doi:10.22100/ijhs.v11i4.1244
Original Article

SJMS 2025;11(4):11-17
sjms.shmu.ac.ir

SJMS
Shahroud Journal of Medical Sciences

The Effect of Cryopreservation on Human Sperm Growth Factors Expression: An Experimental Study

Naghmeh Ahmadiankia[1,2], Mozhgan Fazli[1], Maryam Shabani Nashtaei[3,4], Abolfazl Babakhani[5], Sara Saeednia[1,6*]

[1] School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran.
[2] Cancer Prevention Research Center, Shahroud University of Medical Sciences, Shahroud, Iran.
[3] School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[4] Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran.
[5] Imam Hossein Hospital, Shahroud University of Medical Sciences, Shahroud, Iran.
[6] Tissue Engineering and Stem Cells Research Center, Shahroud University of Medical Sciences, Shahroud, Iran.

Received: 17 May 2025
Accepted: 8 September 2025

Abstract

Background: *Cryopreservation is a widely employed assisted reproductive technology (ART) for the treatment of infertility. However, numerous sperm functions are compromised during the cryopreservation process. Considering the pivotal roles of nerve growth factor (NGF) and fibroblast growth factor (FGF) in sperm physiology and function, this study aimed to investigate the impact of cryopreservation on the expression and quality of these growth factors and their respective receptors.*

Methods: *Semen samples were collected from 30 normozoospermic donors, with each sample divided into fresh and frozen-thawed groups. Sperm parameters were evaluated using computer-assisted sperm analysis (CASA). Real-time polymerase chain reaction (PCR) and Western blotting techniques were employed to quantify the gene and protein expression levels of growth factor receptors. Apoptosis was assessed using Annexin V staining, and enzyme-linked immunosorbent assay (ELISA) was conducted to measure levels of NGF and FGF2 in seminal plasma.*

Results: *The findings demonstrated that the frozen-thawed group exhibited significantly reduced levels of fibroblast growth factor receptor 2 (FGFR2) and a trend towards decreased expression of tropomyosin receptor kinase A (TrkA) compared to the fresh group, although the latter was not statistically significant. Notably, the percentage of cells undergoing late apoptosis was markedly higher in the cryopreservation group relative to the fresh group. Furthermore, cryopreservation resulted in substantial reductions in NGF and FGF2 concentrations within the seminal plasma.*

Conclusions: *The detrimental effects of freezing and thawing on the expression of growth factors and their receptors in sperm cells suggest that the integration of these factors during the cryopreservation process may enhance sperm quality post-thaw. Further research is warranted to explore the potential clinical applications of these findings.*

Keywords: Cryopreservation, Normozoospermic, Nerve growth factors, Fibroblast growth factors, Growth factor receptors.
***Corresponding to:** S Saeednia, **Email:** sarasaeednia@yahoo.com

Please cite this paper as: Ahmadiankia N, Fazli M, Shabani Nashtaei M, Babakhani A, Saeednia S. The Effect of Cryopreservation on Human Sperm Growth Factors Expression: An Experimental Study. Shahroud Journal of Medical Sciences 2025;11(4):11-17.

Introduction

Sperm cryopreservation is a fundamental technique employed in assisted reproductive technology (ART) [1, 2]. This procedure is crucial for men with low sperm concentrations due to factors such as chemotherapy, radiotherapy, or surgical interventions like testicular sperm extraction (TESE) [1, 2]. Although semen cryopreservation has been successfully implemented in ART for decades [3], it is not without limitations, and cryoinjury remains an inevitable consequence of the process [3]. Multiple studies have demonstrated that cryopreservation adversely affects various sperm characteristics, including viability, motility, morphology, DNA integrity, and fertilization capacity [1, 4]. It has been suggested that sperm cryopreservation and thawing may elevate reactive oxygen species (ROS) levels while diminishing antioxidant defenses [5]. Consequently, oxidative stress (OS), resulting from an imbalance between ROS and antioxidants, could contribute to the damage incurred by spermatozoa during cryopreservation [5].

