# Human oocytes express ATP-sensitive K+ channels

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# Human oocytes express ATP-sensitive $K^+$ channels

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**BACKGROUND:** ATP-sensitive $K^+$ ($K_{ATP}$) channels link intracellular metabolism with membrane excitability and play crucial roles in cellular physiology and protection. The $K_{ATP}$ channel protein complex is composed of pore forming, Kir6.x (Kir6.1 or Kir6.2) and regulatory, SURx (SUR2A, SUR2B or SUR1), subunits that associate in different combinations. The objective of this study was to determine whether mammalian oocytes (human, bovine, porcine) express $K_{ATP}$ channels.

**METHODS:** Supernumerary human oocytes at different stages of maturation were obtained from patients undergoing assisted conception treatments. Bovine and porcine oocytes in the germinal vesicle (GV) stage were obtained by aspirating antral follicles from abattoir-derived ovaries. The presence of mRNA for $K_{ATP}$ channel subunits was determined using real-time RT–PCR with primers specific for Kir6.2, Kir6.1, SUR1, SUR2A and SUR2B. To assess whether functional $K_{ATP}$ channels are present in human oocytes, traditional and perforated patch whole cell electrophysiology and immunoprecipitation/western blotting were used.

**RESULTS:** Real-time PCR revealed that mRNA for Kir6.1, Kir6.2, SUR2A and SUR2B, but not SUR1, were present in human oocytes of different stages. Only SUR2B and Kir6.2 mRNAs were detected in GV stage bovine and porcine oocytes. Immunoprecipitation with SUR2 antibody and western blotting with Kir6.1 antibody identified bands corresponding to these subunits in human oocytes. In human oocytes, 2,4-dinitrophenol (400 μM), a metabolic inhibitor known to decrease intracellular ATP and activate $K_{ATP}$ channels, increased whole cell $K^+$ current. On the other hand, $K^+$ current induced by low intracellular ATP was inhibited by extracellular glibenclamide (30 μM), an oral antidiabetic known to block the opening of $K_{ATP}$ channels.

**CONCLUSIONS:** In conclusion, mammalian oocytes express $K_{ATP}$ channels. This opens a new avenue of research into the complex relationship between metabolism and membrane excitability in oocytes under different conditions, including conception.

**Key words:** oocytes / ATP-sensitive $K^+$ channels / Kir6.1/2 / SUR2A/B / membrane potential

## Introduction

ATP-sensitive $K^+$ ($K_{ATP}$) channels were originally described in cardiomyocytes (Noma, 1983), where they perform a cardioprotective role during metabolic stress. Physiological (high) levels of intracellular ATP maintain the channels in a closed state. However, upon a fall in intracellular ATP concentration, such as during hypoxia, the channels are released from inhibition, causing hyperpolarization of membrane potential. This hyperpolarization inhibits voltage operated $Ca^{2+}$ channels and prevents intracellular $Ca^{2+}$ overload (reviewed by Jovanović and Jovanović, 2009). $K_{ATP}$ channels are also expressed in pancreatic β-cells and smooth muscle cells, where they play crucial physiological roles. Pancreatic $K_{ATP}$ channels control insulin secretion, whereas in smooth muscle cells they regulate muscular tone.

Structurally, $K_{ATP}$ channels are composed of pore-forming inward rectifiers, Kir6.1 or Kir6.2, and regulatory, ATP-binding subunits, SUR1, SUR2A or SUR2B. It is generally accepted that four Kir6.x and four SURx are physically associated with each other to form a functional $K_{ATP}$ channel. The properties of these channels are different in various tissues due to the combinations of the subunits forming the channel. SUR1 and Kir6.2 form the pancreatic type of $K_{ATP}$ channels, SUR2A and Kir6.2 form the cardiac type of $K_{ATP}$ channels, and SUR2B and Kir6.1 form the vascular smooth muscle type of $K_{ATP}$ channels and

others (reviewed by Nichols, 2006). There are studies suggesting that further diversity may be generated by a combination of more than one type of Kir6.x and SURx subunits within an individual channel (Shyng and Nichols, 1997; Cui *et al*., 2001; Chan *et al*., 2008).

In any tissue where expressed, $K_{ATP}$ channels regulate membrane potential and, by regulating membrane potential, regulate intracellular $Ca^{2+}$ levels (reviewed by Nichols, 2006). It is well established that dynamics of the intracellular $Ca^{2+}$ in oocytes play a crucial role in fertilization and embryo development, including oocyte maturation. Research so far has been focused on intracellular factors and signalling molecules that regulate intracellular $Ca^{2+}$ dynamics, but there has been little focus on the membrane events (reviewed by Halet *et al*., 2003; Jones, 2005; Machaca, 2007). $K_{ATP}$ channels couple intracellular metabolism with membrane excitability and intracellular $Ca^{2+}$ signalling (reviewed by Nichols, 2006). The presence of $K_{ATP}$ channels in oocytes could play crucial roles in oocyte function, including fertilization and early embryonic events, and may provide protection during metabolic stress. Surprisingly, their presence in mammalian oocytes has never been elucidated. Here we describe the first step towards a better understanding of $K_{ATP}$ channels in mammalian (including human) oocytes, demonstrating both the expression of Kir6.x and SURx subunits and the presence of functional, ATP-regulated $K^+$ conductances in these cells.

# Materials and Methods

## Human, bovine and porcine oocytes

Supernumerary human oocytes that would otherwise have been discarded as a part of routine assisted conception treatments were used in these experiments. The oocytes were at different stages of maturity, namely germinal vesicle stage (GV), metaphase I (MI) and metaphase II (MII). Oocytes were collected from healthy women undergoing assisted reproduction treatment at Ninewells Assisted Conception Unit, Dundee, Scotland (HFEA centre # 0004). Ethical approval was provided by NHS Tayside Research Ethics committee (number 08/S1402/23).

Oocytes were donated for research at GV stage, MI and MII, and had been kept in culture in the clinical programme for varying lengths of time to comply with the constraints of the clinical programme. GV oocytes were characterized by the presence of the GV. MI oocytes had no GV and no polar body. MII oocytes were characterized by the presence of the first polar body. In ICSI treatment cycles, following injection of the mature MII oocytes, the uninjected immature oocytes were donated for research on Day 0, Day 1 or up to Day 5. By the time of use they may have matured further *in vitro*. Oocytes which had been inseminated but had failed to fertilize in both IVF and ICSI treatment cycles were donated to research on Day 2 to Day 5, which is a minimum of 24 and a maximum of 96 h in culture following assessment of fertilization. These were a mixture of GV, MI and MII; some of them had further matured *in vitro*. From the point of assessment of fertilization to being donated for research, all oocytes were cultured in 10 μl drops of cleavage medium under oil at 37°C in an atmosphere of 6% $CO_2$ in air. Oocytes which had failed to fertilize were defined as those showing no pronuclei and no cleavage.

