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Research Article

Investigation of the therapeutic effect of in vitro Ceratonia siliqua application on DNA damage caused by human sperm cryopreservation

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Abstract

Objectives: The main aim of this study was to investigate the effect of Ceratonia siliqua L (carob) plant added to the culture medium to reduce the negative effect on sperm parameters as a result of cryodamage occurring in sperm during human sperm cryopreservation.

Materials and methods: Semen samples obtained with consent from 50 infertile men aged 25-40 years were subjected to spermiogram test. Spermatozoa were divided into normospermia and asthenospermia. Semen was washed with gradient method to separate spermatozoa according to motility and vitality with the least risk of cotamination. The groups were divided into 6 groups. normospermic group with gradient method, normozoospermic group with gradient method and freeze-thawed, normozoospermic group with gradient method and freeze-thawed and Ceratonia siliqua L., normozoospermic group with only gradient method, asthenozoospermic group with gradient method, asthenozoospermic group with gradient method and freeze-thawed, asthenozoospermic group with gradient method and freeze-thawed and added Ceratonia siliqua L. Semen samples were frozen at -196 °C with liquid nitrogen and stored in a nitrogen tank. Then the samples were thawed at 37 °C and vitality test was performed with eosin Y stain in all groups.

Results: In order to investigate the effect of freeze-thawing on sperm parameters, only semen samples washed by gradient method were compared with the frozen and thawed group without the addition of Ceratonia siliqua L. Cryopreservation had a significant ($p<0.05$) negative effect on all sperm parameters. In the investigation of the effect of Ceratonia siliqua L. on cryodamage, sperm vitality parameter was compared in the groups in which Ceratonia siliqua L. was added to the incubation medium in vitro. Ceratonia siliqua L. concentration was adjusted as 0.01%. A positive difference was observed between the groups to which Ceratonia siliqua L. was added compared to the groups to which Ceratonia siliqua L. was not added.

Conclusion: According to the results of this study, the negative effects of sperm freezing on sperm vitality are clearly demonstrated. Considering the positive differences in the groups in which Ceratonia siliqua L. was added in vitro, we think that Ceratonia siliqua L. will contribute to male infertility.

Keywords: sperm, freezing vitality, Ceratonia siliqua L

INTRODUCTION

The most basic element for fertilization is the vitality of the sperm. Due to different reasons, sperm structure and vitality can be damaged and this situation negatively affects the reproductive health of people. Cryopreservation of sperm and testicular tissue has been used as a treatment for male infertility since 1953. Cryopreservation of sperm cells is a frequently used method during infertility treatments. In cancer patients, sperm cells can be frozen and stored for many years in order to preserve reproductive cells before chemotherapy and radiotherapy, in diseases that may lead to testicular damage such as diabetes, auto immune diseases, in oligospermia and ejaculatory irregularities, in patients who do not have cells in the ejaculate of the person but testicular sperm can be obtained (azoospermia) in order to be used in subsequent ART (Assisted Reproductive Treatment) applications [1]. Studies have confirmed that the functional parameters of semen samples frozen with cryoprotectants are adversely affected after thawing. When cryopreserved sperm cells are used in assisted reproduction techniques, low fertilization rates and embryo development, embryos that do not reach day 5 and implantation potential decrease, and miscarriage risk rates increase in pregnant patients.

Ceratonia siliqua L. (carob) is a perennial herb belonging to the family Fabaceae (Leguminosae) in the form of evergreen leafy shrub-tree. The fruits and seeds of carob have different uses. The fruits of carob, which are rich in

minerals such as potassium, calcium, magnesium, phosphorus, sodium, selenium, iron and copper, are consumed directly in the food industry and are used in nutrition as additives. Carob fruits are used in human nutrition as sugar, molasses, cocoa, alcohol and protein product, dietary fibre. Besides all these, it is also used in pharmacy [2]. The phenolic substance in carob, which is rich in phenolic substances, is gallic acid and is a very effective antioxidant [3] Studies show that gallic acid is very effective in slowing down the oxidation of fats [4]. In a study conducted by Souli et al. on mice, it was revealed that carob extract had a protective effect on liver cells against oxidative stress damage caused by ethyl alcohol [5].