The cryopreservation process exacerbates lipid peroxidation in the sperm plasma membrane due to elevated ROS levels. Mammalian sperm are particularly vulnerable to OS due to the significant presence of unsaturated fatty acids in their plasma membranes [6]. Exposure to OS during cryopreservation alters the lipid composition of the plasma membrane [7], reduces head size, and exposes phosphatidylserine (PS) residues on the outer membrane [8]. The translocation of PS from the inner to the outer plasma membrane is one of the earliest indicators of apoptosis. Excessive ROS production and OS, stemming from the inhibition of oxidative phosphorylation or glycolysis, results in ATP depletion and impaired sperm motility [9]. Moreover, OS can damage DNA, leading to fragmentation, base oxidation, cross-linking of chromatin, and other deleterious changes [10]. Sperm physiological processes, including capacitation [11], membrane permeabilization, acrosomal reaction, and fertilization, are heavily reliant on the glycocalyx and carbohydrate components of the sperm membrane, all of which may be compromised by cryopreservation.

Nerve growth factor (NGF) is a neurotrophin implicated in male reproductive function and sperm physiology [12], acting via its receptor, tropomyosin receptor kinase A (TrkA; NTRK1). TrkA, a transmembrane protein, is present in both developing and adult mammalian neurological systems [13]. In mammals, NGF and TrkA have been detected in ejaculated sperm, with evidence indicating that NGF influences sperm viability and

apoptosis. Furthermore, NGF regulates sperm physiology and may contribute to male infertility [14]. NGF and TrkA are localized in distinct regions of mammalian sperm, and their interplay facilitates sperm motility and promotes the acrosome reaction [15]. Additionally, NGF is essential for Leydig cell proliferation, differentiation, and testosterone synthesis [16]. Preclinical studies have shown that exogenous NGF enhances sperm survival, motility, and intracellular nitric oxide (NO) levels while reducing apoptosis in men with asthenozoospermia and normospermia [2, 4].

Fibroblast growth factor 2 (FGF-2) is the most extensively characterized member of the fibroblast growth factor family, which plays a critical role in reproduction. FGFs interact with various receptors known as fibroblast growth factor receptors (FGFRs), which exhibit tyrosine kinase activity and include FGFR1, FGFR2, FGFR3, and FGFR4 [17]. Both FGF2 and functional FGFRs are present in the male reproductive tract, testes, and sperm, where they are implicated in spermatogenesis and sperm motility. Importantly, the pretreatment of human sperm with recombinant FGF2 (rFGF2) has been shown to enhance the percentage of motile cells [18].

This study aimed to investigate the effects of cryopreservation on semen parameters in men with normal sperm quality. Additionally, it sought to evaluate the gene and protein expression of sperm growth factor receptors, quantify apoptosis, and assess the levels of NGF and FGF2 in seminal plasma.

Materials and Methods

Study design and sample collection: Between November 2020 and September 2021, semen samples were collected from 30 normozoospermic donors (age range 19–50 years) with confirmed fertility, who were referred to the Mehr Laboratory (Shahroud, Iran). Samples were collected via masturbation into a sterile container after at least 72 hours of sexual abstinence. Standard semen parameters, including sperm concentration, motility, and morphology, were assessed in accordance with the 5th edition of World Health Organization (WHO) guidelines, following 30 minutes of liquefaction at 37°C in 5% CO_2 (World Health Organization 2010). Individuals with leukocytospermia ($\geq 1 \times 10^6$ white blood cells/mL), varicocele, or other hormonal disorders were excluded, as were those who had recently used medications or antioxidants, engaged in heavy smoking or alcohol consumption, or undergone chemotherapy or radiotherapy. Each sample was divided into two portions: fresh sperm and frozen-thawed sperm without treatment. Sperm parameters and apoptosis were evaluated using computer-assisted sperm analysis (CASA) and Annexin V staining, respectively. Real-time polymerase chain reaction (PCR) and Western blotting were employed to assess the expression of FGFR2 and TrkA mRNAs and proteins, respectively. Moreover, levels of NGF and FGF2 in seminal plasma were quantified using enzyme-linked immunosorbent assay (ELISA). In the cryopreservation group, all procedures were conducted following the freezing-thawing process. Written informed consent was obtained from all participants, and the study was approved by the Ethics Committee of Shahroud University of Medical Sciences (ref. no. 95106, approved 25 July 2017; Ethics Committee Code IR.SHMU.REC.1396.59).

