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SAMBUCUS NIGRA: BLACK FRUITS THAT MAKES SPERMS COME OUT AHEAD IN IN VITRO FERTILIZATION

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ABSTRACT

Freezing is a non-physiological method that includes a series of processes such as dilution, incubation, cooling, freezing and thawing, which affect the structure, biochemistry and function of sperm. Human sperm freezing has overcome many spatial and temporal limitations and is now an integral part of assisted reproductive technologies (ART). Freezing causes biochemical, functional and structural changes in sperm. These changes

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include plasma and sperm acrosome membranes, which increase their permeability and leads to disruption of morphology, motility and chromatin structure. Therefore, freezing causes irreversible damage to sperm, which leads to the loss of movement and biological function of most sperm. Sperm health is one of the important factors in the fertility of couples. Therefore, reducing the harmful effects caused by freezing and properly performing the in vitro Fertilization (IVF) process, the development of useful solutions is inevitable. The extract from black fruits of Elderberry (*Sambucus nigra*) has potent in vitro effect on the sperm motility, vitality and oxidative profile. This extract can largely improve the sperms parameters and stereological parameters (spermatogonia, primary spermatocyte, round spermatid, and Leydig cells), decrease of TNF-a and caspase-3 expression, increase of the serum testosterone, increase of mitochondrial activity, and reduction of reactive oxygen species. So, it can also be used as in vivo status. We suggested that black fruits of *Sambucus nigra* makes sperms come out ahead in IVF techniques.

KEYWORDS: *Sambucus nigra*; Fruit; Assisted Reproductive Technologies; In vitro Fertilization; Sperm Parameters.

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SAMBUCUS NIGRA: FRUTOS NEGROS QUE HACEN QUE LOS ESPERMATOZOIDES SALGAN ADELANTE EN LA FECUNDACIÓN IN VITRO

RESUMEN

La congelación es un método no fisiológico que incluye una serie de procesos como la dilución incubación, enfriamiento, congelación y descongelación, que afectan a la estructura, bioquímica y función de los espermatozoides. La congelación de espermatozoides humanos ha superado muchas temporal y espacial, y ahora forma parte integrante de las tecnologías de reproducción asistida (TRA). La congelación provoca cambios bioquímicos, funcionales y estructurales en los espermatozoides. incluyen las membranas plasmáticas y acrosómicas de los espermatozoides, lo que aumenta su permeabilidad y conduce a la alteración de la morfología, la motilidad y la estructura de la cromatina. Por tanto, la congelación daño irreversible a los espermatozoides, lo que conduce a la pérdida del movimiento y la función biológica de la mayoría de ellos biológica de la mayoría de los espermatozoides. La salud de los espermatozoides es uno de los factores importantes en la fertilidad de las parejas. Por lo tanto, reducir los efectos nocivos causados por la congelación y realizar adecuadamente la Fecundación in Vitro (FIV), es inevitable desarrollar soluciones útiles inevitables. El extracto de los frutos negros del saúco (*Sambucus Nigra*) tiene un potente efecto in vitro sobre la motilidad, la vitalidad y el perfil oxidativo de los espermatozoides. Este extracto puede mejorar en gran medida los parámetros espermáticos y estereológicos (espermatogonía, espermatocito primario

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espermátidas redondas y células de Leydig), la disminución de la expresión de TNF-a y caspasa-3, el aumento testosterona sérica, aumento de la actividad mitocondrial y reducción de las especies reactivas del oxígeno. Por lo tanto, también puede utilizarse como estado in vivo. Sugerimos que los frutos negros de *Sambucus Nigra* hacen que los espermatozoides salgan adelante en las técnicas de FIV.

PALABRAS CLAVE: *Sambucus Nigra*; Fruta; Técnicas de reproducción asistida; Fertilización in vitro; Parámetros espermáticos.

BACKGROUND

About 10-15% of couples around the world are trying to treat infertility, and 50% of the problems of these infertile couples can be related to sperm gametes (Mocé, Fajardo, & Graham, 2016). The frozen sperm bank is used for several purposes: Sperm preservation against complications of therapeutic approaches such as chemotherapy, radiation therapy, orchiotomy, vasectomy, testicular and epididymal sampling in azoospermic patients, gender reassignment, injury spinal cord, and hypogonadotropic hypogonadism. In addition, freeze banks can be used to store semen samples in the following cases:

1- In cases of infertility in which the man cannot provide a sufficient or appropriate amount of sperm for use in ART.

