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The expression profile of *AQP1*, *AQP5* and *AQP9* in granulosa and theca cells of porcine ovarian follicles during oestrous cycle and early pregnancy

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ABSTRACT. Aquaporins (AQP) are hydrophobic integral membrane channel proteins that facilitated water transport across the plasma membrane. In this study, the reverse transcription real-time polymerase chain reaction (Real-Time RT-PCR) assay was used to determine the expression of genes encoding *AQP1*, *AQP5* and *AQP9* in porcine ovarian follicles, separated theca and granulosa cells of six experimental groups: early-luteal (days 2–4), mid-luteal (days 10–12 of the cycle, coinciding with a period of full active corpora lutea corresponding to the activity of corpora lutea in the period of pregnancy), late-luteal (days 14–16 of the cycle, coinciding with a period of luteal regression and development of a new cohort of follicles) and follicular group (days 18–20) of the oestrous cycle, as well as early implantation (days 14–16) and post-implantation, placentation group (days 30–32) of gestation. Significant differences in the *AQP1*, *AQP5* and *AQP9* genes expression between studied groups appeared only in the separated theca and granulosa cells. In the present study it is implied that the AQP1, 5 and 9 participate in the formation of follicular fluid and follicular development. These three examined AQPs appear to act interdependently, thereby maintaining tissue homeostasis.

Introduction

Growth of ovarian follicles in pigs is characterized by continuous activation, slow growth to the antral stage and rapid growth to 4 to 5 mm followed by atresia. Moreover, the gentle balance of follicular cell proliferation and apoptosis determines the destiny of the follicles (Craig et al., 2007). These physiological processes depend on endocrine and paracrine control as well as communication between the follicular compartments including oocyte, granulosa (GC) and theca cells (TC) (Guthrie and Garrett, 2001). However, the mechanisms regulating the selection of ovulatory follicles and the exact mechanism of fluid transport from the vasculature into the avascular granulosa cells layers are not well understood. According to Rodgers et al. (2001) follicular fluid accumulation determines oocyte quality and proper ovulation. Therefore, the transport of water in ovarian follicles seems to be an

important process for the effective reproduction. There is sufficient evidence that extracellular matrix proteins produced by granulosa cells generate an osmotic gradient that drives transcellular water transport by the actions of aquaporins (Rodgers and Irving-Rodgers, 2010).

The cell membrane channel proteins – aquaporins (AQPs) – are responsible for selective movement of water molecules between the cell and the external environment (Preston and Agre, 1991). Thirteen mammalian aquaporin isoforms with a unique tissue-specific pattern of expression have been identified so far. Of these, AQP1 and AQP5 are the members of classical water channels, which are expressed extensively in the reproductive system, and AQP9 belongs to aquaglyceroporins (Zhang et al., 2012). The limited results about AQPs in the ovary suggest that their main function is in antrum formation and expansion in follicles of rodents and humans (Rodgers et al., 2001; Rodgers and Irving-Rodgers, 2010; Thoroddsen et al., 2011). Furthermore, the Aqp5 and 7–9 (McConnel et al., 2002; Starowicz et al., 2014), and Aqp7 and 8 (West-Farell et al., 2009) have been intensively expressed in rat and mouse granulosa cells, respectively. With the respect to their mediation function, the presence of AQPs in granulosa cells suggests that water permeability of antral follicles occurs primarily through transcellular mechanism (McConnel at al., 2002; West-Farrel et al., 2009; Rodgers and Irving-Rodgers, 2010) and this mechanism might be important in folliculogenesis in different species including pigs (Skowronski et al., 2009). Subsequent studies revealed that steroid hormones produced by follicles regulate the expression of ovarian AQPs (West-Farrel et al., 2009; Grzesiak et al., 2013).

In our previous investigations the presence of AQP1, AQP5 and AQP9 proteins in the ovarian follicles collected from gilts on days 17–19 (follicular phase) of the oestrous cycle (Skowronski et al., 2009) was revealed. In our very recent studies with the use of Western-blot analysis and immunohistochemistry there was shown a marked increase in AQP1 and AQP5 levels on days 18–20 of the oestrous cycle, suggesting that these AQPs may be responsible for fluid balance within the pig ovary, at the time when preovulatory follicles are developed (Skowronska et al., 2015). However, the mRNA expression has not been extensively studied. So, to allow for a more comprehensive interpretation of the role of AQPs and physiological potential of ovary for transcription of these genes, the aim of the study was to evaluate, by reverse transcription real-time polymerase chain reaction (Real-Time RT-PCR), the levels of mRNA of *AQP1*, *AQP5* and *AQP9* in the ovarian follicle cells of cycling and early pregnant pigs.

