SPERM CRYOPRESERVATION FOR IMPAIRED SPERMATOGENESIS

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Introduction Sperm cryopreservation for men with severely impaired spermatogenesis is one of the commonest reasons for short term sperm storage, usually in advance of fertility treatment. Cryopreservation is generally very effective, although not all spermatozoa survive the process of freezing and thawing. This review considers various aspects of freezing sperm, including an overview of methods, appropriate use of cryoprotectants and practical considerations, as well as oxidative stress and mechanisms of cell cryodamage.

- Cryopreserved spermatozoa are extensively used in assisted reproductive technology (ART) worldwide.
- Historical scientific advancements have contributed to the evolution of cryopreservation, including the use of liquid nitrogen, glycerol as a cryoprotectant, and fructose solutions for partial dehydration.
- The fortuitous discovery of cryoprotectants significantly increased spermatozoa survival, shaping the field of sperm cryobiology.

Mainstream Cryopreservation:

- Mainstream cryopreservation of human spermatozoa began in the 1960s, with the first human sperm banks established in Iowa, USA, and Tokyo, Japan.
- Fertility treatment using donor sperm led to public outcry, shifting clinics' focus to fertility preservation for men.
- Development of semen processing techniques like swim-up or density-gradient centrifugation (DGC) was a result of the progress in IVF

Autologous Sperm Cryopreservation:

- Sperm banking allows cryopreservation and autologous use of gametes, making it relevant for patients facing gonadotoxic treatment.
- Impaired spermatogenesis is a common reason for short-term sperm freezing.
- The timing and criteria for offering autologous sperm cryopreservation remain undefined, leading to variations in practices.

Sperm Cryodamage and Oxidative Stress:

- Oxidative stress (OS) plays a crucial role in sperm cryodamage, induced by increased ROS and RNS generation.
- OS leads to lipid peroxidation, DNA damage, alterations in DNA methylation, and changes in gene and protein expression.
- Supplementation of cryopreservation medium (CPM) with antioxidants shows potential benefits in minimizing DNA damage and improving sperm characteristics.

Table 1 Composition of selected commercial CPM in clinical use (2022).

	Origio sperm freezing medium	Life global sperm freezing	Sage Quinn's advantage sperm freeze	Irvine scientific freezing medium with TYB
Glycerol	+	+	+	+
Glucose	+		+	
Sucrose	+	+	+	
Egg yolk				+ (20%)
Human serum albumin	+†	3.95 mg/mL	+†	
Synthetic serum replacement + insulin	+			
Glycine	+	+		
EDTA			+	
Sodium citrate				+
Glutamine			+	
TRIS				+
TES				+
HEPES	+	+	+	
Sodium bicarbonate	+		+	
Sodium lactate	+ (L)	+	+ (D,L)	
Calcium chloride	+		+ (dihydrate)	
Potassium chloride	+		+	
Sodium chloride	+		+	
Magnesium chloride	+			
Sodium pyruvate			+	
Sodium phosphate	+			
Potassium phosphate			+	
Magnesium sulphate			+	
Physiologic salts		+†		
Dextrose monohydrate		+		
Fructose				+
Gentamicin	+		+	+
Phenol red			+	