Bovine and porcine oocytes were obtained by aspirating follicular fluid from large antral follicles of abattoir derived ovaries. Oocyte–cumulus complexes were retrieved and placed in Leibovitz medium containing 600 mIU/ml of hyaluronidase (Sigma Chemicals) to remove cumulus cells. Denuded GV intact oocytes were washed three times before being snap frozen for RNA extraction.

## Real-time RT–PCR

For real-time RT–PCR, total RNA was extracted from pooled oocytes (12 human oocytes in different maturation stages, ~35 bovine oocytes or ~35 porcine oocytes all GV intact) using Absolutely RNA Nanoprep kit (Stratagene, La Jolla, CA, USA) and RNeay plus Micro Kit (Qiagen, Crawley, UK) for human and bovine/porcine oocytes respectively, according to the manufacturer's instructions. RNA from human skeletal muscle, which is the tissue expressing all five subunits of $K_{ATP}$ channels (Jovanović *et al*., 2008), was used as a positive control (RNA was prepared as described in Jovanović *et al*., 2008). For bovine and porcine oocytes, the positive control was RNA from heart embryonic H9C2 cells; these cells also express all five $K_{ATP}$ channel subunits (Jovanović *et al*., 2009a,b). For human oocytes, the specific primers for human $K_{ATP}$ channel subunits (SUR2A, Kir6.2, SUR2B, SUR1 and Kir6.1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; as a control gene) were the same ones as previously described (Jovanović *et al*., 2008). For bovine and porcine oocytes, the full sequences of $K_{ATP}$ channel subunits are not yet known, so primers were designed taking into account the most conservative regions of subunits sequences in mammals with known $K_{ATP}$ channel subunits sequences. The primers were as follows: for SUR1, sense, 5'-TCACACCGCTGTTCCTGCT-3', antisense, 5'-AGAAGGAGCGAG GACTTGCC-3', 412 bp was the size of the PCR product; for SUR2A, sense, 5'-CTGGCTTTCTTCAGAATGGT-3', antisense, 5'-AAATACCC TCAGAAAAGACTAAAAC-3', 500 bp was the size of the PCR product; for SUR2AB (SUR2A+SUR2B together), sense, 5'-CATTGCC TACTTATTTCTCTCAG-3', antisense, 5'-ACCATTCTGAAGAAAGC CAG-3', 474 bp was the size of the PCR product; for Kir6.1, sense, 5'-CTGGCTGCTCTTCGCTATC-3', antisense, 5'-AGAATCAAAACCG TGATGGC-3', 234 bp was the size of the PCR product; for Kir6.2, sense, 5'-CCAAGAAAGGCAACTGCAACG-3', antisense, 5'-ATGCTT GCTGAAGATGAGGGT-3', 449 bp was the size of the PCR product; for GAPDH, sense, 5'-GTCTTCACCACCATGGAGAA-3', antisense, 5'-TTCACCACCTTCTTGATGTCA-3', 488 bp was the size of the PCR product. Reverse transcription and whole transcriptome amplification for individual samples were achieved with the QuantiTect whole transcriptome kit (Qiagen, Valencia, CA, USA). RNA was first transcribed to cDNA using T-Script reverse transcriptase, and then the cDNA was ligated using a high-efficiency ligation mix and then amplified using REPLI-g. The resulting cDNA was used as a template for real-time PCR. An SYBR Green I system was used for the RT–PCR and the 25-μl reaction mixture contained: 12.5 μl of iQ™ SYBR® Green Supermix (2 ×), 7.5 nM each primers, 9 μl of $ddH_2O$ and 2 μl of cDNA. In principle, the thermal cycling conditions were as follows: an initial denaturation at 95°C for 3 min, followed by 40 cycles of 10 s of denaturing at 95°C, 15 s of annealing at 56°C and 30 s of extension at 72°C. This protocol was modified for the m-lactic dehydrogenase-, GAPDH- and CK-specific primers changing the extension temperature to 55°C. The real-time PCR was performed in the same wells of a 96-well plate in the iCycler iQ™ Multicolor Real-Time Detection System (BioRad, Hercules, CA, USA). Data were collected following each cycle and displayed graphically (iCycler iQ™ Real-time Detection System Software, version 3.0A, BioRad). Primers were tested for their ability to produce no signal in negative controls by dimer formation and then with regard to the efficiency of the PCR. Efficiency is evaluated by the slope of the regression curve obtained with several dilutions of the cDNA template. Melting curve analysis tested the specificity of primers. Threshold cycle values, PCR efficiency (examined by serially diluting the template cDNA and performing PCR under these conditions) and PCR specificity (by constructing the melting curve) were determined by the same software. Each cDNA sample was duplicated; the corresponding no-RT mRNA sample was included as a negative control (blank).