Material and methods

Animals and collection of ovaries

All experiments were performed in accordance with the national guidelines and were approved by the Local Animal Ethics Committee, University of Warmia and Mazury in Olsztyn (Poland; approval no. 83/2012/DTN). Porcine ovaries used in the present study were derived from the same experimental animals that were examined previously (Skowronska et al., 2015). In total, 30 gilts were assigned to one of six experimental groups (n = 5, per group): early-luteal (days 2–4), mid-luteal (days 10–12 of the cycle, coinciding with a period of full active corpora lutea corresponding to the activity of corpora lutea in the period of pregnancy), late-luteal (days 14–16 of the cycle, coinciding with a period of luteal regression and development of a new cohort of follicles) and follicular group (days 18–20) of the oestrous cycle, as well as early implantation (days 14–16) and post-implantation, placentation group (days 30–32) of gestation. The stage of the cycle was confirmed by a morphological analysis of ovaries according to Akins and Morrissette (1968). Gilts assigned to the pregnant group were naturally bred on the second day of oestrus and the breeding was repeated after 12 h. The stages of pregnancy were confirmed as previously reported by Skowronska et al. (2015). Ovaries were separated from each gilt and frozen in a liquid nitrogen immediately after dissection and then stored at −80 °C until RNA isolation. Plasma concentrations of oestradiol (E_2) and progesterone (P_4) confirming the stages of the cycle and pregnancy were published already (Skowronska et al., 2015).

Isolation of granulosa and theca cells

The follicles without signs of atresia (n = 12) from two ovaries of each gilt from each examined group were used to obtain granulose and theca cells. Granulosa cells were aspirated with a syringe and then washed out with a strong stream of buffer directed to the internal wall of the follicle. The theca layer was scratch off from granulosa cells and enzymatically dispersed in 0.25% trypsin solution. Diffused cells were polled and centrifuged (800 *g* for 10 min) and washed three times to get clean granulosa and theca cells (Maleszka et al., 2014).

The granulose and theca cells were resuspended in Fenozol reagent (A&A Biotechnology, Gdańsk, Poland) and immediately stored at −80 °C until processing for RNA analysis.

Total RNA isolation and reverse transcription

Total RNA was extracted from the follicles and also from separated granulosa and theca cells using the Fenozol reagent following the manufacturer's instructions. The quantity and purity of RNA were determined spectrophotometrically (NanoDrop ND-1000, Thermo Fisher Scientific, Waltham, MA, USA), then randomly selected RNAs were additionally tested by 1.5% agarose gel electrophoresis. Reverse transcription (RT) was performed using an Enhanced Avian HS RT-PCR Kit (Sigma-Aldrich, St. Louis, MO, USA) and a mix of dNTPs and random hexamers as primers. The RT product was kept frozen at −20 °C for PCR analysis.

Real-Time RT-PCR and sequencing

The expression of genes coding aquaporins was determined by Real-Time RT-PCR method with the use of 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) and 18S rRNA (*RN18S*) were used as normalization controls (Dziekoński et al., 2015; Martyniak et al., 2016). Primers for *AQP1*, *AQP5* and *AQP9* and primers for *GAPDH* and *RN18S* genes were applied (Table 1). Each amplification reaction consisted of SYBR Green MIX (12.5 µl), specific primers at the concentration 10 nM (1µl for *AQP1*, *AQP5* and *AQP9*), cDNA (2 µl) and filled up with RN-ase free water to the final volume (25 µl). Real-Time PCR was carried out in duplicates for each sample using the following parameters: one cycle of an initial denaturation (10 min at 95 °C), followed by 40 cycles of denaturation at 95 °C for 15 s; primers annealing at specific temperatures for 1 min and elongation (1 min at 60 °C). The last PCR cycle included a final extension step (10 min at 72 °C). The standard curves were prepared for tested and reference genes. All runs included negative controls. After reaction, randomly selected samples for each gene were sequenced (Genomed, Warsaw, Poland) to confirm the specificity of amplicons of studied genes. The levels of gene expression were calculated with the $\Delta\Delta C_t$ method and normalized using the geometrical means of two reference gene (*GAPDH* and *RN18S*) expressions.

Statistical analysis

All data were analysed using one-way analysis of variance (ANOVA) followed by the Duncan's least significant difference post hoc test and are reported as means ± standard error of the mean (SEM) from five independent observations. Statistical analysis was performed using Statistica 13 software (StatSoft Inc., Tulsa, OK, USA). Differences were considered statistically significant at $P < 0.05$.

