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Water Permeability in Rat Oocytes at Different Maturity Stages: Aquaporin-9 Expression

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Abstract. Important functional and structural modifications occur in mammalian oocytes during their arrival to maturity. In this process, oocytes switch from a high activity level, implying an important metabolic rate and a coordinated movement of water and solutes, to a lower functional state. The aim of this work was to study the mechanisms involved in water movements during oocyte arrival to maturity. Volume changes, induced by an osmotic gradient, were followed by video microscopy in rat oocytes. The water osmotic permeability (P_{osm}) of immature oocytes (proestrus) was sensitive to $HgCl_2$ and phloretin. In contrast, mature oocytes (estrus) had a reduced P_{osm} that was not sensitive to these compounds. When proestrus oocytes were incubated in vitro at 37°C they spontaneously arrived at maturity and its P_{osm} decreased between four and six hours of incubation. RT-PCR experiments were performed using specific primers for all rat aquaporins that had been cloned. We found that aquaporin-9 transcript (AQP9) is present in proestrus oocytes but not in estrus oocytes. AQP9 has been recently described as a "broad selective channel" responsible for solute and water transfers in highly active cells. Our experiments showed that proestrus oocytes, but not estrus, are permeable to mannitol. It is concluded that during the process of maturation, P_{osm} decreases and AQP9 transcripts disappear. We report here the first study correlating water permeability and aquaporin mRNA expression in mammalian oocytes.

Key words: Proestrus — Estrus — Osmotic Permeability — Aquapoins — $HgCl_2$, Diabetes mellitus

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Introduction

The mammalian ovary contains pools of nongrowing and growing oocytes arrested in the first meiotic prophase (immature oocytes). Only fully grown oocytes resume meiosis, arrive at metaphase II (mature oocytes), and are released during each reproductive cycle. Resumption of meiosis can be mediated by a hormonal stimulus in vivo (Downs, Daniel & Eppig, 1998) or simply by the release of oocytes from their ovarian follicles into a suitable culture medium in vitro (Pincus & Enzmann, 1935; Edwards, 1965). During growth, a mouse oocyte undergoes a 300-fold increase in volume becoming one of the largest cells of the body (Wassarman, 1988). This tremendous cell enlargement is a result of a period of intense metabolic activity during which enhancement of solute and water transport mechanisms are presumably required. The follicle, filled with follicular fluid, grows concomitantly with the oocyte. Before ovulation this fluid increases in volume and changes its composition (Espey & Lipner, 1994). After ovulation mature oocytes are deposited in the oviduct, which is an environment designed to facilitate their final maturation. In summary, throughout their life, the oocytes as well as the environment that surrounds them, are subject to very important changes. However, how oocytes adapt to all these changes is poorly understood.

The aim of this work was to study the mechanisms involved in water movements during oocyte arrival to maturity. It is well accepted that water can cross cell membranes through two different pathways: (i) the lipid bilayer and (ii) specific water channels (aquaporins). After the initial discovery of aquaporin-1 (AQP1) in red cells (Preston et al., 1992), nine different aquaporins have been, until now, identified in mammalian tissues (AQP1–AQP9 (King & Agre, 1996; Ishibashi et al.,

1997; Koyama et al., 1997; Ishibashi et al., 1998; Tsukaguchi et al., 1998; Yasui et al., 1999)). Most reports support the hypothesis that water channels, situated in the plasma membrane, play a central role in the hydrosmotic balance regulation at the cellular and total body level.

We have now tested the characteristics of water permeability and the putative expression of aquaporins in rat oocytes at different maturity stages. Our results showed that immature oocytes have higher water and mannitol permeabilities than mature ones. These results correlated with mRNA expression of a specific water channel: AQP9. After maturation, AQP9 mRNA disappeared at the same time that sensitivity to $HgCl_2$ and phloretin was lost. The presence of a broad selectivity water neutral solute channel during the oocyte arrival to maturity may have important implications in the adaptation to osmotic stress. We have found that in streptozotocin-induced diabetic rats (that present a high activity metabolic state) proestrus oocyte P_{osm} was significantly lower than that in control oocytes.

Materials and Methods

Animals

To test cycle evolution daily vaginal smears in Albino Wistar rats, weighing between 200–300 g, were examined. Proestrus (before endogenous LH peak) or estrus rats were killed, in the morning, by cervical dislocation.

