Summary of Safety and Clinical Performance

SpermFreeze (with phenol red) / SpermFreeze SSP

This Summary of Safety and Clinical Performance (SSCP) is intended to provide public access to an updated summary of the main aspects of the safety and clinical performance of the device. The SSCP is not intended to replace the Instructions For Use (IFU) as the main document to ensure the safe use of the device, nor is it intended to provide diagnostic or therapeutic suggestions to the intended users.

1 Device identification and general information

1.1 Device trade name(s)

SpermFreeze
SpermFreeze with phenol red
SpermFreeze SSP

1.2 Manufacturer's name and address

FertiPro NV
Industriepark Noord 32
8730 Beernem
Belgium

1.3 Manufacturer's single registration number (SRN)

BE-MF-000000313

1.4 Basic UDI-DI

5411967SPF152

1.5 Medical device nomenclature description/text

Applicable EMDN code: U08020501 (Materials/solutions for freezing/thawing for assisted reproduction)

1.6 Class of device

Class III devices according to Annex VIII of the MDR

1.7 Year when the first certificate (CE) was issued covering the device

SpermFreeze: 2012
SpermFreeze with phenol red: 2016
SpermFreeze SSP: 2016

1.8 Authorised representative if applicable; name and the SRN

Not applicable

1.9 NB's name and single identification number

BSI Group The Netherlands BV
NB identification number: 2797

2 Intended use of the device

2.1 Intended purpose

Sperm Freezing media are cell culture media for the cryopreservation of human spermatozoa. The media are designed to ensure the recovery of sperm suitable for Assisted Reproductive Technologies (ART) upon cryopreservation. SpermFreeze SSP is a more concentrated cryopreservation medium than SpermFreeze (with phenol red) which allows lower dilution of the sperm samples before freezing.

2.2 Indication(s) and intended patient groups

Sperm Freezing media are used to store donor and partner spermatozoa before assisted reproduction treatments (e.g. intrauterine insemination (IUI), in vitro fertilization (IVF) or intracytoplasmatic sperm injection (ICSI)), to preserve spermatozoa before therapy for malignant diseases, vasectomy or surgical infertility treatments and to ensure the recovery of small number of spermatozoa in severe male factor infertility. Therefore, sperm cryopreservation is an important component of fertility management and is routinely used in ART clinics/andrology labs.

There is no direct patient contact, Sperm Freezing media only come in direct physical contact with human spermatozoa.

2.3 Contraindications and/or limitations

There are no known contraindications and/or limitations identified for Sperm Freezing media.

3 Device description

3.1 Description of the device

Sperm Freezing media are cell culture media for the cryopreservation of human spermatozoa.

The media are designed to cryopreserve sperm before further use in ART and consist of water, salts, glycine and cryoprotectants (i.e. glycerol, sucrose and Human Serum Albumin) to protect sperm during cryopreservation. The inclusion of Human Serum Albumin (which is a medicinal substance derived from human blood plasma) in ART media from FertiPro is approved by the EMA (European Medicine Agency).

The difference between SpermFreeze (with phenol red) and SpermFreeze SSP is the amount of the cryoprotectant glycerol in the medium: SpermFreeze SSP contains 27% of glycerol, SpermFreeze (with phenol red) contains 15%. Importantly, when the end-user uses the correct dilution factor with the semen sample as indicated in both IFU's, the final glycerol concentration in the semen sample to be frozen is almost identical (i.e 6.7% and 6.2% respectively). SpermFreeze SSP is used mainly for the cryopreservation of sperm samples with lower concentrations of spermatozoa, because a much lower dilution of the semen is required when compared to SpermFreeze (with phenol red).

The device is not intended for single use. Multiple single-procedures can be performed with one bottle of Sperm Freezing media. The media can be used up to 7 days after bottle opening (when sterile conditions are maintained and the products are stored at 2-8°C).

Sperm Freezing media are sterilized using aseptic processing techniques (filtration).

The lifetime for Sperm Freezing media is at least 10 years.