Results

Using a quantitative Real-Time RT-PCR, the presence of aquaporins *AQP1*, *AQP5* and *AQP9* mRNA in porcine ovarian follicles during the oestrous cycle and early pregnancy was confirmed. The basal expression of *AQP1* was maintained almost at the same level during the examined days of the oestrous cycle and early pregnancy (Figure 1A,B). No significant changes were observed in the *AQP1* mRNA levels when levels of mRNA during the cycle and the early pregnancy were compared (Figure 1C). Differences in *AQP1* gene expression

Table 1. Forward and reverse primers sequences, amplicons length and GeneBank accession numbers of genes used during Real-Time PCR analysis

Name of the gene	Primer sequence forward/reverse	Amplicon length, bp	Accession number
Aquaporin 1 (*AQP1*)	5'-CAGCGAGTTCAAGAAGAAG-3' 5'-GCGACACCTTCACGTTATC-3'	161	NM_214454.1
Aquaporin 5 (*AQP5*)	5'-CTATGAGTCCGAGGAGGATT-3' 5'-GCTTCGCTGTCATCTGTT-3'	147	NM_001110424.1
Aquaporin 9 (*AQP9*)	5'-TCTGGTGGATTCCTGTAGTG-3' 5'-GGTTTGTCCTCCGATTGTTC-3'	130	NM_001112684.1
Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*)	5'-GACCTCCACTACATGGTCTA-3' 5'-AAGATGGTGATGGCCTTTC-3'	116	NM_001206359.1
18S ribosomal RNA (*RN18S*)	5'-GGCTACCACATCCAAGGAAG-3' 5'-TCCAATGGATCCTCGCGGAA-3'	149	AK393333.1

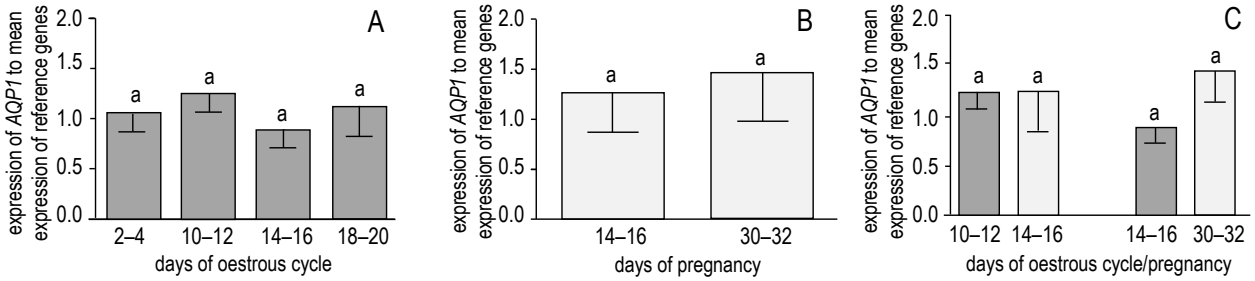

Figure 1. Expression of *AQP1* mRNA in ovarian follicles (A) on days 2–4, 10–12, 14–16 and 18–20 of oestrous cycle; (B) on days 14–16 and 30–32 of pregnancy, and (C) as comparison of *AQP1* expression in ovarian follicles between two stages of cycle (dark bars) and two periods of early pregnancy (bright bars)

were found in the separated theca and granulosa cells (Figure 2A,D). During the oestrous cycle the markedly higher amount of *AQP1* transcripts was noted on days 14–16 and 18–20 in theca cells and on days 14–16 in granulosa cells in comparison with the remaining days 2–4 and 10–12 ($P < 0.05$). There were observed no significant changes in the *AQP1* mRNA levels in the theca cells and granulosa cells during the early pregnancy (Figure 2B,E). When comparing mRNA transcript levels of *AQP1* in the theca cells during the cycle (days 14–16) to the days of the pregnancy (days 30–32), a significantly higher expression on days 14–16 of the cycle (84-fold higher, $P < 0.05$) was noted (Figure 2F).

