

HUMAN SPERM MOTILITY, VIABILITY, AND MORPHOLOGY DECREASE AFTER CRYOPRESERVATION

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ABSTRACT

The aim of this study was to analyze human sperm motility, viability, and morphology before and after cryopreservation. This true laboratory experimental study had pre and post randomized one group design. The study was conducted at the Embryology, Andrology, and Genetics Laboratory, Department of Medical Biology, Faculty of Medicine, Universitas Airlangga from August to November 2017. The eighteen samples of fresh semen were collected from male volunteers who agreed and signed the informed consent of the study. Samples were analyzed their motility, viability, and morphology before and after cryopreservation. Results of this study indicated differentiation between motility before and after cryopreservation. Cryopreservation process decreased progressive motility ($42.22 \pm 9.46\%$; $17.83 \pm 6.24\%$; $p < 0.0001$) and increased the number of immotile spermatozoa ($35.44 \pm 10.15\%$; $60.11 \pm 12.53\%$; $p < 0.0001$). Cryopreservation also decreased human sperm viability ($73.78 \pm 8.91\%$; $40.83 \pm 12.89\%$; $p < 0.0001$) and morphology ($10.94 \pm 4.96\%$; $7.39 \pm 3.90\%$; $p < 0.0001$). Cryopreservation of human spermatozoa caused the decreased of motility, viability, and morphology.

Keywords: Cryopreservation; sperm morphology; sperm motility; sperm viability

ABSTRAK

Penelitian ini bertujuan untuk menganalisis motilitas, viabilitas, dan morfologi spermatozoa manusia sebelum dan sesudah kriopreservasi. Penelitian eksperimental laboratorium murni ini memiliki desain penelitian pre and post randomized one group design serta dilakukan di Laboratorium Embriologi, Andrologi, dan Genetika Departemen Biologi Kedokteran Fakultas Kedokteran Universitas Airlangga pada Agustus hingga November 2017. Sebanyak delapan belas sampel semen segar didapatkan dari sukarelawan yang telah menyetujui dan menandatangani informed consent. Kedelapan belas sampel tersebut dilakukan analisis semen berupa pemeriksaan motilitas, viabilitas, serta morfologi sebelum dan sesudah kriopreservasi. Hasil penelitian ini menunjukkan adanya perbedaan motilitas sebelum dan sesudah kriopreservasi. Proses kriopreservasi menyebabkan penurunan motilitas progresif ($42.22 \pm 9.46\%$; $17.83 \pm 6.24\%$; $p < 0.0001$) dan peningkatan jumlah spermatozoa immotil ($35.44 \pm 10.15\%$; $60.11 \pm 12.53\%$; $p < 0.0001$). Kriopreservasi juga menyebabkan penurunan viabilitas ($73.78 \pm 8.91\%$; $40.83 \pm 12.89\%$; $p < 0.0001$) dan morfologi ($10.94 \pm 4.96\%$; $7.39 \pm 3.90\%$; $p < 0.0001$) spermatozoa manusia. Kriopreservasi spermatozoa manusia dapat menyebabkan penurunan motilitas, viabilitas, dan morfologi.

Kata kunci: Kriopreservasi; morfologi spermatozoa; motilitas spermatozoa; viabilitas spermatozoa

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INTRODUCTION

Cryopreservation has become an important part of the development of assisted reproduction technology (ART). Nowadays, cryopreservation can be performed on spermatozoa, oocytes, and embryos at various stages of embryonic development (Di Santo et al. 2012). Cryopreservation of human spermatozoa, although it has a detrimental effect on the spermatozoa quality,

remains an option in the application of ART, for example in intra uterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI) (Moce et al. 2016).

Cryopreservation indications include sperm storage of severe oligoasthenoteratozoospermia (OAT) to prevent the possibility of decreased quality and number of spermatozoa to optimizing the ART results (Di Santo et

al. 2012), male fertility preservation before chemo or radiotherapy (McLaughlin and Pacey 2009, Di Santo et al. 2012), and sperm storage from epididymal or testicular (Di Santo et al. 2012, Boitrelle et al. 2012).