3.2 A reference to previous generation(s) or variants if such exist, and a description of the differences

No previous generation of the devices have been brought on the market by FertiPro.

3.3 Description of any accessories which are intended to be used in combination with the device

No accessories for Sperm Freezing media are identified.

3.4 Description of any other devices and products which are intended to be used in combination with the device

Sperm Freezing media are intended to be used with the following devices:

- Sperm freezing straws (e.g. CBS high security sperm straws, classified as Class IIa Medical Devices)
- Freezing tank with liquid nitrogen

4 Risks and warnings

4.1 Residual risks and undesirable effects

The only remaining residual risk is the inclusion of Human Serum Albumin in Sperm Freezing media. The inclusion of this medicinal substance derived from human blood plasma in the devices is approved by the EMA. A potential risk associated with Human Serum Albumin is the transmission of viral or prion-carried diseases and the batch-to batch variation.

- **Batch-to-batch variation** is still a problem because of the inherent variability in donor blood. Due to this fluctuation, standardization of procedures remains difficult.
  - ↔ For this reason, a mouse embryo assay is routinely performed as part of the batch release criteria of HSA (incoming inspection) and human sperm survival assays are routinely performed as part of Sperm Freezing media batch release criteria.
- Secondly; with the use of a human-derived protein source, a potential risk exists of **transmitting viral or prion-carried diseases**.
  - ↔ HSA is manufactured with a pasteurization procedure that has led to an excellent viral safety record over the 50 years of clinical use. Only Plasbumin-25 or alternatively, Albunorm 25 will be used as a source of albumin, as these products are covered by a valid Plasma Master File, and the EMA has positively evaluated the usefulness, safety and benefit of the inclusion of these products in FertiPro ART-media.
  - ↔ On the other hand, despite the rigorous quality controls, all cell culture media should still be treated as potentially infectious. At present, there is no known test method that can offer full assurance that products derived from human blood will not transmit infectious agents. The use of Sperm Freezing media is restricted to the cryopreservation of human sperm and is not intended to be in direct contact with users or patients. Even so, the instructions for use / MSDS clearly warn that the medium contains human albumin solution and that protective clothing should be worn.

The major benefit of HSA in Sperm Freezing media is clear:

- Inhibition of lipid peroxidation that can be damaging to sperm.
- Detoxification by binding waste products from cell metabolism.
- HSA prevents cell aggregation and adherence to laboratory equipment and promotes the ease of gamete handling and manipulation.

Based on this analysis it is concluded that the benefit of adding HSA to the media outweighs the risk and the overall residual risk related to the use of Sperm Freezing media for cryopreservation of human sperm has been judged acceptable.

With respect to the above, following information is provided to the customer:

- Product composition is clearly indicated on the labels and instructions for use

- Instructions for use contains the following warnings:
  - Standard measures to prevent infections resulting from the use of medicinal products prepared from human blood or plasma include selection of donors, screening of individual donations and plasma pools for specific markers of infection and the inclusion of effective manufacturing steps for the inactivation/removal of viruses. Despite this, when medicinal products prepared from human blood or plasma are administered, the possibility of transmitting infective agents cannot be totally excluded. This also applies to unknown or emerging viruses and other pathogens. There are no reports of proven virus transmissions with albumin manufactured to European Pharmacopoeia specifications by established processes. Therefore, handle all specimens as if capable of transmitting HIV or hepatitis.
  - All blood products should be treated as potentially infectious. Source material used to manufacture this product was tested and found non-reactive for HbsAg and negative for Anti-HIV-1/-2, HIV-1, HBV, and HCV. Furthermore, source material has been tested for parvovirus B19 and found to be non-elevated. No known test methods can offer assurances that products derived from human blood will not transmit infectious agents.

No other known undesirable side-effects are identified.