Oocyte Isolation

Rat oocytes were isolated in two different stages: before ovulation (proestrus) and after ovulation (estrus). Proestrus ovaries were isolated, trimmed of fat and placed in Petri dishes containing phosphate-buffered saline (PBS) (Gibco BRL, 20012-027 plus $CaCl_2$ 0.1 g/l). Oocyte-cumulus complexes were isolated under a stereoscopic microscope by puncturing antral follicles with a 25-gauge needle. After isolation the complexes were denuded of their cumulus corona investments by repeated pipetting through a narrow bore glass pipette. Estrus oviducts were examined for the presence of oocytes. The dilated ampoule were excised and oocytes were placed in a 100 μl drop of Dulbecco's phosphate-buffered saline containing hialuronidase (1 mg/ml, 1 min), to disperse granulosa cells and the cumulus.

Oocyte in vitro Maturation

Oocyte-cumulus complexes obtained by puncturing antral follicles from proestrus ovaries were placed in a 200 μl drop of M16 (Sigma M7292) in dispensable Petri dishes and covered with paraffin oil. Oocytes were cultured for 1, 2, 4, 6, 8 or 16 hr at 37°C under a humidified atmosphere of 5% CO_2 in air as previously described (Jawerbaum et al., 1996). After incubation, oocyte-cumulus complexes were denuded of their cumulus corona investments as previously mentioned.

Volumetric Assays

Rat oocytes (diameter about 80 μm) were viewed with an inverted microscope and a video camera as previously described (Capurro et al., 1994). Images were analyzed by a computed program. Volumes at time zero were measured in an isotonic medium (phosphate-buffered PBS).

To measure the osmotic water permeability, the oocytes were exposed to a hypotonic medium (30 mosm/l). P_{osm} was calculated from volume changes during the first 90 sec after the osmotic challenge. Data were processed as previously reported (Capurro et al., 1994; Ford et al., 1996). All the experiments were run at 20°C to reduce the possible role of active transports in the observed phenomena.

To examine the effects of mercurial agents, oocytes were incubated in PBS buffer containing 3×10^{-4} mol/l $HgCl_2$, for 5 min before the swelling experiments. Reversibility was tested incubating the oocytes 15 min after $HgCl_2$, in 5×10^{-3} mol/l β-mercaptoethanol. In some experiments water permeability was measured in oocytes incubated during 10 min with phloretin (0.1 mM).

To estimate mannitol movements through the oocyte membrane, iso- and hypo-osmotic experiments were performed. In iso-osmotic assays, oocyte volume changes were followed after their transfer from standard PBS buffer to a modified PBS one in which 270 mosmol/l NaCl was replaced by 270 mosmol/l mannitol (final osmolarity 300 mosmol/l). For hypo-osmotic assays, oocytes were transferred from a standard PBS buffer to a modified PBS buffer in which 135 mosmol/l mannitol was added after subtraction of 270 mosmol/l NaCl (final osmolarity 165 mosmol/l).

Diabetic Rats

To induce experimental diabetes, rats were injected with streptozotocin (65 mg/kg, Sigma, St. Louis, MO) in citrate buffer (0.05 M, pH 4.5). Seven days after the injection animals exhibiting glycosuria higher than 500 mg/dl (Diastix reagent strips, Bayer Diagnostics) were considered diabetic.

RT-PCR Experiments

Total RNAs from kidney, colon and lung (positive controls) were isolated using SV total RNA Isolation System (Promega). Reverse transcription was performed either on 2 μg of total RNA of control tissues or directly on 10 isolated oocytes using the SuperScript Preamplification System for First Strand cDNA Synthesis (Gibco BRL). Control RNAs or oocytes were placed in 50 μl of "RT reaction buffer" containing: 1× PCR buffer, 0.5 μg oligo-dt primer, 0.1 mg/ml BSA, 10 mM DTT, 2.5 mM $MgCl_2$ and 10 U/μl RNAsin. The reaction was heated 3 min at 80°C and cooled at 45°C. PCR buffer (25 μl) containing: 1 × PCR buffer, 0.1 mg/ml BSA, 10 mM DTT, 2.5 mM $MgCl_2$, 400 μM dNTP and 100 units SuperScript II RT, was added to half of the reaction. Control experiments in the absence of the enzyme SuperScript II RT, were performed on the other 25 μl. RT reaction was carried out for 1 hr at 45°C and stopped by heating 2 min at 95°C.

PCR experiments (30 sec at 95°C, 30 sec at 56°C, 45 sec at 72°C for 35 cycles) were done on 5 μl of the RT reaction using 10 pmol of specific primers for rat AQP1 to AQP9 (Table 1). Internal positive control was included in each experiment using β-actin specific primers (sense: 5′ CGG AAC CGC TCA TTG CC 3′; antisense: 5′ ACC CAC ACT GTG CCC ATC TA 3′).

