Glucose import efficiency is reduced in transcriptionally active mouse GV oocytes

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Introduction

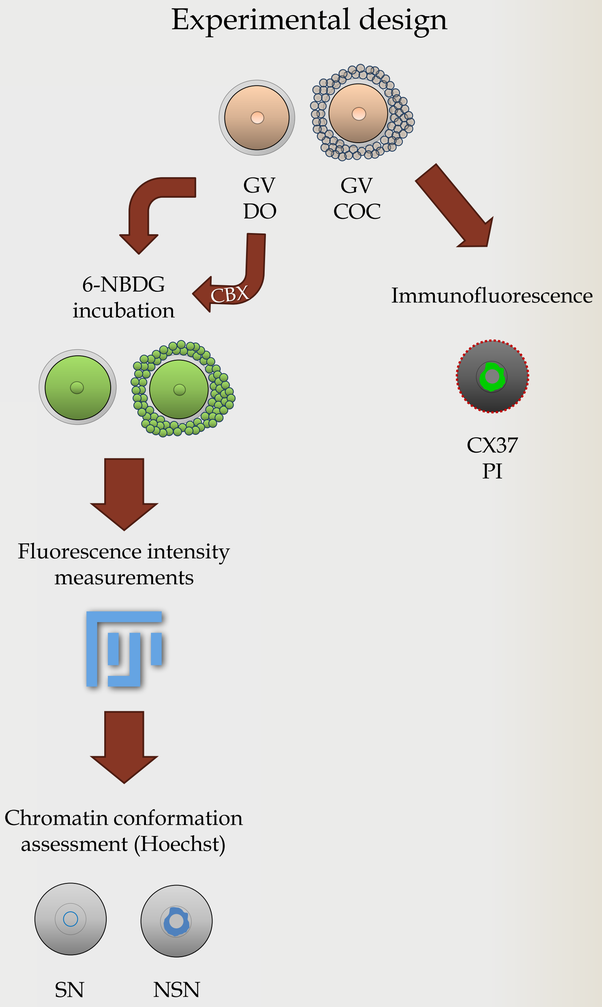
Immature mammalian oocytes (germinal vesicle oocytes or GVs) during their growth remain connected via gap-junctions with neighbouring granulosa cells (GCs), which supply them with nutrients, amino acids and other significant factors. In GVs the glycolysis pathway activity is low and they lack high affinity glucose transporters. For energy, they utilize pyruvate provided by GCs. However, glucose is not only an indirect energy source in oocyte development, but also a substrate in the pentose phosphate pathway, which might take part in meiotic maturation.

In fully grown ovarian follicles, both transcriptionally active (NSN) and inactive (SN) GV oocytes are present. NSN oocytes display lower competence to resume meiosis and potential to develop into a healthy embryo after fertilization. The lower developmental capabilities of NSN oocytes might be caused by insufficient accumulation of factors required for normal development, including glucose. Here we show that denuded NSN oocytes are characterized by reduced glucose transport efficiency in relation to SN oocytes. We also investigate the differences in the distribution of gap-junction forming protein Connexin 37 (CX37) in the two GV populations and show that NSN oocytes exhibit a higher intensity CX37 signal on the membrane. We demonstrate that gap-junction blocker CBX does not inhibit glucose transport in cumulus-oocyte complexes, though it may interfere with CX37 hemichannels (channel halves, connexons) in denuded SN oocytes. Decreased glucose transport in NSN oocytes may be a reason for their reduced potential in *in vitro* culture.