RNA extraction and real time PCR: Real-time PCR was conducted according to previously established protocols [19]. Total RNA was extracted using RNX-PLUSTM (Sinnacolon, Tehran, Iran), and cDNA synthesis was performed using a cDNA synthesis kit (Sinnacolon, Tehran, Iran). Real-time quantitative PCR was conducted using Real Q Plus 2x Master Mix Green (Amplicon, Denmark). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the control housekeeping gene. The forward and reverse primers for GAPDH, FGFR2, and TrkA are summarized in Table 1. All transcript measurements were conducted in duplicate using an Applied Biosystems Real-Time PCR System (Darmstadt, Germany). Relative gene expression levels were calculated using the Livak method ($2^{-\Delta\Delta Ct}$) (Livak and Schmittgen, 2001).

Table 1. Forward and reverse primers for FGFR2, TrkA and GAPDH

Primers		Sequence	Product size
FGFR2	F	5′-AAC GGG AAG GAG TTT AAG CA-3′	99bp
	R	5′-CTT GTC AGA TGG GAC CAC AC-3′	
TrkA	F	5′-TTG GCA TGA GCA GGG ATA TCT-3′	101bp
	R	5′-ACG GTA CAG GAT GCT CTC GG-3′	
GAPDH	F	5′-AAG GTG AAG GTC GGA GTC AAC-3′	102bp
	R	5′-GGG GTC ATT GAT GGC AAC AAT A-3′	

Western blot: Western blotting was performed as previously described [20]. Total protein extraction began with the collection and lysis of cells using RIPA buffer. Protein concentrations were determined using the Bradford assay. Subsequently, proteins were separated by SDS/PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. After a 1-hour blocking step with 5% milk, the membrane was incubated at room temperature for 1 hour with primary antibodies: anti-FGFR2 (Cat No: ab10648; Abcam), anti-TrkA (Cat No: ab76291; Abcam), and anti-beta actin (loading control, Cat No: ab8227; Abcam). Following extensive washing, a goat anti-rabbit IgG H&L (HRP) secondary antibody (Cat No: ab6721; Abcam) was added for another hour at room temperature. Protein bands were visualized using chemiluminescence (Bio-Rad), and band intensity was quantified using ImageJ.

Enzyme-linked immunosorbent assay (ELISA): Seminal plasma was analyzed for NGF and FGF2 using ELISA kits (Abcam; Cat No: ab99986 and ab99979, USA) according to the manufacturer's guidelines. This assay utilizes antibodies specific to human NGF and FGF2, which are coated on a 96-

well plate. In brief, 100 µl of standards and samples were added to each well and incubated for 2.5 hours at room temperature. Following this, 100 µl of biotin-conjugated antibody was added to each well and incubated for 1 hour. The wells were subsequently washed to remove any unbound biotinylated antibody. HRP-conjugated streptavidin was then added and incubated for 45 minutes at room temperature. After additional washing, 100 µl of TMB substrate solution was introduced to each well, with color intensity correlating to the amount of bound NGF. A Stop Solution was added to shift the color from blue to yellow, and the color intensity was measured at 450 nm.

Apoptosis assay: Fresh and freeze-thawed sperm cells were analyzed utilizing the annexin-V FITC/propidium iodide (PI) double-staining method as described by Bagheri et al. [21]. A cell suspension was prepared by adding 500 µl of 1X binding buffer. Cells were then incubated in the dark for 15 minutes at room temperature with 5 µl of annexin V-FITC and 5 µl of PI. Quantification of annexin-V/PI incorporation was performed using a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). Early and late apoptotic, as well as necrotic cell populations, were visualized via dot plot analysis. Detection channels FL1 and FL2 were utilized for annexin-V FITC and PI staining, respectively. Data analysis was conducted using WinMDI software.

Statistical analysis: The Kolmogorov-Smirnov test was employed to assess the normality of the data. Results are presented as mean±standard deviation (SD). Statistical comparisons were made using the independent sample t-test or paired student's t-test, implemented via SPSS version 22 with a significance threshold set at P-value<0.05.

Results

The semen parameters of 30 normozoospermic men whose semen samples underwent freeze-thaw processing are summarized in Table 2. The mean age of participants was 31.28±6.53 years, and the average ejaculate volume was 3.30±0.99 ml.

Gene expression analysis revealed significant reductions in TrkA (P-value=0.01, Figure 1) and FGFR2 (P-value=0.03, Figure 1 A, B) levels in freeze-thawed samples compared to fresh samples.