The AQP9 amplified cDNA fragment (~374 bp) was blunt ended using Klenow fragment of DNA polymerase I, digested with the endonuclease Pst1 (unique site encoded in the AQP9 PCR product) and

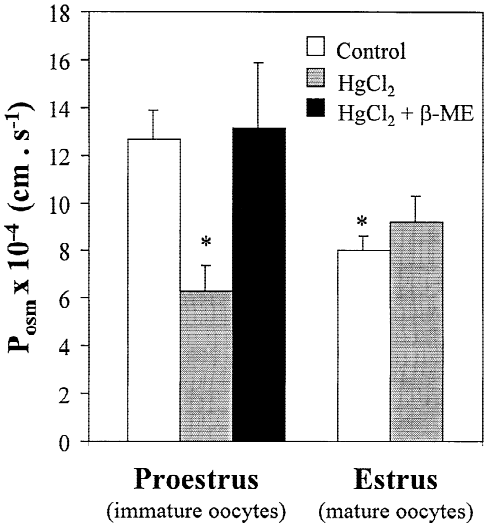

Fig. 1. Osmotic water permeability (P_{osm}) in proestrus and estrus rat oocytes. Mean values of rat oocyte osmotic permeability at two different maturity stages. Proestrus: immature oocytes extracted from ovary; estrus: matured oocytes extracted from oviduct. Proestrus P_{osm} value was higher than estrus. The inhibitory effect of $HgCl_2$ in proestrus oocytes was reversed when they were exposed during 15 min to β-mercaptoethanol. $^*P < 0.001$ when compared to proestrus control values.

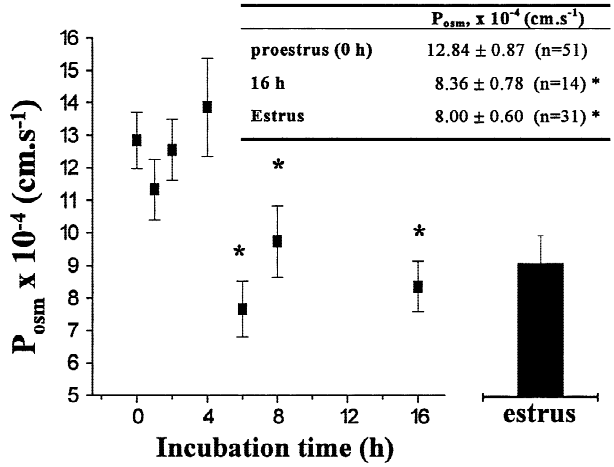

Fig. 2. Oocytes osmotic permeability variation during in vitro maturation. When oocytes were incubated in vitro (37°C) a significant P_{osm} reduction occurred between 4 and 6 hr. After 16 hr, the P_{osm} values were not significantly different from those observed in estrus oocytes. $^*P < 0.01$, when compared to control proestrus values ($t = 0$).

cloned between the Pst1, Sma I sites of the pSport II plasmid (Gibco BRL Life Technologies). The cloned 284 bp fragment was sequenced using T7 polymerase and chain terminating inhibitors.

Statistical Analysis

Data are expressed as means ± SE. Statistical analyses were performed using Student *t*-test for unpaired data.

Results

Oocyte Water Permeability Studies at Different Maturity Stages

Mammalian oocytes undergo a series of recognized stages, including germinal vesicle breakdown and extrusion of the first polar body as meiotic maturation proceeds to ovulation, and these changes have been linked with alterations in membrane permeability to various ions and solutes (Powers, 1982). However, mechanisms involved in water movements during oocyte arrival to maturity are unknown. We have tested the characteristics of water permeability in rat oocytes at different maturity stages.

Figure 1 summarizes mean values of osmotic water permeability obtained in mature and immature oocytes (proestrus and estrus). It can be observed that P_{osm} values were significantly higher in proestrus oocytes when compared to estrus oocytes [P_{osm}, (cm · sec^{-1} × 10^{-4}): 12.9 ± 0.9 (proestrus, $n = 51$) and 8.0 ± 0.6 (estrus, $n = 31$), $P < 0.001$]. Moreover, preincubation with 3×10^{-4} mol/l $HgCl_2$, a water channel blocker, significantly inhibited osmotic permeability in proestrus oocytes while P_{osm} values were not affected by this agent in estrus oocytes. This inhibition was fully reversible after treatment with β-mercaptoethanol (5×10^{-3} mol/l) indicating the specificity of the response.

