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Boosting Fertility: The Impact of Flaxseed Extract on Sperm Quality during Storage in an In vitro study

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Abstract

Sperm preservation is vital for the success of assisted reproductive techniques (ART). Laboratory stress can compromise sperm structure and function, leading to oxidative damage to proteins, lipids, and nucleic acids, which impairs fertilization. Recently, plant extracts have emerged as a cost-effective, natural method to enhance semen quality during storage. Linseed, rich in phenolic compounds, polyunsaturated fatty acids (70%, mainly alpha-linoleic and linoleic acids), and vitamin E, serves as an antioxidant. This study aimed to assess the impact of different concentrations of flaxseed extract on human sperm parameters during storage. Semen samples with normal parameters were collected from healthy participants and treated with varying concentrations (0%, 100 µg/ml, and 200 µg/ml) of flaxseed extract while stored at 37°C for 0, 6, and 10 hours. Improvements in sperm parameters were recorded and compared to control groups. Treatment groups exhibited increased total motility (LIN, STR, and progressive movement) compared to controls ($p < 0.05$), particularly at higher concentrations. Linseed extract also significantly reduced sperm mortality compared to controls ($p < 0.05$), especially at lower concentrations. This study demonstrates that antioxidant-rich plant extracts like flaxseed can enhance semen quality during storage by improving sperm motility and reducing oxidative stress.

I. INTRODUCTION

In various regions across the globe, medicinal plants have proven to offer numerous health benefits to individuals and continue to be a crucial resource for uncovering novel bioactive compounds [1]. Research has shown that many of these plants exhibit significant biological activities, which have been validated through in vitro and in vivo studies involving both animals and humans [2]. These findings highlight the potential of medicinal plants as valuable sources of natural remedies and therapeutic agents for various health conditions. Medicinal plants have been shown to be a promising alternative approach to sperm quality improvement in the treatment of infertility. [3]. The World Health Organization (WHO) defines infertility as the inability to conceive after two years of regular, unprotected intercourse [4] . According to data spanning from 1990 to 2021, the latest global infertility prevalence estimates for 2022 reveal that around one in six individuals have encountered infertility at some point in their lives on a worldwide scale. The lifetime prevalence of infertility is projected to be approximately 17.5%, with a confidence interval of 95% ranging from 15.0% to 20.3% [5]. This rise in infertility has driven rapid advancements in reproductive medicine and research, leading to significant developments and innovative technologies for treating infertile couples globally. Assisted reproductive technology (ART) has become a widely recommended option, offering hope to those struggling to conceive. ART advancements, including in vitro fertilization (IVF), intra-cytoplasmic sperm injection (ICSI), and pre-implantation genetic testing, have greatly improved success rates for couples undergoing fertility treatments. Improved cryopreservation techniques also enhance the preservation of gametes and embryos [6]. Preserving sperm is essential for the effectiveness of

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assisted reproductive technology (ART). Regardless of the type of extender and storage conditions employed, the handling and preservation processes can have a detrimental impact on sperm quality [7]. Oxidative stress (OS) occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defenses that neutralize them [8]. Excessive ROS production can harm all cellular components of gametes and embryos, including proteins, lipids, and nucleic acids, ultimately affecting fertility [9]. Oxidative stress is recognized as a major factor in male and female infertility and is a critical cause of defective gametes and poorly developing embryos in assisted reproductive techniques (ART)[9]. Physiological ROS are produced in cells during respiration and can also originate from abnormal or dead spermatozoa and phagocytic cells in the ejaculate and female reproductive tract [10]. These ROS negatively impact the quality of post-thawed sperm, inhibiting motility, capacitation, and the acrosome reaction through lipid peroxidation of the sperm membrane [11]. Additionally, the handling of sperm in ART under elevated oxygen levels, as well as the removal of seminal plasma and high glucose concentrations, can lead to excessive ROS production [12]. Other external sources of ROS include metal cations, visible light, centrifugation, pH, and temperature changes [13, 14]. To mitigate the adverse effects of ROS overproduction, biological systems rely on antioxidants. The generation of ROS due to pathological conditions in the genital tract or the handling of gametes and embryos in high oxygen environments during ART necessitates the use of antioxidants to protect cells from oxidative stress [15].