The main aim of this study was to show the effects of in vitro Ceratonia siliqua L. application on sperm vitality.

MATERIAL AND METHODS

Study Design

50 (25 normospermia, 25 asthenozoospermia) male patients aged between 25 and 40 years were included. After semen sampling with the consent of 50 volunteer patients, demographic characteristics of 50 infertile male patients (smoking, accessory gland infection, alcohol intake, recent hormonal treatment, vitamin consumption and varicocele disorder) were analysed. not included in the study. .Virtual azoospermia and azoospermic samples with sperm volume less than 1.0 ml, leucocyte cell count more than 1 million per ml were excluded from the study, while sperm volume more than 1.5 ml, count more than 15 million, motility more than 40% and morphology >4% normozoospermic semen samples and asthenozoospermia semen samples with sperm volume more than 1.5 ml, sperm count over 15 million, motility less than 40% and morphology >4% were included in the study. Semen samples will be obtained by masturbation after 3-4 days of ideal sexual abstinence. The semen sample obtained by masturbation will be collected in a sterile, wide-mouthed container and the semen will be left at room temperature for 30 minutes for surfactant. After macroscopic evaluation, microscopic evaluation of sperm function parameters will be performed in all cases.

Determination of study groups

Semen samples of 50 patients are divided into 2 groups as normozoospermia (n=25) and asthenozoospermia (n=25) and then semen samples are divided into 6 groups among themselves.1The 1st group is normozoospermic group washed only with gradient method, the 2nd group is normozoospermic group washed with gradient method and freeze-thawed, the 3rd group is normozoospermic group washed with gradient method and freeze-thawed and added Ceratonia siliqua L., the 4th group is normozoospermic group, and the 4th group is normozoospermic group. asthenozoospermic group washed with gradient method, 5th group was washed with group gradient method and freeze-thawed asthenozoospermic group and finally 6th group was washed with gradient method and freeze-thawed asthenozoospermic group with Ceratonia siliqua L. added.

Sperm Washing (Density-Gradient Method)

It is the most frequently used sperm washing method to select the best sperm in terms of quality, motility and vitality. In the Density-Gradient Method, sperm are passed through colloidal silica coated particles to obtain sperm for in vitro fertilisation (IVF) and intra cytoplasmic sperm injection (ICSI). There are different branded products for this method. In this washing procedure, firstly, a 2-layer solution is prepared by adding 1 ml of 90% gradient solution and 1 ml of 45% gradient solution to a conical centrifuge tube without mixing. The semen sample is slowly added to the gradient solutions. The conical tube is centrifuged at 1200 rpm for 10 minutes. The supernatant accumulated on the sperm pellet is removed from the sample. The pellet remaining at the bottom is pipetted and 5 ml of sperm washing medium is added to homogenise the pellet and centrifuged at 1800 rpm for 10 more minutes. The structure remaining at the bottom and containing sperm is evaluated to be used in the culture process [6].

Sperm Cryopreservation

The semen sample to be analysed is allowed to liquefy in an oven for 15-30 minutes. The likeified sample is evaluated in terms of sperm parameters. The sperm freezing medium divided into 5 mL conical tubes is brought to room temperature before sperm freezing. The gradient washed semen sample is transferred to a sterile 15 mL conical centrifuge tube, the ejaculate volume is determined and the freezing medium is added slowly dropwise over 30 seconds with a semen to freezing medium ratio of 1:1. Mix thoroughly after adding each drop. The most important point to be considered in sperm cryopreservation is not to create air bubbles. After slowly adding all the freezing medium to the semen sample within 30 s, the mixture is allowed to equilibrate at room temperature for approximately 10 minutes. The sperm freezing medium mixture is transferred to the vial. The vials are not overfilled to allow for expansion. Seal the vials tightly. The vials are exposed to liquid nitrogen vapour for a minimum of one hour and then replaced in the tank.