2-On the day of ovulation, it is not possible to collect seminal fluid for any reason.

3-Azoospermic people whose sperm is obtained by surgical techniques (Bahmyari, Zare, Sharma, Agarwal, & Halvaei, 2020; O'connell, Mcclure, & Lewis, 2002).

Freezing includes the deposition of water in the form of ice crystals, the result of which is the separation of water from its

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dissolved substances. In this process, the formation of intracellular ice crystals and the concentration of dissolved substances are problematic, and the survival of frozen cells depends on the type of cell, freezing speed, type of antifreeze, and freezing method (Ozkavukcu, Erdemli, Isik, Oztuna, & Karahuseyinoglu, 2008). Sperm freezing methods can be divided into several categories: slow freezing, rapid freezing, programmed freezing, and dry freezing (lyophilization). Rapid freezing causes water to form a glass-like structure instead of ice (Chian & Quinn, 2010). This technique uses a fast rate, which causes less cell damage and has greater benefits than slow freezing. The sperm frozen in this technique has an even greater quality than sperm frozen using the slow freezing approach, but it can be done with less equipment and in less time. If the freezing speed is too high, the intracellular water does not entirely flow out and freezes inside the cell, causing ice crystals form in the cytoplasm and eventually lead to cell damage (Muldrew & McGann, 1994). The concentration of cryoprotective agents (CPA) in the freezing media affects the production of ice crystals inside the cell in addition to the freezing rate. When these protective factors are used, the formation of ice crystals inside the cell is reduced. However, if the freezing process is carried out gradually, water has time to leave the cell, resulting in complete dehydration of the cell, a reduction in the volume of the cell and its organelles, and an increase in the concentration of intracellular solutes before the temperature reaches the freezing point. This condition affects the protein and lipid complex of the membrane and results in macromolecule denaturation, a reduction in the size of the channels, the irreversible induction of diffusion, and, as a result, hypertonic stress. Hypertonic stress can disturb the balance of electrolytes and cause excessive swelling of the normal volume (isotonic swelling), which in subsequent can result in cell lysis. But if freezing is done at an ideal speed, less damage will be occurred to the cell. The freezing

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speed used in this method is low enough to prevent ice forming inside the cell. Moreover, it is high enough to reduce any damage that would result from the cell's electrolyte and solute balance being upset. Although frozen/thawed sperm has several advantages for reproduction, numerous reports indicate that the freezing and thawing processes seriously decrease sperm function (Colás, Junquera, Pérez-Pé, Cebrián-Pérez, & Muiño-Blanco, 2009). Sperm kept at low temperatures loses some of its capacity to become pregnant when compared to fresh sperm. Liquid nitrogen, or LN2, which is used in the freezing process, lowers the temperature of the cells or the entire tissue to below 0ºC, which is typically -196 °C (Di Santo, Tarozzi, Nadalini, & Borini, 2011). Sperm is kept in storage for a long time by inhibiting intracellular metabolism; in fact, at -196 °C, virtually no biochemical activity occurs since there isn't enough thermal energy for chemical reactions to happen. Additionally, no aqueous environment is required for metabolic activities (Anger, Gilbert, & Goldstein, 2003; Justice & Christensen, 2013). However, freeze-thaw processes have the potential to damage living tissues and cells (Martins, Agarwal, & Henkel, 2019). On sperm function, freezing has the following negative effects: Disruption of sperm plasma membrane, chromatin structure, motility, vitality, the ability to fertilize, early embryo growth and development, implantation, and ultimately a reduction in pregnancy rate (Li, Zhou, Lv, Ge, Liu, & Zhou, 2019; Majzoub & Agarwal, 2020; Martins, Agarwal, & Henkel, 2019). The process that could endanger the health of sperm during freezing is apoptosis. Also, freezing procedures can seriously damage human sperm due to oxidative stress (ROS) (A. Najafi, Asadi, Moawad, Mikaeili, Amidi, Adutwum, et al., 2016). The production of reactive oxygen species (ROS), including superoxide anion (O2-), hydrogen peroxide (H2O2), and hydroxyl radicals (OH), is essential for sperm function during sperm capacity, acrosomatic reaction, and zona pellucida bonding