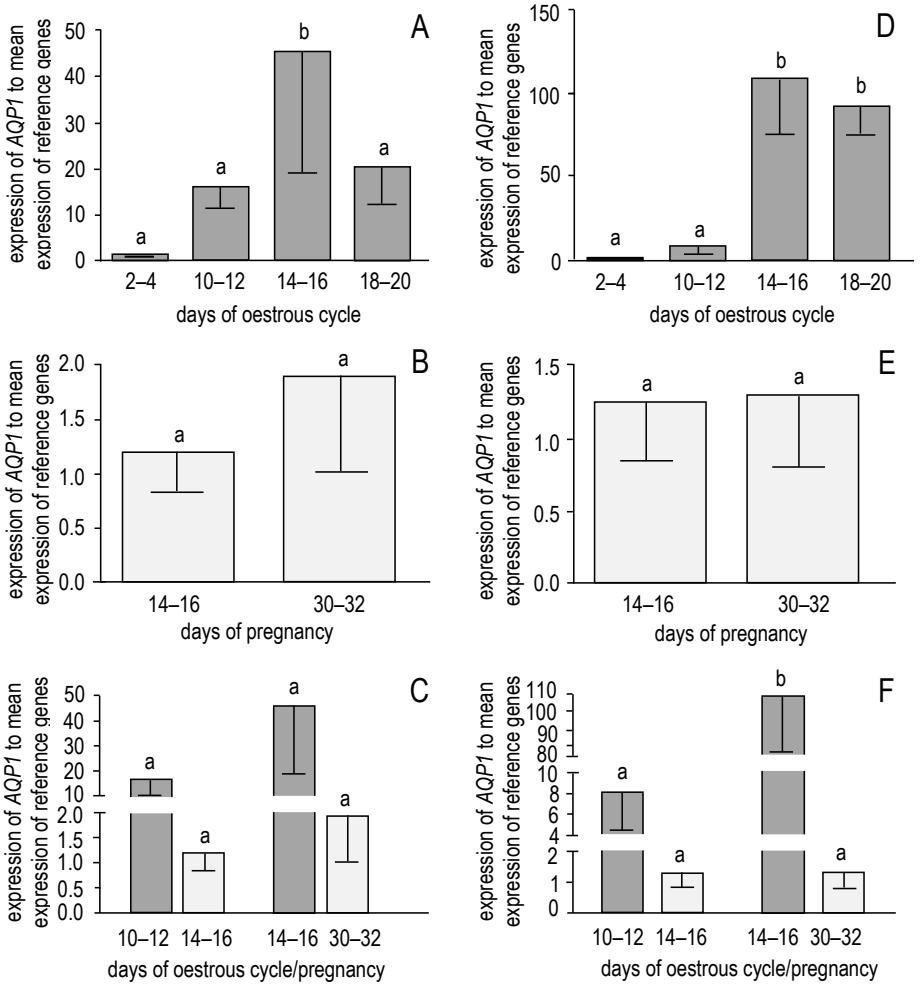

Figure 2. Expression of *AQP1* mRNA in granulosa and theca cells, respectively (A and D) on days 2–4, 10–12, 14–16 and 18–20 of oestrous cycle, and (B and E) on days 14–16 and 30–32 of pregnancy. Comparison of *AQP1* expression in granulosa and theca cells (C and F) between two stages of cycle (dark bars) and two periods of pregnancy (bright bars). Differences in transcript levels within the experimental groups were assumed as statistically significant at $P < 0.05$ and marked with the different letters (a–b)

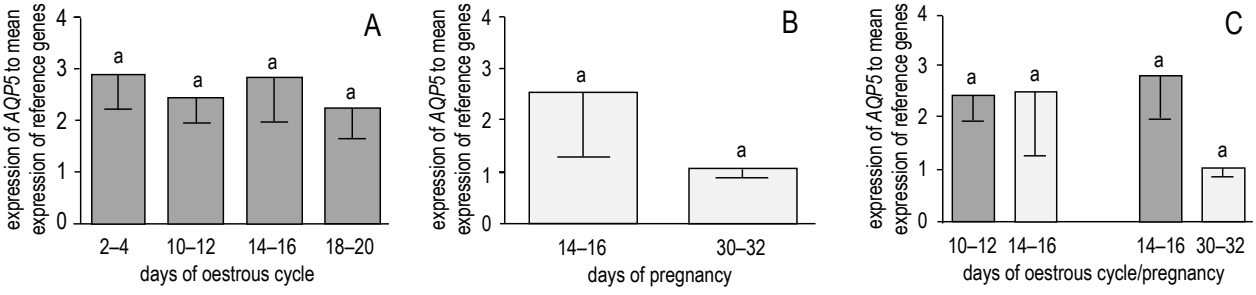

Figure 3. Expression of *AQP5* mRNA in ovarian follicles (A) on days 2–4, 10–12, 14–16 and 18–20 of oestrous cycle; (B) on days 14–16 and 30–32 of pregnancy, and (C) as comparison of *AQP5* expression in ovarian follicles between two stages of cycle (dark bars) and two periods of early pregnancy (bright bars)

The basal expression of *AQP5* mRNA in the porcine ovarian follicles did not significantly differ between studied days of the oestrous cycle and early pregnancy (Figure 3). During the oestrous cycle *AQP5* mRNA level in granulosa cells was significantly higher ($P < 0.05$) on days 10–12 than on remaining days (Figure 4A). In theca cells, a significantly higher level of *AQP5* expression on days 18–20 of the cycle (with 44-fold higher, $P < 0.05$) and a tendency to increase expression on days 10–12 in comparison with days 2–4 and 14–16 of the cycle were reported ($P < 0.05$) (Figure 4D). In pregnant gilts, significant differences in the expression of this gene were neither found in granulosa nor theca cells (Figure 4B,E). The mRNA levels were low in both periods of early pregnancy.