Sperm Thawing

For sperm thawing, the vials are removed from liquid nitrogen and kept at room temperature for 1 min and then thawed with the help of 37 °C water bath. After the sample is completely thawed, the washing solution is taken into a conical tube, approximately 5 ml of washing medium is added on it and centrifuged at 1600 rpm for 10 min and the supernatant is discarded and removed from the cryoprotectant. The sperm samples obtained after thawing are analysed for sperm function parameters.

Preparation of Ceratonia siliqua L. Extract

The plant samples used in our study were purchased from herbalists. Since the organoleptic properties of the purchased plant samples were considered to be appropriate, those obtained from the samples collected by growing in nature were preferred. Ceratonia siliqua L. fruit samples were first dried in the shade. The dried

samples were first crushed into coarse pieces using a mortar and pestle. Then, with the help of a blender, the coarse pieces were thoroughly crushed into powder. The obtained carob powders used in the study were weighed 0.001g. They were macerated in 10 ml of Ham's F-10 (Irvine, USA) solution at room temperature for 6 hours with a final concentration of 0.01%. At the end of maceration, the solution was filtered through Whatman No.1 filter paper and plant extracts with a final concentration of 0.01% were obtained [7]. The obtained plant extracts were divided into tubes to be used in the study and the tube mouths were wrapped with parafilm and stored at +4 °C. Samples brought to 37 °C before the study were used.

Eosin Y staining for sperm viability assessment

After 10 µl of Ceratonia siliqua L was added to the samples of all groups, 10 µl of Eosin-Y solution (1:1 ratio) was added to the slide, washed with gradient washing method for 30 minutes, covered with a coverslip and evaluated after 30 seconds. At least 100 spermatozoa were analysed with 40X objective. The spermatozoa that did not take the stain were grouped as live and the spermatozoa that took the stain were grouped as dead and the ratio of live spermatozoa was determined as % vitality. Eosin Y stain was used in our study.

RESULTS

When we evaluated vitality, we observed that freezing and thawing had a negative effect on vitality. While 828±.5% vitality was observed in normospermic semen samples that underwent only gradient, this rate decreased to 27±6% in normospermic k semen samples after freezing and thawing, and a statistically significant ($p< 0.05$) decrease in plasma membrane integrity was observed when all cryopreserved groups were compared with fresh semen. When we look at the comparison between the frozen and thawed groups, 38.6±55.151% vitality was observed in the normospermic Semen + Freezing Medium + Locust bean group, which is the highest value among the other frozen and thawed groups and a significant difference was observed ($p<0.05$). However, no significant ($p=0.997$) difference was found between the normospermic and asthenospermic groups which were washed only by gradient method in terms of vitality. There was no significant difference between the 2nd and 5th groups ($p=0.784$, $p=0.213$) in % vitality values.