4.2 Warnings and precautions

Besides the above, attention should be paid to the following warnings and precautions (as described in the instructions for use):

- Do not use the product if:
  - it becomes cloudy or shows any evidence of microbial contamination
  - seal of the container is opened or defect when the product is delivered
  - expiry date has been exceeded
- Do not freeze before use
- Do not re-sterilize after opening
- Aseptic technique should be used to avoid possible contamination. Sperm Freezing media do not contain any antibiotics.
- Always wear protective clothing when handling specimens.
- Any serious incident (as defined in European Medical Device Regulation 2017/745) that has occurred should be reported to FertiPro and the competent authority of the Member State in which the user and/or patient is established.

4.3 Summary of any field safety corrective action (FSCA including FSN) if applicable

No field safety corrective actions with regard to SpermFreeze (with phenol red) / SpermFreeze SSP were needed.

5 Summary of clinical evaluation and post-market clinical follow-up (PMCF)

5.1 Real-word evidence analyses

A literature search is performed to investigate:

- whether clinical data obtained during literature search are consistent with the embryological and clinical ART outcomes described in the benchmark papers from the ESHRE (European Society of Human Reproduction and Embryology) (see tables below)
  OR
- whether clinical data obtained during literature search is within the acceptable defined outcomes concerning the different sperm parameters (motility, vitality, DNA fragmentation and fertilization potential)
  OR

- whether the results regarding motility and vitality are not be significantly lower than the results from cryopreservation media of competitors

- The embryological outcomes must be consistent with the competency limits as reported by the ESHRE Vienna consensus group in 2017 (ESHRE Special Interest Group of Embryology 2017).

  The Vienna consensus report published in 2017 is the result of a 2 day consensus meeting of expert professionals from Sweden, Turkey, UK, Australia, Italy, Spain, Belgium, Austria, Ireland, Canada, USA, and Norway. As a starting point for the discussion, two surveys were organized to collect information on indicators used in IVF laboratories worldwide. During the meeting, the results of the surveys, scientific evidence (where available), and personal clinical experience were integrated into presentations by experts on specific topics. After presentation, each proposed indicator was discussed until consensus was reached within the panel (ESHRE Special Interest Group of Embryology 2017).

Competency limits reported by the ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine in 2017. The Vienna consensus: report of an expert meeting on the development of art laboratory performance indicators (ESHRE Special Interest Group of Embryology 2017).	IVF normal fertilization rate: **≥60%** (lower range: 50%) ICSI normal fertilization rate: **≥65%** (lower range: 60%) ***Since multiple factors can have an influence on the embryology outcomes, (ART policy, approach of the clinic, patients characteristics), a value 10% lower than the competency limit is acceptable.***

- **Clinical ART data** obtained from IVF centers should be consistent with the clinical outcomes described in the benchmark paper from the ESHRE (Wyns et al. 2021).

  Each year, the ESHRE publishes a peer-reviewed report, which collects, analyses and reports ART data generated in Europe. The most recent report includes data from 1382 institutions in 39 countries, with a total of 940 503 treatment cycles (covering the time period from 1 January to 31 December 2017) (Wyns et al. 2021) and is summarized in the table below:

	In vitro fertilization (IVF):	**Intra cytoplasmic sperm injection (ICSI):**	**Frozen embryo replacement (FER):**	**Intrauterine insemination(IUI):**
ART in Europe, 2017: results generated from European registries by ESHRE (Wyns C, C De Geyter, C Calhaz-Jorge, MS Kupka, et al. 2021. 'ART in Europe, 2017: results generated from European registries by ESHRE', *Hum Reprod Open*, 2021: hoab026.)	Clinical pregnancy rate per aspiration: **28.3%** *(range: 21.0 – 42.3%)* Clinical pregnancy rate per transfer: **36.2%** *(range: 28.4 – 47.1%)* Delivery rate per aspiration: **21.5%** *(range: 10.4 – 40.9%)* Delivery rate per transfer: **27.5%** *(range: 11.2 – 41.5%)*	Clinical pregnancy rate per aspiration: **26.3%** *(range: 18.4 – 38.2%)* Clinical pregnancy rate per transfer: **35.2%** *(range: 27.4 – 45.1%)* Delivery rate per aspiration: **20.2%** *(range: 12.2 – 36.8%)* Delivery rate per transfer: **26.8%** *(range: 17.5 – 37.4%)*	Pregnancy rate per thawing: **34.4%** *(range: 22.0 – 49.1%)* Pregnancy rate per transfer: 35.3% (range: 23.5 – 51.3%) Delivery rate per thawing: **23.3%** *(range: 3.2 – 37.8%)* *Delivery rate per transfer:* *24.3%* *(range: 3.2 – 38.6%)*	**using husband semen (IUI-H):** Delivery rate per cycle: **8.8%** *(range: 4.2 - 24.4%)* **using donor semen (IUI-D):** Delivery rate per cycle: **13.8%** *(range: 4.3 – 42.0%)*