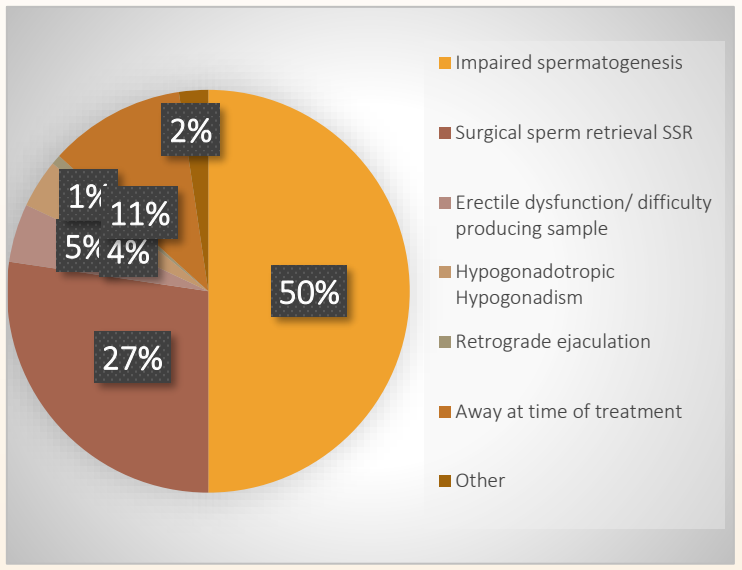

Figure1: Excluding samples in long-term storage for fertility preservation (n=279). 50% of samples were stored due to significantly impaired spermatogenesis (n=140)

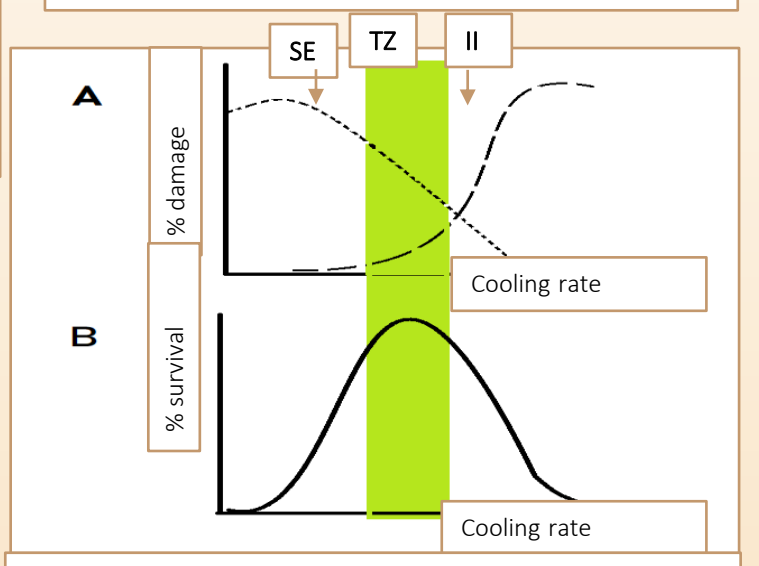

Figure 2: Cryodamage and cell survival during cryopreservation. (A) High cell damage due to solution effect (SE) when cooling rate is slow (dotted line). High cell damage due to intracellular ice (II) when cooling rate is high (dashed line). (B) Overall cell survival (solid line). Transition zone (TZ), where negative effects of II and SE are minimized, describes optimal cooling conditions for cryopreservation (green-shaded area).

Cryostorage: Methodology and Practical Aspects:

- Semen samples are collected and analyzed before cryopreservation.
- The debate lies in whether to prepare samples before freezing or freeze unprepared samples, utilizing the antioxidant properties of seminal plasma.
- Cryostorage tanks, either liquid or vapor phase, require stringent conditions and continuous monitoring to ensure sample quality.

Vitrification of Sperm:

- Vitrification, involving ultra-rapid cooling and warming rates, shows promise for sperm cryopreservation.
- It may become a faster alternative method with significant benefits for fertility clinics.

Thawing Sperm:

- After cryopreservation, a test thaw is performed to assess sperm survival.
- Different thawing techniques are used for ejaculate and testicular sperm to preserve sample quality.

How Long Should We Freeze Sperm?:

- Recent UK legislative changes allow sperm storage for up to 55 years.
- Utilization of cryostored sperm remains low, despite no impact on clinical outcomes.

Conclusion:

- Cryopreserved spermatozoa have revolutionized fertility treatments and preservation.
- While advancements have been made, challenges remain, such as improving post-thaw sperm quality and understanding the long-term effects on sperm and offspring

Reference :Process and Pitfalls of Sperm Cryopreservation, Sperm cryopreservation for impaired spermatogenesis G Hughes[1] and S Martins da Silva