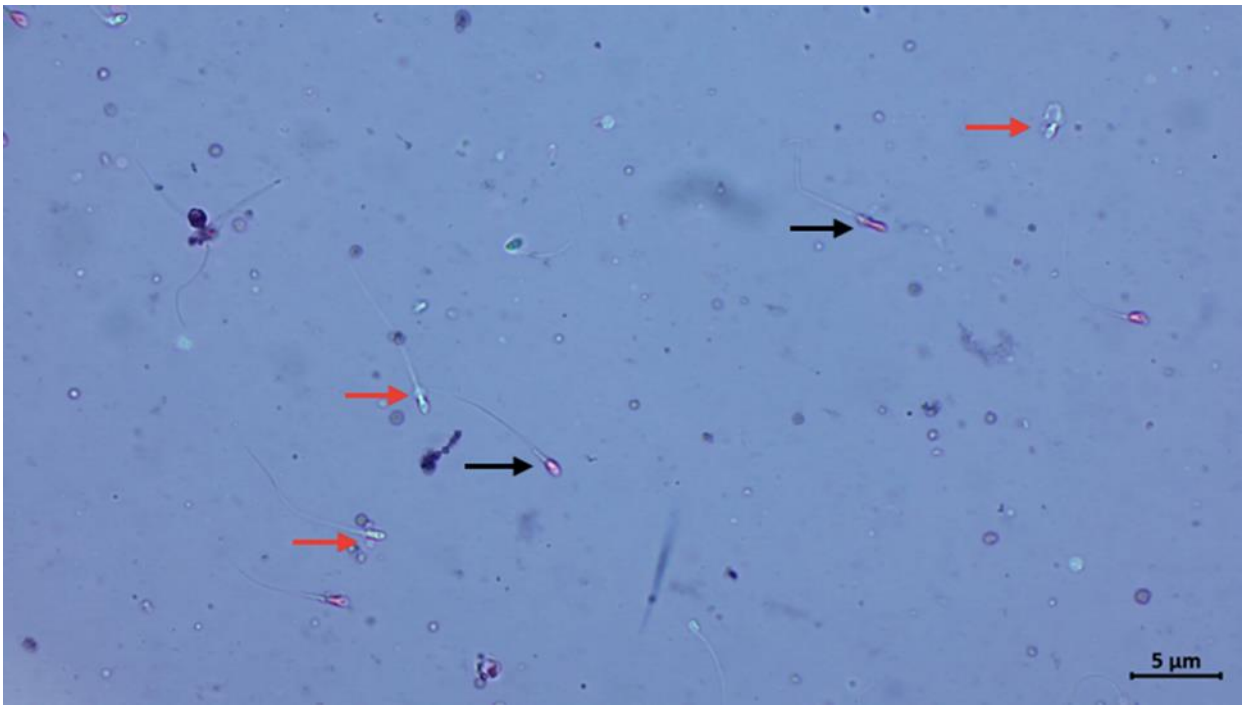

Figure 1: This image shows spermatozoa treated with eosin Y for vitality determination. Black arrows indicate non-viable sperms and red arrows indicate viable sperms.

DISCUSSION

The use of therapeutic effects of plants has started with the history of mankind. Almost all of the drugs used in modern medicine are derived from plants. In almost all cultures, certain plants have been used for therapeutic purposes. While plants are frequently used alone in herbalism in Western societies, plant mixtures are predominantly used in Chinese Medicine [8]. Herbal therapies are widely utilised in infertility treatment due to their lower cost, easy accessibility, lack of concern about the side effects of chemical drugs and lack of invasive intervention. The general profile of the patients who resorted to herbal treatment in infertility treatment is low-educated, low-income and very young couples. According to the study of Zini et al. 31% of infertile men

used complementary therapy and 64% of these were multivitamin capsules and 20% were herbal products [9].

The first studies were carried out on trying to increase the success by adding seminal plasma proteins to the medium. With the addition of these proteins to the medium, changes in sperm motility, acrosome and membrane integrity and ROS level were observed. However, the increase in success varies depending on the volume of seminal plasma added, the medium used and the incubation temperature [10]. Condorelli et al. conducted an in vitro study on patients with normozoospermia. In this study, the effects of myoinositol on infertility were investigated. in the study, myoinositol was exposed to sperm in vitro at 2 mg/mL for 2 hours. as a result, it was observed that myoinositol did not affect mitochondrial functions and increased sperm count [11].

In 2012, in another in vitro study conducted by Condorelli et al., this time myo-inositol (2 mg/mL, for 2 hours) was administered to the sperm of 40 patients diagnosed with 20 normozoospermia and 20 oligoastheno-teratozoospermia. While sperm motility increased in both groups, it was observed that mitochondrial function improved only in the sperm of patients with oligoastheno-teratozoospermia [12]. In another in vitro study, semen samples obtained from infertile men were analysed and antioxidant ratios of seminal plasma and low levels of zinc and selenium minerals were detected [13].

Although high doses of antioxidant supplementation have been observed to improve semen quality in many in vitro studies, the number of studies showing the dietary intake of antioxidants and sperm quality parameters in infertile men is very limited. Considering these studies, it has been shown that there is a positive correlation between low dietary antioxidant intake and low sperm quality [14]. In our study, we have examined the cryoharvest formed in sperm during the sperm cryopreservation process on the vitality, and we have shown that we can improve these parameters by supplementing Ceratonia siliqua L. in vitro. Most of our results are consistent with the literature and there is no previous study on the addition of Ceratonia siliqua L silymarin to the medium as a cryopreservative for cryopreservation of human sperm. Our results are a pioneering study proving that Ceratonia siliqua L. can be used effectively in this field.