Herbal medicine has been found to be a promising alternative approach in improving sperm quality. For example, a study conducted by Ambiye et al. [16] found that herbal supplements containing L-carnitine, coenzyme Q10, and Vitamin E significantly increased sperm motility and concentration in infertile males. Additionally, another study by Ambiye et al. [16] demonstrated that the herb ashwagandha improved semen parameters such as sperm count and motility in men with infertility issues. These findings suggest that herbal medicine can play a pivotal role in enhancing sperm quality through its antioxidant and anti-inflammatory properties. Therefore, integrating herbal supplements into traditional treatments may offer a safe and effective strategy for addressing male infertility concerns and optimizing reproductive health outcomes [17].

Most plant species are considered powerful sources of antioxidants, which can act as reactive oxygen species (ROS) scavengers. Plant extracts have recently emerged as a cheap and natural source of additives to preserve and enhance sperm function during semen storage [7].

Linseed (Linum usitatissimum), commonly known as flaxseed, belongs to the Linaceae family and is recognized as a functional food due to its nutritional benefits and positive impact on health, as noted in traditional medicine [1]. Its biologically active properties have piqued the interest of nutritionists and medical researchers, who have highlighted its potential health benefits [18]. Numerous studies have documented flaxseed's health-promoting effects, including reduced cardiovascular disease risk, lower cancer incidence, decreased inflammation, improved gastrointestinal regularity, and relief from menopausal symptoms [19]. Flaxseed is rich in nutrients and biologically active compounds, such as fatty acids (notably alpha-linolenic acid or ALA), phytoestrogenic lignans (secoisolariciresinol diglycoside or SDG), high-quality proteins, dietary fiber, and phenolic compounds [20].

This study aimed to investigate the effect of the alcoholic extract of flaxseed on the parameters of sperm in the laboratory.

II. MATERIAL AND METHODS

A. Study design

Experienced technicians collected normal semen samples from 50 infertile volunteers, all of whom had healthy sperm. These samples were frozen with varying concentrations of flaxseed herb extract without any additional chemicals. Sperm quality was evaluated at different time intervals through tests detailed in the results section below.

B. Collocation of flaxseed and extract preparation

Brown flaxseeds were sourced from a local market in Misurata, Libya. They were washed with tap water and then distilled water, dried, and ground into powder using a manual mill. A total of 40 g of flaxseed powder was extracted for 6 hours in 200 ml of 85% ethanol, following the protocol by Elgenaidi [1] with a Soxhlet apparatus. The solution was collected in Petri dishes and allowed to evaporate at room temperature. Subsequently, 1 ml of the extraction was diluted in 1 ml of dimethylsulfoxide, filtered through a sterile 0.45 µm syringe filter, and stored in flasks at 4°C.

C. Sperm samples collection

Samples of healthy sperms were collected in collaboration with the Centre for Assisted Fertility and Genetic Diagnosis at Al-Amal Hospital in Misurata. A semen sample was obtained from a healthy male (normal sperm) and incubated at 37 degrees for 20 minutes. The size and viscosity of the samples were recorded, and the sperm's quantity, motility, morphology, and vitality were analyzed. All semen parameters matched the normal ranges established by the World Health Organization.

D. Sperm preparation

The semen sample was processed using the density gradient technique outlined by [1] to isolate highly motile spermatozoa. This method employed 1 ml each of 90% and 45% Puresperm solutions in a 15 ml conical tube. First, 1 ml of 90% solution was added, followed by 1 ml of 45% solution and then 2 ml of semen. The mixture was centrifuged at 1600 rpm for 20 minutes. The clear semen plasma rose to the top, separating white blood cells and debris at the bottom. Immature and abnormal sperm were distributed along the gradient by density and motility, while highly motile normal sperm collected as a pellet at the bottom.

E. In vitro incubation of spermatozoa with flaxseed extract

After selecting healthy sperm samples, we added various concentrations of flaxseed extract to evaluate its effectiveness in maintaining sample quality and enhancing functionality. The samples were treated with an aqueous extract of linseed at room temperature at different concentrations 0%, 0.1%, and 0.2% (0, 100 µg/ml and 200 µg/ml). The sample was divided into a control group and two experimental groups. Ham's F10 medium was added to the control group. The extract was added at a concentration of 0.1% in the first group and 0.2% in the second group. The groups were incubated at 37°C and 5% carbon dioxide for 6 hours and the results were recorded. Sperm motility and vitality were assessed after treatment using an Olympus microscope equipped with a camera (SCA1024) and Sperm Class Analyzer software (SCA, Microptic, Barcelona, Spain).