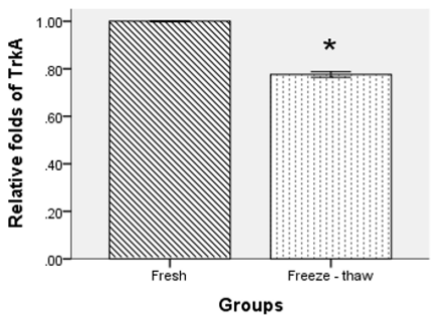
The presence of FGFR2 and TrkA proteins in ejaculated human spermatozoa was assessed via Western blotting using specific antibodies. While the expression levels of both proteins decreased in freeze-thaw samples relative to fresh samples, a statistically significant reduction was observed for FGFR2 (P-value<0.05); however, the decrease in TrkA was not statistically significant (Figure 1 C, D).

Table 2. Semen characteristic of 30 normozoospermic men before and after freeze – thaw

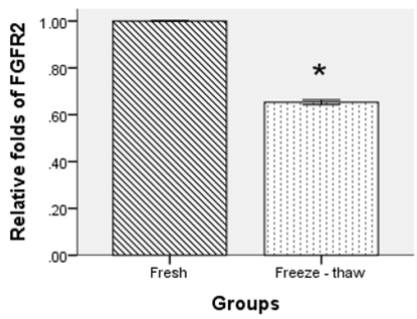
Characteristics / variables	Fresh semen samples (n=30)	Frozen-thawed semen samples (n=30)	P-value
Sperm density (mill/ml)	101.38±51.17	86.57±41.62	0.000
Motile sperms (%)	78.40±16.21	68.18±15.81	0.000
Progressive sperms (%)	62.18±17.75	53.76±17.42	0.000
Normal morphology (%)	53.25±27.40	46.89±26.19	0.000

Values are expressed as Mean±SD.

P-value<0.05 was considered significant.



A



B

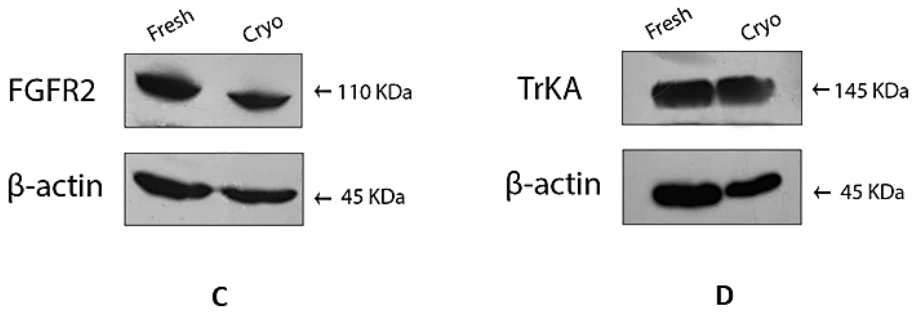


Figure 1. illustrates the fold changes in levels of TrkA and FGFR2, along with Western blot analysis of their protein expression normalized to β-actin.

Panels A and B show that the fold changes in TrkA and FGFR2 levels were significantly lower in freeze-thaw samples compared to fresh samples (P-value<0.05).

Panels C and D indicate reduced protein expression levels of both TrkA and FGFR2 in freeze-thaw samples, with a notable lack of significance for TrkA.

Differences between groups are denoted by * (P-value<0.05).

The concentrations of NGF and FGF2 in seminal plasma were quantified using ELISA kits. Standard curves were established to calculate protein concentrations in nanograms per milligram of total protein. The mean concentration of NGF in fresh samples was 92.07±9.91 ng/ml, while the concentration following freeze-thaw processing was 77.67±12.18 ng/ml, representing a statistically significant difference (P-value=0.001). The average concentrations of FGF2 in fresh and freeze-thaw samples were 55.78±18.64 ng/ml and 46.45±16.90 ng/ml, respectively, also showing statistical significance (P-value=0.04).

Figure 2 depicts the mean percentage of apoptotic alterations following cryopreservation. Compared to fresh samples, the mean percentage of viable cells in the freeze-thaw group was significantly lower (P-value=0.02). There were no substantial differences in early apoptotic rates between the fresh and freeze-thaw groups (P-value>0.05). However, the percentage of late apoptotic cells was significantly higher in the cryopreserved samples (48.12±13.63) compared to fresh samples (24.81±23.98) (P-value<0.05). No statistically significant variations in necrosis rates were observed between freeze-thaw and fresh samples (P-value>0.05).