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(Agarwal, Sharma, Nallella, Thomas Jr, Alvarez, & Sikka, 2006). The key factor causing sperm destruction is an imbalance between the presence of ROS and the antioxidant activity of sperm (Wang, Zhang, Ikemoto, Anderson, & Loughlin, 1997). Sperm are susceptible to free radical damage due to their unique plasma membrane structure, high mitochondrial density, limited cytoplasm, and low antioxidant content (Bollwein, Fuchs, & Koess, 2008). (Figure 1)

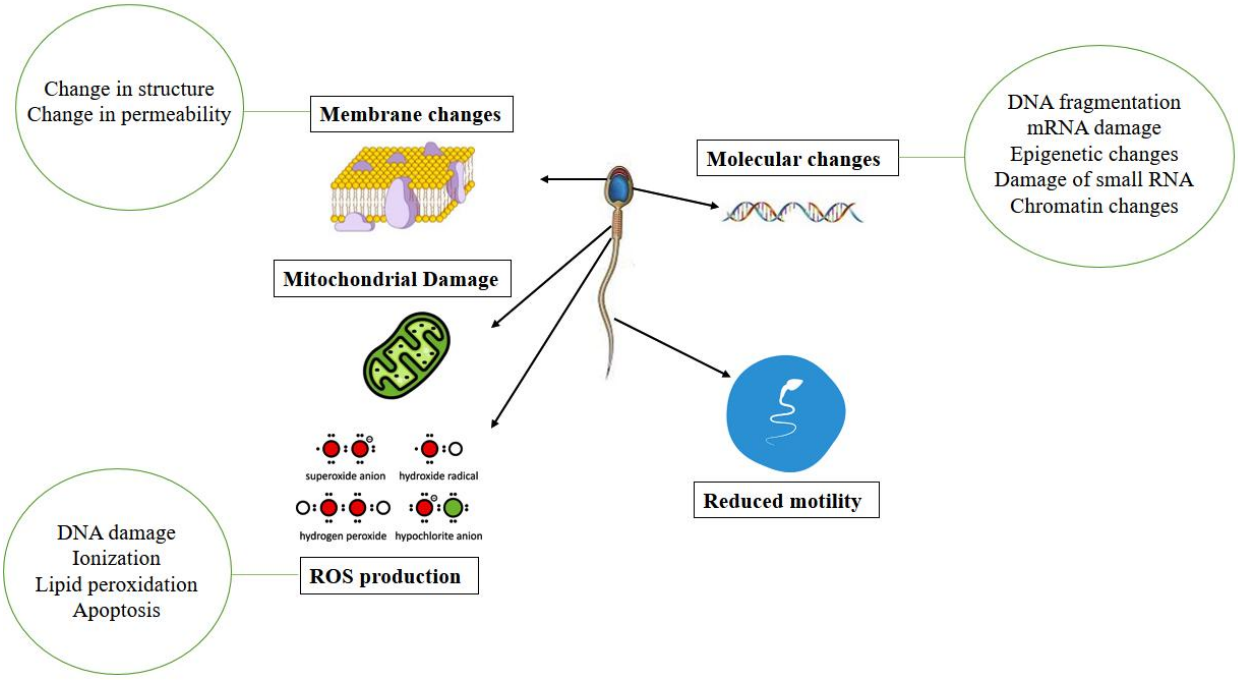

Figure 1. Possible changes of sperm parameters after freezing process.

Antioxidants are the main defense factors against oxidative stress caused by free radical (Silva, Soares, Batista, Almeida, Nunes, Peixoto, et al., 2011). Since many years ago, medicinal plants from all over the world have been analyzed in an effort to find reliable and secure components for regulating fertility. This strategy has little

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negative effects compared to synthetic medications, making it an excellent substitute. The effects of different medicinal plant extracts on both male and female animal reproduction have been studied (RK Sharma, Goyal, & Bhat, 2013). Research has been done on the extracts of various plants, including Ferollago angulate (Bohlouli, 2019), *Sambucus nigra* (Abdramanov, Massanyi, Sarsembayeva, Usenbayev, Alimov, & Tvrdá, 2021), Moringa oleifera (Abdramanov, Massanyi, Sarsembayeva, Usenbayev, Alimov, & Tvrdá, 2021), and etc.