F. Statistical analysis

Statistical analysis was performed using Medical statistical software (version 12.1.3.0, Mariakerke, Belgium). The Kolmogorov-Smirnoff test was used to test for normal distribution, followed by an independent samples t-test. If the data were not normally distributed, the Mann-Whitney test was performed. Data were expressed as mean ± standard deviation, and a p-value < 0.05 was considered statistically significant. Analysis of variance (ANOVA) was also performed between groups and a p-value < 0.05 was considered statistically significant. The TUKEY outlier detection test was also performed. Graphs were analysed using Graph Pad Prism version 6.

G. Informed Consent

In this study, all participants were fully informed about the objectives and procedures involved in providing semen samples, which were exclusively used for diagnostic purposes. Verbal consent was obtained from each participant, acknowledging that their samples would be destroyed after the completion of the experiment. The study strictly adhered to ethical guidelines and ensured the confidentiality and respect for all participants' contributions.

III. RESULTS

Initially, there was no significant difference in sperm concentration between the control group and the groups treated with 100 µg/ml and 200 µg/ml flax extract. However, after 6 hours, a significant increase in sperm concentration was observed in the group treated with 100 µg/ml, while a marginally significant increase was observed in the group treated with 200 µg/ml compared to the control group. This suggests that linseed extract may have a beneficial effect on sperm concentration over time. It was also noted that the increase in sperm concentration at 10 hours was significantly valued at the concentrations of 100 µg/ml and 200 µg/ml compared to the control group, but to a lesser amount (Figure 1).

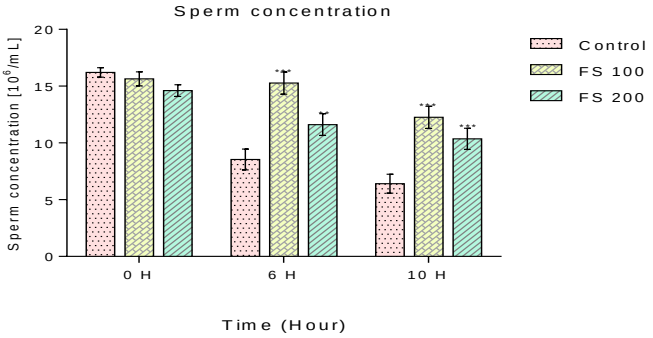

Figure 1: Effect of flaxseed extract on sperm concentration

In this study, it was found that there was no significant difference in progressive motility at zero hour when comparing different concentrations of linseed with the control group. However, a significant increase in progressive motility was observed at a concentration of 200 µg/ml, whereas no significant difference in progressive motility was observed at a concentration of 100 µg/ml after the same period. Interestingly, after 10 hours, there was still no significant difference in progressive motility in the 100 µg/ml concentration group compared to the control group. These results suggest that higher concentrations of flax extract may have a more pronounced effect on sperm motility over time. (Figure 2).

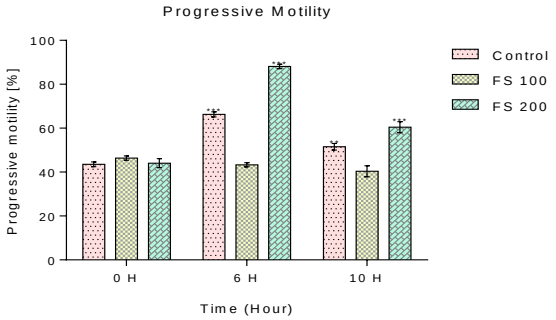

Figure 2: Effect of ethanol extract for flaxseed on progressive motility

In the different combinations of concentrations, no significant change in nucleus penetration was observed at zero hour compared to the control group. However, there was a highly significant increase at 200 µg/ml and a slight increase at 100 µg/ml after 6 hours. These results suggest that flaxseed extract may have a positive effect on the penetration of nuclei into sperm parameters over time (Figure 3).