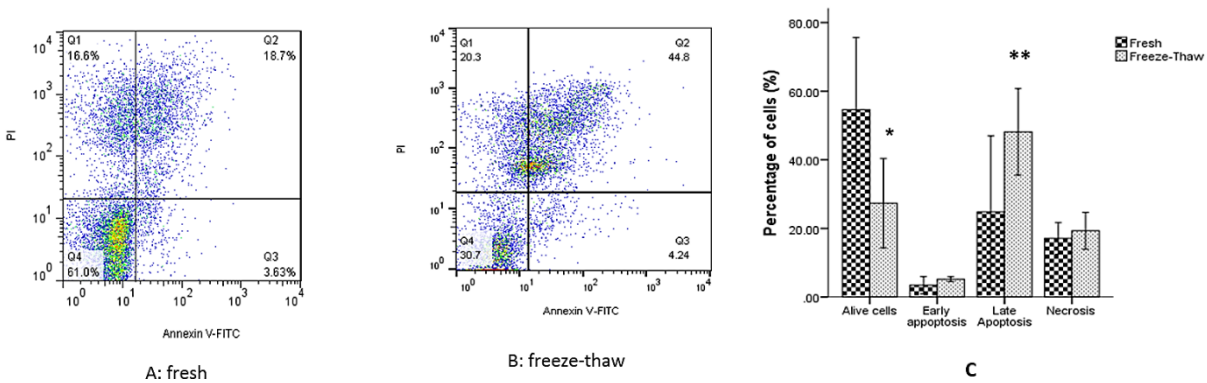


Figure 2 A, B illustrates the assessment of apoptotic changes in cells using flow cytometry.

Viable, apoptotic, and necrotic cell populations were visualized by constructing a dot plot with the assistance of FACS.

The FL1 channel detected annexin-V FITC staining, while the FL2 channel was used for PI staining.

Panel A represents the fresh group, while Panel B details the freeze-thaw group.

Panel C summarizes the mean percentage of apoptotic changes following cryopreservation in normal human sperm.

The percentage of alive cells in the freeze-thaw group was significantly decreased compared to fresh samples (P-value=0.02).

Late apoptosis rates were significantly increased in the cryopreservation group compared to the fresh group (P-value<0.05).

A significance threshold of P-value<0.05 was considered statistically significant.

Correlation analysis between cell membrane asymmetry and sperm motility revealed that after freezing-thawing, the Pearson correlation coefficients for motile sperm and progressive motility with apoptotic cells were -0.16 and -0.04, respectively. These coefficients indicate an inverse relationship, suggesting that as the proportion of apoptotic cells increased, both motile sperm and progressive motility decreased, albeit this correlation did not reach statistical significance (Table 3).

Table 3. Presents the correlation between apoptotic cells and sperm motility across study groups

Variables	Fresh group			Freeze - thaw group		
	Mean±SD	Correlation coefficient	P-value	Mean±SD	Correlation coefficient	P-value
Motile sperms (%)	78.40±16.21	-0.22	0.26	68.18±15.81	-0.16	0.41
Progressive sperms (%)	62.18±17.75	-0.13	0.50	53.76±17.42	-0.04	0.80

Values are expressed as Mean±SD.

P-value<0.05 was considered significant.

Limitations: The study encountered limitations due to restricted access to normal samples and the small volumes of certain specimens.

Future Research: Future investigations should focus on analyzing the status of growth factors and the effects of the freeze-thaw process in samples with sperm disorders.

Discussion

Cryopreservation adversely affects sperm viability, motility, and fertilization capability. Mechanisms of cryoinjury in spermatozoa include hyperosmotic stress, mitochondrial dysfunction, apoptosis, DNA damage, and OS, all of which contribute to alterations in membrane fluidity and lipid peroxidation [22]. The findings of the current study demonstrate that the freezing-thawing process significantly reduces sperm concentration, motility, and morphological integrity, consistent with previous research examining the effects of cryopreservation on human sperm [23].