The following acceptable outcomes of the **sperm parameters** are set (based on results found in the literature):

- a reduction in sperm motility is set on 50-60% after cryopreservation with SpermFreeze media*
- a reduction in sperm vitality is set on 50% after cryopreservation with SpermFreeze media*
- effect on sperm DNA fragmentation is comparable with that of similar devices with identical intended use as SpermFreeze media on the market
- fertilization potential of sperm cryopreserved with SpermFreeze media is comparable with that of fresh sperm

* In case of a comparative study with similar devices with identical intended use as Sperm Freezing media: the reduction of sperm motility/vitality is not significantly lower than the reduction in sperm motility/vitality of the cryopreservation media of competitors.

Sperm vitality, motility and Sperm DNA fragmentation are important parameters to evaluate the quality of the spermatozoa upon cryopreservation, but importantly, reduction of these parameters do not exclude that the thawed semen sample will result in a successful ART procedure. Based on these parameters, the physician will determine which ART, i.e. IUI, IVF or ICSI, will be used to have the highest chance to succeed. As indicated above, the ICSI technique circumvents the natural selection process in fertilization and enables the successful use of spermatozoa with severely impaired characteristics to achieve clinical pregnancy. The devices included in this clinical evaluation report are for professional use only, and do need the knowledge of these professionals to interpret the semen samples upon thawing.

An overview of the articles studying the performance of Sperm Freezing media is indicated in the table below. Overall, it can be concluded from these papers that embryological and ART outcomes, when Sperm Freezing media are used, are consistent with the clinical outcomes described in the benchmark papers from the ESHRE (Wyns et al. 2021; ESHRE Special Interest Group of Embryology 2017). Also the effects on motility, viability, DNA fragmentation and fertilization potential after cryopreservation of sperm with Sperm Freezing media are within acceptable limits and comparable with other products on the market.

Selected articles describing the performance and/or safety of Sperm Freezing media[1]				
(Zribi et al. 2010)	(Prisant et al. 2010)	(Montagut et al. 2015)	(Falah 2019)	(Karacan et al. 2013)
(Thomson et al. 2009)	(Rahana et al. 2011)	(Fabozzi et al. 2016)	(Seifi et al. 2020)	
(Donnelly, McClure, and Lewis 2001)	(Freour et al. 2012)	(Lusignan et al. 2018)	(Lierman et al. 2021)	
(Vutyavanich, Piromlertamorn, and Nunta 2010)	(Ahmad et al. 2010)	(El-Ahwany, Samir, and Alahwany 2018)	(Hezavehei et al. 2021)	
(Punyatanasakchai et al. 2008)	(Satirapod et al. 2012)	(Awaga et al. 2019)	(Valipour et al. 2021)	
(Desrosiers et al. 2006)	(Zribi et al. 2012)	(Reignier et al. 2018)	(Santonastaso et al. 2021)	
(Bhattacharya et al. 2006)	(Moubasher et al. 2013)	(O'Neill et al. 2019)	(Kumari et al. 2021)	
(Bandularatne and Bongso 2002)	(Bizet et al. 2012)	(Taher-Mofrad et al. 2020)	(Tvrda et al. 2021)	
(Donnelly et al. 2001)	(Gatimel, Leandri, and Parinaud 2013)	(Valipour et al. 2020)	(Dayal et al. 2021)	
(O'Connell, McClure, and Lewis 2002)	(Boitrelle et al. 2012)	(Tvrda et al. 2020)	(Hosseinmardi et al. 2021)	