Sambucus nigra, also known as elderberry or black elderberry, is a member of the Adoxaceae family and a source of bioactive chemicals that have gained popularity due to their advantageous effects in the prevention and treatment of a number of diseases. This plant has many applications in homeopathy and traditional medicine, and it has also drawn interest from researchers in the field of modern medicine. This plant's astringent, diuretic, and antiviral characteristics are common consequences (Abdramanov, Massanyi, Sarsembayeva, Usenbayev, Alimov, & Tvrdá, 2021). Pomegranate extract has been discovered to have antioxidant activity, much like other small, dark-fleshed fruits like raspberries and blackberries. These plants' flavonoids, which include flavanone, flavone, anthocyanins, and derivatives of isoflavones, are thought to have antioxidant activity and a protective role against oxidative stress agents such 2-amidinopropane, hydrogen peroxide, ascorbic acid, dihydrochloride, and ferrous sulfate (Dawidowicz, Wianowska, & Baraniak, 2006; Demo, Petrakis, Kefalas, & Boskou, 1998). Under controlled laboratory circumstances, elderberry extract powder has a very strong antioxidant activity (Bratu, Doroftei, Negreanu-Pirjol, Hostina, & Porta, 2012). In addition, flavonoids are mostly found in leaves, which make up the majority of a plant's biomass (Dawidowicz, Wianowska, & Baraniak, 2006). According to studies, elderberry also has anti-inflammatory, antiviral, and

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anti-diabetic properties due to its high anthocyanin and polyphenol content. Elderberry is also effective against some significant pathogenic microorganisms that cause wounds, deformities, skin infections, enteritis, typhoid, and candidiasis. Elderberry extract can therefore be used to treat various illnesses. Elderberry extract is efficient against Pseudomonas aeruginosa, Escherichia coli, and Salmonella typhi but less so against Staphylococcus aureus, Bacillus subtilis, and Candida albicans in low quantities. Elderberry can be used to cure illnesses brought on by a few certain experimental pathogens. It can certainly be considered as a healthy diet and also contains sterols, tannins, and essential oils. Additionally, it functions well as a food preservative (Mohammadsadeghi, Malekpour, Zahedi, & Eskandari, 2013). Elderberries contain a lot of flavonoids, which are easily able to take part in intracellular and extracellular oxidation-reduction events. Flavonoids play their antioxidant role by interacting with free radicals and inhibiting the oxidation process (reduction of activity of oxidase enzymes). In both animals and people, studies have shown that a number of plants, including elderberry, may have an impact on the male reproductive system. One or even multiple of this plant's bioactive components are principally responsible for these effects (D'cruz, Vaithinathan, Jubendradass, & Mathur, 2010).

In some limited studies, results showed that the fruits of elderberry can improve the sperm parameters. These fruits can increase the testosterone and decrease the TNF-a and caspase-3 expression in mice with hyperthermia- induced testis dysfunction. So, elderberry diet may be regarded as suitable diet for improvement of sperm and reproductive variables (Moghaddam, Farrokhi, Hasani, Khosravi, Pirani, Vakili, et al., 2022). Abdramanov et al assessed the in vitro effects of the elderberry (*Sambucus nigra*) extract on the motility, viability and reactive oxygen species (ROS) production of bovine spermatozoa in different time periods (0, 2, 6 and 24

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hours). This group showed that the elderberry extract had a considerable in vitro effect on the sperm motility, vitality and oxidative profile and indicate that *Sambucus nigra* extract possesses activity promoting properties at 10 and 5 µg/mL.(Abdramanov, Massanyi, Sarsembayeva, Usenbayev, Alimov, & Tvrdá, 2017). This extract also increased the mitochondrial activity. These two studies are the only relevant studies about the effect of *Sambucus nigra* extract on improvement of sperm function. Only one study has been conducted to investigate the effect of this extract on frozen sperm in the laboratory environment. The different capacities of the fruits of *Sambucus nigra* including antimicrobial and antioxidant activity, changing the gene expression of proteins involved in apoptosis, reducing the production and negative function of ROS, and protective effect on the membranes of mammalian cells in the field of sperm preservation and storage in laboratory has been neglected (Figure 2).

