Effect Of *Amomum Granum Paradise* Culture Medium In Activating The Sperms Of Infertile Patients With Mild Asthenozoospermia Using Two Different Techniques In Vitro.

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ABSTRACT

The current study included the examination and activation of 25 semen samples of men with asthenemia. The study aimed to improve reproductive ability and activation of spermatophores using Amumum granum Janna two different techniques represented by swimming and gradient centrifugation, with an incubation period of 30, 45 , 60 minutes. The results showed a significant ($p < 0.05$) increase in the percentage of motile sperm, the degree of sperm activity, and the coefficient of sperm motility, with a significant decrease in the concentration of sperm, the percentage of abnormal sperm, and the concentration ,We conclude from the current study that Amomum granum has a clear role in stimulating sperm transactions and thus has a role in increasing the chances of successful external fertilization.

INTRODUCTION

Plants playing an increasingly role in maintaining human health due to their medicinal value. The World Health Organization estimates that about 80% of the world's population still relies on bioactive components of plants as folk medicine in traditional remedies The availability of phytochemicals in the plant extract, reduction of their side effects, also their ability to target the biochemical pathway, and the low cost of medicinal plants have led to their worldwide spread and use for the prevention and treatment of human diseases (1) Medicines in traditional settings are mainly prepared from a single plant or a combination of plants, and their effectiveness depends largely on the use of an appropriate plant part in addition to the biological effectiveness, which in turn depends on the presence of the quantity and the nature of the secondary extract in a crude drug (2), therefore, traditional societies and ethnic nationalities have used medicinal plants over the years in ethnic medicine to treat various diseases without any scientific knowledge of the physiologically active components known as phytochemicals, which were responsible for the medicinal and pharmacological potential of the plant (3).

Aframomum melegueta is one of the most important plants commonly used in the rainforest region of Africa, especially Nigeria, as a spice in food, and it is a medicinal plant that belongs to the Zingiberaceae family. In Nigeria, local names include: “ose-oji” (Igbo), “Atari” (Yoruba), and “Sita” (Hausa). (4)

Advances in industry and biotechnology have led to improvements in human life, but have produced many physical, chemical and biological factors that affect our environment, as 15% of couples around the world suffer from infertility, and the rate of the male factor ranges between 40-50% of all cases of marital infertility, as the There are many causes of male infertility for infertility, including blockages, varicocele, infection, exposure to toxins and radiation, despite progress in our understanding of the physiology of human reproductive organs, 23% of the causes of infertility remain unknown, as some of the causes of male infertility can be hereditary. (5).

the importance of the reproductive system in preserving the type of the organism, studies related to the reproductive aspects continued in order to identify the foundations for preserving the type, the integrity and completeness of the genetic material, and the continuation of survival. And the wife did not show any clear reason why the pregnancy did not occur, so the man is considered sterile (6). Therefore, the term infertility is defined as the failure to achieve pregnancy after one year of continuous marriage without the use of contraceptives (7) , Infertility occurs in 3.5-16.7%

of marriages worldwide (8) About 30-40% of infertility causes are caused by the wife, 10-30% are caused by the husband, and 15-30% are contributed by both spouses (9), while unexplained infertility represents about 5-25% (10).

Active movement and good progressive movement is what is necessary for normal sperm so that they have to penetrate the cervical mucus and migrate through the female genital tract to reach the fallopian tube, and complete the process to fertilizing the egg (11).

A patient is considered to have asthenospermia when the percentage of motile sperm is less than 50% within one hour of ejaculation (12).

Cultures used to stimulate human semen, which results in good semen quality, is one of the various important factors in the success of in vitro infertility treatment (Kaewnoonual et al., 2008), and sperm preparation by using a medium containing glucose, lactate and bicarbonate results a Significant increase in the progressive motility of sperm by 16-40% of spermatogonia (Holt and Harrison, 2002).

Infertility cases represented by the syndrome of lack of weakness and distortion of sperm are treated by artificial insemination using the sperm of the husband (AIH), as well as by using the process of microinjection into the cytoplasm of the egg, as its results showed a good success rate in the treatment of explained and unexplained infertility (Motazedian et al., 2010).