[1] *Two additional articles were retrieved that describe safety and performance of Sperm Freezing media. Due to reasons of confidentiality, these papers are not listed in the table. Note however that all outcomes described in these additional articles are consistent with the outcomes as described in the benchmark papers.*

Selected articles describing the performance and/or safety of Sperm Freezing media[1]				
(Saritha and Bongso 2001)	(Philippon et al. 2015)	(Karthikeyan et al. 2019)	(Hezavehei et al. 2022)	
(Menon et al. 2009)	(Tongdee et al. 2015)	(Konc, Kanyo, and Cseh 2008)	(Androni et al. 2021)	

5.2 Device registries

In addition, ART outcomes of one IVF clinic located in Europe is included in the clinical evaluation report of Sperm Freezing media. It could be concluded that the ART outcomes of the IVF clinic using Sperm Freezing media, are consistent with the clinical outcomes, as described in the benchmark paper from the ESHRE (European Society of Human Reproduction and Embryology) (Wyns et al. 2021).

5.3 Analysis complaints, customer/market feedback, vigilance

Based on the analysis performed in 2019, two changes are made to the IFU:

- ✓ Literature search showed that the reported viability and motility after cryopreservation with Sperm Freezing media resulted in better results when native semen samples are frozen compared to the situation where semen was washed before freezing. Based on this finding, the following is added to the method section in the IFU of Sperm Freezing media: '*Ideally, cryopreservation is performed on native semen samples.*'

- ✓ During literature search, it was noted that some publications use SpermFreeze with a 1:1 semen SpermFreeze dilution instead of 1:0.7. This does results in a higher concentration, i.e. 7.5% of glycerol in the frozen semen sample instead of 6.2 when the 1:0.7 dilution is used. This is still in line with the recommended use of 5-10% glycerol for maximum cryosurvival of human spermatozoa by (Mahadevan and Trounson 1983) and (Di Santo et al. 2012). Based on the satisfying outcome reported in the studies using SpermFreeze in a 1:1 dilution, we made the following change to the method section in the IFU of SpermFreeze: *Mix 1 ml of sperm with 0.7 – 1 ml of SpermFreeze.*

No other additional actions were initiated, based on the cumulative nature and/or occurrence of all complaints, customer/market feedback and vigilance (if any) during the PMCF analysis.

5.4 An overall summary of the clinical performance and safety

Cryopreservation of human semen samples is an essential step in the management of fertility and is routinely used in ART clinics and andrology labs. Despite the success of this technique, cryopreservation of semen samples is associated with damage to the frozen/thawed semen sample, which is inherent to the cryopreservation method. An optimal cryopreservation method for human spermatozoa which would circumstance these advantages is not yet determined. With this in mind, it is important to evaluate the damaging effects after cryopreservation with Sperm Freezing media and to evaluate if these effects are comparable with other cryopreservation media on the market. The main damaging effects after cryopreservation are decreased motility, decreased viability and increased DNA fragmentation.

All these aspects are studied in the clinical evaluation report of the Sperm Freezing media. It could be concluded that Sperm Freezing media function as stated by the manufacturer and that no complications or problems have been reported.

This is established by clinical data which demonstrate that

- ART outcomes (from literature or received from IVF clinic) of procedures in which the Sperm Freezing media are used fall within the normal range of published outcomes as reported by the ESHRE

- The results found in literature are within the acceptable defined outcomes concerning the different sperm parameters (motility, vitality, DNA fragmentation and fertilization potential)
- The results regarding motility and vitality found in literature are not significantly lower than the results from cryopreservation media of competitors

Moreover, there is no evidence from the clinical data, as well as from the registered complains, market/customer feedback and/or vigilance that Sperm Freezing media are toxic for gametes and embryos, nor that the media have risk for mutagenity, oncogenicity, teratogenity, carcinogenity, cytotoxicity, allergenicity and irritancy for patients and users.

