The survey of the hydro-alcoholic extract of Ceratonia siliqua L on human sperm parameters and DNA Fragmentation after cryopreservation

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Introduction

Human sperm banking is an important procedure in the assisted reproductive technique centers. It entails sperm damage. The aim of this study was to investigate beneficial effect of Ceratonia siliqua (C. siliqua) supplement in freezing/thawing media on post thaw sperm parameters and sperm chromatin quality in normozoospermic samples.

Methods

Forty normozoospermic specimens were included in this prospective study. Each sample was divided into ten groups. In groups one to five, 0 (as control group) 5, 10, 20 and 30 µg/ml C. siliqua were added to freezing medium and in groups six to ten, similar concentration of C. siliqua were added to thawing medium for 30 min incubation. Sperm concentration, progressive motility, normal morphology, viability, aniline blue (AB), toluidine blue (TB) and sperm chromatin dispersion (SCD) staining tests were evaluated before vitrification and after thawing.

Results

The results showed that 10 and 20 µg/ml supplementation of C. siliqua in freezing/thawing media significantly increased progressive motility, normal morphology and viability of sperm ($p < 0.05$) as well as decreased AB, TB and SCD ($p < 0.05$). Also, 20 µg/ml had significantly higher improvement compared to 10 µg/ml C. siliqua ($p < 0.05$).

Conclusions

The present study showed that C. siliqua supplemented freezing/thawing media can improve sperm quality of normozoospermic samples after freezing/thawing.

Table 1 Fresh semen analysis of the patients before vitrification in normozoospermic specimens

Variables	Sperm analysis in men
Volume	3.3 ± 1.1
pH	7.5 ± 0.1
Concentration (10^6/ml)	111.3 ± 29.21
Progressive motility (%)	65.55 ± 8.23
Normal morphology (%)	10.07 ± 1.5
Viability (%)	72.71 ± 8.5

Data presents as mean ± SD

Table 2 Effect of *C. siliqua* concentrations 5, 10, 20 and 30 µg/ml added to vitrification/thawing media on sperm progressive motility, normal morphology and viability

Treatment in normozoospermic specimens	Progressive motility (%)	Normal morphology (%)	Viability (%)
Control (vitrification medium + 0 µg/ml *C. siliqua*) (group I)	45.13 ± 1.72[a]	7.01 ± 0.7[a]	58.3 ± 7.12[a]
Vitrification medium + 5 µg/ml *C. siliqua* (group II)	48.28 ± 8.88	7.65 ± 1.12	61.6 ± 3.1
Vitrification medium + 10 µg/ml *C. siliqua* (group III)	55.12 ± 2.21[ac]	8.12 ± 0.87[ac]	64.12 ± 4.2[ac]
Vitrification medium + 20 µg/ml *C. siliqua* (IV)	64.05 ± 2.63[ac]	10.02 ± 1.65[ac]	71.82 ± 6.24[ac]
Vitrification medium + 30 µg/ml *C. siliqua* (V)	44.01 ± 2.41	5.24 ± 1.1	47.12 ± 5.01
Control (thawing medium + 0 µg/ml *C. siliqua*) (VI)	45.02 ± 3.21[b]	6.12 ± 1.2[b]	57.24 ± 6.1[b]
Thawing medium + 5 µg/ml *C. siliqua* (VII)	47.01 ± 1.51	7.32 ± 0.96	60.12 ± 5.14
Thawing medium + 10 µg/ml *C. siliqua* (VIII)	51.16 ± 2.14[bd]	8.62 ± 1.31[bd]	62.41 ± 4.91[bd]
Thawing medium + 20 µg/ml *C. siliqua* (IX)	60.11 ± 3.14[bd]	10.01 ± 0.69[bd]	69.12 ± 3.06[bd]
Thawing medium + 30 µg/ml *C. siliqua* (X)	46.20 ± 01	6.35 ± 1.51	57.01 ± 3.21

Data presents as percentage ± SD

[a]Difference between group I and group III/IV

[b]Difference between group VI and group VIII/IX

[c]Difference between group IV and group III

[d]Difference between group IX and group VIII

Table 3 Effect of *C. siliqua* concentrations 5, 10, 20 and 30 µg/ml added to vitrification/thawing media on sperm chromatin/DNA evaluation

Treatment in normozoospermic specimens	AB (+) (%)	TB (+) (%)	SCD (+) (%)
Control (vitrification medium + 0 µg/ml *C. siliqua*) (group I)	27.12 ± 3.44[a]	26.47 ± 5.26[a]	26.14 ± 1.41[a]
Vitrification medium + 5 µg/ml *C. siliqua* (group II)	25.21 ± 4.10	22.41 ± 3.11	25.31 ± 2.14
Vitrification medium + 10 µg/ml *C. siliqua* (group III)	19.21 ± 2.21[ac]	19.23 ± 4.01[ac]	18.31 ± 2.31[ac]
Vitrification medium + 20 µg/ml *C. siliqua* (IV)	16.21 ± 6.31[ac]	15.3 ± 2.21[ac]	15.31 ± 1.19[ac]
Vitrification medium + 30 µg/ml *C. siliqua* (V)	25.31 ± 3.26	25.12 ± 3.21	25.63 ± 2.10
Control (thawing medium + 0 µg/ml *C. siliqua*) (VI)	21.12 ± 3.25[b]	25.71 ± 7.12[b]	26.16 ± 2.14[b]
Thawing medium + 5 µg/ml *C. siliqua* (VII)	26.36 ± 1.19	25.74 ± 6.02	22.30 ± 1.09
Thawing medium + 10 µg/ml *C. siliqua* (VIII)	19.31 ± 5.32[bd]	19.01 ± 4.41[bd]	20.04 ± 1.13[bd]
Thawing medium + 20 µg/ml *C. siliqua* (IX)	17.12 ± 2.21[bd]	16.02 ± 2.14[bd]	16.30 ± 1.08[bd]
Thawing medium + 30 µg/ml *C. siliqua* (X)	26.26 ± 4.54	25.43 ± 4.23	26.12 ± 2.01

Data presents as percentage ± SD

AB aniline blue, *TB* toluidine blue, *SCD* sperm chromatin dispersion

[a]Difference between group I and group III/IV

[b]Difference between group VI and group VIII/IX

[c]Difference between group IV and group III

[d]Difference between group IX and group VIII