MATERIALS AND METHODS

The research was conducted in private laboratories / Karbala Governorate, semen samples were collected in clean, sterile dry Petri-dishes by hand masturbation after an abstinence period of not less than three days and not more than five days, then the samples were placed before being examined ,The incubator was at a temperature of 37 °C to allow it to have a natural bowel movement, and it was examined macroscopically and microscopically after fixing the liquefaction time as follows:

1 - Macroscopic Examination

1-1 Liquefaction Time: The newly ejaculated semen is characterized by being liquid and quickly turns into a semisolid or coagulated state under the influence of the Protienkinase enzyme secreted by the seminal vesicles. Normal effusion occurs within 15-20 minutes after collecting the sample.

1-2 PH: The pH was measured after ejaculation with special strips for this purpose (Germany) PH Universalindkator numbered from 1-14, and the pH of semen is somewhat basic, ranging between 7.0 – 8.5 .

1-3 Semen volume: The sample size was measured using volumetric test tubes

1-4 Viscosity: The viscosity of semen was estimated by observing the mucous thread, by pouring out the sample from a Pasteur pipette. The consistency is abnormal when the sample is a thread of more than 3 .

2- Microscopic Examination: One drop was taken from each sample, mixed well after complete liquefaction, and the drop was placed in the center of the Mackler chamber (sperm meter) and covered with the lid of the device. Then the samples were examined under force X 10 and then under force X40 before and after stimulation to determine the following sperm parameters:

2-1 Sperm concentration: Sperm concentration was estimated by placing 20 microliters of semen sample in a special hole in the center of the counter designated for this purpose, and the number of sperms was counted in ten squares in a straight line out of 100 squares.

2-2 Percentage of Sperm Motility and Grade of Sperm Activity: The percentage of motile sperm was determined using a sperm meter and according to the following equation:

The percentage of motile sperm=$\frac{The\ number\ of\ motile\ sperms}{Total\ sperm\ count} \times 100$

The degree of sperm activity was determined based on what was stated in the guide of the World Health Organization (12), according to the following gradation:

0 non-motile sperms, 1- sperms with local movement, - 2 medium-slow progressive movement, 3 - good straight progressive movement, 4- very good progressive linear straight movement.

2-3 Sperm Motility Index(SMI):

Some samples have a high percentage of sperm motility but have a low level of activity, while other samples show a low percentage of moving sperm and a high degree of activity. Therefore, research has sought to measure the sperm motility coefficient to link these two important parameters (Bartoov et al., 1991) and calculate Sperm motility coefficient multiplied by:

SMI= Percentage of motile × Degree of sperm activity

2-4 Percentage of Normal Sperm Morphology: The shape of the sperm was characterized using Spermac stain kit before and after activation (Oettle, 1986), and it was examined with a light microscope under a large lens (x100) using oil immersion, and the percentage of normal sperm was calculated according to the following equation:

Percentage of Normal Sperm Morphology=$\frac{The\ number\ of\ normal\ sperm}{Total\ sperm\ count} \times 100$

And the patient is considered to have abnormal sperm if the percentage of normal sperm is less than 30% (12).

3- Stimulation of sperm parameters: - Patients with moderate and severe asthenospermia were identified and the sperm stimulation process was performed and prepared for artificial insemination with three different techniques and incubation periods (35-45-60), as follows:

3-1 Swim up Technique: : a 0.5 ml of the prepared culture was placed in a graduated test tube, then 0.2 ml of semen was pushed by a Pasteur pipette to the bottom of the tube gently and incubated tilted at 37 °C in the incubator for the previous periods referred to above. After that, a drop was taken from the upper surface for the purpose of examination at each incubation period (Al Morshedy, 2006).

3-2 Centrifugation Wash-Out Technique: The washing and centrifugation technique was used (Al-Hadi, 1997), where 0.5 ml of culture medium was placed with 0.2 ml of semen, then the centrifugation process was carried out at a force of 2000 rpm for 5 minutes. The supernatant was removed after the centrifugation process, and the sperm pellet was covered with 0.5 ml of culture medium, then the tube was placed in the incubator obliquely, and its pre-incubation period was examined by taking a drop from the upper surface.

Statistical analysis :

The results of the research were analyzed statistically using Design Randomized Completely, then the significant differences between the rates were tested using the least significant difference (L.S.D) at the significance level (P>0.05) (Rawi, 2000).