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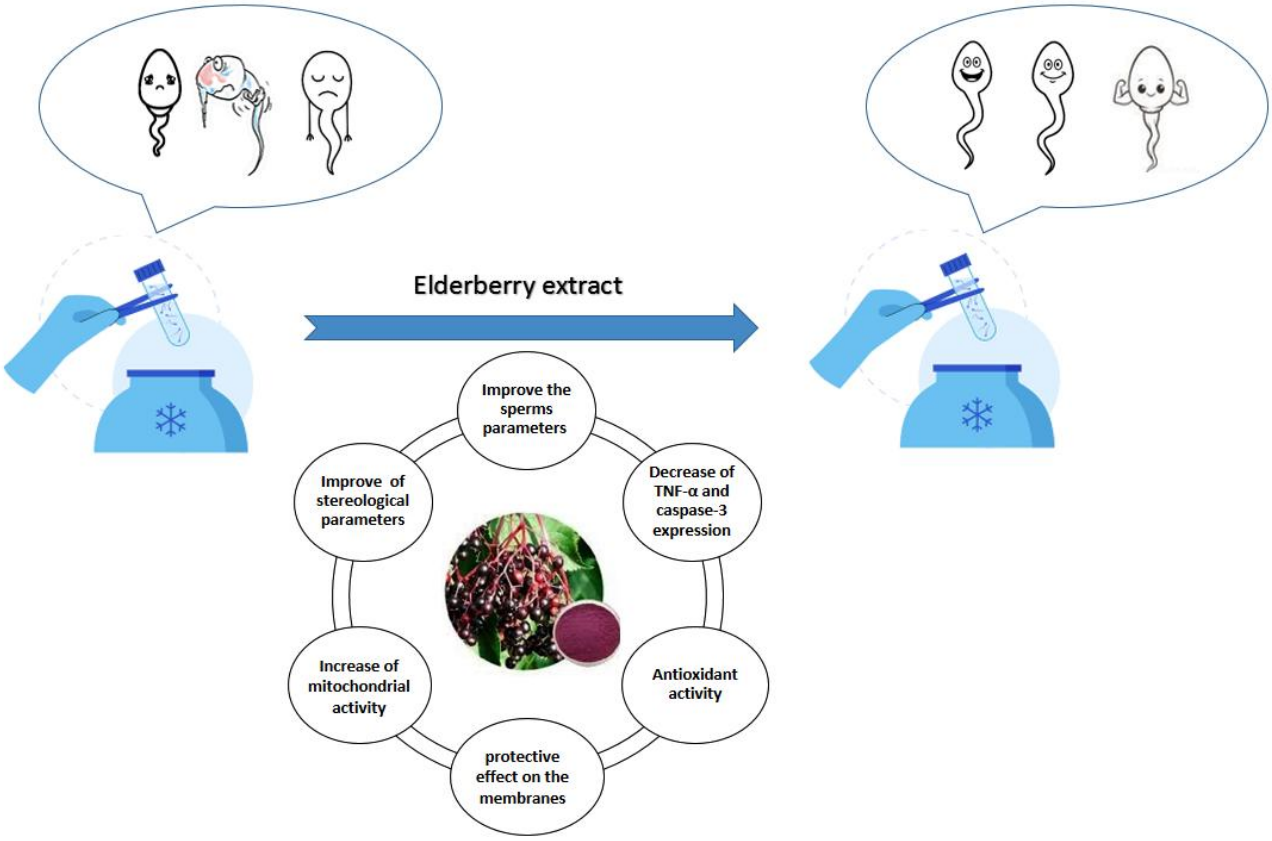

Figure 2. The effects of Elderberry extract on sperm preservation under freezing process.

There are some limitations to using *Sambucus nigra* extract to increase the stability of frozen sperm: low permeability into the cell, low solubility in different solvents, sensitivity in high concentrations, and low effectiveness in low concentrations. Using the capabilities of nanotechnology can solve these limitations. Protein nanostructures are the best option due to their high biocompatibility and unique biological activities (Vojdani Nejad Yazdi, Zare-Zardini, Miresmaeili, & Fesahat, 2022; Zare-Zardini, Soltaninejad, Ghorani-Azam, Nafisi-Moghadam, Haddadzadegan, Ansari, et al., 2022). Therefore, we suggest to define studies on the prodcution and use of crude extract

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from *Sambucus nigra* and extract loaded in different protein nanoparticles as an adjuvant to increase the stability of frozen sperm in the laboratory environment..

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