5.5 Ongoing or planned post-market clinical follow-up

Post-market clinical follow-up for Sperm Freezing media (including PMCF for the HSA component included in Sperm Freezing media) will be performed at least yearly and will include analyses of real-world evidence by performing a literature search, screening of device registers for clinical data, as well as analysis of all complaints, customer/market feedback, vigilance.

The Summary of Safety and Clinical Performance will be updated with information from the post-market clinical follow-up, if this is needed to ensure that any clinical and/or safety information described in this document remains correct and complete.

6 Possible diagnostic or therapeutic alternatives

Cryopreservation is a necessary tool in ART. Two different methods, i.e. conventional slow-freezing and vitrification of spermatozoa exist. Conventional slow-freezing as performed with Sperm Freezing media is still the most commonly used technique and almost all products currently on the market by competitors are based on this technique.

Sperm vitrification is described in literature but is still under development, and does have some remaining challenges to tackle (for further reading see reviews: (Sharma et al. 2015) and (Li et al. 2019). This is reflected in the fact that no commercial media are currently available on the market for sperm vitrification.

7 Suggested profile and training for users

Sperm Freezing media are used in specialized laboratories performing fertilization techniques, including IVF, ICSI and sperm preparation/analysis. The intended users are IVF professionals (lab technicians, embryologists, or medical doctors).

8 Reference to any applicable common specification(s), harmonized standard(s) or applicable guidance document(s)

The following guidance document was used:

- **MDCG 2019-9**: Summary of safety and clinical performance A guide for manufacturers and notified bodies (August 2019).

The following technical standards apply to Sperm Freezing media:

- **MDR 2017/745**: European Medical Device Regulation 2017/745 of 5 April 2017.
- **(EN) ISO 13485:2016/EN ISO13485:2016/Ac:2018**: Medical devices — Quality management systems — Requirements for regulatory purposes.
- **(EN) ISO 20417:2021**: Medical devices: information supplied by the manufacturer
- **ISO 10993-1:2018 / EN ISO 10993-1:2020**: Biological evaluation of medical devices -- Part 1: Evaluation and testing.

- **(EN) ISO 10993-18:2020**: Biological evaluation of medical devices – Part 18: Chemical characterization of medical device materials within a risk management process
- **ISO 13408-1:2008 (Amd 1:2013)/EN ISO 13408-1:2015**: Aseptic processing of health care products – Part 1: general requirements.
- **(EN) ISO 13408-2:2018**: Aseptic processing of health care products – Part 2: Filtration.
- **ISO 13408-6:2005 (Amd 1:2013)/EN ISO 13408-6:2011**: Aseptic processing of health care products – Part 6: Isolator systems.
- **ISO 14644-1:2015/EN ISO 14644-1:2016**: Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness by particle concentration.
- **(EN) ISO 14644-3:2019**: Cleanrooms and associated controlled environments - Part 3: Test methods.
- **(EN) ISO 14971:2019**: Medical devices – Application of risk management to medical devices.
- **ISO 15223-1: 2021/(EN) ISO 15223-1:2016**: Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied - Part 1: General requirements.
- **(EN) ISO 17665-1:2006**: Sterilization of health care products – Moist heat – Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices.
- **ISO 23640:2011/EN ISO 23640:2015**: In vitro diagnostic medical devices: Evaluation of stability of in vitro diagnostic reagents (Applicable with exclusion of the following sections: No standard is available for the evaluation of stability of Medical Devices, therefore this standard is used as guideline for the set-up of the stability testing)
- **EN 556-2:2015**: Sterilization of medical devices – Requirements for medical devices to be designated 'STERILE' –Requirements for aseptically processed medical devices.
- **IEC 62366-1:2015 (Amd 1:2020)**: Medical devices - Part 1: Application of usability engineering to medical devices.
- **NBOG BPG 2014-3**: Guidance for manufacturers and Notified Bodies on reporting of Design Changes and Changes of the Quality System.
- **EMA/CHMP/578661/2010**: EMA recommendation on the procedural aspects and dossier requirements for the consultation to the EMA by a notified body on an ancillary medicinal substance or an ancillary human blood derivate incorporated in a medical device or active implantable medical device.