RESULTS AND DISCUSSION

Table (1) Effect of using the aqueous extract of Amomum granum paradis seeds

FertiCult Flushing Medium in stimulating sperm parameters for patients with severe asthenospermia (DGC) Density gradient centrifugation with an incubation period of 45 minutes.

Amomum granum paradis	**FertiCult Flushing Medium**		**studied standards**
Density gradient centrifugation	Density gradient centrifugation	**before activation**	
d **18.1** **3.1±**	**D** **8.1** **1.9±**	**a** **29** **6.8±**	**Sperm concentration 10^6×/ ml**
b **16.4** **3.4 ±**	**d** **23.3** **3.6±**	**a** **14** **2.1±**	**percentage** **for moving sperm**
C **1.8** **0.2±**	**c** **2.3** **0.3±**	**a** **1.5** **0.6±**	**The degree of sperm activity**
D **48** **7.5±**	**d** **56.3** **9.7±**	**a** **42.6** **6.4±**	**Percentage of abnormal sperm**

The values represent the mean ± standard error S.E

The number of samples = 11

($P < 0.05$) the different letters indicate the significance.

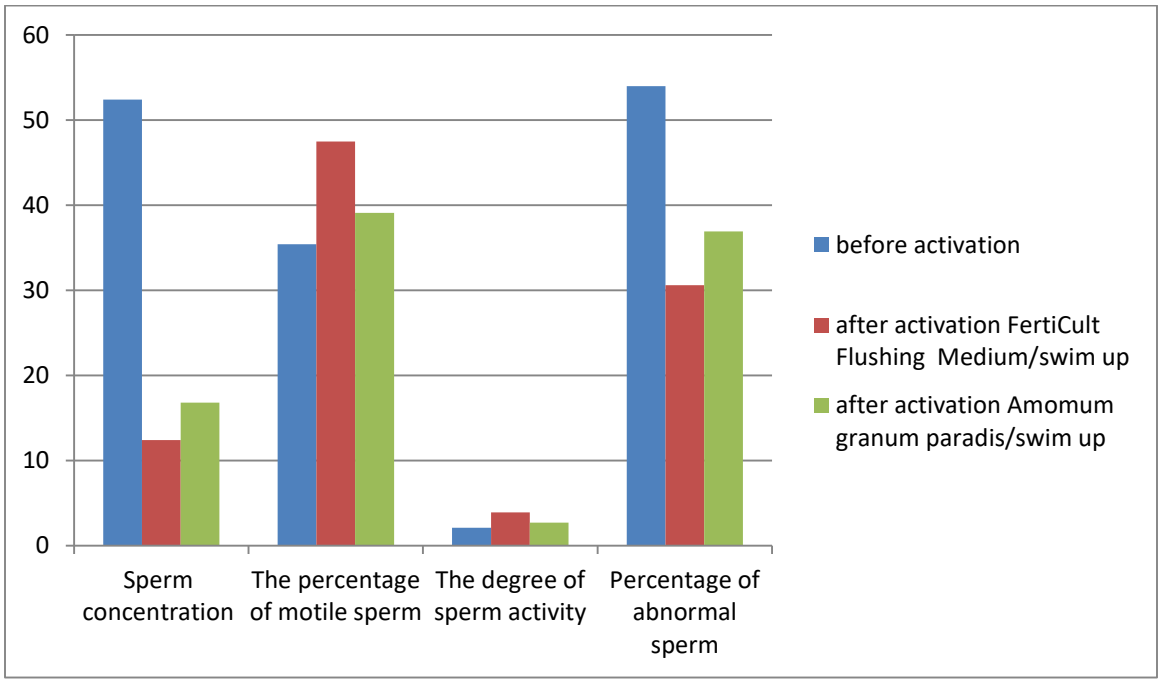

Figure (1) Effect of using the aqueous extract of Amomum granum paradis seeds

FertiCult Flushing Medium in stimulating sperm parameters for patients with severe asthenospermia (DGC) Density gradient centrifugation with an incubation period of 45 minutes.

