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The Effect of Ceratonia siliqua L. on Semen Parameters in Idiopathic Male Infertility: A Systematic Review

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ABSTRACT

Background & aim: At least 50% of infertile couples' problems are related to the male factor infertility. There is widespread use of herbal medicine among men for infertility problems. This study was conducted to determine the effect of Ceratonia siliqua L. (Carob) on semen parameters in idiopathic male infertility.
Methods: This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Articles were searched without time restrictions till September 2023 through searching English databases including PubMed, Scopus, Web of Science, Cochrane Library, EMBASE and ProQuest using MeSH terms of male infertility, clinical trial, herbal medicine, idiopathic male infertility, Ceratonia siliqua L, Carob, semen parameters and their equivalent words in Persian, which were searched individually or in combination. Two reviewers independently assessed eligibility and quality of included articles and any disagreements were resolved by consensus with a third researcher.
Results: Out of 140 articles, 97 documents were removed due to duplication. After screening 43 remaining articles, 30 articles were excluded based on the title and abstract, and eight articles due to being conducted on animals. One article was the study protocol, which was also excluded. Finally, four articles were included in systematic review. All studies reported that Carob is effective in improving some or all parameters of semen including count, motility, and morphology.
Conclusion: Considering small number of human studies related to the effect of Carob on semen parameters, conducting more robust clinical trials are recommended.

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Introduction

Approximately 15% of couples are unable to conceive after 1 year of unprotected intercourse; assuming that 50% of all infertility cases are due to female factors alone, 20-30% are due to male factors alone, and the remaining 20-30% are due to a combination of male and female factors (1). The prevalence of primary infertility based on the clinical, epidemiological and demographic characteristics defined by WHO were 20.2, 12.8 and 9.2%, respectively (2, 3). In addition, the secondary infertility rate was 4.9% (4).

Infertility causes anxiety and depression in couples (5). The infertility rate is as high as 40 to 50% in developing countries, including Nigeria and India. Infertility is a common

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problem affecting one per six couples, most of whom are now seeking medical attention; although diagnostic problems make it difficult to determine a male factor infertility (3).The major causes of infertility and the etiology of male infertility are multifactorial. This could be due to genetic, physical abnormalities, injuries, drugs, infections of the genital tract, radiation, toxins, or unexplained. The major causes of male factor infertility in Nigeria are infection and hormonal abnormalities (3). Other studies have focused on the contributions of environmental factors, such as diet and toxic elements, cultural behaviors, and genetic factors (6).

The major cause of male infertility is idiopathic. Medical management of male infertility is typically empirical when the causes are idiopathic or genetic; in most instances, medical therapy represents off-label use that is not specifically approved by the FDA. Understanding the hypothalamic-pituitary-gonadal (HPG) axis and the effect of estrogen excess is critical for the assessment and treatment of male infertility. The use of certain medical treatments has been associated with an increase in sperm production or motility, and primarily focuses on optimizing testosterone (T) production from the Leydig cells, increasing follicle-stimulating hormone (FSH) levels to stimulate Sertoli cells and spermatogenesis, and normalizing the T to estrogen ratio (6).

Medicinal herbs traditionally treat types of diseases (7). The use of herbal medicines has been increased and due to the poisoning and side effects of allopathic medicines, the number of herbal medicine manufacturers is increasing and herbal medicines are produced on a large scale in pharmaceutical units (6). In the last few decades, there has been exponential growth in the field of herbal medicine. It is popularizing in developing as well as in developed countries due to its natural origin and low side effects.

The Ceratonia siliqua L. tree, traditionally used from 5,000 years ago, is native to the Mediterranean countries (8). Medicinal plants and herbal products with an antioxidant capacity can enhance male reproductive system functions. Experimental use of Carob due to its antioxidant properties is common among infertile men in many countries like Palestine and Iran (8-10). In olden times, carob was used to treat patients on an individual basis and drug was prepared based on the patient's need. In traditional medicine, Carob is known as a fertility-promoting plant. Its tree is evergreen with fruits that have antioxidant activity (11).

Reactive oxygen species (ROS) are ever-present in the body, acting as signal transducers in the complex biochemical cascade required for sperm maturation. At physiological levels, they play an important role in sperm maturation, capacitation, hyper activation, acrosome reaction, and sperm-oocyte function. An excessive amount of ROS results in oxidative stress which is one of the leading causes of male infertility (12).

In recent years, most idiopathic male infertility is due to high levels of ROS, which can damage proteins, fats, and DNA, slow the progress of sperm, damage the acrosome and prevent sperm fertilization with oocytes. Since oxidative stress processes play an important role in idiopathic male infertility, Carob was used due to its potent antioxidant properties to treat idiopathic male infertility (13).