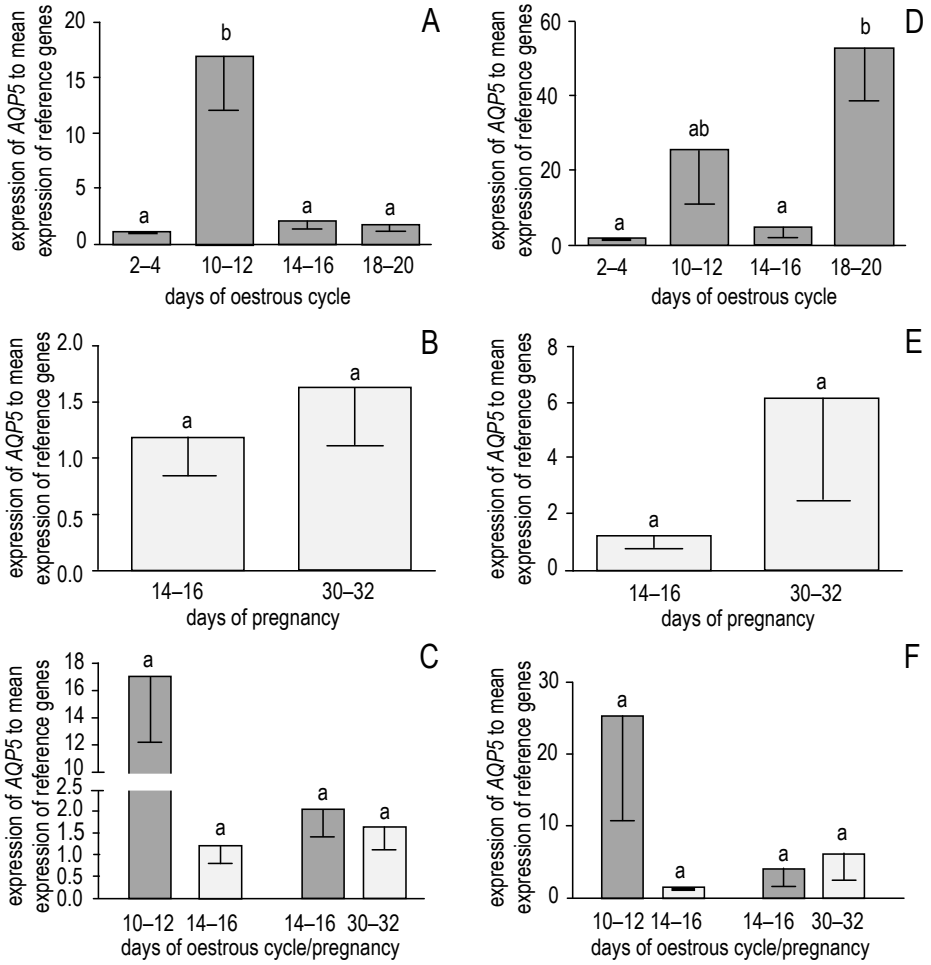

Figure 4. Expression of *AQP5* mRNA in granulosa and theca cells, respectively (A and D) on days 2–4, 10–12, 14–16 and 18–0 of oestrous cycle, and (B and E) on days 14–16 and 30–32 of pregnancy. Comparison of *AQP5* expression in granulosa and theca cells (C and F) between two stages of cycle (dark bars) and two periods of pregnancy (bright bars). Differences in transcript levels within experimental groups were assumed as statistically significant for $P < 0.05$ and marked with different letters (a–b)

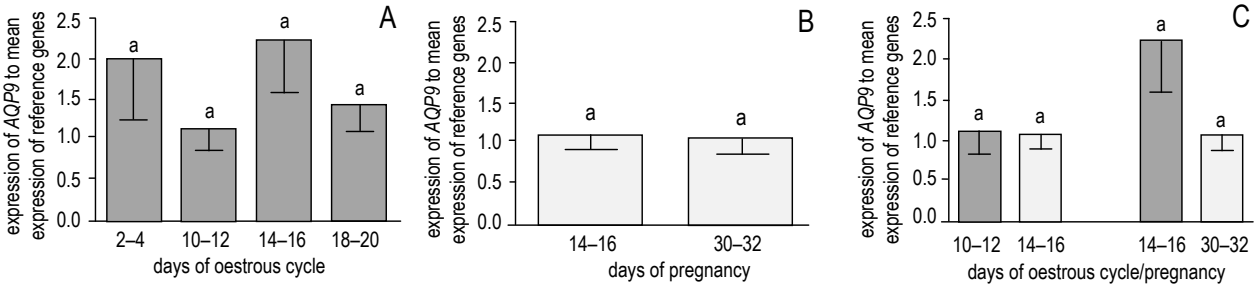

Figure 5. Expression of *AQP9* mRNA in ovarian follicles (A) on days 2–4, 10–12, 14–16 and 18–20 of oestrous cycle; (B) on days 14–16 and 30–32 of pregnancy, and (C) as comparison of *AQP9* expression in ovarian follicles between two stages of cycle (dark bars) and two periods of early pregnancy (bright bars)