The plasma membrane plays a crucial role in sperm function and survival during the freezing process [24]. Cryopreservation is associated with morphological degradation of spermatozoa [23], disturbances in plasma membrane properties [24], and the loss of phospholipid asymmetry due to the translocation of PS from the inner to the outer leaflet, which may signal the onset of apoptosis [25]. Our study observed a reduction in live cell populations alongside an increase in apoptotic cells following freezing and thawing, corroborating previous reports [25, 26]. Additionally, we noted a negative correlation between cell membrane asymmetry and sperm motility, indicating that increased apoptotic cells were associated with decreased motile and progressively motile sperm. This result was comparable to that of Sion et al [26].

Given the critical roles of NGF and FGF2 in sperm physiology, coupled with the adverse effects of cryopreservation, we further assessed the impact of this process on the expression and quality of these factors and their receptors in human sperm. NGF, a neurotrophin, mediates its actions through the activation of the TrkA receptor [27]. Evidence indicates its presence in germinal cells at all developmental stages. Moreover, studies in animal models suggest that NGF positively influences reproductive systems and sperm functions, including testicular development, sperm differentiation, maturation, viability, and motility [12, 28].

Since the identification of NGF in male genital secretions since the 1980s, its presence has been documented in the seminal plasma of various livestock species [29]. Initial assessments of NGF levels in humans indicated that oligoasthenozoospermic men have lower NGF levels in seminal plasma compared to asthenozoospermic and fertile counterparts [30]. Our current investigation confirms decreased TrkA gene and protein expression in the freeze-thaw group versus the fresh group, alongside a significant reduction in NGF concentration post-cryopreservation. In preliminary studies, exogenous NGF was shown to enhance sperm survival and motility while reducing apoptotic content in both astheno- and normozoospermic males [2, 4].

The current study also identified reduced FGFR gene and protein expression levels in the freeze-thaw group compared to fresh samples, along with a significant decrease in FGF2 concentrations within seminal plasma. Evidence from rodent models suggests that FGF2 is produced by Sertoli and germ cells [31], indicating that Sertoli cell function is modulated by germ cell-secreted ligands [32]. FGF2 has been implicated in promoting the growth and survival of perinatal gonocytes and modulating the transformation of gonocytes to spermatogonia [33]. Still, its role in adult testicular spermatogenesis remains underexplored. To protect sperm from OS, FGF2 stimulates glutamyl transpeptidase activity, maintaining low glutathione levels and safeguarding sperm from oxidative damage [34].

Previous studies have confirmed the presence of FGF-2 and its receptors in human testes and sperm, signifying their crucial roles in spermatogenesis and motility regulation. Activation of signaling pathways related to FGF2/FGFRs has been demonstrated to influence human sperm motility, and FGF2 levels have been correlated with sperm concentration and motility [18, 35]. Research by Lucía Saucedo et al. on FGF2 knockout mice indicated that insufficient FGF2 levels significantly impaired sperm structure and function, accompanied by alterations in the biochemical processes underlying sperm capacitation [17]. Given the detrimental effects

of cryopreservation on growth factors and their receptors in sperm cells, integrating these factors into the cryopreservation protocol may prove beneficial in enhancing the quality of frozen sperm cells. Further investigations are warranted to evaluate the potential clinical applications of this approach.

Ethical Considerations

The Ethics Committee of Shahroud University of Medical Sciences approved the project (Ethics committee reference number: IR.SHMU.REC.1396.59). All the procedures were followed in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Written consent was obtained from all participants.

Acknowledgment

Not applicable.

Conflict of Interest

The authors declare no conflicts of interest.

Funding

This study was financially supported by the Shahroud University of Medical Sciences, Shahroud, Iran.

References

1. L. K. Thomson, S. D. Fleming, R. J. Aitken, G. N. De Iuliis, J. A. Zieschang and A. M. Clark. Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis. Human reproduction (Oxford, England) 2009;24:2061-70. doi: 10.1093/humrep/dep214

2. S. Saeednia, et al. Nerve growth factor in human semen: Effect of nerve growth factor on the normozoospermic men during cryopreservation process. Iran J Basic Med Sci 2015; 18:292-9.