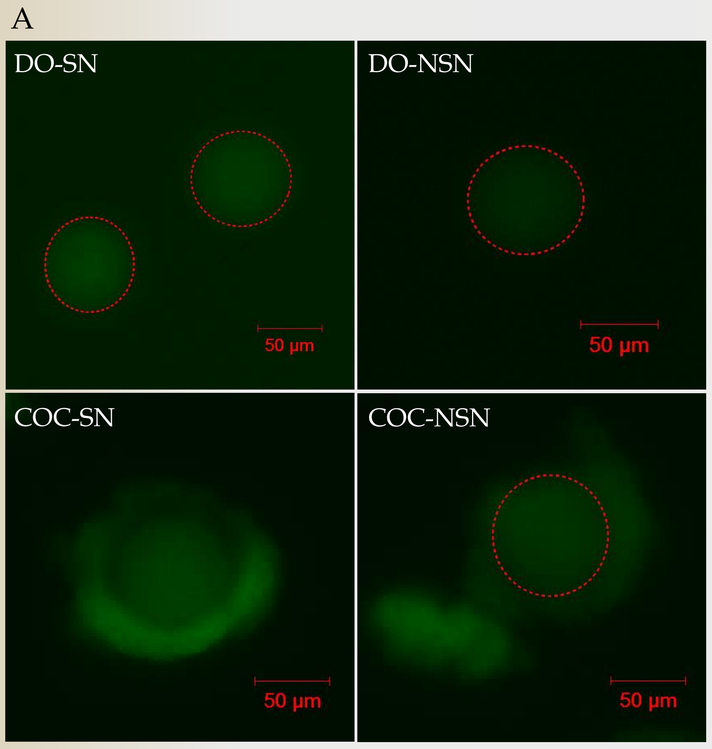
Objectives

- investigating whether SN and NSN oocytes differ in their ability to transport glucose;
- exploring the differences in gap-junction transport between the oocyte and granulosa cells in SN and NSN oocytes.

Methods



Results



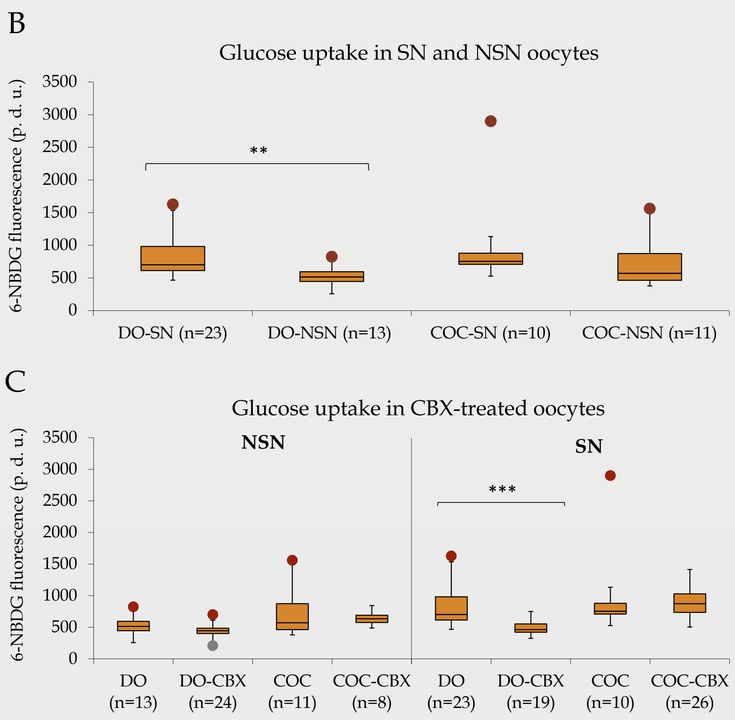


Fig. 1. Transcriptionally active oocytes (NSN) import the glucose analogue (6-NBDG) less effectively than transcriptionally inactive oocytes (SN). **(A)** Representative images of 6-NBDG uptake by denuded (DO) and granulosa cells-enclosed (COC) NSN and SN oocytes and **(B)** comparative chart showing that denuded NSN oocytes import less 6-NBDG than their SN counterparts, while the presence of granulosa cells mitigates the differences. **(C)** Pre-incubation in a gap-junction blocker, carbenoxolone (CBX), reduces 6-NBDG uptake by denuded SN oocytes, but cumulus-enclosed SN oocytes and all NSN oocytes import the same amounts of 6-NBDG regardless of CBX-pre-incubation. CBX is a well-known, but ultimately moderately potent gap-junction inhibitor. These results suggest that CBX treatment might be ineffective in inhibiting glucose uptake in GV oocytes. ** = p<0.01; *** = p<0.001.

