AQPS5 was ovari- not estradiol- or progesterone-dependent. The authors suggested that other ovarian signals are involved in the expression of this AQP. However, it is known that human AQPS5 gene contains androgen-response element (Moehren et al., 2008). Therefore, it cannot be excluded that the expression of AQPS5 is androgen-dependent, the more that in the epithelial cells of the epididymis male reproductive system, a structure that is homologous to the female oviduct, also expresses AQPS5 (Da Silva et al., 2006; Hermo et al., 2008). Such a possibility was supported by the fact that in the rat oviduct AR mRNA expression was found in the cycling and pre-implantation rats (Okada et al., 2003), while AR immunoreactivity was present in the oviduct of epithelial, stromal and muscle cells (Pelletier et al., 2000). Also the granulosa cells of preantral follicles feature AR, while cumulus granulosa cells were found to express AR during their whole lifespan, including the postovulatory period (Szoltys and Slomczynska, 2000; Szoltys et al., 2010). Moreover, preovulatory follicular fluid (Szoltys et al., 2001) and postovulatory ampulla contain androgens (our unpublished data). The presence of androgens and AR is suggestive of androgen action via AR. However, it cannot be excluded that the follicular expression of AQPS5 is estrogen-dependent. As found by Kobayashi et al. (2006) the mouse uterine promoter of AQPS5 gene contains a functional estrogen response element that is activated by estradiol. Thus far, however, there are not reports on the presence of estrogen receptors in the preovulatory cumulus granulosa cells.

Conclusions

In the present study immunofluorescence assay was used to depict and decipher the spatiotemporal expression of AQPS5 in the developing and maturing rat ovarian follicle, with special reference to the COC, including its presence in the oviduct ampulla. The data obtained revealed several structures that feature positive AQPS5 immunostaining. The first one appeared in the preantral follicles and persisted almost until ovulation. This structure formed a fluorescent ring that encircled and made direct contact with the oolemma and most likely represent AQPS5 molecules, at the ends of projections of corona radiata cells facilitating the access of oocytes to water. Other AQPS5 immunopositive staining appeared during the cumulus expansion and included expanding cumulus granulosa cells and the ECM produced by them. The source of the ECM staining is likely to involve numerous structures, protruding from cumulus cells blebs and extensions equipped with AQPS5 and providing water for hydration of hyaluronan. The late structures found to demonstrate AQPS5 immunofluorescent staining were postovulatory oocytes and ampullary epithelial cells, in which the investigated AQPSs could be involved in the transcellular movement of water in the oviduct ampulla and oocytes.

Acknowledgements

This work was financially supported by DS/MND/WBiNoZ/I2/24/2011. The authors express sincere acknowledgements to the Confocal Microscopy Laboratory (Institute of Zoology, Jagiellonian University, Krakow, Poland) for the use of microscope LSM 510 META, Axiovert 200M, ConfoCor 3 (Carl Zeiss Microimaging GmbH, Jena, Germany). M. Grzesiak is supported by funding from the Jagiellonian University within the SET project. The SET project is co-financed by the European Union within the European Social Fund.

References

Aralla A, Borromeo V, Groppetti D, Secchi C, Cremonesi F, Arrighi S. A collaboration of aquaporins handles water transport in


Lindsay LA, Murphy CR. Redistribution of aquaporins 1 and 5 in the rat uterus is dependent on progesterone: a study with light and electron microscopy. Reproduction 2006;131:369–78.

McConnell NA, Yunus RS, Gross SA, Bost KL, Clemens MG, Hughes FM. Water permeability of an ovarian antral follicle is predominantly transcellular and mediated by aquaporins. Endocrinology 2002;143:2905–12.