9 Summary of the safety and clinical performance of the device intended for patients

A summary of the safety and clinical performance of the device intended for patients, is not applicable as the device is for professional use only.

10 Revision history

SSCP revision number	Date issued	Change description	Revision validated by the Notified Body
A.1	19/12/2019	Initial version	Date: not yet Validation language: English
A.2	06/05/2020	CC191017-01: HSSA testing	Date: not yet Validation language: English
A.3	02/11/2020	Implementation remarks BSI during MDR conformity assessment	Date: not yet Validation language: English
A.4	05/03/2021	Update 2021	Date: not yet Validation language: English

A.5	05/04/2021	Remarks BSI question round 3	Date: not yet Validation language: English
A.6	04/06/2021	Remarks BSI question round 3 on SSCP	Version A.6 is validated by the Notified Body Validation language: English
A.7	03/02/2022	Update 2022	Date: not yet Validation language: English

11 References

Ahmad, L., S. Jalali, S. A. Shami, Z. Akram, S. Batool, and O. Kalsoom. 2010. 'Effects of cryopreservation on sperm DNA integrity in normospermic and four categories of infertile males', *Syst Biol Reprod Med*, 56: 74-83.

Androni, D.A., S. Dodds, M. Tomlinson, and W.E. Maalouf. 2021. 'Is pre-freeze sperm preparation more advantageous than post-freeze?', *Reproduction and Fertility*, 2: 17-25.

Awaga, H. A., S. Lymperi, J. K. Bosdou, A. Makedos, A. Mitsoli, M. G. Bazioti, D. Savvaidou, D. G. Goulis, K. Chatzimeletiou, M. N. Salem, S. R. Ahmed, G. Grimbizis, B. C. Tarlatzis, and E. M. Kolibianakis. 2019. 'Addition of procyanidine to semen preserves progressive sperm motility up to three hours of incubation', *Reprod Biol*, 19: 255-60.

Bandularatne, E., and A. Bongso. 2002. 'Evaluation of human sperm function after repeated freezing and thawing', *J Androl*, 23: 242-9.

Bhattacharya, J. , S. Kundu, C. Varghese, M. Bhattacharya, A. Bhattacharyya, and A. Agarwal. 2006. 'A comparative study on TEST-Yolk buffer and human sperm preservation medium on post thaw characteristics of human sperm from pre-freeze specimens', *Conference paper of ASRM 2006*.

Bizet, P., J. Saias-Magnan, E. Jouve, J. M. Grillo, G. Karsenty, C. Metzler-Guillemain, and J. Perrin. 2012. 'Sperm cryopreservation before cancer treatment: a 15-year monocentric experience', *Reprod Biomed Online*, 24: 321-30.

Boitrelle, F., M. Albert, C. Theillac, F. Ferfouri, M. Bergere, F. Vialard, R. Wainer, M. Bailly, and J. Selva. 2012. 'Cryopreservation of human spermatozoa decreases the number of motile normal spermatozoa, induces nuclear vacuolization and chromatin decondensation', *J Androl*, 33: 1371-8.

Dayal, R., S. Singh, A.K. Thakur, P. Kumar, K. Singh, and P. Purkait. 2021. 'Effect of semen freezing and thawing on sperm survival and motility rate: A comparative analysis ', *International Journal of Scientific Research in Science and Technology*, 8.

Desrosiers, P., C. Legare, P. Leclerc, and R. Sullivan. 2006. 'Membranous and structural damage that occur during cryopreservation of human sperm may be time-related events', *Fertil Steril*, 85: 1744-52.

Di Santo, M., N. Tarozzi, M. Nadalini, and A. Borini. 2012. 'Human Sperm Cryopreservation: Update on Techniques, Effect on DNA Integrity, and Implications for ART', *Adv Urol*, 2012: 854837.

Donnelly, E. T., N. McClure, and S. E. Lewis. 2001. 'Cryopreservation of human semen and prepared sperm: effects on motility parameters and DNA integrity', *Fertil Steril*, 76: 892-900.

