Title

Calcium response during human oocyte activation: the effect of *in vitro* maturation and cryopreservation

Keywords

calcium oscillations, in vitro maturation, oocyte activation, oocyte freezing, cryopreservation

Authors

D. Nikiforaki,
F. Vanden Meerschaut,
C. Qian,
E. Van den Abbeel
B. Heindryckx,
P. De Sutter

[1]University Hospital Ghent, Department for Reproductive Medicine, Ghent, Belgium.

Introduction

Oocyte activation is mediated by a particular pattern of prolonged oscillations of intracellular free calcium concentration ($[Ca^{2+}]_i$), an ability acquired during oocyte maturation. $[Ca^{2+}]_i$ oscillations are necessary for oocyte activation and embryo development. Nevertheless, the mechanism in humans remains largely unknown. Oocyte cryopreservation is a promising procedure for preserving human reproductive potential. However, the unique sensitivity of human oocytes to cryodamage may compromise their developmental competence because of damage to organelles involved in calcium modulation. Furthermore, analysis of $[Ca^{2+}]_i$ oscillations is important for evaluating the efficiency of cytoplasmic maturation. The aim of this study was to compare the calcium responses after ICSI of *in vivo* matured, *in vitro* matured (IVM), and *in vitro* aged human oocytes and to investigate the effect of cryopreservation on calcium responses.

Materials and methods

The study was approved by Local (2009/130) and Federal Ethical Committees (Adv020) and written informed consent was obtained. Human germinal vesicle (GV), metaphase I (MI), and *in vivo* matured (IVO-MII) oocytes were obtained from stimulated cycles. GV oocytes were matured for 24 hours (GV-MII24h) in supplemented TCM199 medium and MI oocytes were matured for 3 hours (MI-MII3h) in Sydney IVF Cleavage medium (Cook). Slow freezing was performed according to a two-step 1,2-propanediol-sucrose protocol. The study groups were: GV-MII24h (fresh: n=14 and frozen-thawed: n=15), MI-MII3h (fresh: n=17 and frozen-thawed: n=11), *in vitro* aged oocytes with no signs of fertilization 20 hours after IVF (IVF FF, n=12) and IVO-MII oocytes when no sperm was available on the day of ICSI/IVF (n=11). Oocytes loaded with fura-2-AM were injected with frozen-thawed control sperm from a single donor. Calcium imaging started within 5-15 min after ICSI and continued for 16 hours at 37^{o}C and 6% CO_2. The Student t-test and Mann-Whitney U-test were used to determine statistical significance ($P \leq 0.05$) where appropriate.

Results
Calcium responses in IVO-MII oocytes initiated 48 ± 34.1 min after ICSI with an average frequency of 1 peak / 23 ± 7.2 min over the first 3 hours of $[Ca^{2+}]_i$ oscillations and lasted for 8 ± 4.1 hours. IVF FF oocytes differed significantly from IVO-MII oocytes (latency 95 ± 44.8 min, 1 peak / 33 ± 10.5 min, duration 14 ± 4.1 hours, P≤0.05). The average frequency over the first 3 hours was higher in IVO-MII oocytes compared to GV-MII24h (1 peak / 36 ± 9.9 min, P≤0.05) and MI-MII3h oocytes (1 peak / 35 ± 12.2 min, P≤0.05). $[Ca^{2+}]_i$ oscillations initiated earlier in IVO-MII oocytes compared to GV-MII24h oocytes (89 ± 45.9 min, P≤0.05). The total duration of $[Ca^{2+}]_i$ oscillations was significantly shorter in IVO-MII oocytes compared to MI-MII3h (12 ± 3.4 hours, P≤0.05). The other parameters were not statistically different between the study groups. In a second set of experiments, $[Ca^{2+}]_i$ oscillations were compared between fresh and cryopreserved IVM oocytes. The average frequency during the first hour was significantly higher in fresh compared to frozen-thawed GV-MII24h oocytes (1 peak / 29 ± 10.3 min versus 1 peak / 40 ± 15.7 min, P≤0.05). $[Ca^{2+}]_i$ oscillations initiated earlier in fresh MI-MII3h compared to frozen-thawed MI-MII3h (69 ± 45.6 min versus 108 ± 35.7 min, P≤0.05). The other parameters were not statistically different between the study groups.

Conclusions
The effectiveness of oocyte freezing and IVM in maintaining developmental competence is still under debate. Our findings demonstrate that cryopreservation and especially IVM influence the ability of human oocytes to elicit calcium responses after ICSI. *In vivo* matured human oocytes exhibit a specific pattern of $[Ca^{2+}]_i$ oscillations distinct from IVM and *in vitro* aged oocytes. Further research should address the specific role of abortive calcium signaling in developmental failure.