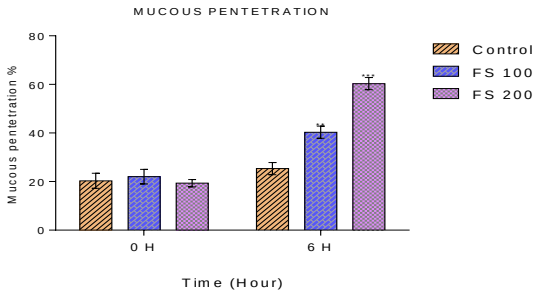

Figure 3: Effect of ethanol extract for flaxseed on mucus penetration

Straightness (STR) remained relatively stable at zero hours in all combinations compared to the control group. However, a notable increase was observed at concentrations of 100 µg/ml and 200 µg/ml after 6 hours, indicating a positive effect of flax extract on sperm straightness (Figure 4).

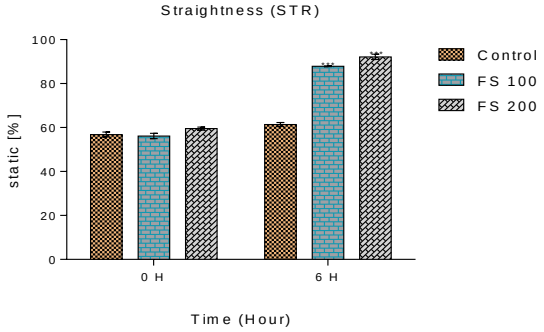

Figure 4: Effect of ethanol extract for flaxseed on straightness (STR)

As shown in Figure 5, After 6 hours, sperm linearity at the concentrations of 100 µg/ml and 200 µg/ml was noticeably higher than in the control group. This suggests that the flax extract positively affected sperm linearity at these particular concentrations. No significant changes were observed at the zero hour, indicating that it took some time for the extract to exert its effects on sperm motility. These results highlight the potential benefits of using flax extract as a natural supplement to improve sperm quality and fertility.

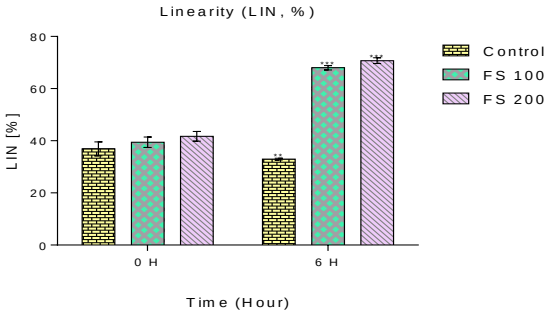

Figure 5: Effect of ethanol extract for flaxseed on linearity

IV. DISCUSSION

When handling semen samples in the laboratory, germ cells are subject to damage during storage due to increased oxidative stress. Therefore, antioxidants should be added to sperm storage media. In this study, we sought to evaluate the effectiveness of linseed extract on sperm parameters during storage with high oxidative activity. A positive effect of the extract was observed on overall motility (progressive motility, STR and LIN) compared to the control group ($P < 0.005$), and a higher concentration of sperm was also observed in the presence of the extract compared to the control group. This effect can be attributed to the presence of many antioxidants in linseed. This finding is in line with several studies showing that plant extracts with antioxidant activity contribute to the improvement of sperm parameters [21-23]. Our study aligns with Yuan et al. [24], who found that dietary linseed oil (FO) and vitamin E (VE) enhance semen quality in Simmental bulls. FO and VE boost sperm motility and antioxidant capacity in frozen-thawed semen, linked to increased aconitic acid levels influenced by ABAT and HADHA genes. This research emphasizes the importance of a balanced diet of FO and VE to improve reproductive performance.

In a vitro study by Ashrafi et al. [25] demonstrated that antioxidants eliminate free radicals like superoxide ions, hydrogen peroxide, hydroxyl radicals, and peroxyl, resulting in reduced mitochondrial infection and adequate

ATP for sperm movement [26]. Flaxseed is recognized as a rich source of phenolic compounds, including flavonoids, which possess strong antioxidant properties that reduce malondialdehyde and nitric oxide production and prevent programmed cell death [27, 28]. Our findings align with numerous studies linking improved sperm parameters to the antioxidant effects of phenolic compounds and flavonoids [29, 30]. In contrast, Mohanraj et al. [31] found that A. Marmelos extract, despite its high phenolic content, had an inhibitory effect.