It is well known that immature oocytes undergo spontaneous arrival to maturity when incubated in appropriated conditions (Edwards, 1965; Pincus & Enzmann, 1935). We have now evaluated whether water permeability of proestrus rat oocytes is modified during the in vitro arrival to maturity. P_{osm} values were measured at time zero and after 1, 2, 4, 6, 8 or 16 hr of in vitro incubation at 37°C. Figure 2 shows a significant reduction in P_{osm} values between 4 to 6 hr [P_{osm}, (cm · sec^{-1} × 10^{-4}): 13.9 ± 1.5 (4 hr, $n = 6$) and 7.7 ± 0.9 (6 hr, $n = 14$), $P < 0.01$]. After 16 hr (maintained in vitro) the P_{osm} values were not significantly different from those in vivo maturated (estrus oocytes).

Aquaporins in Rat Oocytes

PCR amplification with specific primers for aquaporins was carried out to identify the putative expression of water channels in rat oocytes. Figure 3 summarizes experiments where specific primers for aquaporins 1 to 8 were tested by RT-PCR from oocytes or total RNA of positive controls (rat kidney, colon and lung). As expected RT-PCR of rat kidney RNA produced fragments of the proper size (Table 1) with AQP1, AQP2, AQP3,

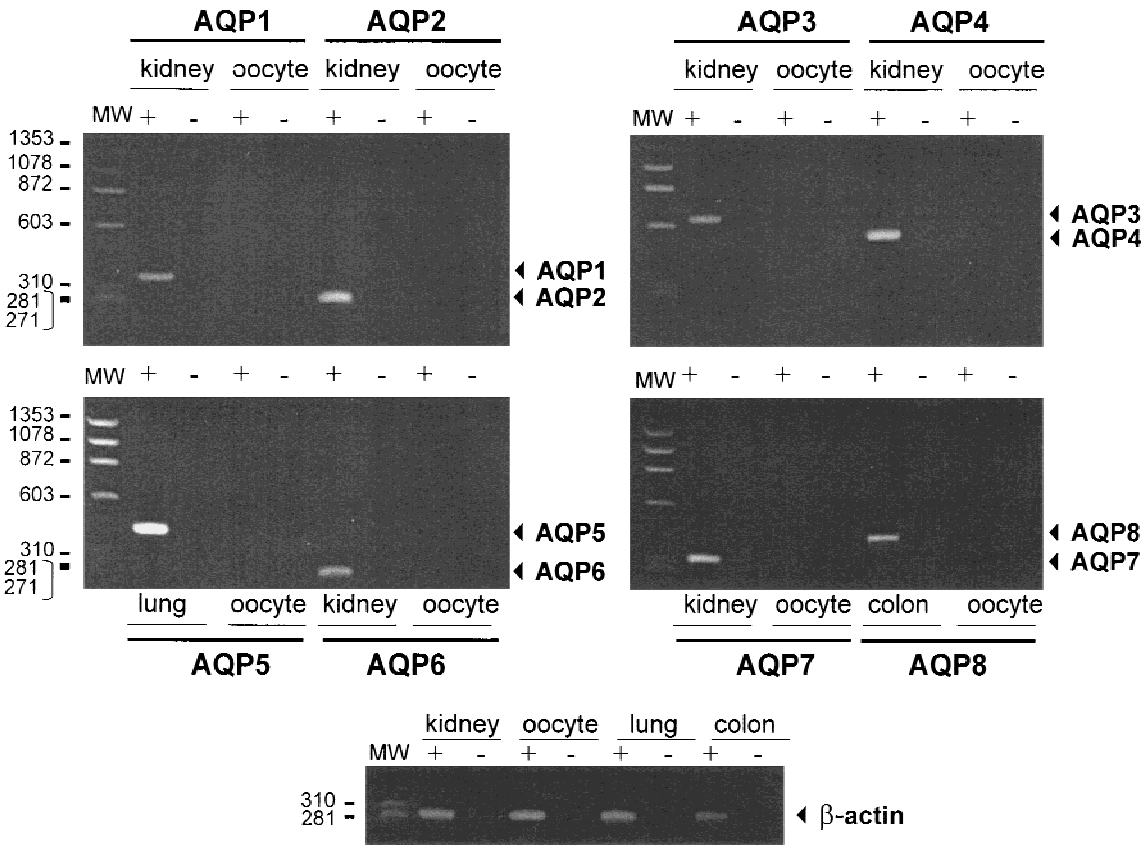

Fig. 3. Rt-PCR experiments using specific primers for AQP1-8. Total RNAs from oocytes, kidney (as positive control of AQP1, AQP2, AQP3, AQP4, AQP6, AQP7), lung (as positive control of AQP5) and colon (as positive control of AQP8), were reverse transcribed and the PCR amplified with aquaporin and β-actin primers (*see* Table 1). After 35 PCR cycles, an aliquot (10 μl) of each reaction was electrophoresed through a 2% agarose gel stained with ethidium bromide. Assays were carried out in the presence (+) or absence (−) of RT enzyme. Left lane, molecular weight marker (MW, ϕ X174 *Hae III* digested). Specific signals can be observed in all the positive controls but no amplification occurs in the oocyte.

AQP4, AQP6, AQP7. AQP5 was amplified in lung and AQP8 in colon. In contrast, no amplifications for any aquaporins were obtained in immature oocytes. β-actin was used as an internal control in all tissues and oocytes. Figure 4 shows the results of a representative RT-PCR experiment using specific primers for AQP-9 and β-Actin. A positive band of ~374 bp for AQP9 was found in proestrus but not in estrus oocytes (five and three different experiments, respectively). The amplified fragment, obtained from proestrus oocytes cDNA, was subcloned and sequenced confirming 100% homology with the previously AQP9 reported in liver and testis (Tsukaguchi et al., 1998). Immature oocytes are surrounded by follicular cells before the experiment's oocytes are manually desfolliculated. Therefore, RT-PCR was also performed in follicular cells in order to test whether the specific signal corresponds to oocytes and not to follicular cells. The absence of AQP9 in follicular cells confirms the specificity of AQP9 expression in immature oocytes (Fig. 4).