3. O. A. Bogle, et al. Identification of protein changes in human spermatozoa throughout the cryopreservation process. Andrology 2017; 5:10-22. doi: 10.1111/andr.12279

4. S. Saeednia, M. Shabani Nashtaei, H. Bahadoran, A. Aleyasin and F. Amidi. Effect of nerve growth factor on sperm quality in asthenozoosprmic men during cryopreservation. Reproductive biology and endocrinology: RB&E 2016; 14:29. doi: 10.1186/s12958-016-0163-z

5. S. Chatterjee and C. Gagnon. Production of reactive oxygen species by spermatozoa undergoing cooling, freezing, and thawing. Molecular Reproduction and Development: Incorporating Gamete Research 2001; 59:451-8. doi: 10.1002/mrd.1052

6. C. Aurich. Factors affecting the plasma membrane function of cooled-stored stallion spermatozoa. Animal reproduction science 2005; 89:65-75. doi: 10.1016/j.anireprosci.2005.06.025

7. J. Schiller, J. Arnhold, H. J. Glander and K. Arnold. Lipid analysis of human spermatozoa and seminal plasma by MALDI-TOF mass spectrometry and NMR spectroscopy - effects of freezing and thawing. Chemistry and physics of lipids 2000; 106:145-56. doi: 10.1016/S0009-3084(00)00148-1

8. N. K. Duru, M. S. Morshedi, A. Schuffner and S. Oehninger. Cryopreservation-Thawing of fractionated human spermatozoa is associated with membrane phosphatidylserine externalization and not DNA fragmentation. Journal of andrology 2001; 22:646-51. doi: 10.1002/j.1939-4640.2001.tb02225.x

9. D. Dominguez-Fandos, M. I. Camejo, J. L. Ballesca and R. Oliva. Human sperm DNA fragmentation: correlation of TUNEL results as assessed by flow cytometry and optical microscopy. Cytometry Part A: the journal of the International Society for Analytical Cytology 2007; 71:1011-8. doi: 10.1002/cyto.a.20484

10. J. K. Critser, B. W. Arneson, D. V. Aaker, A. R. Huse-Benda and G. D. Ball. Cryopreservation of human spermatozoa. II. Postthaw chronology of motility and of zona-free hamster ova penetration. Fertility and sterility 1987; 47:980-4. doi: 10.1016/S0015-0282(16)59233-4

11. N. L. Cross and J. W. Overstreet. Glycoconjugates of the human sperm surface: distribution and alterations that accompany capacitation in vitro. Gamete research 1987; 16:23-35. doi: 10.1002/mrd.1120160104

12. W. Jin, A. Tanaka, G. Watanabe, H. Matsuda and K. Taya. Effect of NGF on the motility and acrosome reaction of golden hamster spermatozoa in vitro. Journal of Reproduction and Development 2010:1005270273-. doi: 10.1262/jrd.09-219N

13. R. T. Uren and A. M. Turnley. Regulation of neurotrophin receptor (Trk) signaling: suppressor of cytokine signaling 2 (SOCS2) is a new player. Frontiers in molecular neuroscience 2014; 7:39. doi: 10.3389/fnmol.2014.00039

14. C. Li, et al. Detection of nerve growth factor (NGF) and its specific receptor (TrkA) in ejaculated bovine sperm, and the effects of NGF on sperm function. Theriogenology 2010; 74:1615-22. doi: 10.1016/j.theriogenology.2010.06.033

15. C. Li, L. Zheng, C. Wang and X. Zhou. Absence of nerve growth factor and comparison of tyrosine kinase receptor A levels in mature spermatozoa from oligoasthenozoospermic, asthenozoospermic and fertile men. Clinica chimica acta; international journal of clinical chemistry 2010; 411:1482-6. doi: 10.1016/j.cca.2010.06.002

16. L. Zhang, et al. NGF induces adult stem Leydig cells to proliferate and differentiate during Leydig cell regeneration. Biochemical and Biophysical Research Communications 2013; 436:300-5. doi: 10.1016/j.bbrc.2013.05.098

17. L. Saucedo, et al. Deficiency of fibroblast growth factor 2 (FGF-2) leads to abnormal spermatogenesis and altered sperm physiology. J Cell Physiol 2018; 233:9640-51. doi: 10.1002/jcp.26876

18. L. Saucedo, et al. Fibroblast growth factor receptors (FGFRs) in human sperm: expression, functionality and involvement in motility regulation. PLoS One 2015;10: e0127297. doi: 10.1371/journal.pone.0127297

19. S. Asadian, et al. Circulating endothelial progenitor cells in pregnant women with premature rupture of membranes: potential association with placental disorders. Reproduction, Fertility and Development 2018;30:1689-98. doi: 10.1071/RD17523