Research indicates that polyunsaturated fatty acids (PUFAs) and omega-3 supplements can enhance sperm membrane integrity, reduce oxidative stress damage, and maintain the physical properties of sperm cells [32]. A specific study found that adding egg yolk containing PUFAs, particularly from flaxseed or fish oil, helps preserve the quality of rabbit semen during storage [33]. Additionally, including vitamin E at a concentration of 5 IU in rabbit semen diluents during cold storage has been shown to improve sperm survival and reduce oxidative damage [34]. Flaxseed is noted as a rich source of these beneficial compounds [18].

While improved mucus penetration might be attributed to enhanced overall caliber, our study found that a lower concentration of flaxseed extracts better-affected sperm concentration at 6 and 10 hours for reasons that remain unclear. Conversely, research by Skorkowska-Telichowska et al. [35] suggests that high antioxidant levels in linseed extract could intensify oxidative effects on certain cells, potentially impacting V79 cell protection against oxidative stress from linseed oil emulsions [35].

V. CONCLUSION

This laboratory study confirms that plant extracts high in antioxidants, like linseed, can enhance semen quality during storage. Our findings indicate a positive impact of this extract on sperm motility and penetration. Antioxidants help mitigate oxidative stress during sperm processing. Future research should focus on developing standardized protocols and identifying effective combinations that improve sperm quality.

VI. ACKNOWLEDGMENTS

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VII. CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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تعزيز الخصوبة: دراسة مختبرية لتأثير بذور الكتان على نوعية الحيوانات المنوية اثناء التخزين

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الكلمات المفتاحية

تقنيات الإنجاب المساعدة؛ بذور الكتان؛ الإجهاد التأكسدي؛ تخزين الحيوانات المنوية؛ مضادات الأكسدة العشبية.

الملخص

تعتبر حفظ الحيوانات المنوية اثناء عملية التخزين من الاشـــياء الحيوية والمهمة لنجاح ثقنيات التلقيح الصناعي. يمكن ان يتسبب الاجهاد في المختبر اثناء مناولة السائل المنوي عند تجهيزه للتخزين اختلال في تركيب وظيفة الحيوانات المنوية حيث يمكن ان يؤدى الى تلف في مكوناته من البروتين والدهون والحامض النووي ، الذي ينتج عنة انخفاض القدرة على الاخصاب. في الآونة الأخيرة أشا رت العديد من الابحاث بأن المستخلصات النباتية تعتبر كطريقة طبيعية غير مكلفة وفعالة للتعزيز جودة الســـائل المنوي اثناء التخزين . بذور الكتان، الغنية بالمركبات الفينولية والأحماض الدهنية المتعددة غير المشـــبعة (70٪، (حمض ألفا-لينولينيك وحمض اللينوليك)، وفيتامين اي تعمل كمضـــاد اكســدة. هدفت هذه الدراســة إلى تقييم تأثير تركيزات مختلفة من مســتخلص بذور الكتان على معايير الحيوانات المنوية البشرية أثناء التخزين . تم جمع عينات السائل المنوي ذات معايير طبيعية من مشـــاركين أصـــحاء، وتم معالجتها بتركيزات مختلفة (0%، 100 ميكروجرام/مل، و200 ميكروجرام/مل) من مســـتخلص بذور الكتان أثناء تخزينها عند درجة حرارة 37 درجة مئوية لمدة 0 و6 و10 ساعات. تم تسجيل التحسينات في معايير الحيوانات المنوية ومقارنتها بالمجموعات الضـــابطة. أظهرت النتائج بأن هناك زيادة في الحركة الكلية والحركة التقدمية في المجموعات المعالجة ، لا ســـيما عند التركيزات الأعلى. كما قلل مستخلص بذور الكتان بشـكل ملحوظ من معدل موت الحيوانات المنوية مقارنة بالمجموعات الضـــابطة خاصـــة عند التركيزات الأقل. الاســـتنتاج: تظهر هذه الدراسة أن مستخلصات النباتات الغنية بمضادات الأكسدة مثل بذور الكتان يمكن أن تعزز جودة الســـائل المنوي أثناء التخزين من خلال تحســـين حركة الحيوانات المنوية وتقليل الإجهاد التأكسدي