Ceratonia siliqua L. (Carob) Pods are rich in calcium, iron, potassium, phosphorus, sodium, and sulfur, and also contain zinc, copper, and selenium, which are cofactors for antioxidant enzymes. It also consists of fatty acids and phenolic compounds with antioxidant effects. It is also a good source of vitamins C, D, E, B6, niacin, and folic acid (13). It is a herbal medicine used in traditional Tunisian medicine to treat gastrointestinal disorders (11). This plant improves the function of reproductive system through antioxidant and androgenic activity, and affects the levels of luteinizing hormone [LH] and follicle-stimulating hormones (FSH), decreasing peroxidation products of lipids, improvement of motility, and total number of sperm(14). There are few randomized trials which evaluated the effect of Carob on the improvement of semen parameters in male infertility cases (10, 14-16). Carob was investigated in the studies on mice (17) and its efficacy has been reported on male rats spermatogenesis (18) and improving their reproductive function (16, 19). Considering the widespread use of carob to improve male infertility, it appears necessary to review evidence-based literature to examine its effect

on male infertility (20, 21). Therefore, this systematic review was designed to evaluate the findings of clinical trials regarding the effects of Ceratonia siliqua L. (carob) on semen parameters in idiopathic male infertility.

Materials and Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 Statement. In this study, all randomized controlled trials on the effect of carob on idiopathic male infertility following the PICO criteria (population, intervention, control, and outcomes) were examined without any time limitations till September 2023. Idiopathic male infertility was defined as men with disorders in terms of sperm count (oligospermia), abnormal morphology (teratospermia) and reduced sperm motility (asthenospermia) without any pathological cause of male infertility such as varicocele, infection of the reproductive system, obstruction of the sperm pathway, radiotherapy and genetic disorders (22). They used Carob in the form of a capsule, or syrup with different doses. The control group received the placebo or vitamin E, or Lactose. The outcomes were mean changes in semen parameters including count, motility, and morphology. The semen parameters were analyzed based on the WHO criteria (2010; 5th edition guidelines) (23-24).

The search was performed using Medical Subject Headings (MeSH) terms. The researchers used the keywords separately or in combination with other words. These keywords were male infertility OR idiopathic male infertility OR sperm parameters AND Carob OR Ceratonia siliqua L AND herbal medicine or medicinal plants AND randomized clinical trial OR randomized controlled trial OR randomized trial OR RCT. in English databases including PubMed, Scopus, Web of Science, Cochrane Library, EMBASE and ProQuest. The inclusion criteria were:

Men with disorders including decreased sperm count (oligospermia), abnormal morphology (teratozoospermia), and reduced sperm motility (asthenospermia). They had no systemic diseases such as diabetes, thyroid and cerebrovascular disease, no history of chemotherapy, no smoking, alcohol, and drug addiction, the absence of male infertility factor including obstruction of the sperm pathway, sexually transmitted infections, ejaculation disorder, descent of the testes, and varicocele.

The articles retrieved from the databases were evaluated for eligibility criteria by two researchers (S.A. and M.M.). At first, the duplicates were removed. Two researchers independently reviewed the title and abstract of the articles and then reviewed the full text. During the evaluation of the articles, in case of disagreements, discussion and consensus were performed with the third senior researcher (R. L.R.).

The studies were reviewed in three stages including assessing eligibility in terms of inclusion criteria, quality assessment of the studies, and data extraction by all members of the research team. Data related to each study including author, year, study setting, sample size, participants in both intervention and control groups, outcomes, and findings were extracted and presented in an extraction table (Table 1).

The characteristics of the included studies and the risk of bias in studies are presented in table 2. Two researchers (S.A. and F.S.), separately, evaluated the risk of bias according to the Cochrane risk of bias tool (25) **i**n terms of random sequence generations, allocation concealment, blinding of participants and personnel, outcome assessments, incomplete outcome data, and selective reporting. In case of any disagreements, discussion was performed with two senior researchers (R. L.R. and A.T.).

Assessment of risk of bias

Random sequence generation (assessing the possible selection bias)

The studies which were performed with nonrandom process (such as date of birth, file number, ID card number, day or date of admission) were considered high risk, those with random process (such as computer random number generator, random numbers table, dice, and coin) as low risk, and those with insufficient information as unclear risk.

