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Title:

Effect of ionomycin and calcium in the culture medium on the calcium releasing pattern of mouse oocytes and their subsequent embryonic development

Authors and affilitiations

Lu, Y. [a]; Nikiforaki, D. [a]; Vanden Meerschaut, F. [a]; Neupane, J. [a]; Qian, C. [a]; De Vos, W. H. [b, c]; Ozil, J. P. [d]; Deroo, T. [a]; De Sutter P. [a]; Heindryckx, B. [a]

[a]Department for Reproductive Medicine, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium
[b]Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, 2020 Antwerp, Belgium
[c]Cell Systems and Imaging research Group (CSI), Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium
[d] Unité de Biologie du Développement et Reproduction, INRA, 78352 Jouy-en-Josas cedex, France

Abstract

Study question
Does the concentration of ionomycin and Ca^{2+} ($[Ca^{2+}]$) in the culture medium affect the Ca^{2+}-releasing pattern of mouse oocytes and their subsequent embryonic developmental potential when activated by ionomycin?

Summary answer
The concentration of both ionomycin and $[Ca^{2+}]$ in the culture media influence the Ca^{2+}-rise and further embryonic development of mouse oocytes. From all combinations tested, 10 μM ionomycin dissolved in 1X-Ca^{2+}culture medium provoked a Ca^{2+}-rise with the highest amplitude and area under the curve (AUC) and resulted in the highest blastocyst rate.

What is known already?
Ionomycin is a Ca^{2+}-selective ionophore frequently used as an agent for assisted

oocyte activation (AOA) in clinical practice to overcome failed fertilization after ICSI. It has been shown recently in the bovine that ionomycin combined with a high [Ca^{2+}] in the culture medium increases the activation rate and improves embryonic development. However, neither the precise mechanism of action of ionomycin nor the possible factors influencing its efficiency has been studied.

Study design, size, duration

The effect of ionomycin concentration was investigated by applying 5 μM, 10 μM and 15 μM ionomycin dissolved in KSOM media. The effect of [Ca^{2+}] was analyzed by using 10 μM ionomycin dissolved in either Ca^{2+}-free KSOM, 1X-Ca^{2+} KSOM (1.71mM), 3X-Ca^{2+} KSOM, 6X-Ca^{2+} KSOM and three commonly used commercial IVF media.

Participants/materials, setting, methods

MII oocytes were collected from 6- to 10- week-old B6D2F1 mice. The absolute amplitude and the AUC (total amount of calcium released) of the ionomycin-induced Ca^{2+}-rises were measured by fluorescence time-lapse imaging. The embryo development was assessed at the two-cell, morula and blastocyst stage.

Main results and the role of chance

The amplitude, AUC and blastocyst formation rates in the 10 μM ionomycin group were significantly higher compared to the 5 μM and 15 μM ionomycin groups (P<0.01). In the Ca^{2+}-free KSOM group, the Ca^{2+}-rise had a similar amplitude but a significantly decreased AUC compared to the 1X-Ca^{2+} KSOM group (P<0.01), and no blastocyst formation was observed. When the [Ca^{2+}] in the culture media increased 3 or 6 times, the amplitude and the AUC were significantly reduced (P<0.01) compared to 1X-Ca^{2+} KSOM; however, no significance was found in blastocyst formation rate. Similarly, different patterns of Ca^{2+}-rises were observed when ionomycin was dissolved in three different commercial IVF media, with one medium containing the highest [Ca^{2+}], but exhibiting the lowest AUC (P<0.05).

Limitations, reasons for caution

The effect of ionomycin and [Ca^{2+}] can be tested reliably with mouse oocytes, yet the findings should be extended to the humans with caution.

Wider implications of the findings

Both the Ca^{2+}-releasing pattern and the embryonic developmental potential after ionomycin exposure were influenced by the concentration of ionomycin and [Ca^{2+}] in the culture medium. These results may explain the observed differences in the efficiency of AOA in a clinical setting. Further, these parameters have to be taken into

account when optimizing or applying AOA protocols. Further studies are needed to determine the optimal ionomycin and [Ca^{2+}] for human oocytes.

Study funding/competing interest(s)

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