The survey of the hydro-alcoholic extract of Ceratonia siliqua L on viability and fertilization after cryopreservation in mice

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Introduction

Oocyte banking is a vital step for safekeeping and spreading genetic resources of animals. It is also used for fertility preservation of human. Oocyte vitrification is closely related to the lower developmental competence which includes the cryo-injury arisen during vitrification. The aim of the present study was to evaluate the maturation, embryonic development and production of reactive oxygen species (ROS) of mice oocytes following the supplementation vitrification media with different concentrations of Ceratonia siliqua (carob) extracts.

Methods

In this experimental study, germinal vesicle oocytes collected from 8 to 10 week-old female NMRI mice (30–40 gr) were randomly divided into six groups of vitrification media supplemented with 0 (control), 5, 10, 20, 30 and 50 lg/ml C. siliqua. After thawing, oocytes were put in an in vitro maturation medium (IVM) (a-MEM: Alpha Minimum Essential Medium). 3–4 and 24 h (hr) later, the oocyte nuclear maturity was checked. Standard in vitro fertilization was performed on the matured oocytes (MII), and embryonic development was followed. Extra- and intracellular ROS was measured in IVM medium after 24 h of oocyte incubation.

Results

The addition of 20 and 30 lg/ml C. siliqua extract to vitrification media improved normal morphology of warmed germinal vesicle (GV) oocytes, rate of germinal vesicle break down (GVBD), and metaphase 2 (MII) oocyte formation significantly ($p<0.05$). Fertilization rate, (embryonic development to 2 cells stage, 4–8 cells stage, and[8 cells stage increased in the 30 lg/ml C. siliqua group significantly ($p < 0.05$). Furthermore, supplementation of 30 lg/ml C. siliqua in vitrification media significantly decreased extra- and intra-cellular of ROS as well as embryonic fragmentation ($p < 0.05$).

Conclusions

In conclusion, supplementation of GV oocyte vitrification media with carob extract improved maturation, fertilization, and embryonic development rate and decreased extra- and intra-cellular ROS levels.

Table 1 Effect of Carob concentrations (0, 5, 10, 20, 30 and 50 µg/ml) added into vitrification solutions on in vitro maturation of vitrified mouse GV oocytes

Treatments	No. of GV oocytes vitrified	No. of GV oocytes recovered	GV Oocytes with normal morphology N (%)	No. of GV oocytes that matured to	
				GVBD stage (%)	MII stage (%)
Control	127	120	86 (71.66 ± 1.85)[b]	61 (70.93 ± 2.78)[b]	52 (60.46 ± 3.57)[b]
5 µg/ml	127	119	88 (73.94 ± 4.54)[b]	68 (77.27 ± 1.66)[b]	57 (64.77 ± 3.85)[b]
10 µg/ml	127	122	93 (76.22 ± 2.13)[b]	72 (77.41 ± 4.16)[b]	61 (65.59 ± 3.33)[b]
20 µg/ml	127	120	95 (79.16 ± 3.60)[a]	76 (80.00 ± 4.18)[a]	63 (66.31 ± 1.45750)[b]
30 µg/ml	127	122	115 (94.26 ± 1.53)[a]	106 (92.17 ± 2.92)[a]	100 (86.95 ± 4.41)[a]
50 µg/ml	127	120	77 (64.16 ± 1.72)[c]	46 (59.74 ± 2.68)[c]	39 (50.64 ± 3.14)[c]

Table 2 Effect of Carob concentrations (0, 5, 10, 20, 30 and 50 µg/ml) added into vitrification solutions on early embryonic development

Treatment	Fertilized oocytes/ MII n (%)	2-cell (cleavage) n (%)	4–8-cell/fertilized oocytes n (%)	> 8-Cell/fertilized oocytes n (%)	> 10% fragmentation n (%)
Control	31 (59.61 ± 3.80)[b]	18 (58.89 ± 2.32)[b]	13 (41.93 ± 2.24)[b]	12 (38.70 ± 5.99)[b]	20 (64.51 ± 2.51)[b]
5 µg/ml	35 (61.40 ± 0.95)[b]	21 (60.00 ± 4.75)[b]	18 (51.42 ± 1.17)[b]	13 (40.00 ± 2.97)[b]	22 (62.85 ± 2.01)[b]
10 µg/ml	39 (63.93 ± 1.71)[b]	25 (64.10 ± 3.66)[b]	21 (53.84 ± 1.02)[b]	17 (43.58 ± 1.00)[b]	22 (56.41 ± 1.52)[b]
20 µg/ml	43 (68.25 ± 1.35)[a]	28 (65.11 ± 4.65)[b]	24 (55.81 ± 1.52)[b]	19 (44.18 ± 1.00)[b]	24 (55.81 ± 2.08)[b]
30 µg/ml	91 (91.07 ± 5.12)[a]	79 (86.81 ± 5.83)[a]	61 (67.61 ± 3.41)[a]	56 (61.53 ± 2.22)[a]	35 (38.46 ± 1.38)[a]
50 µg/ml	20 (51.28 ± 2.21)[c]	8 (40.55 ± 2.21)[c]	6 (30.67 ± 5.49)[c]	5 (25.00 ± 2.87)[c]	15 (75.00 ± 3.72)[c]