Sperm cryopreservation can cause damage to spermatozoa which results in decreased sperm motility and viability (McLaughlin and Pacey 2009). A decrease in the motility and viability may lead to a decline in fertilization ability (Zribi et al. 2010). The ice crystals formation of sperm cryopreservation cause the buildup of electrolytes and other solutes in cells that damage the sperm intracellular (Yeste 2016). This intracellular damage is difficult to detect but causes damage the sperm function.

The aim of this study is to analyze sperm quality factors that lead to the sperm ability to fertilize oocyte before and after cryopreservation. That factors are sperm motility, viability, and morphology analyzed using semen analysis (WHO 2010).

MATERIALS AND METHODS

This true laboratory experimental study had pre and post randomized one group design. The study was conducted at the Embryology, Andrology, and Genetics Laboratory, Department of Medical Biology, Faculty of Medicine, Universitas Airlangga from August to November 2017. The study was agreed by local ethical commission (Komite Etik Penelitian Kesehatan Fakultas Kedokteran Universitas Airlangga) and shown by ethical clearance certificate.

Eighteen samples of fresh semen were collected from male volunteers that agreed and signed the informed consent of the study. Samples taken using WHO (2010) standard and completed the study criterias, semen volume >2 ml and sperm concentration >5 x 10⁶/ml.

The samples were analyzed their motility, viability, and morphology right after liquefaction. Sperm motility, morphology, and viability analyzed using WHO (2010) protocol. All of this examination took two times, before and after freezed-thawed process of cryopreservation.

Sperm cryopreservation with rapid freezing technique used 0.5 ml straw and mixed of 1:1 sample and cryoprotectant (SpermFreeze™, Vitrolife Inc.). The mixed specimen intra straw then steamed 3 cm apart from the liquid nitrogen for 30 minutes and stored in liquid nitrogen (-196°C) tank for 24 hours. The straws then thawed in waterbath (37°C) for 30 seconds and placed in room temperature for 20 minutes before the second sperm quality examinations.

Data analysis was compare the sperm motility, viability, and morphology before and after sperm cryopreservation used SPSS 21 statistic software and was described as significant different if p value < α (5%). Normal distribution analyzed using One Sample Kolmogorov Smirnov test and the different between before and after cryopreservation analyzed using Paired T-test.

RESULTS

The range of eighteen sperm concentration was 9.04 - 122 x 10⁶/ml with the average was 40.66 ± 25.34 x 10⁶/ml. All the datas showed normal distribution analyzed using One Sample Kolmogorov Smirnov test. The results of sperm progressive motility showed significantly decreased after cryopreservation, the sperm immotile number showed significantly increased, while the sperm non progressive motility after cryopreservation was decreased but not significant (Table 1). Whereas, The paired T-test results showed that the sperm viability and morphology before and after cryopreservation was significantly different (Table 2).

DISCUSSION

The most influential factor of the sperm motility is the availability of energy generated by disassembling adenosine triphosphate (ATP) into adenosine diphosphate (ADP), thus driving microtubule doublets in axonema (Cooper and Yeung 2010). Mitochondria are located in the midpiece between the membrane and nine spindle threads, having the function of providing the energy needed for sperm motility. The largest energy used by spermatozoa is the ATP molecules that are made in cytoplasm by glycolysis or phosphorylative oxidation in mitochondria. The ATP then will be transferred to spindles or microtubules to move the sperm tail (Di Santo 2012).

Sperm progressive motility before and after cryopreservation showed statistically significant decrease outcomes, accompanied by a significant increase in immotile numbers. This change may be explained that in the cryopreservation process, sperm plasma and mitochondria membrane are very susceptible to be disturbed. Changes in fluidity of the mitochondrial membrane potentially produce and release reactive oxygen species (ROS) (Agarwal et al. 2014), indirectly, thus significantly decrease sperm motility (Di Santo 2012).