Insemination in the living body depends mainly on the movement of sperm and their number, because fertilization inside the body requires the passage of sperm within the cervical mucus, which naturally depends on the linear progressive movement of sperm, and that studying the effect of using various culture media and various techniques in glass to further improve the parameters of sperm and isolate them from The surrounding environment was found as an opportunity to treat infertility for infertile patients The techniques used to stimulate human sperms in vitro or outside the body are designed in a way that simulates or imitates the processes that occur in the living body in terms of separating sperm from seminal plasma, and selecting sperms with normal and good movement. (13)

Our findiny a decrease in sperm concentration in oligozoospermia and asthenozoospermia compared with the normozoospermia group. This may be due to (chromosomal abnormalities, disturbed gene regulation, environmental factors, factors associated with infection and immunity, endocrine dysfunction (14) and asthenozoospermia, which is defined as less than (40 percent) total semen motility The current study showed that patients with normal sperm morphology reduced sperm concentration after apheresis, as shown in the results. Swimming reduced sperm count with head and tail ultrastructural defects. This is consistent with (15) & (16). The results of the current study showed a significant increase in liquefaction time in the asthenozoospermia group compared with the normal group. Our results also agreed with the study that showed the abnormal group, the mean pH and liquefaction time were statistically higher than the normal group (17). The increase in sperm after swimming affects the total number of sperm and motility. The swimming method was associated with an increased pregnancy rate, sperm concentration, and sperm motility, (18)., After sperm preparation, the concentration increase of low normal polymorphisms is greater than the GC method (19) The data revealed increased mobility, vitality, and normal formation as measured by swimming (20). There was a significant decrease of $P<0.05$ in sperm concentration after in vitro activation by FertiCult medium due to the inability of dead animals and abnormal sperm to swim and migrate from the pellt to the top layer of culture medium. These results were in agreement with other studies using culture medium as the medium for activation. Sperm in vitro With

regard to sperm motility, there was a significant increase in sperm motility compared to pre-stimulation. These culture media (FertiCult & aqueous extract) contain many ions such as sodium, potassium, calcium, magnesium, phosphate, pyruvate and lactate. These ions act as an energy source to activat (21).

the sperms that Increase its movement This was consistent with the study of (21) and (22). After in vitro sperm stimulation (ISA), all sperm parameters of all groups in this study were improved except for sperm concentration and non-motile sperm (%). The sperm concentration of all groups before activation was higher than after activation, due to The inability of dead and abnormal sperm morphology with impaired motility to swim and migrate to the upper layer of the culture medium as supported by (23). The results showed that the SU method was more effective than the DGC method in improving sperm count and motility (1). The study showed a significant decrease in anti-spermatozoa. There was a significant decrease in sperm immobility (D-score may indicate that only motile sperm swim to the top of the layer and are dead and stationary. The sperm remained in the lower part of the middle. This was consistent with the results of (25) Our study found a significant increase ($P < 0.05$) in morphologically normal spermatozoa (MNS) compared to the results before activation. It may be because spermatozoa are considered normal forms through their greater activity during spermatozoa with abnormal shapes that are characterized by It has poor movement at the bottom (26). A significant decrease in sperm concentration after in vitro activation was observed in both media,

It may be due to the inability of dead and weakly active spermatozoa to move upwards and travel from the bottom layer upon top layer of the implant medium, similar results have been recorded by other studies (24) There was an improvement in the percentage of sperm motility and the proportion of morphologically normal sperm after activation. This finding may be due to the rapid movement of normal sperm from the bottom layer of the culture medium, where both media consist of Ca++ and energy supplements to enhance motility, and other factors to cut motility, which leads to In turn, the sperms are removed from the stress factor and the production of reactive oxygen species responsible for that weak sperm and DNA damage (27). This in turn leads to a significant decrease in abnormal symptoms, While the high level of active sperm motility was observed when Amomum granum paradise aqueous extract medium was used to activate spermatozoa in vitro, this in turn may be related to the composition of FertiCult, which contains a mixture of HEPES, bicarbonate, physiological salts, glucose, lactate and albumin (28).). The media acts as a buffering system that has increased buffering capacity and pH stability in the range (7.2 to 7.6). This allows the media to be better resistant to fluctuations in pH caused by changes in cellular metabolism and thus, cellular metabolism does not require a carbon dioxide incubation to avoid pH drops below 7.0 (22).

CONCLUSION

The study aimed to improve reproductive ability and activation of spermatophores using Amumum granum by two different techniques represented by swimming and gradient centrifugation, with an incubation period of 45 minutes The results showed a significant ($p < 0.05$) increase in the percentage of motile sperm, the degree of sperm activity, and the coefficient of sperm motility, and a significant decrease in the concentration of sperm, the percentage of abnormal sperm, and the concentration .

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