TITLE

Vitrification causes a redox imbalance in MII human *in vitro aged* and "good" oocytes but apparently can be counteracted by the crocin antioxidant.

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Objective

Determine the effect of the extreme conditions of vitrification on the human oocyte depending on the maternal age and evaluate the possible beneficial effect of the crocin antioxidant.

Design

Case control experimental study

Materials and Methods

Determine after the vitrification and depending on the maternal age different intracellular features:

- the distribution of the chromosomes in the meiotic plate and the configuration of the spindle by using αtubulin antibody and Hoechst on *in vitro* aged MII oocytes;
- the redox balance on *in vitro* aged MII oocytes and on "good" MII oocytes by autofluorescence;
- the effect of the crocin antioxidant modulating the redox balance.

Quantitative data obtained from fresh and vitrified oocytes were compared, considering the maternal age. The data were analyzed using a univariate linear regression performed using Sigma Plot and Sigma Stat software packages (Statistics Package for Social Sciences, Germany). P-Values >0.05 were considered statistically significant.

Results

Age was responsible of a decreased normal chromosomal distribution, but not vitrification. Vitrification caused an oxidation of the metabolic couples FAD++/$FADH_2$ and NAD(P)++/NAD(P)H determined through the redox ratio FAD++/NAD(P)H, in both *in vitro* aged and "good" MII human oocytes (Table 1). The adition of 400µg/ml of crocin in the vitrification, warming and 2h post warming culture medium avoided this effect, reducing almost half of the oxidation observed after the vitrification.

	Young women < 26 years			**Reproductively older women > 36 years**	
Oocyte treatment	MII *In vitro* aged Fresh	MII *In vitro* aged Vitrified	"Good" MII Vitrified	MII *In vitro* aged Fresh	MII *In vitro* aged Vitrified
Oocyte number (1st exp + 2nd exp + 3rd exp)	40 + 40	46 + 46	0 + 15	40 + 52	46 + 52
Oocytes with normal chromosomal distribution	61,1%	57,14%	-	24,14%	26,67%
Ratio FAD++/NADH	0.938±0.045	1,295±0.034*	1,131±0.026	1,057±0.098	1,263±0.087**

* p-value<0.001; **p-value<0.05.

Table 1.

Conclusions

Although the incredible ability of the oocyte not only to survive to vitrification but to preserve its reproductive potential as the excellent clinical outcomes reflect, vitrification can cause a subtle damage in the oocyte as is causing an oxidation of the metabolic coenzymes $FADH_2$ and NAD(P)H, slowing down the mitochondrial activity and penalizing the antioxidant system of the oocyte. This could be the reason of the This could be the cause of the slowdown observed in the embryonic development.

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