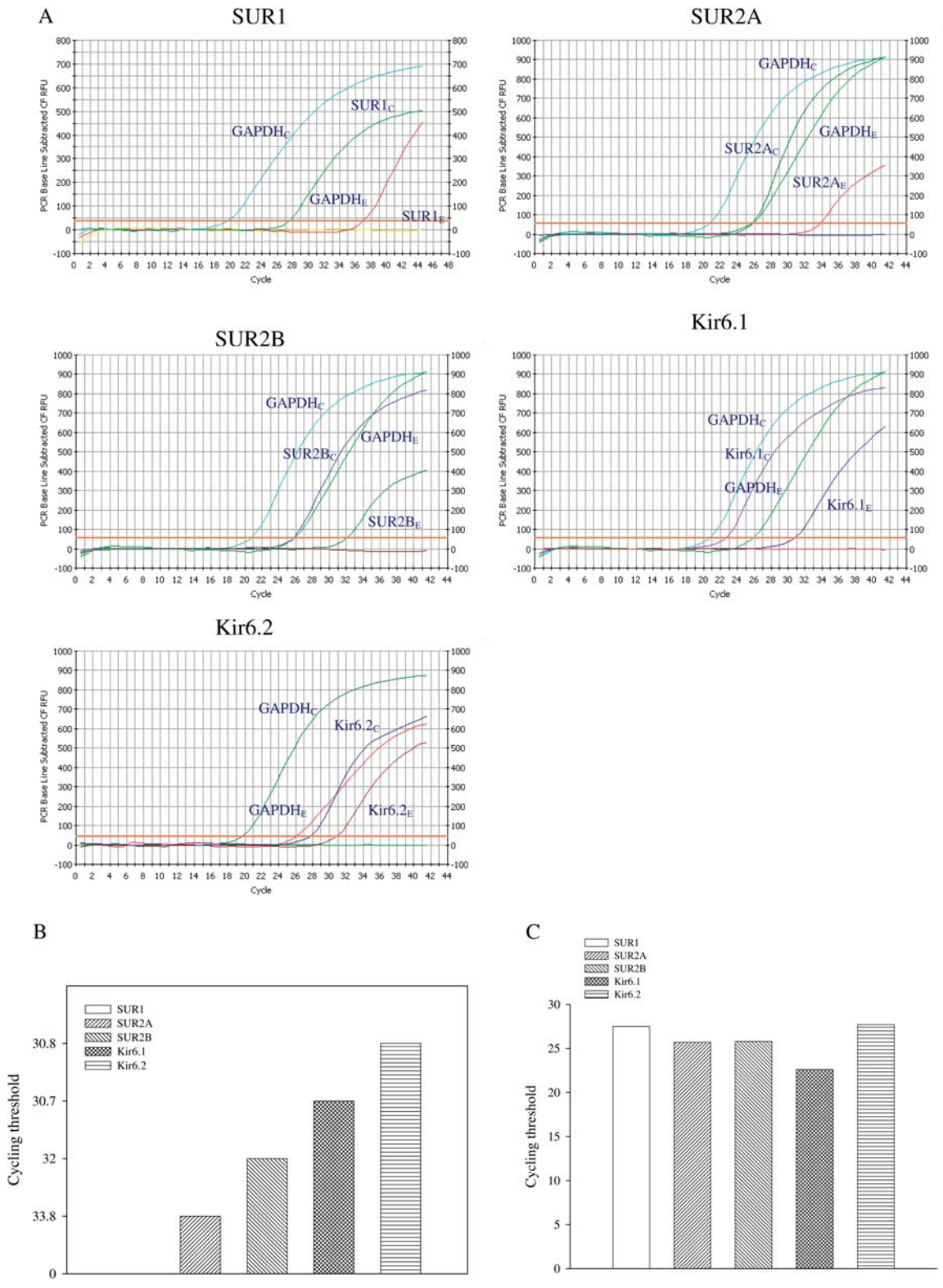


**Figure 1** $K_{ATP}$ channel subunits mRNAs in human oocytes. (**A**) Original progress curves for the real-time PCR amplification of SUR1, SUR2A, SUR2B, Kir6.1 and Kir6.2 cDNA in human oocytes. Curves labelled with subscripts C and E correspond to control and oocyte curves respectively. (**B** and **C**) Bar graphs showing cycle threshold for the real-time PCR amplification of SUR1, SUR2A, SUR2B, Kir6.1 and Kir6.2 from human oocytes (B) and human skeletal muscle (C) that served as a positive control. Absence of a bar pattern in graphs that is depicted in symbols means that no PCR product was obtained for this particular gene.

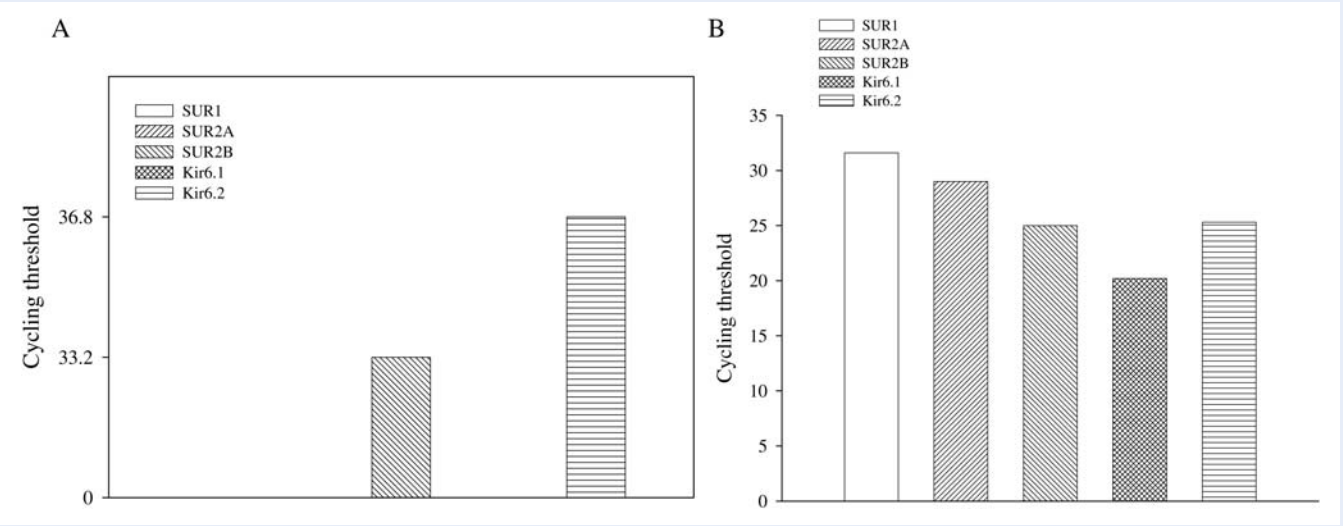


**Figure 2** $K_{ATP}$ channel subunits mRNAs in bovine oocytes. Bar graphs showing cycle threshold for the real-time PCR amplification of SUR1, SUR2A, SUR2B, Kir6.1 and Kir6.2 from bovine oocytes (**A**) and H9C2 cells (**B**) that served as a positive control. Absence of a bar pattern in graphs that are depicted in symbols means that no PCR product was obtained for this particular gene.

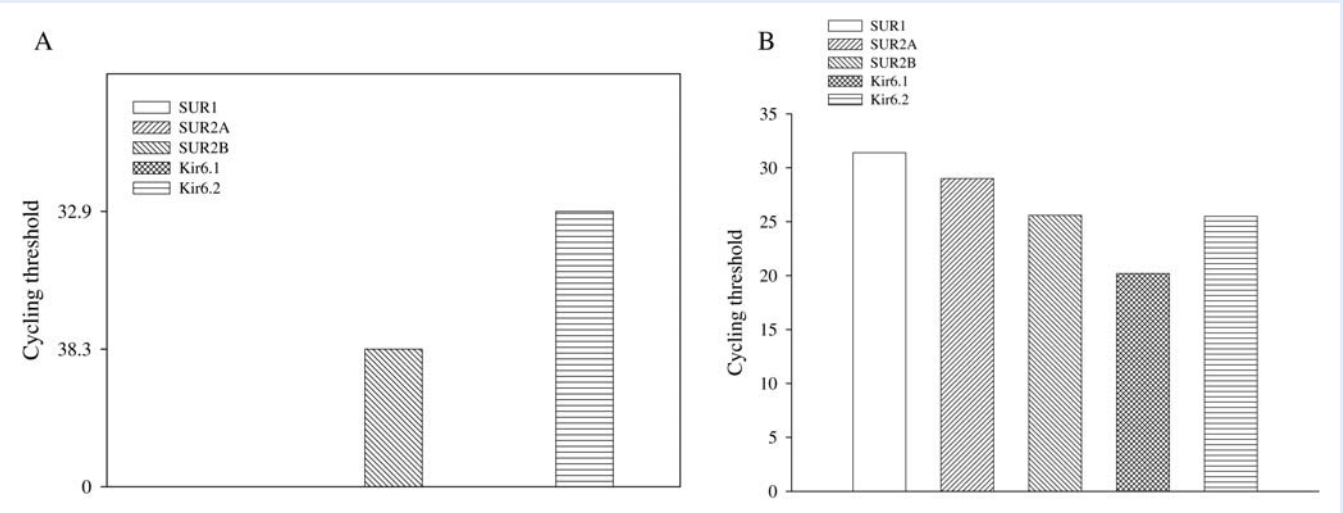


**Figure 3** $K_{ATP}$ channel subunits mRNAs in porcine oocytes. Bar graphs showing cycle threshold for the real-time PCR amplification of SUR1, SUR2A, SUR2B, Kir6.1 and Kir6.2 from porcine oocytes (**A**) and H9C2 cells (**B**) that served as a positive control. Absence of a bar pattern in graphs that are depicted in symbols means that no PCR product was obtained for this particular gene.

