

Role of alcoholic extract of Black Carrot in improvement of human sperm parameters during in vitro activation

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Abstract:

Background

The increasing prevalence of infertility cases is becoming a major public health problem in developing countries due to changes in diet and lifestyle.

Objectives

Black Carrot Extract BCE (*Daucuscarota*) plays an important role as fertility agent due to its antioxidant and anti-proliferative properties. Therefore, the aim of the present study was to identify the effects of different concentrations of BCE on the outcome of in vitro human sperm activation of infertile patients

Methods

Thirty two semen samples were prepared which served as a control, using direct swim-up technique. Modified Earl's medium (MEM) was used only in group 2 (G2) and two concentrations of BCE (10µg/1ml and 20µg/1ml) were added to culture media MEM of groups 3 (G3) and 4 (G4) for *in vitro* sperm activation (ISA).

Results

a highly significant ($P<0.001$) correlation was noticed between G4 with G2 and G3 in progressive motility when activation by adding 20µg/1ml of BCE to culture media as compared to G3 (which adding 10µg/1ml of BCE to culture media) and G2 (activation with culture media only). Moreover, swim-up activation technique with culture media and by both adding (10µg/1ml and 20µg/1ml of BCE respectively) to culture media enhancement in the (sperm motility, progressive sperm activity and sperm morphology).

Conclusion

The high concentration of BCE is effective in the enhancement of progressive sperm activity during in vitro human sperm activation.

Key words: Black Carrot Extract (BCE), Beta- carotene, swim-up technique.

Introduction

Infertility is defined as a year of unprotected intercourse in the fertile phase of the menstrual cycles in women under 35 years, or after 6 months in women 35 years or older without achieving pregnancy (1). The origin of infertility is similarly due to male and female factors resulted from multiple causes (2). Accuracy of diagnosis is the most important factor to solve the infertility problem (3). Semen analysis is one of the first tests done to

evaluate a man's fertility. It can help determine if a problem in sperm production or quality of the sperm is causing infertility. Approximately half of couples unable to have children have a male infertility problem (4).

Therefore, the treatment can be determined by medicine and/or assisted reproductive technologies (5). Because the successful reproduction requires complete provisions of nutrients, like; zinc (6), vit A

(retinol)(7), vit B12, vit B9, vit E(8), vit D(9), selenium(10), nickel(11), manganese(12), copper(13), fatty acid(14), and protein(15). Carrot (*Daucus carota*) is one of the top sources of minerals (16) and provitamin A(17). It contains carotenoids which are dark-colored dyes (pigments) found in plant foods that can turn into a form of vitamin A. There are more than 500 known carotenoids. One such carotenoid is beta-carotene (18) which is an antioxidant (16). In black carrots BC, the beta-carotenes are in fact double as compared to what you will find with other carrot varieties. So, it's good for the overall health and specially organs like the skin, eyes, teeth, digestive system and reproductive system. Particularly, black carrots have more added benefits than orange carrot (19).

The present study aims to evaluate the effect of direct swim-up activation technique with culture media MEM and by both adding (10µg/1ml and 20µg/1ml of BCE respectively) to culture media.

Subjects, Material and Methods

This study was carried out at High Institute of Infertility Diagnosis and ART, Al-Nahrain University. Thirty two semen samples were obtained from infertile patients.

1. Preparation of culture medium

Modified Earl's Medium (MEM) was used in this study with earl's salts, glutamine and other groups of minerals. Powder of MEM (0.88g) was dissolved in 100 mL of deionized distilled water and 5% of human serum albumin was added. After that, pH of solution was adjusted between (7.2-7.4). Then, the prepared culture medium was filtrated by using Millipore filter (0.22µm) and stored in refrigerator until use.

2. Semen samples collection

The sample of seminal fluid was collected after 3-5 days of abstinence directly in a clean, dry and sterile disposable Petri-dish by masturbation in a private and quite room adjacent to the semen analysis laboratory. The container was labeled with the following information, name, age, abstinence period and time of sample collection. The specimens were placed in an incubator at 37°C for 30 minutes to allow liquefaction(20). The liquefied semen was carefully mixed for few seconds, and then the specimen was examined in detail by macroscopic and microscopic examinations within one hour of collection (21).

3. Techniques of *in vitro* human sperm activation (Direct swim-up technique)

One mL of the prepared culture medium either alone or supplied with two different doses of BCE (10µg/1ml and 20µg/1ml) was added to the test tube, and then 1mL of the liquefied semen was layered beneath a culture medium. After incubation for 30 minute at 37°C, one drop (10µL) of the mixture was aspirated by Pasteur pipette from upper layer which separate semen than culture media. Then, examined under light microscope at (400X) magnification for assessment parameters of sperm function.

4. Experimental design

Semen samples were divided after liquefaction into four groups, each sample was divided into four

aliquots. The first aliquot (G1) was without addition, the second aliquot (G2) was put beneath (1:1 v: v) of modified Earl's medium (MEM) in Falcon tube. The third aliquot (G3) was put in a falcon tube under of MEM with (10µg/1ml) of BCE and the fourth aliquot (G4) was put under MEM with (20µg/1ml) of BCE. The samples were placed in an incubator in oblique position and sperm parameters were determined after 30 minutes.

5. Statistical analysis:

Means and standard error of mean (mean + SEM) were determined using descriptive statistical methods. The data were statistically analyzed by multiple analysis of variance (MANOVA) and LSD to compare among different means of groups using special statistical computerized package SPSS (statistical Package of Social Science version 15).

Results

Macroscopic and microscopic parameters of SFA for subjects involved in this study are shown in Table (1). Ordinarily, most parameters of SFA were within normal ranges according to the criteria of WHO (2010).