Table 3 Effect of Carob concentrations (0, 5, 10, 20, 30 and 50 µg/ml) added into vitrification solutions on ROS level

Treatments	ROS Level (Relative light units/s)
Baise medium	(51.33 ± 1.08)[c]
Control	(70.33 ± 1.15)[b]
5 µg/ml	(68.00 ± 1.00)[b]
10 µg/ml	(61.33 ± 1.52)[b]
20 µg/ml	(58.33 ± 0.57)[b]
30 µg/ml	(50.66 ± 0.58)[c]
50 µg/ml	(80.33 ± 1.15)[a]

[a,b,c]Data are presented absolute values (mean ± SD), and different superscript letters (a, b, c) indicate significant differences among experimental groups ($p < 0.05$)

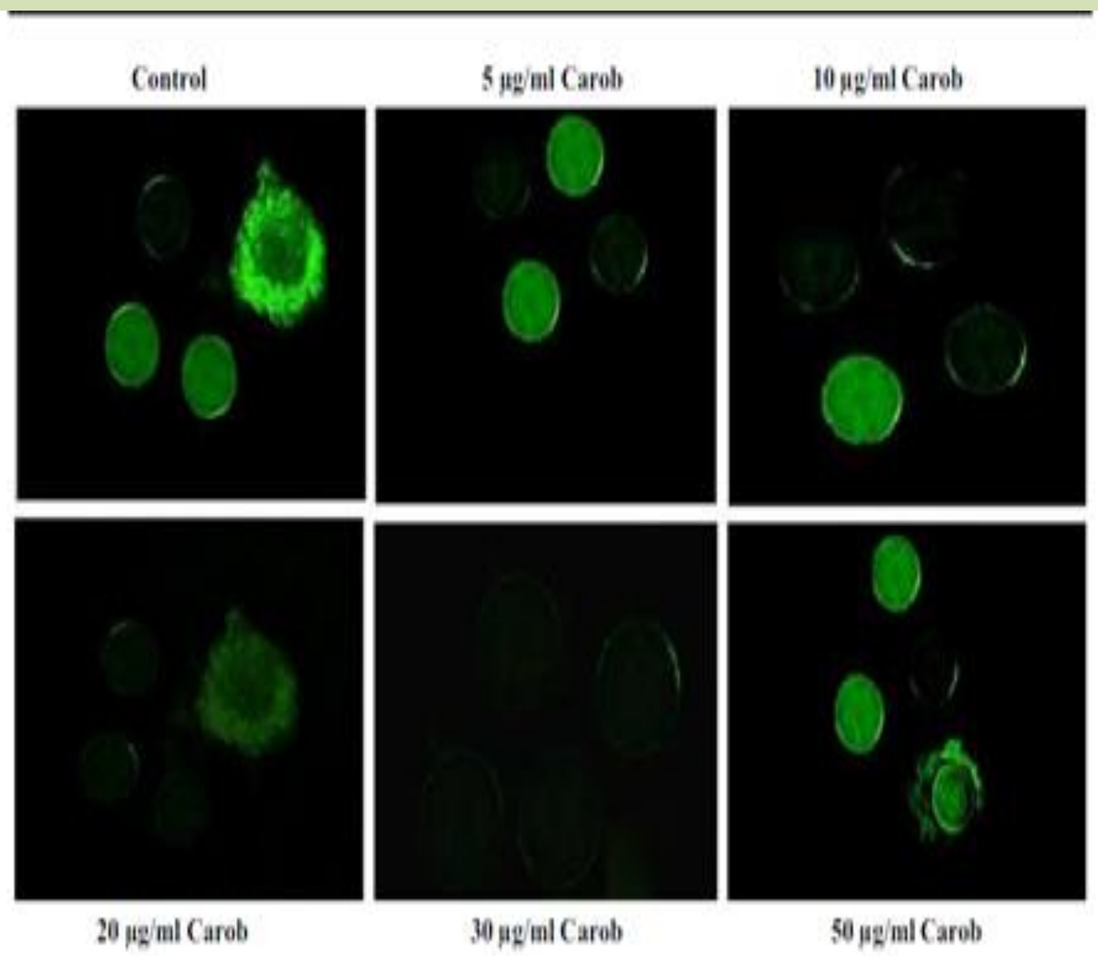

Fig. 1 Effect of Carob concentrations (0, 5, 10, 20, 30 and 50 µg/ml) added to freeze media on ROS level