## Immunoprecipitation/western blotting

Sheep anti-SUR2 and anti-Kir6.1 antibodies were used for immunoprecipitation and western blotting. To obtain sufficient amounts of oocyte proteins, we pooled 87 human oocytes (in GV, MI and MII stages) in buffer containing 1% Triton X-100 (w/w), 5 mM Tris, pH 7.4, 1 mM EDTA, 1 mM EGTA and SigmaFAST-TM protease inhibitor cocktail. The buffer was concentrated using Vivaspin 500 columns (Sartorius Stedium Biotech). Ten micrograms of the epitope-specific SUR2 antibody (an antibody that recognizes both SUR2A and SUR2B subunits) were pre-bound to Protein-G Sepharose beads and used to immunoprecipitate from protein extract. The pellets of this precipitation were run on SDS polyacrylamide gels for western analysis. Western blot probing was performed using 1/500 dilution of anti-Kir6.1 antibody and detection was achieved using Protein-G HRP and ECL reagents. Cells that do not natively express $K_{ATP}$ channels (A549 cells; Crawford *et al.*, 2002b; Jovanović *et al.*, 2003) were processed as described for oocytes and used as a control.

## Patch clamp electrophysiology

Before patch clamp experiments, the zona pellucida was removed as described in Lai *et al.* (1994). In total, seven oocytes were patched. Experiments were performed while oocytes were immersed in Tyrode solution (in mM: 136.5 NaCl; 5.4 KCl; 1.8 $CaCl_2$; 0.53 $MgCl_2$; 5.5 glucose; 5.5 HEPES–NaOH; pH 7.4). For standard whole cell recording (where pipette solution replaces the cytosol), pipettes (resistance 3–5 MΩ) were filled with (in mM): KCl 140, $MgCl_2$ 1, ATP 0.01, EGTA–KOH 5,

HEPES–KOH 5 (pH 7.3). For experiments with 2,4-dinitrophenol (DNP), we used the perforated patch clamp electrophysiology (where the intracellular milieu remains intact). For this method the same pipette solution was used except with ATP omitted and amphotericin B (Sigma, 240 μg/ml) added. The membrane potential was normally held at −40 mV and the currents were evoked by a series of 400 ms depolarizing and hyperpolarizing current steps (−100 mV to +80 mV in 20 mV steps) recorded directly to hard disk using an Axopatch-200B amplifier, Digidata-1321 interface and pClamp8 software (Axon Instruments, Inc., Forster City, CA, USA).

### Statistical analysis

Data obtained by patch clamp electrophysiology are presented as mean ± SEM, with *n* representing the number of experiments. Mean values were compared using the paired or unpaired *t*-test, where appropriate. $P < 0.05$ was considered statistically significant.

## Results

### mRNA of $K_{ATP}$ channel subunits in human, bovine and porcine oocytes

We have examined the presence of $K_{ATP}$ channel subunits in human, bovine and porcine oocytes using real-time RT–PCR. In each run, there were positive controls with RNA from either human skeletal muscle (for human oocytes) or H9C2 cells (for bovine and porcine oocytes) and the technical soundness of the experiments was verified by the presence of GAPDH mRNA. In human oocytes, real-time RT–PCR revealed that SUR1 is not expressed whereas SUR2A, SUR2B, Kir6.1 and Kir6.2 mRNAs were detected in human oocytes (Fig. 1). In bovine GV stage oocytes, it was found that SUR2B and Kir6.2 subunits are expressed, but there was no evidence of the presence of SUR1, SUR2A and Kir6.1 (Fig. 2). Similar findings were obtained with porcine GV stage oocytes (Fig. 3).

### Kir6.1 protein is associated with SUR2A/SUR2B proteins in human oocytes

SUR2A/B immunoprecipitate was obtained from pooled human oocytes. Western blotting of this precipitation with anti-Kir6.1 antibody has revealed a signal that was just below 50 kDa (Fig. 4). This signal was absent in A549 cells that do not natively express $K_{ATP}$ channels. The size of Kir6.1 is 47 kDa, and as such the signal is where it is expected for this subunit, showing both that Kir6.1 protein is expressed and that it is associated with SUR2A/B subunits, consistent with the formation of functional $K_{ATP}$ channels.

### Whole cell $K^+$ current in human oocytes

To confirm the presence of functional $K_{ATP}$ channels in human oocytes, we have performed patch clamp experiments. $K_{ATP}$ channels are activated by a decrease in intracellular ATP levels. Using the perforated patch clamp technique, where the oocytes are dependent upon endogenous generation of ATP, we exposed oocytes to 400 μM of DNP, a metabolic inhibitor known to decrease intracellular ATP and activate $K_{ATP}$ channels (Jovanović *et al.*, 2009a,b). DNP (400 μM) increased the whole cell $K^+$ current (Fig. 5A, $n = 4$). The difference between $K^+$ currents in the presence and absence of DNP (400 μM) was statistically significant (Fig. 5A1; current at 80 mV was 901 ± 93 pA in the absence and 1718 ± 236 pA in the presence of DNP, $P = 0.04$, $n = 4$). We further examined the effect of reduced cytoplasmic (ATP) by using standard whole cell recording (conditions where the pipette saline replaces the cytoplasm) with a pipette solution containing only 10 μM ATP. This low concentration of ATP does not inhibit $K_{ATP}$ channel opening, but is sufficient to prevent a channel 'run-down'. The current recorded under these conditions (current at 80 mV = 1628 ± 178 pA; $n = 3$) was significantly greater than the current recorded by the perforated patch clamp technique, when intracellular ATP was at its physiological level (current at 80 mV = 901 ± 93 pA; $n = 4$, $P = 0.01$; Fig. 5). This whole cell $K^+$ current observed with low intracellular ATP was inhibited by extracellular glybenclamide (30 μM), an oral antidiabetic known to block the opening of $K_{ATP}$ channels (Fig. 5B; $n = 3$, $P = 0.01$).