No significant changes in the basal expression level of *AQP9* mRNA in the porcine ovarian follicles were seen between all days of the oestrous cycle (Figure 5A) and pregnancy (Figure 5B). Moreover, no significant changes were observed in the *AQP9* mRNA transcript levels in theca cells (Figure 6D). In granulosa cells basal expression of *AQP9* mRNA was the greatest on days 10–12 ($P < 0.05$) in comparison with days 2–4, 14–16 and 18–20 (Figure 6A). Moreover, a significant increase in *AQP9* mRNA levels in these cells was also observed on days 14–16 and 18–20 as compared with days 2–4 ($P < 0.05$) (Figure 6A). The higher expression of *AQP9* in granulosa cells was observed at days 10–12 of the oestrous cycle ($P < 0.05$), then was the level of expression in the implantation period (days 14–16),

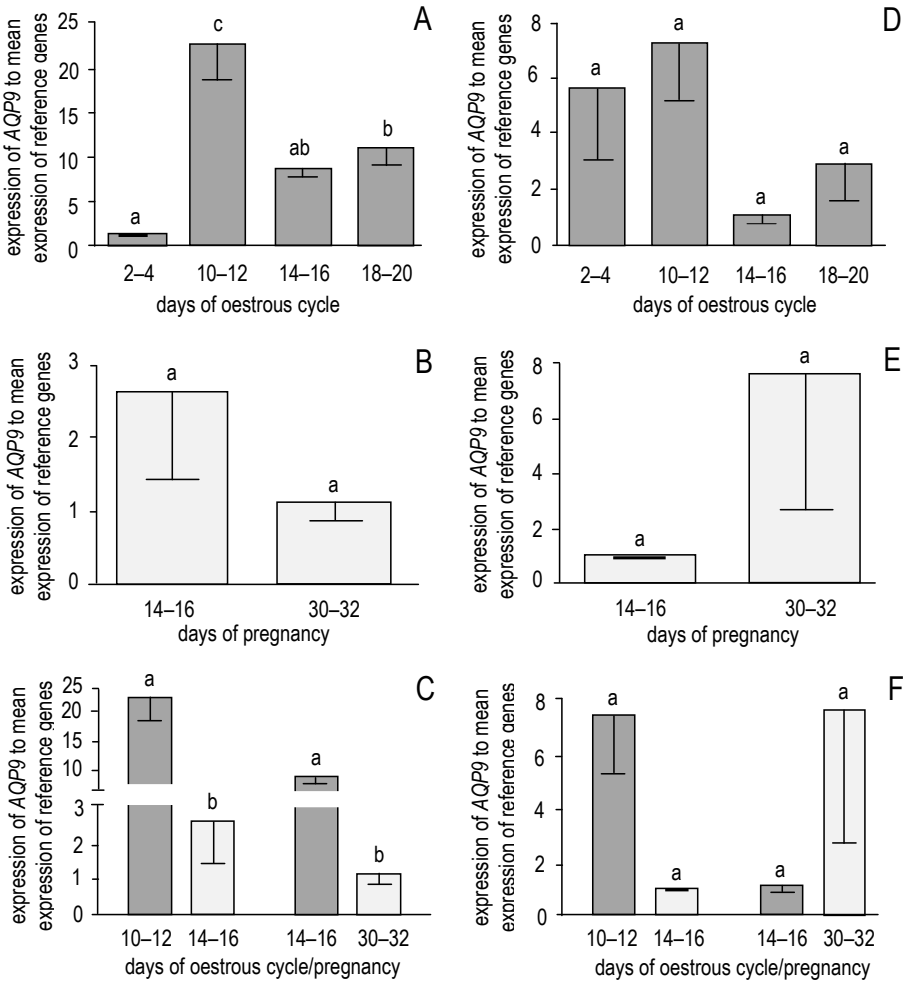

Figure 6. Expression of *AQP9* mRNA in granulosa and theca cells, respectively (A and D) on days 2–4, 10–12, 14–16 and 18–20 of oestrous cycle and (B and E) on days 14–16 and 30–32 of pregnancy. Comparison of *AQP9* expression in granulosa and theca cells (C and F) between two stages of cycle (dark bars) and two periods of pregnancy (bright bars). Differences in transcript levels within the experimental group were assumed as statistically significant for $P < 0.05$ and marked with the different letters (a–c)

similarly, the expression of *AQP9* was significantly higher at days 14–16 of the oestrous cycle than at the end of the implantation (days 30–32, $P < 0.05$), (Figure 6C). In theca cells the relative expression of *AQP9* was not significant between the examined days of the cycle and early pregnancy (Figure 6F).

Discussion

In the present study it was documented that mRNA of three isoforms of AQPs (*AQP1*, *AQP5* and *AQP9*) are expressed in the pig ovarian follicles, separated granulosa and theca cells of the precise stages of the oestrous cycle and early pregnancy.