20. M. Bagheri, M. Fazli, S. Saeednia, M. G. Kharanagh and N. Ahmadiankia. Sulforaphane modulates cell migration and expression of β-catenin and epithelial mesenchymal transition markers in breast cancer cells. Iranian Journal of Public Health 2020; 49:77. doi: 10.18502/ijph.v49i1.3054

21. M. Bagheri, M. Fazli, S. Saeednia, A. Kor and N. Ahmadiankia. Pomegranate peel extract inhibits expression of β-catenin, epithelial mesenchymal transition, and metastasis in triple negative breast cancer cells. Cellular and molecular biology (Noisy-le-Grand, France) 2018;64:86-91. doi: 10.14715/cmb/2018.64.7.15

22. J. Kopeika, A. Thornhill and Y. Khalaf. The effect of cryopreservation on the genome of gametes and embryos: principles of cryobiology and critical appraisal of the evidence. Human reproduction update 2015; 21:209-27. doi: 10.1093/humupd/dmu063

23. M. O'connell, N. Mcclure and S. Lewis. The effects of cryopreservation on sperm morphology, motility and mitochondrial function. Human reproduction 2002; 17:704-9. doi: 10.1093/humrep/17.3.704

24. M. Giraud, C. Motta, D. Boucher and G. Grizard. Membrane fluidity predicts the outcome of cryopreservation of human spermatozoa. Human Reproduction 2000; 15:2160-4. doi: 10.1093/humrep/15.10.2160

25. H.-J. Glander and J. Schaller. Binding of annexin V to plasma membranes of human spermatozoa: a rapid assay for detection of membrane changes after cryostorage. MHR: Basic science of reproductive medicine 1999; 5:109-15. doi: 10.1093/molehr/5.2.109

26. B. Sion, L. Janny, D. Boucher and G. Grizard. Annexin V binding to plasma membrane predicts the quality of human cryopreserved spermatozoa. International journal of andrology 2004; 27:108-14. doi: 10.1046/j.1365-2605.2003.00457.x

27. D. Mutter, R. Middendorff and M. S. Davidoff. Neurotrophic factors in the testis. Biomedical Reviews 1999; 10:25-30. doi: 10.14748/bmr.v10.4

28. W. Jin, et al. Cellular localization of NGF and its receptors trkA and p75LNGFR in male reproductive organs of the Japanese monkey, Macaca fuscata fuscata. Endocrine 2006; 29:155-60. doi: 10.1385/ENDO:29:1:155

29. C. Castellini, et al. Role of NGF on sperm traits: A review. Theriogenology 2020; 150:210-4. doi: 10.1016/j.theriogenology.2020.01.039

30. C. Li, L. Zheng, C. Wang and X. Zhou. Absence of nerve growth factor and comparison of tyrosine kinase receptor A levels in mature spermatozoa from

oligoasthenozoospermic, asthenozoospermic and fertile men. Clinica Chimica Acta 2010; 411:1482-6. doi: 10.1016/j.cca.2010.06.002

31. M. Regueira, et al. FSH and bFGF regulate the expression of genes involved in Sertoli cell energetic metabolism. General and Comparative Endocrinology 2015; 222:124-33. doi: 10.1016/j.ygcen.2015.08.011

32. M. Gómez, et al. Sertoli-secreted FGF-2 induces PFKFB4 isozyme expression in mouse spermatogenic cells by activation of the MEK/ERK/CREB pathway. American Journal of Physiology-Endocrinology and Metabolism 2012;303: E695-E707. doi: 10.1152/ajpendo.00381.2011

33. H. P. Pui and Y. Saga. Gonocytes-to-spermatogonia transition initiates prior to birth in murine testes and it requires FGF signaling. Mechanisms of development 2017; 144:125-39. doi: 10.1016/j.mod.2017.03.002

34. Z.-J. Lan, J. C. Labus and B. T. Hinton. Regulation of gamma-glutamyl transpeptidase catalytic activity and protein level in the initial segment of the rat epididymis by testicular factors: role of basic fibroblast growth factor. Biology of Reproduction 1998; 58:197-206. doi: 10.1095/biolreprod58.1.197

35. D. G. Azúa, et al. Fibroblast growth factor 2 (FGF2) is present in human spermatozoa and is related with sperm motility. The use of recombinant FGF2 to improve motile sperm recovery. Andrology 2017; 5:990-8. doi: 10.1111/andr.12398