Allocation concealment (assessing the possible selection bias)

The studies which were performed with alternate allocation (based on hospital file

Table 1. Characteristics of studies that examined the effect of Carob on the treatment of idiopathic male infertility

First Author	Year	Country	Title of the article	Intervention group	Control group	Outcomes Studied (Diagnostic Criteria)	Quality assessment of articles
Mahdiani et al (16)	2018	Iran	Effect of Carob (Ceratonia siliqua L.) oral supplementation on changes of Semen parameters, oxidative stress, inflammatory biomarkers and reproductive hormones in infertile men	30 men with Astheno spermia received carob	30 men with Astheno spermia received placebo (lactose)	Differences in the changes in count, concentration and the percentage of motile sperms, total antioxidant capacity, concentration	Fair (Low risk in five RoB items, unclear in two items)
Pilehvari et al (14)	2023	Iran	Effect of Carob Supplement on Spermogram Parameters and Sexual Function of Infertile Men Referred to the Infertility Center, Hamadan, Iran, 2019: A Randomized Controlled Trial	30 men with Astheno spermia received Carob	30 men with Astheno spermia received placebo (lactose)	The highest rate of normal viscosity was in the Carob group (92.6%), motility 74.1 % and 100% of the subjects had normal sperm shape, and viscosity was 82.6% in the placebo group.	Fair (Low risk in five RoB items, unclear in two items)
Aghajani et al (15)	2021	Iran	Effect of Ceratonia siliqua (Carob) syrup and vitamin E on semen parameters, oxidative stress index and sex hormones in infertile men: Protocol for a randomized controlled trial	30 men with Astheno spermia received Carob	30 men with Astheno spermia received Vit. E	The primary outcomes of this Study were the semen parameters (count, motility, and morphology).	Fair (Low risk in five RoB items, unclear in two items)
Sanagoo et al(10)	2021	Iran	Comparison of the effect of Ceratonia siliqua L. fruit oral capsule and vitamin E on semen parameters in men with idiopathic infertility: A triple blind randomized controlled clinical trial	30 men with Astheno spermia received Carob	30 men with Astheno spermia received Vit. E	sperm count increased from 49.08 to 60.22 million/mL in the carob , motility, normal morphology.There was no serious side effect during the treatment course	Good (Low risk in all RoB items)

Table 2. Characteristics of included studies and their risk of bias.

Sanagoo et al. 2021		
Method: RCT		
	This study was a triple blind controlled trial including 50 participants between the ages of 20 and 45 who had idiopathic infertility In two groups, including the Carob group (n = 25) and the control group (vitamin E) (n = 25), one participant was excluded from the Carob group (her husband's pregnancy).	
Participants	Inclusion criteria: Participants with idiopathic infertility, without any chronic disease and medication. Exclusion criteria included genital infection and anatomical abnormalities such as duct obstruction, having Varicocele, Pelvic surgery or need for it, cancer, thyroid, diabetes, kidney failure, testicular atrophy, history of receiving chemotherapy drugs, testosterone, anti-androgen drugs	
Interventions	They were randomly divided into two groups. First group Carob capsules received 1500 mg and the second group received 1500 mg vitamin E capsules three times a day for 90 days.	
Outcomes	The results of this study was semen parameters (count, motility and morphology).	
Results	Use 1500 mg oral Carob capsules per day for 90 day improved sperm motility, but had a significant effect on the count and development Morphology compared to vitamin E capsules	
Risk of bias	**Authors' judgment**	**Support for judgment**
Random sequence generation (Selection bias)	Low risk	The allocation sequence was generated by the person not involved in the research
Allocation concealment (Selection bias)	Low risk	The envelopes were sealed, opaque, closed which contained 90 capsules, and their preparation was based on the allocation sequence by the person not involved in the research.
Blinding of participants and personnel (Performance bias)	Low risk	This study was a triple blind controlled trial
Blinding of outcome assessor (Detection bias)	Low risk	This study was a triple blind controlled trial
Incomplete outcome data (Attrition bias)	Low risk	Only one person was exclude due of pregnant his woman in Carob group
Selective reporting (Reporting bias)	Low risk	All out come have been reported
Aghajani et al. 2021		
Method: RCT		
	This study was a parallel, randomized controlled trial on 60 idiopatic infertile participants with asthenospermia, teratospermia and oligozoospermia with 30 men in the experimental group taking Carob syrup while 30 men in the comparison group receiving vitamin E.	
Participants	Men with disorders in terms of sperm count (oligospermia), abnormal morphology (teratospermia) and decreased sperm motility (asthenospermia), statistical results of semen analysis based on WHO criteria (2010; Fifth Edition Guidelines), age between 20 to 45 Year, infertility lasts more than 1 year, BMI less than 30 and having a normal female partner, normal testis volume, absence of systemic illness diseases, no history of chemotherapy, absence of smoking, alcohol, and substance or drug addiction and lack of history of vasectomy or obstructive azoospermia included in the study. Patients with history of obstructive azoospermia or vasectomy, recurrent or residual varicocele and history of cryptorchidism or chromosomal abnormalities such as Kleinfelter's syndromeand individuals with hypogonadism or pituitary disorders, patients with azoospermia, genital tract infection, and a history of chemotherapy or any fertility treatment were not included in the study.	