Table 1. Human sperm motility before and after cryopreservation

Type of sperm motility	Before cryopreservation (mean \pm SD)	After cryopreservation (mean \pm SD)	P value
Progressive	42.22 \pm 9.46 %	17.83 \pm 6.24 %	< 0.0001
Non progressive	22.33 \pm 9.17 %	22.06 \pm 7.91 %	0.929
Immotile	35.44 \pm 10.15 %	60.11 \pm 12.53 %	< 0.0001

Sperm motility was analyzed using WHO (2010) protocol

Table 2. Human sperm viability and morphology before and after cryopreservation

Sperm parameters	Before cryopreservation (mean \pm SD)	After cryopreservation (mean \pm SD)	P value
Viability	73.78 \pm 8.91 %	40.83 \pm 12.89 %	< 0.0001
Morphology	10.94 \pm 4.96 %	7.39 \pm 3.90 %	< 0.0001

Sperm viability and morphology were analyzed using WHO (2010) protocol

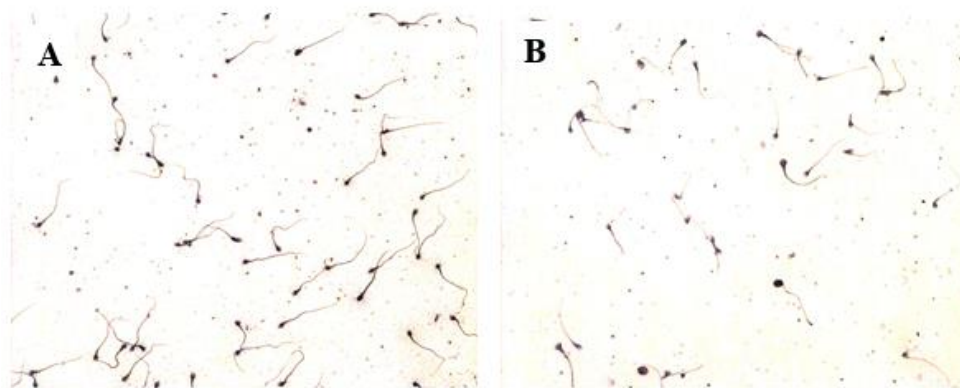


Fig. 1. Sperm morphology different between before (A) and after (B) cryopreservation

Sperm viability before and after cryopreservation showed statistically significant decreased. Cryopreservation produces ROS in spermatozoa (Agarwal et al. 2014) that can cause deviation of sperm structures such as disturbances in the plasma membrane. This change is associated with decreased motility, viability, and fertilization ability of spermatozoa (Boitrelle et al. 2012). Sperm plasma membrane contains rich phospholipid and cholesterol that can be modified by cryopreservation process (Di Santo 2012). The death of spermatozoa after cryopreservation may be caused by cryoinjury. Cryoinjury can occur by the intracellular ice formation or electrolyte imbalance due to extracellular unfrozen fraction (Yeste 2016).

Sperm morphology after cryopreservation significantly decreased. The sperm morphological changes are also possible due to the cryopreservation process. This mechanism is related to osmotic stress and intracellular ice crystal formation (Yeste 2016). Morphological changes are also occurred during rehydration and

increasing temperature after thawing. Ice crystals during sperm cryopreservation cause structural and membrane integrity changes then form of spermatozoa morphological change.

The important factor that contribute to sperm survival during cryopreservation is cryoprotecting agent (CPA). CPA will act as protective agents for cell membranes and organelles in order to avoid damage to cell integrity during freezing and thawing process (Yeste 2016). Intracellular CPA use different osmotic pressure between intra and extracellular to enter the intracellular to provide cytoplasmic protection. Extracellular CPA cause intracellular dehydration due to different osmotic pressure without entering into cells. Commonly used as intracellular CPAs are glycerol (glycerin), dimethylsulfoxide (DMSO), ethylene glycol, 1,2 propanediol. Extracellular CPA which may be used are polyvinyl pyrrolidone (PVP), sugars (sucrose, glucose, raffinose), proteins and lipoproteins, egg yolks, blood serum, and milk (Hossain 2008).

The sperm intracellular component can increase sperm survival. This is due to the amount of macromolecules dissolved in the spermatozoa cytosol is high so the amount of water is less (Di Santo et al. 2012). This condition resulting the sperm cytosolic viscosity becomes high and can serve as natural cryoprotectant which is not toxic. The sperm ability to maintain its viability in frozen storage also influenced by CPA.

CONCLUSION

This study conclude that there is different of sperm motility, viability, and morphology before and after cryopreservation. The cryopreservation process leads to a decrease in sperm progressive motility and elevated sperm immotiles. The cryopreservation process also leads to decreased sperm viability and morphology.

Furthermore, it is necessary for further studies about the sperm ability for fertilize oocyte and also the sperm genetic materials condition after cryopreservation.

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