Table (1) shows the sperm concentration in (G1) before activation, it was (51.88 ± 4.69) highly significant ($P < 0.001$) increased compared to (15.84 ± 1.6 (G2)) without adding BCE in the media that used for activation and (14.78 ± 1.39 in (G3) and 14.06 ± 1.29 in (G4)) by adding BCE (10µg/1ml and 20µg/1ml respectively) to culture media and to. Moreover, highly significant ($P < 0.001$) decreased shown in immotile sperm (%) for activation groups (G2, G3 and G4) when compared with before activation group (G1)

While, in the same table shows correlation between the percentages of sperm motility and normal sperm morphology in (G2, G3 and G4) that was highly significance different ($P < 0.001$) increase after swim-up activation (with and without adding BCE to culture media) compared to (G1) before activation.

A highly significant ($P < 0.001$) increase was reported in progressive motility (%) after activation between G2, G3 and G4 in compared with G1. Also, a highly significance increased was noticed in G4 when compared with G2 and G3 in progressive motility (%) after activation by adding 20µg/1ml of BCE to culture media G3 and G2.

Noticed in non-progressive motility percentage, a significant ($P < 0.05$) increase between G2 (after activated) with G1 (before activated) after used direct swim-up activation technique, but showed highly significance ($P < 0.001$) decrease between G4 (after in vitro activation by adding 20µg/1ml of BCE to culture media) with G1 (before activation), G2 (activation with culture media only) and G3 (activation by adding 10µg/1ml of BCE to culture media).

Table (1): In vitro sperm activation using direct swim-up technique and modified Earl's medium (MEM) enriched with two concentrations of black carrot extract (10µg/1ml and 20µg/1ml).

Parameters	Before activation G1	After activation with culture media G2	After activation with 10µg/1ml BCE G3	After activation with 20µg/1ml BCE G4
Concentration	51.88 ± 4.69	15.84 ± A. 1.61	14.78 ± A. 1.39	14.06 ± A. 1.29
Sperm motility	66.88 ± 2.35	100 ± B. 0.00	100 ± B. 0.00	100 ± B. 0.00
Progressive motility	28.44 ± 2.60	53.28 ± B. .00	59.53 ± B. 2.32	70.78 ± B C. 2.06
Non-progressive motility	39.84 ± 2.15	46.56 ± D. 2.61	40.47 ± 2.32	29.28 ± E. 2.09
Immotile sperm	33.44 ± 2.34	0.00 ± A. 0.00	0.00 ± A. 0.00	0.00 ± A. 0.00
Normal sperm morphology	38.59 ± 1.42	59.38 ± B. 1.91	61.25 ± B. 2.07	61.56 ± B. 1.99

A :Means highly significance different (P<0.001) decrease between G2,G3,G4 with G1.

B :Means highly significance different (P<0.001) increase between G2,G3,G4 with G1.

C :Means highly significance different (P<0.001) increase between G4 with G2 & G3.

D : Means significant difference (P<0.05) increase between G2 with G1.

E : Means highly significance different (P<0.001) decrease between G4 with G1, G2,G3.

Discussion

In vitro sperm activation is a very important step in laboratory technique that plays an important role in determining the outcome of assisted reproductive technologies (22). By using in vitro human sperm activation technique (direct swim-up technique) with modified Earl's medium (MEM), a marked decrease in sperm concentration and immotile sperm for all infertile men was reported after activation as compared to before activation. This result may be due to the beneficial effects of the preparation technique in removing the dead, immotile spermatozoa and semen debris in such a way that only superior quality motile spermatozoa were harvested and unfortunate quality spermatozoa were absent in the after activation medium (23). This seems to agree with Allaw (24).

Swim-up activation technique improves the percentages of human sperm motility, progressive sperm motility (A+B) and normal sperm morphology, because the culture media and procedures for sperm washing can improve sperm function in the assisted reproductive technologies (25). Furthermore, it was reported that only the active motile sperms will swim-up to the superior area or the equatorial zone during in vitro human sperm activation using swim-up technique (23). Additionally, during sperm preparation techniques the motile normal sperm with the best morphology will swim-up successfully (26, 27 and 28).

In the current study, the increased percentage of

progressive motility is directly proportional to treatment with BCE. This may be due to the chemical composition and physical properties of the carrot (*Daucus carota*), especially the fatty acid and mineral contents (Ca, Cu, Fe, K, Mg, Mn, Ni, Se and Zn) of seeds (17 and 29). Several studies demonstrated the protective anti-oxidant properties of Selenium (Se), an essential trace element fundamental to human health. Selenium participates in the processes of cell growth, apoptosis and modification of cell signaling systems and transcription factors (30). Zinc (Zn) Enhances sperm motility (6). Deficiencies of zinc and selenium cause sperm morphological defects (30). Nickel is believed to be important in enhancing sperm motility and the biochemical and physiological significance of nickel in spermatogenesis is poorly understood (31).

Ca⁺⁺ ion (which is one of the content BCE), increases sperm motility by preventing the degradation of cAMP through inhibition of phosphate diesterase enzyme. (32).

Furthermore, BCE also contain protein and free sugars like: glucose, sucrose and fructose (33 and 34). All of these substances stimulate sperm motility and the grade activity of forward movement. Protein maintains sperm osmolarity and in turn integrity of sperm cell membrane (35). BCE is rich in Beta-carotene which is an antioxidant (16). It also contains vitamins E and C which are the major chain-breaking antioxidant in sperm membranes and appears to have a dose dependent protective effect (36). Recent randomized control trials have reported that vitamin E is effective in treating infertile males with high ROS levels (37). The decrease in the level of these free radicals is associated with the decrease of abnormal sperm forms and leukocytes (38). Therefore, a high concentration of BCE is effective in the enhancement of progressive sperm activity during in vitro human sperm activation.

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