The expression of AQPs genes was demonstrated in the ovary tissues of different species: *AQP3* in ewes (Sales et al., 2014), *AQP3*, *AQP7* and *AQP9* in the ovine prenatal follicles (Sales et al., 2015), *Aqp8* in mice (Su et al., 2010) and *AQP1-4* in humans (Thoroddsen et al., 2011). Whereas, the presence of *AQP5* mRNA in porcine ovarian follicles was reported recently by Grzesiak et al. (2016). In that study diminished AQP5 mRNA and protein expressions within pre-antral and large antral follicles of adult pigs were presented. In earlier studies, the same group revealed *in vitro* that testosterone acting *via* intracellular androgen receptors increases water permeability of porcine granulosa cells, through the regulation of AQP activity (Grzesiak et al., 2013). In the light of these reports AQP5 appears to be a potential regulator of follicular fluid accumulation during follicular growth under androgen control.

In our recent studies it was demonstrated that AQP5 was immunolocalized in primary, pre-antral and antral follicles (Skowronska et al., 2015), and high level of this protein expression was stated on days 18–20 of the oestrous cycle. In the present study the basal expression of *AQP5* mRNA in the porcine follicles was relatively constant during luteal phase with the marked 2-fold decrease on days 18–20 during follicular phase. West-Farrel et al. (2009), who cultured *in vitro* mice follicles, showed unchanged *Aqp7* and *Aqp8* mRNA levels suggesting that AQPs involved in antrum formation should be expressed at constant level in developing follicles instead of an induction of gene expression before antrum formation. In the present study, higher levels of *AQP5* gene expression during the luteal phase and lower expression during the follicular phase could denote stimulatory effect of progesterone and the inhibitory influence of oestradiol on ovarian AQPs mRNA expression. In contrast to the present study, in our previous study (Skowronska et al., 2015) it was shown that protein concentrations are not exactly correlated with the abundance of the corresponding mRNA levels. This finding, however, is with agreement with several proteomic studies (Vogel and Marcotte, 2013) evaluating mRNA and protein concentrations. It is hypothesized that variation between these molecules can be explained by post-transcriptional mechanism (Sales et al., 2016). Nonetheless, in the theca cells, higher transcript levels of *AQP5* mRNA were observed during the mid-luteal (days 10–12) and during the follicular phase (days 18–20), and in granulosa cells on days 10–12 of the cycle. It is well known that a co-operation between these two types of cells under gonadotrophic stimulation is possible. Granulosa cells like endocrine cells are actively involved in follicle growth and development (Basini et al., 2016), whereas theca cells are needed for folliculogenesis to synthesize androgen and provide cross-talk with granulosa cells and oocyte (Canipari et al., 2012). In the present study, mRNA encoding *AQP5* during the early luteal phase (days 2–4) was the lowest in both granulosa and theca cells, which corresponds with the stage of formation of primordial follicles. At this time of the cycle, a primordial follicle is composed of an oocyte and surrounding granulosa cells, and thecal layers are not formed until the follicle is activated and reaches the secondary stage of development. Although no significant difference in expression of *AQP5* gene in theca cells was found on days 2–4 and 14–16, a significant increase was observed in the middle of luteal phase and during follicular phase, at the time when pre-antral and antral follicles are developed. This suggests that basal expression of *AQP5* mRNA in thecal cells is submitted to the development of ovarian follicles, e.g., formation and expansion of antrum and probably follicular rupture. Similar relationship was found by Thoroddsen et al. (2011) in the study concerning *AQP3* in granulosa and theca cells of the preovulatory follicles in women.

In the present study it was supposed that increased potency of ovary to produce the *AQP5* transcripts during the oestrous cycle supports the concept that AQP5 participates in water transport across the entire follicular development, including the transition from pre-antral to antral stage and is required for immediate increase in fluid transport. Also, it was shown that the transcription and translation of AQP5 undergo changes during the oestrous cycle. More recently, in the *in vitro* study of Sales et al. (2015) changes in expression patterns of *AQP3, 7* and *9* were demonstrated and it was indicated that

during follicular development transcription and translation might be also altered.

Rather stable ovarian expression of *AQP1* gene during the oestrous cycle was demonstrated in the present study. In granulosa cells the lowest expression was noted on days 2–4 with the significant increase on days 14–16. The establishment and continual remodelling of a complex vascular system influence the follicle development. According to Basini et al. (2016) this angiogenic process is modulated by the biosynthetic activity of granulosa cells. In the follicles of all stages, this layer of cells remains avascular up to ovulation (Binelli and Murphy, 2010). Although the granulosa cells are connected by a gap junctions in a functional syncytium (Tong et al., 2006). In our previous study on the pig ovaries, the AQP1 immunoreactivity in the endothelium of capillaries surrounding the follicle was demonstrated (Skowronski et al., 2009). Western blot analysis of AQP1 expression tended to be higher on days 2–4 and 14–16 (Skowronska et al., 2015). In the present study *AQP1* mRNA show high expression in pre-antral and antral follicle. The low levels of *AQP1* transcript in the early luteal phase and the mid-luteal phase and higher expression during the late luteal phase and during follicular phase correspond to the high protein expression at this stage of the cycle. Also, in the present study some differences in *AQP1* mRNA expression in granulosa and theca cells were observed, e.g., markedly higher expression of *AQP1* in theca cells on days 14–16 and 18–20. It is thought that this protein may play a role in the process of follicle enlarging, follicular fluid formation and expansion of the antrum, e.g., thecal microvasculature with the presence of AQP1 transports water into the antral cavity.