It was previously reported that rat AQP9 mediates the passage of water and a wide variety of noncharged solutes in a phloretin- and mercury-sensitive manner (Tsukaguchi et al., 1998). Because of this we have evaluated phloretin sensitivity and mannitol movements in immature and mature oocytes. Figure 5*A* shows that P_{osm} was reduced by phloretin (0.1 mM) in proestrus oocytes but not in estrus oocytes.

To test whether mannitol moves across the oocyte membrane we measured the oocyte's volume change in buffers that contain mannitol in iso- and hypo-osmotic conditions. After 20 sec of exposure to iso-osmotic PBS in which 270 mosmol/l NaCl was replaced by 270 mosmol/l of mannitol, the volume of the proestrus oocytes increased significantly in comparison with control oocytes maintained in standard PBS buffer (8 ± 1%, $n = 5$ *vs.* 0.7 ± 0.4%, $n = 10$; $P < 0.001$). On the contrary, no difference was observed in estrus oocytes.

Figure 5*B* and *C* shows proestrus and estrus oocyte volume changes, in the presence of hypo-osmotic conditions. In control experiments (filled circles) medium osmolarity was 165 mosmol/l being NaCl the principal

Table 1. Primers used for aquaporins expression

	Primers	cDNA sequence location (nt)	Amplified fragment size (bp)	Data bank accession number
AQP1	up: 5′-GTC CCA CAT GGT CTA GCC TTG TCT G-3′	983–1008	362	107268
	dw: 5′-GGG AAG GGT CCT GGA GGT AAG TCA-3′	1321–1345		
AQP2	up: 5′-GCC CCT TGC AGG AAC CAG ACA-3′	1079–1100	277	D13906
	dw: 5′-GCC AAA GCG GGA ATG ACA GTC-3′	1335–1362		
AQP3	up: 5′-GGC TAA AAA CGC TCC CTG TAT CCA-3′	974–998	645	d17695
	dw: 5′-GGA GTT TCC CAC CCC TAT TCC TAA A-3′	1594–1619		
AQP4	up: 5′-TGC CAC CCA TTA AGG AAA CAG ATT-3′	1028–1052	540	u14007
	dw: 5′-GAT GCT GAG GGG GAA GAA GGA TTA T-3′	1543–1568		
AQP5	up: 4′-AGC CCC TGG ACC ACT GGA GAA A-3′	935–957	441	u16245
	dw: 5′-CAC CCC ACC CCC ATC CTT GAC-3′	1355–1376		
AQP6	up: 5′-CTG CTT GTA TGG TGT CCC TGG TGT-3′	1003–1026	262	af083879
	dw: 5′-GGC CTT GGA AAA CTA ACT GGA TGG-3′	1241–1264		
AQP7	up: 5′-GCT GGC TGG GGC AAG AAA GTG-3′	931–952	323	ab000507
	dw: 5′-TTT ATT GCA GAA GGG TTG TGG TCA-3′	1230–1254		
AQP8	up: 5′-CCA CTG CCT GGA GTC CTC AGC-3′	913–933	433	af007775
	dw: 5′-AAG AGG AAA GAG GGT GGG GAG AAC-3′	1322–1346		
AQP9	up: 5′-GAA GCT CGA CCC AGA CAT GAA GG-3′	1055–1078	374	af016406
	dw: 5′-GTA AAA AAA GGT CTC TGA GGC TAT CCA-3′	1402–1429		