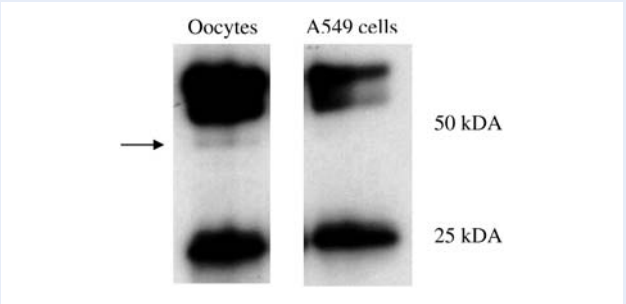


**Figure 4** Kir6.1 protein that physically associates with SUR2A and/or SUR2B in human oocytes. A western blot of anti-SUR2 immunoprecipitate from human oocytes and A549 cells (cells that do not express $K_{ATP}$ channels natively) with anti-Kir6.1 antibody under depicted conditions. The arrow indicates a signal corresponding to Kir6.1.

## Discussion

Human oocytes express four out of five known $K_{ATP}$ channel subunits, strongly suggesting that human oocytes express $K_{ATP}$ channels. $K_{ATP}$ transcripts are also present in oocytes of other mammalian species and in human oocytes the Kir6.1 protein is present and is associated with SUR2A/B (demonstrated by immunoprecipitation/western blotting). The presence in human oocytes of transcripts encoding more subunits than required to form functional $K_{ATP}$ channels in a single cell type is not unusual. In cardiomyocytes, there is mRNA of all five $K_{ATP}$ channel subunits (Du *et al.*, 2006). Originally, it was suggested that SUR2A and Kir6.2 alone form $K_{ATP}$ channels in these cells (Inagaki *et al.*, 1996), but more recent research would suggest that all of the expressed subunits could be a part of sarcolemmal $K_{ATP}$ channels (Cui *et al.*, 2001; Chan *et al.*, 2008). The presence of mRNA of four subunits in human oocytes could indicate that $K_{ATP}$ channels in these cells are composed of more than just a single SURx and Kir6.x subunit type. Since human oocytes at different stages of maturation were pooled, the presence of mRNAs for four subunits may reflect a change in the level of expression of different $K_{ATP}$ channel subunits in oocytes during maturation. This is consistent with the findings that pure populations of bovine and porcine GV oocytes expressed only SUR2B and Kir6.2 mRNAs. These results could imply that $K_{ATP}$ channels in mammalian

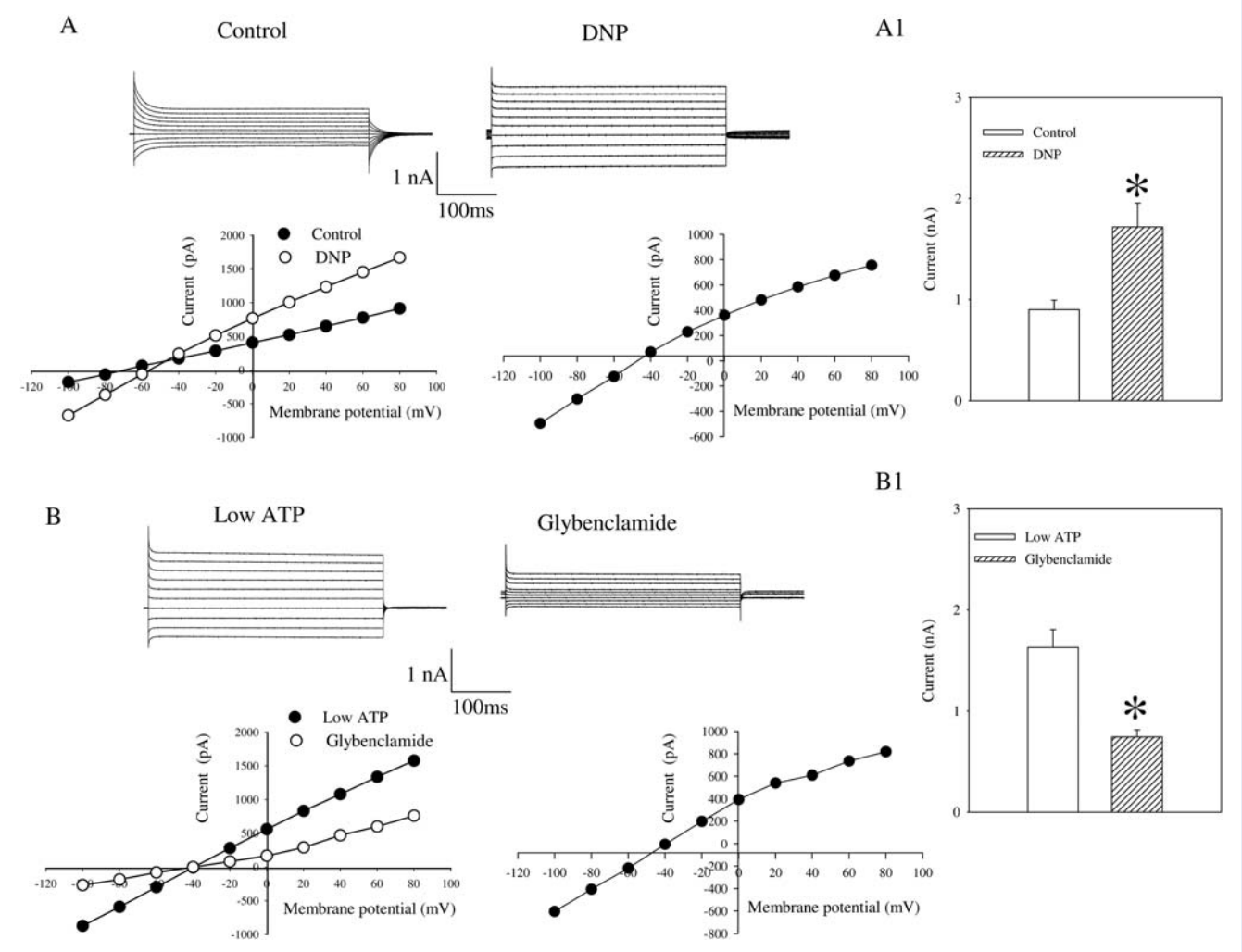


**Figure 5** Whole cell $K^+$ current in human oocytes in response to procedures that alter intracellular ATP levels. (**A**) Original superimposed membrane currents in a human oocyte in the absence (control) and presence (DNP) of DNP (400 μM), and corresponding I–V relationship (left graph) and DNP-sensitive component of the current (right graph; DNP-sensitive component of the current was obtained by digital subtraction of the current in the presence and absence of DNP). (**A1**) Bar graph showing whole cell current at 80 mV in the absence and presence of DNP (400 μM). Bars are mean ± SEM ($n = 4$). *$P < 0.05$. (**B**) Original superimposed membrane currents in a human oocyte in the absence (low ATP) and presence (glybenclamide) of glybenclamide (30 μM), and corresponding I–V relationship (left graph) and glybenclamide-sensitive component of the current (right graph; glybenclamide-sensitive component of the current was obtained by digital subtraction of the current in the absence and presence of glybenclamide). (**B1**) Bar graph showing whole cell current at 80 mV in the absence and presence of glybenclamide (30 μM). Bars are mean ± SEM ($n = 3$). *$P < 0.05$.