CONCLUSION

In the light of the above literature, most of our results are consistent with the literature and there is no previous study on the addition of Ceratonia siliqua L silymarin to the medium as a cryopreservative for cryopreservation of human sperm. Our results are a pioneering study proving that Ceratonia siliqua L. can be used effectively in this field.

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REFERENCES

1. Singh, R., Hamada, A. J., Bukavina, L., & Agarwal, A. Physical deformities relevant to male infertility. Nature reviews. Urology, 2012; 9(3):156-174. https://doi.org/10.1038/nrurol.2012.11 PMid:22349654

2. Tous, J., Romero, A., Battle, I., The carob tree: botany, horticulture, and genetic resources. Horticultural Reviews, 2013; 41:385-456. https://doi.org/10.1002/9781118707418.ch08

3. Kumazawa, S., Taniguchi, M., Suzuki, Y., Shimura, M., Kwon, M., Nakayama, T. Antioxidant activity of polyphenols in carob. Journal of Agriculture and Food Chemistry, 2002; 50:373- 377. https://doi.org/10.1021/jf010938r PMid:11782210

4. Owen, R.W., Haubner, R., Hull, W.E., Erben, G., Spiegelhalder, B., Bartsch, H. Isolation and elucidation of the major individual polyphenols in carob fibre. Food and Chemical Toxicology,2003; 41:1727-1738. https://doi.org/10.1016/S0278-6915(03)00200-X PMid:14563398

5. Ahmed, M.M., Biochemical studies on nephroprotective effect of carob (Ceratonia siliqua L.) growing in Egypt. Nature and Science,2010, 8(3), 41- 47.

6. WHO, 2010. Laboratory manual for the examination of human semen and sperm cervical mucus interaction Cambridge University Press p.

7. Yusuf, M.K., Ibegbu, A.O., Agbon, A.N., Evang, A., Determination of Median Lethal Dose of Ethanolic Extract of Sesamum indicum seeds in adult Wistar Rats., African Journal of Cellular Pathology, 2017; 9:73-76. https://doi.org/10.5897/AJCPath2018.0011

8. Altun R, Özden A. Complementary and alternative medicine.; Güncel Gastroenteroloji 2004;8(3):231-235.

9. Zini A, Fischer MA, Nam RK, Jarvı K. Use of alternatve and hormonal therapies in male infertility.; Urology 2004;63 (1):141-143. https://doi.org/10.1016/j.urology.2003.07.018 PMid:14751367

10. Fraser L, Dziekońska A, Strzeżek R, Strzeżek J, Dialysis of boar semen prior to freezing-thawing: Its effects on post-thaw sperm characteristics. Theriogenology, 2007; 67(5):994-1003. https://doi.org/10.1016/j.theriogenology.2006.12.002 PMid:17218000

11. Condorelli, R.A., La Vignera, S., Di Bari, F., Unfer, V., Calogero, A.E., Effects of myoinositol on sperm mitochondrial function in vitro. Eur Rev Med Pharmacol Sci, 2011; 15(2):129-134.

12. Condorelli, R.A., La Vignera, S., Bellanca, S., Vicari, E., Calogero, A.E., Myoinositol: does it improve sperm mitochondrial function and sperm motility?. J Urol, 2012; 79(6):1290-1295. https://doi.org/10.1016/j.urology.2012.03.005 PMid:22656408

13. Türk, S., Mändar, R., Mahlapuu, R., Viitak, A., Punab, M., Kullisaar, T., Male infertility: decreased levels of selenium, zinc and antioxidants. J Trace Elem Med Biol, 2014; 28(2):179-185. https://doi.org/10.1016/j.jtemb.2013.12.005 PMid:24462254

14. Mendiola, J., Torres-Cantero, A.M., Vioque, J., Moreno-Grau, J.M., Ten, J., Roca, M. A low intake of antioxidant nutrients is associated with poor semen quality in patients attending fertility clinics. Fertil Steril, 2010; 93(4):1128-1133. https://doi.org/10.1016/j.fertnstert.2008.10.075 PMid:19147135