Interventions	Participants were randomly divided into two groups. Carob syrup twice a day or Vitamin E 100 mg twice a day for 90 days.
Outcomes	The outcomes were semen parameters, hormonal levels, oxidative stress markers, and pregnancy rate.
Results	Semen count, morphology and motility parameters were significantly higher in the carob group. No significant difference was detected in post-treatment hormonal parameters and oxidative markers between groups, except for total antioxidant capacity (TAC) which was higher post-treatment in carob group. A significantly higher pregnancy rate was found among the carob group.

Risk of bias	**Authors' judgment**	**Support of judgment**
Random sequence generation (Selection bias)	Low risk	Randomization was performed as permuted block randomization with a 1:1 allocation. The randomization table was kept in a locked file cabinet and in a password-protected computer in the infertility center.
Allocation concealment (Selection bias)	Low risk	The researcher folded two times the paper containing 4-size blocks, put them in the standard thick envelopes with serial numbers. For every eligible patient, the researcher randomly selected one of the envelopes after shuffling and assigned subjects into intervention and comparison group.
Blinding of participants and personnel (Performance bias)	unclear risk	The study has not clarified blinding of the participants.
Blinding of outcome assessor (Detection bias)	Low risk	The outcomes assessor performing and analysing data from the semen samples, endocrine tests, and biomarker analysis were blinded to the participant grouping.
Incomplete outcome data (Attrition bias)	Low risk	There were five drop out of participants in the Carob group three participants were unable to contact and two participants requested to withdraw and in Vitamin E group one person have allergy, one person unable to contact and two participants requested to withdraw.
Selective reporting (Reporting bias)	Unclear risk	All out come have been reported. Insufficient information has been reported for some outcomes.

Mahdiani et al.2018

Method: RCT	
Participants	This study was a randomized double-blind controlled clinical trial which included 60 men with asthenospermia. At least one year after the decision to have children and 45-20 years and the development of asthenotratospermia (idiopathic) were the inclusion criteria. Infertile men with a specific cause (such as hormonal disorders), infertility of the wife, in case of drug and alcohol use, underlying disease such as diabetes, kidney disease (more than twice the creatinine), chronic liver disease and taking antioxidant supplements They have not been included in the study for the past three months.
Interventions	Participants as intervention (Carob) and placebo (lactose) groups (n=30). The intervention group received 1500 mg of Carob per day capsules), and the placebo group received 1500 mg placebo capsules daily for 3 months. One person in Carob group did not use Carob capsule and 2 person in lactose group couldn't complete the study.
Outcomes	Count, concentration and percentage of mobility sperm, total antioxidant capacity, MDA concentration and plasma inflammatory markers were significantly different after the intervention. Changes in sex hormones in the two groups were not significant.
Results	

Risk of bias	**Authors' judgment**	**Support of judgment**

Random sequence generation (Selection bias)	Low risk	The allocation sequence was generated by the person not involved in the research
Allocation concealment (Selection bias)	high risk	The envelopes were not sealed and opaque.
Blinding of participants and personnel (Performance bias)	Low risk	In this study, the patients and the researcher were blind to the groups.
Blinding of outcome assessor (Detection bias)	unclear risk	In this study, the outcome assessors were blind to the groups.
Incomplete outcome data (Attrition bias)	Low risk	One person in Carob group did not use Carob capsule and 2 person in lactose group could not complete the study.
Selective reporting (Reporting bias)	Low risk	All outcomes have been reported.

Pilehvari, et al.2023

Method: RCT	
Participants	This study was designed as a two-group randomized, double-blind, placebo-controlled clinical trial performed on idiopathic infertile men. 60 men were selected. The inclusion criteria were being a man under 40 years of age with primary infertility; abnormality of at least one of the semen parameters), lack of infertility-related disorders, lack of chronic diseases, having a BMI less than 30, lack of the use of narcotic substances, alcohol and any drugs that disrupt spermatogenesis, pituitary suppressants, anti-androgens), use of drugs that cause ejaculation failure, use of drugs that cause dysfunction, long-term use of drugs such as anabolic steroids, cannabis, heroin and cocaine, no history of testicular and vasodilator surgery, no contact with pesticides, heavy metals and solvents, and non-use of antioxidant supplements in the last three months.
Interventions	The first group received 1.5 grams of Carob daily and the second received 1.5 grams Placebo.
Outcomes	The outcomes included semen volume and normal viscosity, sperm count, shape and motility and male sex hormones.
Results	Normal semen volume and sperm count per ml decreased (P> 0.05), normal viscosity and progressive motility increased and also normal sperm shape increased in Carob group compared to placebo group (0.05 P <).