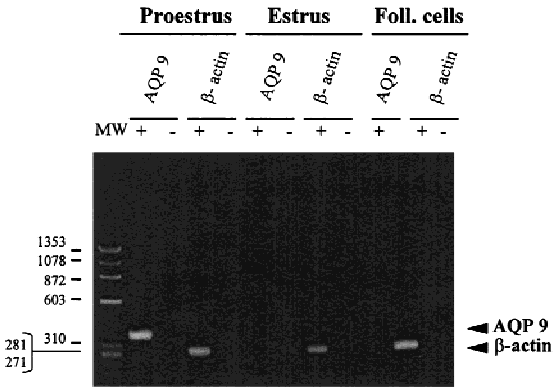

Fig. 4. AQP9 mRNA expression in immature oocytes. This photo shows a typical RT-PCR experiment performed in mRNAs from proestrus oocytes, estrus oocytes and follicular cells. The same samples were analyzed by RT-PCR for AQP9 and β-actin mRNAs expression. After 35 PCR cycles, an aliquot (10 μl) of each reaction was electrophoresed through a 2% agarose gel stained with ethidium bromide. Assays were carried out in the presence (+) or absence (−) of RT enzyme. Left lane, molecular weight marker (MW, ϕ X174 *Hae III* digested). A specific band of ~374 bp can be observed for AQP9 mRNA expression in proestrus oocytes. Control: β-actin mRNAs (~289 bp) were detected in proestrus oocytes, estrus oocytes and follicular cells.

osmolyte. In "mannitol experiments" NaCl was replaced by mannitol (total osmolarity was maintained at 165 mosmol/l). It can be observed that in proestrus oocytes the change of volume was higher in the presence of mannitol (Fig. 5*B*). On the other hand, no difference between mannitol and control solutions was observed in estrus oocytes (Fig. 5*C*). These results indicate that mannitol enters the cell in immature oocytes but not in mature ones.

Water Permeability and Aquaporin 9 mRNA Expression in Oocytes from Diabetic Rats

In another experimental series, we studied oocyte water permeability in a pathological condition: diabetes mellitus type I. No information is available about oocyte permeability properties in animals with this disease. We compared proestrus *vs.* estrus oocytes P_{osm} values, in normal and diabetic rats (Table 2). It can be observed that the diabetic rats presented similar P_{osm} values in estrus and proestrus oocytes. Furthermore, a significant reduction in water permeability appeared when the proestrus oocytes from diabetic rats were compared to those from normal rats. Proestrus oocytes from diabetic rats did not show sensitivity to $HgCl_2$. However, AQP9 mRNA was detected by RT-PCR in diabetic immature oocytes (*data not shown*).

Discussion

Oogenesis represents one of the most highly specialized and regulated biological processes in mammals. To become a "competent oocyte" and to have the possibility of reaching maturation, several changes in oocyte structure and function occur. These changes are accompanied with modifications in the composition of the fluid that bathe these cells. Consequently, important water and solute movements must occur in order to prepare oocytes