It was revealed that AQP1 expression significantly increases after follicular rupture suggesting that AQP1 may act on the process relating to transition of the follicle into corpus luteum (Thoroddsen et al., 2011; Sales et al., 2014). Furthermore, Lee et al. (2016) demonstrated that the mRNA level of *AQP1* is associated with retrieved oocyte number, and that AQP1 may be one of the factors that modulate individual ovarian response to exogenous gonadotrophin.

The Aqp9 expression was previously found in rat granulosa cells (McConnell et al., 2002). Moreover, the fluctuations of *Aqp9* transcripts during growth and oocyte maturation in rats were observed (Ford et al., 2000). In our previous study the subcellular localization of AQP9 within the granulosa cells in the porcine ovary was shown (Skowronski et al., 2009). Also, AQP9 is thought to be present in human granulosa cells (Qu et al., 2010; Thoroddsen et al., 2011). In earlier studies concerning rodents, only Aqp7, 8 and 9 have been detected in the granulosa cells (Brañes et al., 2005; West-Farell et al., 2009). Importantly, AQP9 is the member of aquaglyceroporins subgroup, which is permeable not only to water but also to glycerol, urea and other non-electrolytes. Moreover, in *in vitro* studies of secondary follicles changes in AQP9 expression pattern during antrum formation were stated (Rodger and Irving Rodgers 2010; Sales et al., 2015). In the present study, high expression of *AQP9* mRNA in granulosa cells on days 10–12, 14–16 and 18–20 of the cycle and low expression on days 2–4 were observed. Hence, the expression of *AQP9* in granulosa cells may suggest that transport of above-mentioned substances is also essential for the follicle development, differentiation and improvement of oocyte quality. AQP9 might ensure sufficient supply of granulosa cells with substrates (androgens) for oestrogen production. Very recently, Sales et al. (2016) revealed that AQP9 is essential for cryopreservation process. In the present study the expression of *AQP9* transcripts in the theca cells was demonstrated, but the amount of transcript was too low to compare it with granulosa cells, and the confirmation by immunohistochemistry was impossible. Therefore, further studies should be conducted to explicate the role of AQP9 in these cells.

In this study, it was also shown that *AQP1*, *AQP5* and *AQP9* genes are present in porcine ovarian follicles, granulosa and theca cells during pregnancy. *AQP1* gene expression in theca cells was significantly higher on days 14–16 of the oestrous cycle than on days 30–32 of pregnancy. The content of *AQP9* transcript in granulosa cells was higher on days 10–12 and 14–16 of the cycle than on days 14–16 and 30–32 of pregnancy, respectively. In pigs and in other species follicular development during pregnancy does not seem to be dependent on gonadotrophin status changes. The peripheral level of LH during early pregnancy mimics LH concentrations on the mid-luteal phase of the oestrous cycle. However, Duda et al. (2004) and Knapczyk et al. (2008) indicated that oestrogens and androgens are essential for the maintenance of the pregnancy and that ovarian follicles are necessary during this period. Moreover, Wiesak et al. (1992) found that small follicles in pregnant pigs from day 20 and day 30 produced significantly more progesterone than large follicles. A similar relationship was also evident among follicles on day 12 of the oestrous cycle, but not on day 12 of the pregnancy.

The results of the present study confirm the hypotheses of others authors (Zhang et al., 2012;

Grzesiak et al., 2013; Sales et al., 2015) that AQP expression in the female reproductive tissues is regulated hormonally. Comparing our previous and present studies on AQPs in porcine ovary it was noticed that concentrations of gene transcripts and respective proteins change during follicular development, reflecting a modulation in the activity of these membrane proteins.

Conclusions

The expressions of mRNA for three aquaporin (AQP) isoforms *AQP1*, *AQP5* and *AQP9* in the porcine follicles, granulosa and theca cells from the oestrous cycle and early pregnancy were demonstrated. The basal expression of genes coding these AQPs was different during studied periods and might have been influenced by steroid hormones. The presence of these AQPs may be essential for the provision of water and other solutes during formation of follicular fluid and follicular development. It may be speculated that in the pig ovarian follicle high expression of *AQP1* in the theca cells would be important for the transport of fluid into the interstitium underlying the basal lamina and that water would pass through this membrane passively to be transported by AQP5 and 9 in granulosa cells into the antrum. So, AQP1, 5 and 9 in porcine ovarian tissue appear to act interdependently, thereby maintaining tissue homeostasis.

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