Risk of bias	Authors' judgment	Support of judgment
Random sequence generation (Selection bias)	Low risk	The allocation sequence was generated by the person not involved in the research
Allocation concealment (Selection bias)	Unclear risk	The study has not clarified the method of allocation concealment.
Blinding of participants and personnel (Performance bias)	Low risk	The study was double-blind, Placebo-controlled clinical trial
Blinding of outcome assessor (Detection bias)	unclear risk	The study has not clarified blinding of outcome assessors.
Incomplete outcome data	Low risk	In the Carob group three person loss follow-up, two

(Attrition bias)		people emigrated, one resigned due to illness and the other. In placebo (lactose) group four persons loss follow-up, one person emigrated, one resigned due to illness and the other.
Selective reporting (Reporting bias)	Low risk	All out come have been reported.

number or birth date or ID card number) were considered high risk, those with a central assignment (randomization with web-based or cell phone or phone or, opaque and sealed envelopes) as low risk, and those with insufficient information as unclear risk.

Blinding of participants and personnel (assessing the possible performance bias)

The studies which both blinded participants and personnel were identified as low risk, those with no blinding as high risk, and those with insufficient information as unclear risk.

Blinding of outcome (assessing the possible detection bias)

The method of assessment of each outcome was examined separately. Complete blinding of the outcome's assessor was considered as low risk; otherwise, it was reported as high risk, and the risk of bias was unclear if there was insufficient information.

Incomplete outcome data (assessing the possible attrition bias)

It was identified high risk if there is many missed data or in the case of unbalanced loss in the two groups. Also, it was considered low risk there is no or little missed data, or the two groups have the same reasons for the sample loss. Unclear risk of bias is considered when there is insufficient information.

Selective reporting (assessing the reporting bias)

High risk of bias was reported if all the predicted results are not reported or are incomplete reported and are useless. The low risk of bias was identified if all predicted results of the study are reviewed and reported. Unclear risk of bias is considered when reporting insufficient information.

After reviewing the databases, four articles were finally included in this study, but for some reason, data in this review was reported as descriptive and qualitative through a systematic review.

The selected control group for Carob and the method of analysis were different in the included studies. Vit E was used in the study of Sanagoo and Aghajani(10, 15) as a control group, but Mahdavi and Pilehvari (14, 16) used lactose as a control or placebo group. In the study by Pilehvari, the results of semen parameters were reported in terms of percentage and number(14), but in the other three studies, the mentioned parameters were reported in terms of Mean±SD (10, 15, 16) For these reasons, no meta-analysis was possible. In this research all ethical guidelines, such as avoidance of similarity and expression the right and true finding were considered.

Results

A total of 140 articles were identified by searching, of which 97 were removed due to duplication. After screening of 43 remained articles, 30 articles were excluded because they were not interventional study, as well as eight articles due to being conducted on animals. One article was study protocol (22), which was also excluded. Finally, four articles were included in this systematic review (10, 14-16). The PRISMA flowchart for systematic reviews was used to present the selection process of the studies (Figure 1). These four entered studies were clinical trials and were conducted in Iran (10, 14-16) including a total number of 230 infertile men (50 in the study of Sanagoo et al. (2021), 60 in the study by Aghagani et al. (2021), 60 in the study of Mahdiani et al. (2018), and 60 in the study conducted by Pilehvari et al. (2023) (10, 14-16). Aghajani et al. (2021) used Carob for the intervention group and Vit E for the control group (10, 15). Mahdiani et al. (2018) and Pilehvari et al. (2023) used carob for the intervention group and lactose for the control group (14, 16). The duration of intervention was 12 weeks for two included articles and daily dose of Carob was 1500 mg in the form of capsule (10, 14, 16). In the study of Aghajani et al. (2021), participants were randomly divided

into the two groups of Carob syrup or Vit E 100 mg twice a day for 3 months (15). All four studies reported that Carob is effective in improving some or all semen parameters including count, motility, and morphology (10, 14-16).