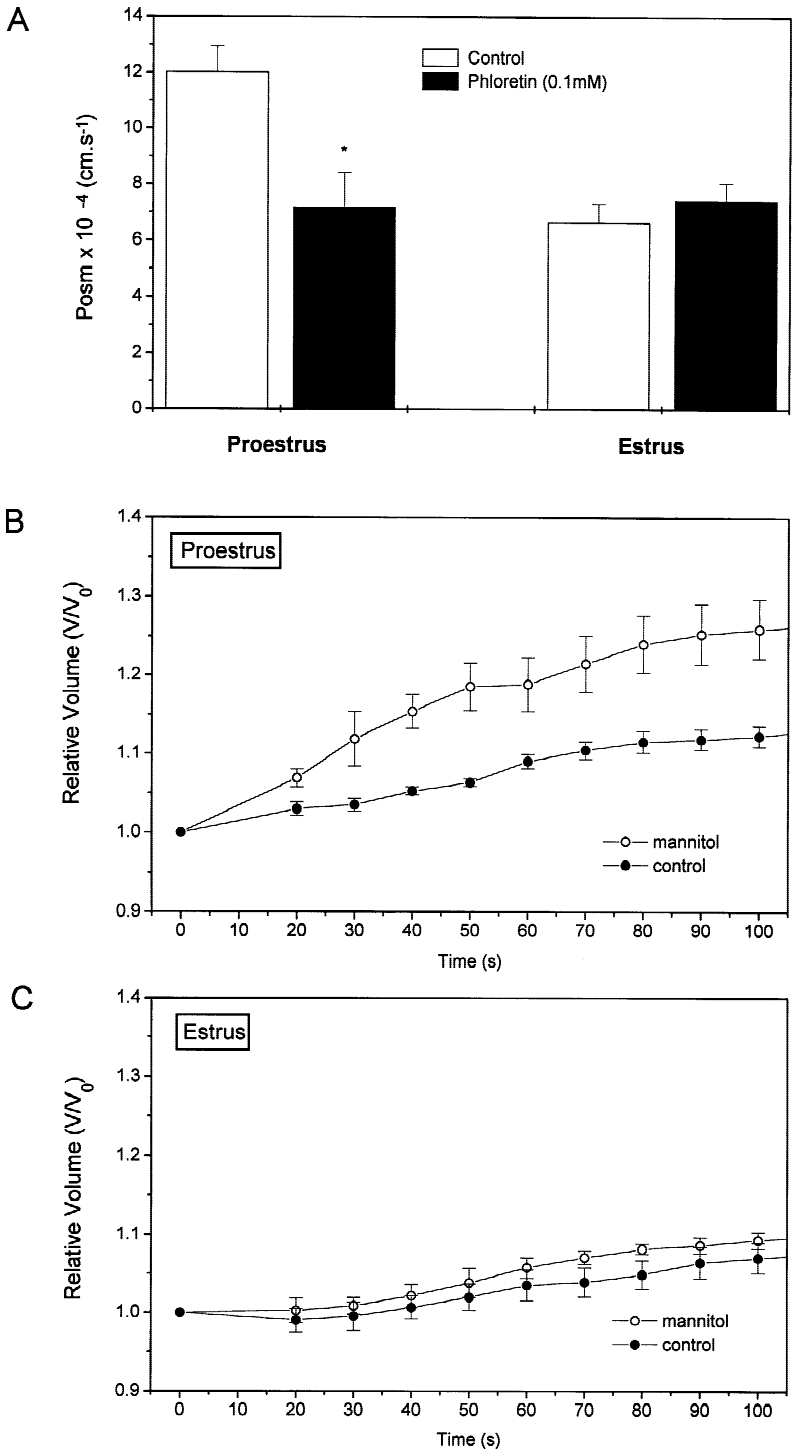

Fig. 5. Functional properties of proestrus and estrus oocytes. (*A*) Water osmotic permeability was measured after 10 min of incubation with 0.1 mM phloretin. P_{osm} was significantly reduced by phloretin in proestrus oocytes but not in estrus oocytes ($P < 0.01$). Data represent the mean ± SE from 9 to 14 oocytes. (*B*) Time course of osmotic swelling of proestrus oocytes in hypo-osmotic conditions. Control experiments (filled circles) correspond to hypo-osmotic PBS solution (165 mosmol/l) with NaCl the principal osmolyte. Mannitol experiments (open circles) correspond to hypo-osmotic PBS solution (165 mosmol/l) with mannitol the principal osmolyte. (*C*) Time course of osmotic swelling of estrus oocytes as described in *B*.

for survival in different conditions. However, at present only very limited information is available about oocyte water permeability at the immature stage and its variation during the maturation process (Ruffing et al., 1993; Le Gal, Gasqui & Renard, 1994). Le Gal (1994) has reported that in the presence of propanediol immature goat oocytes shrink to a greater extent than do mature ones. It can be also mentioned that an irregular shrinkage phenomenon was observed in metaphase I, but not in metaphase II, in primate oocytes under a hyperosmotic shock (Younis et al., 1996). This would indicate a reduction in water permeability during the maturation process.