oocytes change their composition from the SUR2B/Kir6.2 combination at the GV stage (as these subunits were detected in all three species that provided GV oocytes) into SUR2A/Kir6.1 (as these subunits are detected only in human oocytes where MI and MII oocytes were present). Such changes in $K_{ATP}$ channel subunit expression occur in response to changing physiological conditions; e.g. in ischaemically stressed heart cells there is up-regulation of Kir6.1, which apparently replaces Kir6.2 in the sarcolemmal $K_{ATP}$ channel protein complex (Akao *et al.*, 1997). The electrophysiological and regulatory properties of SUR2B/Kir6.2 and SUR2A/Kir6.1 channel types are different (reviewed by Nichols, 2006) and changing expression would have effects on the membrane potential and intracellular $Ca^{2+}$ as the oocyte matures (summarized in Fig. 6).

To date, the presence of transcripts for $K_{ATP}$ channel regulatory and pore-forming subunits has always been associated with the presence of functional $K_{ATP}$ channels in the plasma membrane (Akrouh *et al.*, 2009). We have used immunoprecipitation/western blotting to show that there is a population of Kir6.1 proteins that physically associate with SUR2A and/or SUR2B which are present in human oocytes, as this methodology is suitable to assess the presence of fully assembled $K_{ATP}$ channels in human tissue (Jovanović *et al.*, 2008). To confirm the functional status of such channels in human oocytes, we applied patch clamp electrophysiology. The defining attribute of $K_{ATP}$ channels is their regulation by intracellular ATP levels (Noma, 1983). It is well established that DNP, an uncoupler of oxidative phosphorylation, activates $K_{ATP}$ channels in all cell types

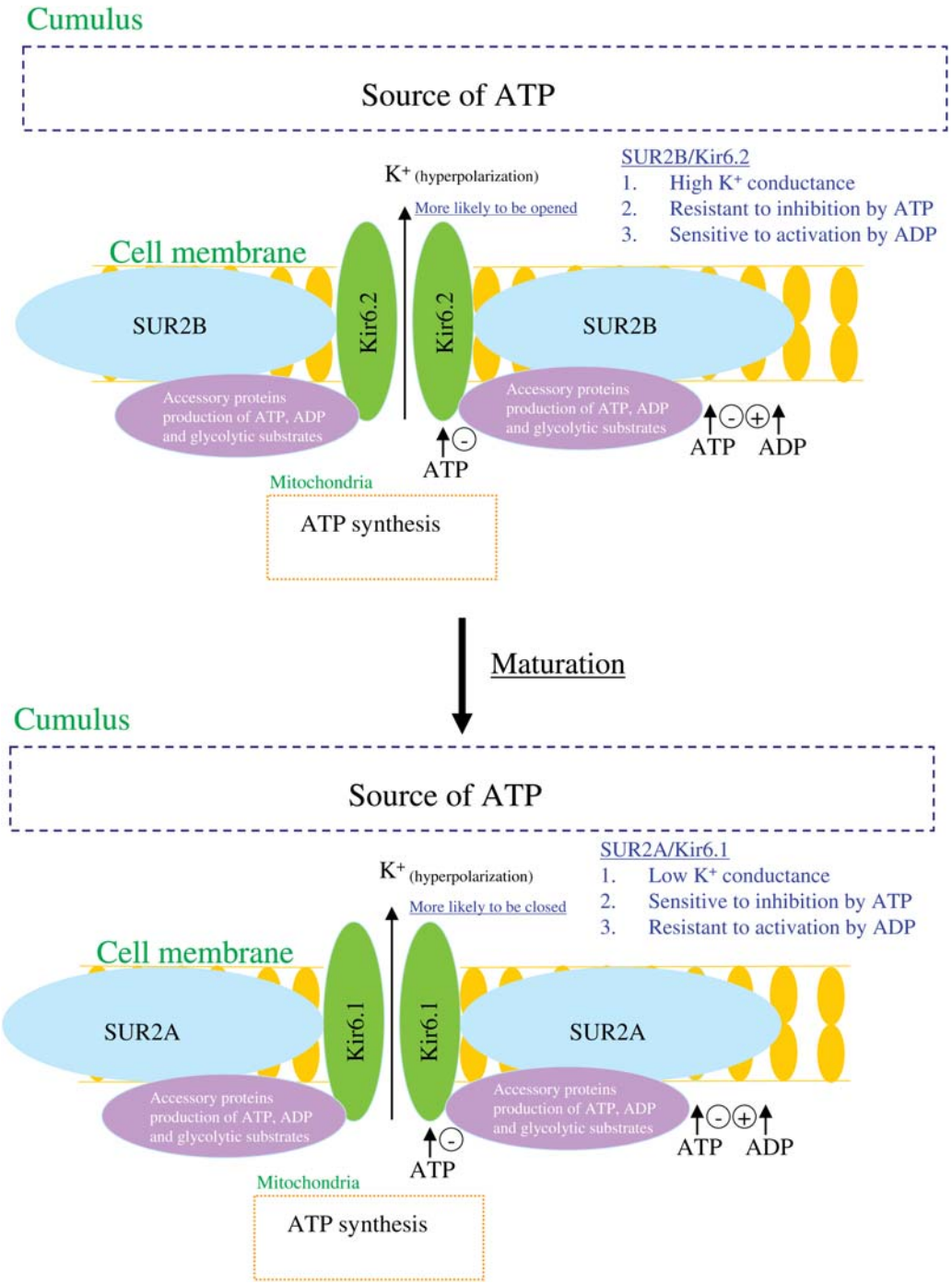


**Figure 6** Cartoon summarizing possible structure, regulation and function of $K_{ATP}$ channels in human oocytes based on the findings from the present study as well as findings from previous studies that have investigated $K_{ATP}$ channels in other cell types and the physiology of oocytes (Carrasco *et al.*, 2001; Crawford *et al.*, 2002a,b; Tosti and Boni, 2004; Jovanović *et al.*, 2005, 2009a,b; Nichols, 2006; Van Blerkom *et al.*, 2008; Van Blerkom, 2009).

tested so far (Jovanović and Jovanović, 2001a,b; Jovanović *et al.*, 2009a). It is also well known that the oral antidiabetic drug glybenclamide inhibits the activity of all known types of $K_{ATP}$ channel (reviewed by Akrouh *et al.*, 2009). In the present study, DNP induced a large, whole cell current which reversed close to $E_K$, which is a typical finding when cells express $K_{ATP}$ channels (Jovanović *et al.*, 2009b). In cells without $K_{ATP}$ channels or where the channel protein complex has been disrupted, DNP does not have this effect (Jovanović and Jovanović, 2001a; Du *et al.*, 2010). Maintenance of low intracellular ATP by using the whole cell patch clamp configuration (pipette

saline containing 10 μM ATP) was also associated with increased whole cell $K^+$ current. This current was glybenclamide-sensitive, further supporting the notion that functional $K_{ATP}$ channels are present in the plasma membrane of human oocytes. Both DNP-induced and glybenclamide-sensitive $K^+$ currents exhibited a reversal potential consistent with $K^+$ conductance and showed weak inward rectification (Fig. 5), a property typical of $K_{ATP}$ channels. Our results, using different, but complementary techniques, suggest that oocytes express functional $K_{ATP}$ channels in the plasma membrane.