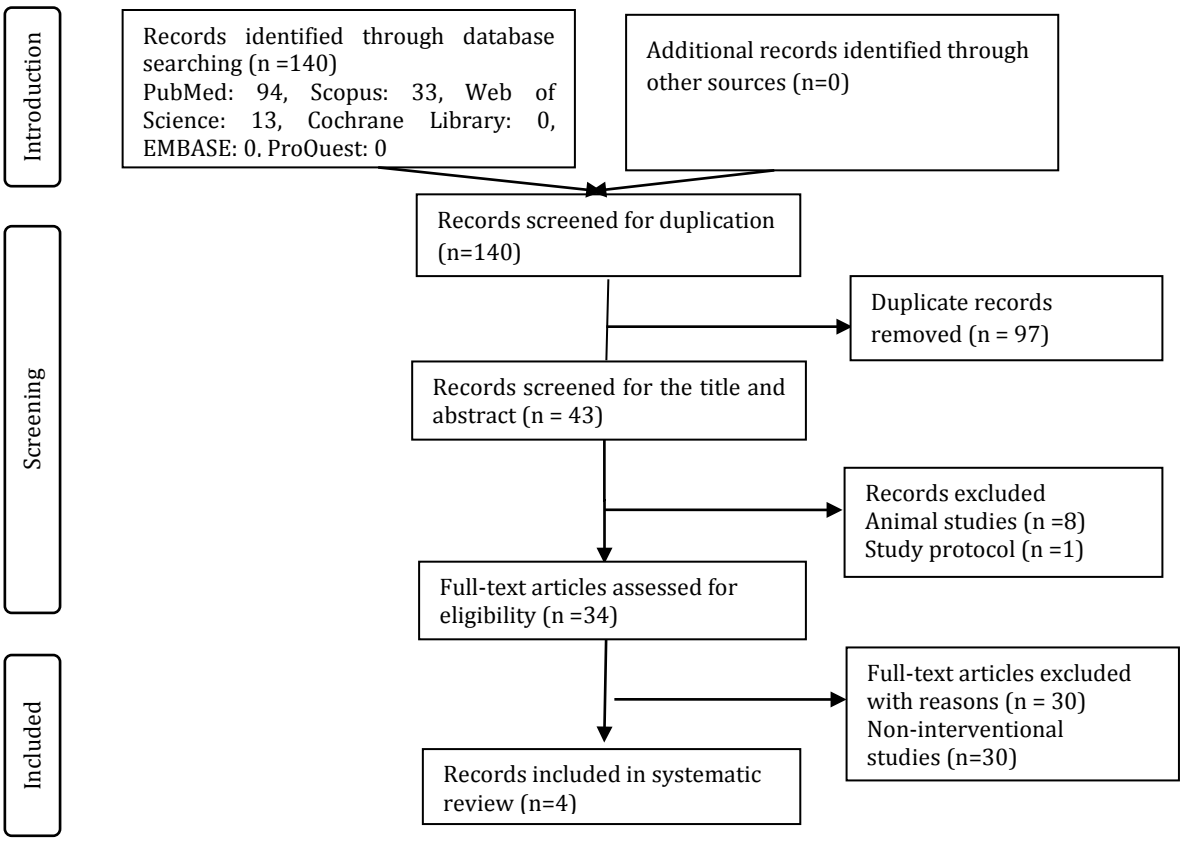

Figure 1. Study selection steps based on the PRISMA flow diagram

Pregnancy rates were significantly higher in the Carob group(15). In the studies conducted by Sanagoo et al. (2021), Aghajani et al. (2021), Mahdiani et al and (2018), Pilehvari et al. (2023), sperm motility increased in the Carob group(10, 14-16), and in the study by Pilehvari et al. (2023), the number of sperm decreased and viscosity of sperm increased, and in study of Sanagoo et al. (2021), morphology and count significantly improved in the Carob group, that was not significant while comparing with the Vit E group(10, 14). Pilehvari et al (2023), showed that after the intervention, normal semen volume and sperm count decreased, normal viscosity and progressive motility increased, and also the normal morphology of sperm increased in the Carob group compared to the placebo group when controlling semen levels in the pre-intervention stage(14). Sanagoo et al. (2021) reported that Carob improves sperm motility but has no significant effect on improving morphology and count compared to the control group, as morphology and count significantly increased in both Carob and Vit E groups(10). The results of these RCTs showed no side effects during the treatment course of Carob (10, 14-16).

Sperm motility

In the study of Sanagoo et al. (2021), a triple-blind trial on 50 participants comparing the effect of the Carob and Vit E capsules, sperm motility significantly increased in the Carob

group compared to the Vit E group (from 45.90% to 52.90% in the Carob group but decreased from 55.23% to 38.10% in the Vit E group; adjusted mean difference (aMD)=17.22%, 95% CI: 8.53 to 25.92) after the intervention(10). In the study of Mahdiani et al. (2018), significant difference was observed in the percentage of sperm motility in the Carob group (5.4±1.02) and in the Lactose group (0.73±0.75) (P=0.019)(16). Aghajani et al. (2021) showed the percentage of total motile sperm (increased to 46.72 ± 17.84 in the Carob group and increased to 29.48 ± 20.22 in the Vit E group (Adjusted mean difference (aMD: 15.82, 95% CI: 6.21 to 25.44)(15). In the study by Pilehvari et al. (2023) it was found that the progressive motility of more than 32.2% in the Carob group was74.1%, and in the lactose group was 60.9% (P = 0.31); also normal sperm shape in the Carob group was 100% and in the lactose group was 82.6% (P = 0.03)(14). Sanagoo et al. (2021) reported that oral Carob improved sperm motility but didn't significantly improved morphology and count. There were no serious side effects during treatment(10).