We report here the first study correlating water per-

Table 2. Comparison of P_{osm} values (mean ± SE, cm · sec^{-1}, $\times 10^{-4}$) in normal (control) and diabetic rats

	Control	Diabetic
0 mM $HgCl_2$		
Proestrus	10.8 ± 0.9 (n = 11)	6.5 ± 0.7* (n = 33)
Estrus	7.1 ± 1.1 (n = 11)	7.3 ± 1.7 (n = 10)
0.3 mM $HgCl_2$		
Proestrus	6.0 ± 1.0 (n = 8)	5.6 ± 0.7 (n = 7)
Estrus	8.1 ± 1.0 (n = 8)	6.2 ± 1.2 (n = 6)

P_{osm} in proestrus oocytes from diabetic rats was significantly lower than that in control rats, *$P < 0.001$. Additionally, in diabetic oocytes no $HgCl_2$ sensitivity was observed.

meability and aquaporin mRNA expression in mammalian oocytes. Our functional studies show that water permeability in rat oocytes was reduced during their arrival to maturity. Oocytes P_{osm} values also decrease during in vitro incubation (between 4 and 6 hr). Therefore, our experiments exhibited a clear parallelism between in vivo and in vitro maturation.

It is generally accepted that reversible inhibition of osmotic water permeability (P_{osm}) with mercurial agents strongly indicates the presence of water channels (aquaporins) in cell membranes (Zhang et al., 1993; Ibarra et al., 1994). In this work, we demonstrated that in proestrus oocytes (immature) P_{osm} could be inhibited by $HgCl_2$. It is then concluded that a water channel disappeared from the oocyte membrane during the process of maturation and this would explain the difference observed in water permeability. To test this hypothesis RT-PCR experiments were performed. The obtained results show the presence of mRNA corresponding to a specific water channel, AQP9, that was recently identified (Tsukaguchi et al., 1998; Ko et al., 1998). Rat AQP9 (rAQP9) was described as a broad selective-neutral solute channel and was detected in brain, lung and testis with the highest levels occurring in liver. This protein allows the passage of water and a wide variety of neutral solutes (i.e., urea, glycerol, mannitol) in a mercury and phloretin-sensitive manner. A human homologue (hAQP9) was described by Ishibashi (1998), but its permeability was found to be restricted to water and urea. Recently, Tsukaguchi et al. (1999) reported a reevaluation of the functional characteristics of hAQP9, and demonstrated that this protein allowed the passage of the same solutes as rAQP9. Our functional results showed that proestrus oocyte water permeability was sensitive to mercury and phloretin. Moreover, when proestrus oocytes were transferred to a PBS solution containing mannitol (iso- or hypo-osmotic) volume rapidly changed indicating mannitol entry. This phenomenon was not observed in estrus oocytes. Sensitivity to mercury and phloretin, water and mannitol permeation and AQP9 mRNA expression, altogether support the presence of AQP9 in immature but not in mature oocytes. The disappearance of AQP9 mRNA at later developmental stages was also observed by Tsukaguchi (1998) in spermatocytes, indicating a parallelism in AQP9 mRNA expression between female and male germinal cells.

We can speculate that AQP9 expression in immature stages would be necessary during oocyte growth. At the moment of ovulation the loss of AQP9 may serve as a defense mechanism to protect the delicate oocyte from the acute changes that occur during follicular wall disruption.

There is no available information about the characteristics of oocyte water permeability in pathological conditions like diabetes mellitus. This disease is characterized by several hydrosaline disorders like severe dehydratation, metabolic acidosis, osmotic stress and alterations of the reproductive function linked to a reduction in the number of matured oocytes (Bitar, 1997; Vescla et al., 1995; Vomatchka & Johnson, 1982). Our functional studies show that proestrus oocytes from streptozotocin-induced diabetic rats, had a lower P_{osm} (not sensitive to $HgCl_2$) than those obtained in normal rats. Surprisingly, AQP9 mRNA was detected in diabetic immature oocytes. To explain these results different alternatives can be proposed: (i) the protein traduction could be altered; (ii) the protein could be present but it might be not functional; or (iii) the protein could retain its capacity to move solutes but not water. This last possibility was demonstrated by Zeuthen and Klaerke (1999) who reported that AQP3 (that belongs to the same subfamily as AQP9) acts as a glycerol and water channel at physiological pH, but predominantly as a glycerol channel at pH around 6.1. Moreover, Tsukaguchi et al. (1998) observed upregulation of AQP9 mRNA in the liver of rats exposed to hypertonic conditions (streptozotocin-treated diabetic rats). They proposed that since AQP9 confers a broad selective aqueous pore, regulation of AQP9 may facilitate secretion of metabolites such as ketone bodies and protect cells from damage caused by unwanted volume changes.

Future experiments will clarify the link between expression and function of oocyte water channels in normal and pathological conditions.

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