As this is the first report to uncover the presence of $K_{ATP}$ channels in oocytes, their possible role is still elusive. In some tissues, $K_{ATP}$ channels are physiologically open, while in others they are normally closed and are activated by a specific signalling pathway (reviewed by Minimi *et al*., 2004). In cell types where $K_{ATP}$ channels are physiologically closed, their opening is associated with clamping membrane potential at the value of $K^+$ equilibrium and counteracting membrane depolarization. In turn, this prevents an increase in intracellular $Ca^{2+}$ if it is driven by $Ca^{2+}$ influx via L-type $Ca^{2+}$ channels (Jovanović and Jovanović, 2001a,b). On the other hand, in cell types where $K_{ATP}$ channels are physiologically open, inhibition of these channels induces membrane depolarization, $Ca^{2+}$ influx and increase in intracellular $Ca^{2+}$. At the moment, we cannot draw a definite conclusion whether $K_{ATP}$ channels are normally closed or open in oocytes, but the increase of $K^+$ current by DNP would favour the idea that these channels were closed in the cell population we tested. In oocytes, the dynamics of intracellular $Ca^{2+}$ concentration is crucial for regulation of oocyte maturation and fertilization (review by Tosti and Boni, 2004). Oocyte development and maturation in mammals are associated with changes in membrane potential and $Na^+$ and $K^+$ permeance ratio; the membrane potential of human and other mammals immature oocytes is approximately −30 to 40 mV and is suggested to decrease to a potential of −15 to 25 mV as the maturation process progresses (Eusebi *et al*., 1984; Feichtinger *et al*., 1988; Mattioli *et al*., 1990; Emery *et al*., 2001; Boni *et al*., 2008). At fertilization, an increase of intracellular $Ca^{2+}$ has been reported which is associated with membrane hyperpolarization. As $Ca^{2+}$ oscillations are synchronized with episodes of membrane hyperpolarization, it has been suggested that $Ca^{2+}$-sensitive $K^+$ channels are responsible for this phenomenon (Miyazaki and Igusa, 1982; Dale *et al*., 1996; reviewed by Tosti and Boni, 2004). The membrane potential of oocytes was shown to be regulated by cumulus that also provides ATP to oocytes and regulates polarity of suboolemmal mitochondria (Albertini *et al*., 2001; Emery *et al*., 2001; Van Blerkom *et al*., 2008; reviewed by Van Blerkom, 2009). It is therefore quite possible that there is a link between ATP levels and regulation of membrane potential in oocytes. If this is the case, it is logical to think that $K_{ATP}$ channels could provide this link. The physical proximity of mitochondria as a source of ATP and $K_{ATP}$ channels is probably crucial in regulating $K_{ATP}$ channels activity, as it has been shown that the level of $K_{ATP}$ channel ligands surrounding the channel is much more important for the channel activity than the overall intracellular concentration of these ligands (Elvir-Mairena *et al*., 1996). Suboolemmal mitochondrias could regulate the probability of the channel opening by feeding ATP into the $K_{ATP}$ channel protein complex that, besides channel subunits, contain enzymes utilizing ATP as a substrate. Specifically, $K_{ATP}$ channel subunits physically associate with adenylate kinase, creatine kinase and five glycolytic enzymes, and substrates of these enzymes and their products (ATP, ADP, AMP, lactate, 1,3-bisphosphoglycerate) serve as $K_{ATP}$ channel ligands that can both open and close the channel (Carrasco *et al*., 2001; Crawford *et al*., 2002a,b; Jovanović and Jovanović, 2005; Jovanović *et al*., 2005). At the same time, the $K_{ATP}$ channel protein complex can also produce ATP itself (Jovanović *et al*., 2009a,b; Du *et al*., 2010) and provide substrates for suboolemmal mitochondria (Fig. 6). Thus, $K_{ATP}$ channels could transduce metabolic changes during maturation and fertilization into changes in membrane excitability and intracellular $Ca^{2+}$ dynamics.

## Conclusion

In conclusion, this study has shown that human, bovine and porcine oocytes express $K_{ATP}$ channels. The property of these channels to transduce metabolic changes into changes in membrane excitability supports the notion that $K_{ATP}$ channels could play important roles in oocyte maturation and fertilization.

## Authors' roles

All authors have contributed to the study design, revised and approved the manuscript. In addition to that, E.B., E.D., K.M.W., V.K., M.M. and E.E.T. were involved in oocyte collection, while Q.D., S.J. and A.S. have performed experiments using these oocytes. A.J. has designed and supervised the study, drafted and approved the manuscript.

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## References

Akao M, Otani H, Horie M, Takano M, Kuniyasu A, Nakayama H, Kouchi I, Murakami T, Sasayama S. Myocardial ischemia induces differential regulation of $K_{ATP}$ channel gene expression in rat hearts. *J Clin Invest* 1997;**100**:3053–3059.

Akrouh A, Halcomb SE, Nichols CG, Sala-Rabanal M. Molecular biology of $K_{ATP}$ channels and implications for health and disease. *IUBMB Life* 2009; **61**:971–978.

Albertini DF, Combelles CM, Benecchi E, Carabatsos MJ. Cellular basis for paracrine regulation of follicle development. *Reproduction* 2001; **121**:647–653.

Boni R, Cocchia N, Silvestre F, Tortora G, Lorizio R, Tosti E. Juvenile and adult immature and *in vitro* matured ovine oocytes evaluated in relation to membrane electrical properties, calcium stores, IP3 sensitivity and apoptosis occurrence in cumulus cells. *Mol Reprod Dev* 2008; **75**:1752–1760.

Carrasco AJ, Dzeja PP, Alekseev AE, Pucar D, Zingman LV, Abraham MR, Hodgson D, Bienengraeber M, Puceat M, Janssen E *et al.* Adenylate kinase phosphotransfer communicates cellular energetic signals to ATP-sensitive potassium channels. *Proc Natl Acad Sci USA* 2001; **98**:7623–7628.

Chan KW, Wheeler A, Csanady L. Sulfonylurea receptors type 1 and 2A randomly assemble to form heteromeric katp channels of mixed subunit composition. *J Gen Physiol* 2008;**131**:43–58.