Sperm count

The study by Sanagoo et al. (2021) showed that sperm normal count significantly increased in both Carob and Vit E group. After the intervention, the increased mean sperm count from 49.08 to 60.22 million/ml was observed in the Carob group, and from 47.64 to 58.88 million/ml in the vitamin E group (adjusted mean difference (aMD)=1.124 million/ml, 95% CI:-12.892 to 15.141)(10). Mahdiani et al. (2018) in their study showed significant differences after the intervention in the number of sperm in the Carob group (12.51±4.88) compared to the Lactose group (0.86±0.42) (P<0.001), and concentration in the Carob group (3.71±2.9) compared to the Lactose group (1.7±0.23) (P=0.01)(16). Aghajani et al. (2021) showed that after the intervention, the number of sperm increased to 41.87 ± 30.59 in the Carob group and increased to 27.28 ± 25.98 in the Vit E group (aMD: 14.93, 95% CI: 0.35 to 29.44)(15). The study by Pilehvari et al. (2023) showed the normal sperm count per ml (million) was 85.2% in the Carob group compared 78.3% in the lactose group, (P=0.71)(14).

Sperm morphology

In the study by Aghajani et al. (2021), normal morphology increased to 11.36 ± 9.22 % in the Carob, but decreased to 4.50 ± 4.71 in the Vit E group (aMD:6.87, 95% CI: 2.78 to 10.95)(15). The study by Sanagoo et al. (2021) showed that normal morphology in the Carob group increased from 11.52% to 67.05%, and from 10.20% to 77.47% in the vitamin E group (aMD=-10.88%, 95% CI: - 22.30 to 0.536)(10). Pilehvari et al. (2023) showed that after the intervention, normal sperm shape in the Carob group was 100 and in the lactose group was 82.6% (P= 0.03)(14). Mahdiani et al. (2018) in their study showed that normal morphology increased to 0.19±0.01 in the Carob group compared to 0.15±0.05 in the Lactose group (P=0.011)(16). In the study by Pilehvari et al. (2023), the normal viscosity in the Carob group was 92.6% and in the lactose group 82.6% (P=0.002). Their results showed that after the intervention the normal semen volume in the Carob group was 92.6% and in the Placebo group was 91.3%, which was significantly different in the two groups (P = 1.00)(14).

Risk of bias

In the terms of risk of bias among the included studies, regarding random sequence generations, all the studies by Sanagoo et al. (2021), Mahdiani et al. (2018), (Pilehvari et al. (2023) and Aghajani et al. (2021) were low risk (10, 14-16). Allocation concealment in two studies (Sanagoo et al. (2021) and Aghajani et al. (2021) (50%) was low risk (10, 15), but in studies by Pilehvari et al (2023) and Mahdiani et al. (2018) were unclear risk (14, 16). The blinding of participants and personnel was low risk in three articles (Mahdiani et al. (2018), Pilehvari et al. (2023) and Sanagoo et al. (2021) (10, 14, 16); but unclear in one study (Aghajani et al. (2021) (15). The blinding of outcome assessors was low risk in two studies of Sanagoo et al. (2021) and Aghajani et al. (2021) were low risk (10, 15), but unclear in two other studies including Mahdiani et al. (2018) and Pilehvari et al. (2023) (14, 16). Incomplete outcome data was low risk in all investigated articles(10, 14-16) (Figure 2, 3). selective reporting was low risk in in three studies of Sanagoo et al. (2021), Pilehvari et al. (2023) and Mahdiani et al. (2018) (10, 14, 16), but unclear

in Aghajani et al. (2021) (15). All studies appeard to be free of other sources of bias (10, 14-16).

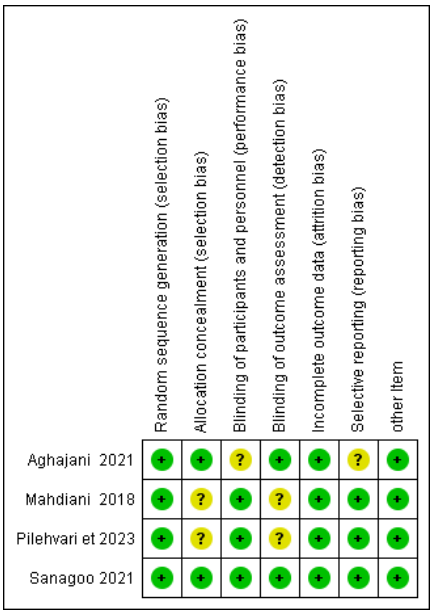

Figure 2. Risk of bias summary: Reviewers' judgment on risk of bias items for each included study