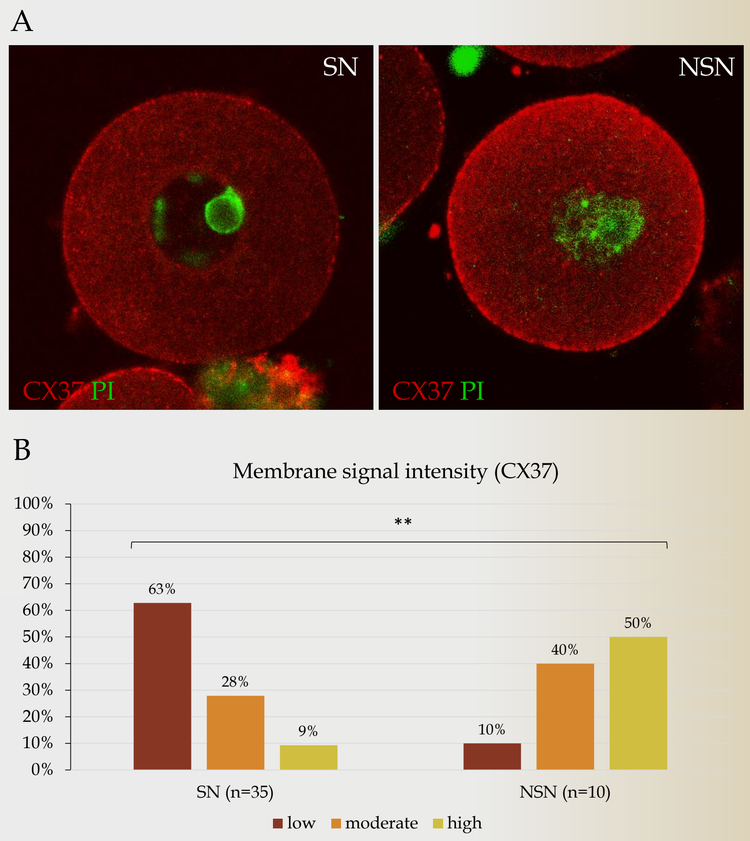


Fig. 2. Transcriptionally active (NSN) oocytes exhibit higher intensity membrane signal of CX37 in comparison with SN oocytes. **(A)** Representative pictures of SN and NSN oocytes. **(B)** NSN oocytes often express more CX37 in the membrane, suggesting that CX37 is not the main transporter of glucose into the oocyte. ** = p<0.01.

Conclusions

- Denuded NSN oocytes import glucose less efficiently compared to SN oocytes.
- CBX treatment is ineffective in inhibiting glucose import in COC and NSN oocytes express more CX37 than SN oocytes, suggesting that connexons may not be the main means of glucose transport in the oocyte.
- Although COC appear to transport glucose normally regardless of the transcriptional status of the oocyte, reduced glucose transport in denuded NSN oocytes may at least partially explain their lower developmental competence while cultured *in vitro*.

Bibliography

Fluks M, Szczepanska K, Ishikawa T, Ajduk A. (2019). *Transcriptional status of mouse oocytes corresponds with their ability to generate Ca^{2+} release.* Reproduction 157: 465–474. doi: 10.1530/REP-18-0625.

This study was supported by the Ministry of Science and Higher Education through the Faculty of Biology, University of Warsaw intramural grant DSM (EM11/2019) to MF.

Ministry of Science and Higher Education
Republic of Poland

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