Crawford RM, Ranki HJ, Botting CH, Budas GR, Jovanovic A. Creatine kinase is physically associated with the cardiac ATP-sensitive $K^+$ channel *in vivo*. *FASEB J* 2002a;**16**:102–104.

Crawford RM, Budas GR, Jovanović S, Ranki HJ, Wilson TJ, Davies AM, Jovanović A. M-LDH serves as a sarcolemmal K(ATP) channel subunit essential for cell protection against ischemia. *EMBO J* 2002b; **21**:3936–3948.

Cui Y, Giblin JP, Clapp LH, Tinker A. A mechanism for ATP-sensitive potassium channel diversity: functional coassembly of two pore-forming subunits. *Proc Natl Acad Sci USA* 2001;**98**:729–734.

Dale B, Fortunato A, Monfrecola V, Tosti E. A soluble sperm factor gates Ca(2+)-activated $K^+$ channels in human oocytes. *J Assist Reprod Genet* 1996;**13**:573–577.

Du Q, Jovanović S, Clelland A, Sukhodub A, Budas GR, Phelan K, Murray-Tait V, Malone L, Jovanović A. Overexpression of SUR2A generates a cardiac phenotype resistant to ischaemia. *FASEB J* 2006; **20**:1131–1141.

Du Q, Jovanović S, Sukhodub A, Jovanović A. Infection with AV-SUR2A protects H9C2 cells against metabolic stress: a mechanism of SUR2A-mediated cytoprotection independent from the $K_{ATP}$ channels activity. *Biochem Biophys Acta-Mol Cell Res* 2010;**1803**:405–415.

Elvir-Mairena JR, Jovanovic A, Gomez LA, Alekseev AE, Terzic A. Reversal of the ATP-liganded state of ATP-sensitive $K^+$ channels by adenylate kinase activity. *J Biol Chem* 1996;**271**:31903–31908.

Emery BR, Miller RL, Carrell DT. Hamster oocyte membrane potential and ion permeability vary with preantral cumulus cell attachment and developmental stage. *BMC Dev Biol* 2001;**1**:14.

Eusebi F, Passeto N, Siracusa G. Acetylcholine receptors in human oocytes. *J Physiol (Lond)* 1984;**346**:321–330.

Feichtinger W, Osterode W, Hoyer J. Membrane potential measurements in human oocytes. *Arch Gynecol Obstet* 1988;**243**:123–129.

Halet G, Marangos P, Fitzharris G, Carroll J. $Ca^{2+}$oscillations at fertilization in mammals. *Biochem Soc Trans* 2003;**31**:907–911.

Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, Seino S. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive $K^+$ channels. *Neuron* 1996;**16**:1011–1017.

Jones KT. Mammalian egg activation: from $Ca^{2+}$ spiking to cell cycle progression. *Reproduction* 2005;**130**:813–823.

Jovanović S, Jovanović A. Delivery of genes encoding $K_{ATP}$ channel subunits in conjuction with pinacidil prevents membrane depolarisation in cells exposed to chemical hypoxia-reoxygenation. *Biochem Biophys Res Commun* 2001a;**282**:1098–1102.

Jovanović S, Jovanović A. Pinacidil prevents membrane depolarisation and intracellular $Ca^{2+}$ loading in single cardiomyocytes exposed to severe metabolic stress. *Int J Mol Med* 2001b;**7**:639–643.

Jovanović S, Jovanović A. High glucose regulates the activity of cardiac sarcolemmal $K_{ATP}$ channels via 1,3-bisphosphoglycerate: a novel link between cardiac membrane excitability and glucose metabolism. *Diabetes* 2005;**54**:383–393.

Jovanović A, Jovanović S. SUR2A targeting for cardioprotection? *Curr Opin Pharmacol* 2009;**9**:189–193.

Jovanović S, Crawford RM, Ranki HJ, Jovanović A. Large conductance $Ca^{2+}$-activated $K^+$ channels sense acute changes in oxygen tension in alveolar epithelial cells. *Am J Respir Cell Mol Biol* 2003;**28**:363–372.

Jovanović S, Du Q, Crawford RM, Budas GR, Stagljar I, Jovanović A. Glyceraldehyde 3-phosphate dehydrogenase serves as an accessory protein of the cardiac sarcolemmal K(ATP) channel. *EMBO Rep* 2005; **6**:848–852.

Jovanović S, Du Q, Mukhopadhyay S, Swingler R, Buckley R, McEachan J, Jovanović A. A patient suffering from hypokalemic periodic paralysisis is deficient in skeletal muscle ATP-sensitive $K^+$ channels. *Clin Transl Sci* 2008;**1**:71–74.

Jovanović S, Du Q, Sukhodub A, Jovanović A. M-LDH physically associated with sarcolemmal $K_{ATP}$ channels mediates cytoprotection in heart embryonic H9C2 cells. *Int J Biochem Cell Biol* 2009a;**41**:2295–2301.

Jovanović S, Du Q, Sukhodub A, Jovanović A. Dual mechanism of cytoprotection afforded by M-LDH in embryonic heart H9C2 cells. *Biochem Biophys Acta-Mol Cell Res* 2009b;**1793**:1379–1386.

Lai AC, Ryan JP, Saunders DM. Removal of the zona pellucida and parthenogenetic activation affect rates of survival of ultrarapidly frozen mouse oocytes. *Reprod Fertil Dev* 1994;**6**:771–775.

Machaca K. $Ca^{2+}$ signalling differentiation during oocyte maturation. *J Cell Physiol* 2007;**213**:331–340.

Mattioli M, Barboni B, Bacci ML, Seren E. Maturation of pig oocytes: observation of membrane potential. *Biol Reprod* 1990;**43**: 318–322.

Minimi K, Miki T, Kadawaki K, Seino S. Roles of ATP-sensitive $K^+$ channels as metabolic sensors: studies of Kir6.x null mice. *Diabetes* 2004;**53** (Suppl 3):S176–S180.

Miyazaki S, Igusa Y. $Ca^{2+}$-mediated activation of $K^+$ current at fertilization of golden hamster egg. *Proc Natl Acad Sci USA* 1982;**79**:931–935.

Nichols CG. $K_{ATP}$ channels as molecular sensors of cellular metabolism. *Nature* 2006;**440**:470–476.

Noma A. ATP-regulated $K^+$ channels in cardiac muscle. *Nature* 1983; **305**:147–148.

Shyng S, Nichols CG. Octometric stoichiometry of the $K_{ATP}$ channel complex. *J Gen Physiol* 1997;**110**:655–664.

Tosti E, Boni R. Electrical events during gamete maturation and fertilization in animals and humans. *Hum Reprod Update* 2004;**10**:53–65.

Van Blerkom J, Davies P, Thalhammer V. Regulation of mitochondrial polarity in mouse and human oocytes: the influence of cumulus derived nitric oxide. *Mol Hum Reprod* 2008;**14**:431–444.

Van Blerkom J. Mitochondria in early mammalian development. *Semin Cell Dev Biol* 2009;**20**:354–364.