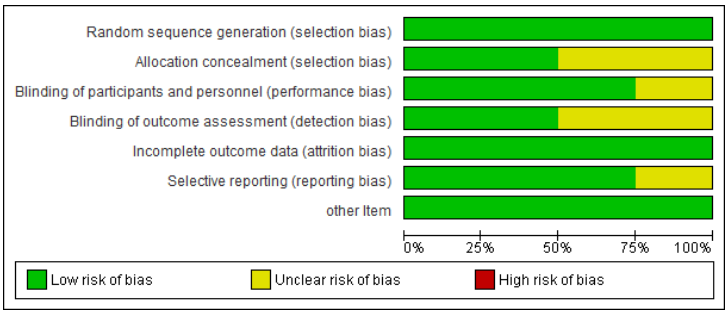

Figure 3. Risk of bias graph: Reviewers' judgment on risk of bias items presented as percentage across all included studies

Discussion

The present study was performed to identify the effects of Carob on idiopathic male infertility through a systematic review of the literature. All four studies reported that Carob is effective in improving some of the semen parameters including count, motility, and morphology (10, 14-16). The research on animals similarly showed that the beneficial effect of Carob extract improves the quality of semen(26). Vafaei et al. (2018) reported that Carob increases sperm count and motility in the infertile rats. Carob extracts improved semen quality, biochemical parameters, germinal epithelium thickness, and testosterone level in busulfan-induced infertile rats(27). Mokhtari et al. (2012) showed that Carob could effectively improve the function of the reproductive system in mice. Also, the extracts of Carob cause changes in the brain and Pituitary-testicular axis. It was affected by the quantity of LH, FSH, testosterone and dihydrotestosterone hormones, and testicular tissue as well as fertility improvement in male rats (18). Soleimanzadeh et al. (2020) showed that co-

administration of Carob fruit hydro-alcoholic extract improved sperm parameters, elevated sexual hormones, TAC (Total antioxidant capacity), Glutathione GSH (linear tripeptide of L-glutamine, L-cysteine, and glycin) content, and antioxidant enzymes activity of serum, and reduced serum MDA (Malondialdehyde) levels (17). Sadeghzadeh et al. (2020) reported that Carob extract can significantly prevent the adverse effects of cyclophosphamide on sperm motility, and decrease tissue Malondialdehyde (MDA) levels, serum total antioxidant and testosterone. Ceratonia extract can modify the reproductive toxicity of cyclophosphamide in rats due to its antioxidant properties(26). Ghorbaninejad et al. (2021) showed that Carob extract can increase semen parameters and induce spermatogenesis in infertile mice. Carob extract improves spermatogenesis by affecting Sertoli and Leydig cells, and may regulate spermatogenic hormones through its amino acid components identified in the extract(28). All the above mentioned studies confirmed the results of the present study (17, 18, 26-28). Ata et al. (2018) reported that Carob could have beneficial influences on sperm concentration in rabbits. However, there were no significant changes in ejaculate volume, pH, progressive motility, head defect, tail defect, and percentage of viable semen. Their findings were not consistent with the results of the present study (29). In all four studies reviewed in the present study, Carob increases count, motility, and morphology of sperm (10, 14-16), but in the study by Ata et al. (2018), it has been reported that it only increases the count of sperm (28). Pilehvari et al. (2022) reported only an increased viscosity rate in the Carob group(14).

Recent studies have shown that antioxidants and proper nutrition improve sexual function, and also increase sperm count and fertility rate. Studies have shown that Carob improves fertility in infertile men (10, 14-16).

This systematic review was the first review research, which investigated the efficacy of Ceratonia siliqua L. as a potential herb on semen parameters in infertile men. This study was done by a thorough and sensitive search strategy with the cooperation of a research librarian.

One of the limitations of this study was small number of studies included in the review, which makes taking a strong conclusion difficult, like previous systematic reviews focused on different issues of infertility (30, 31). The other limitation was that all included studies in the review were performed in Iran and no article was found in other countries to evaluate the effect of Carob on idiopathic male infertility. Another limitation was the existence of bias in the reviewed articles (10, 14-16). Therefore, the reviewed articles had a mediocre quality. In two studies by Aghajani et al. (2021) and Sanagoo et al. (2021), Carob was not compared with placebo and rather compared with Vit E; therefore, conducting a meta-analysis was not possible. So, more robust studies are recommended (10, 15).

Clinical application of Carob may improve semen parameters, which is probably related to both its involvement to change the level of testosterone and its antioxidant properties. Further studies are needed to be performed in order to determine the optimal dose and duration of treatment.

Conclusion

The results of this systematic review of four articles showed improved quality of semen parameters in the Carob group. Semen parameters including sperm count, morphology, and motility significantly improved in the intervention group compared to the control group.

Since there were only four articles which have been clinically done on humans and also the small number of participants in this study, the findings cannot be generalized to the community, therefore, it seems that further studies is needed to be conducted with different doses, more intervention duration, and follow-up periods to investigate stability of its effect as well as its long-term side effects.

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Conflicts of interest

Authors declared no conflicts of interest.

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