Donnelly, E. T., E. K. Steele, N. McClure, and S. E. Lewis. 2001. 'Assessment of DNA integrity and morphology of ejaculated spermatozoa from fertile and infertile men before and after cryopreservation', *Hum Reprod*, 16: 1191-9.

El-Ahwany, A., H. Samir, and H. Alahwany. 2018. 'Using two different thawing temperatures and their effect on the motility recovery of human cryopreserved sperms in cancer patients.', *Middle East Fertility Society Journal*, 23: 331-34.

ESHRE Special Interest Group of Embryology, ESHRE. 2017. 'The Vienna consensus: report of an expert meeting on the development of art laboratory performance indicators', *Hum Reprod Open*, 2017: hox011.

Fabozzi, G., M. F. Starita, E. Rega, A. Alteri, A. Colicchia, C. Piscitelli, and P. Giannini. 2016. 'Evaluation of the Efficiency of Two Different Freezing Media and Two Different Protocols to Preserve Human Spermatozoa from Cryoinjury', *Int J Reprod Med*, 2016: 6059757.

Falah, K. M. 2019. 'Intracytoplasmic sperm injection with fresh versus cryopreserved testicular sperm in azoospermic patients ', *Middle East Fertility Society Journal*, 24.

Freour, T., S. Mirallie, M. Jean, and P. Barriere. 2012. 'Sperm banking and assisted reproductive outcome in men with cancer: a 10 years' experience', *Int J Clin Oncol*, 17: 598-603.

Gatimel, N., R. Leandri, and J. Parinaud. 2013. 'Sperm vacuoles are not modified by freezing--thawing procedures', *Reprod Biomed Online*, 26: 240-6.

Hezavehei, M., M. Mirzaei, M. Sharafi, Y. Wu, V. Gupta, M. Fitzhenry, H. M. Kouchesfahani, P. Eftekhari-Yazdi, H. Baharvand, A. Dalman, P. A. Haynes, A. Shahverdi, and G. H. Salekdeh. 2022. 'Proteomics study reveals the molecular mechanisms underlying cryotolerance induced by mild sublethal stress in human sperm', *Cell Tissue Res*, 387: 143-57.

Hezavehei, M., M. Sharafi, R. Fathi, A. Shahverdi, and M. A. S. Gilani. 2021. 'Membrane lipid replacement with nano-micelles in human sperm cryopreservation improves post-thaw function and acrosome protein integrity', *Reprod Biomed Online*, 43: 257-68.

Hosseinmardi, M., F. Siadat, M. Sharafi, N. H. Roodbari, and M. Hezavehei. 2021. 'Protective Effect of Cerium Oxide Nanoparticles on Human Sperm Function During Cryopreservation', *Biopreserv Biobank*.

Karacan, M., F. Alwaeely, S. Erkan, Z. Cebi, M. Berberoglugil, M. Batukan, M. Ulug, A. Arvas, and T. Camlibel. 2013. 'Outcome of intracytoplasmic sperm injection cycles with fresh testicular spermatozoa obtained on the day of or the day before oocyte collection and with cryopreserved testicular sperm in patients with azoospermia', *Fertil Steril*, 100: 975-80.

Karthikeyan, M., D. Arakkal, A. M. Mangalaraj, and M. S. Kamath. 2019. 'Comparison of Conventional Slow Freeze versus Permeable Cryoprotectant-Free Vitrification of Abnormal Semen Sample: A Randomized Controlled Trial', *J Hum Reprod Sci*, 12: 150-55.

Konc, J., K. Kanyo, and S. Cseh. 2008. 'The effect of condition/state of testicular spermatozoa injected to the outcome of TESE-ICSI-ET cycles', *Eur J Obstet Gynecol Reprod Biol*, 141: 39-43.

Kumari, S., S. R. Salian, A. Rao, S. M. Somagond, R. R. Kamble, A. Nesaragi, J. Das, G. K. Rajanikant, S. Mutalik, S. V. Raghu, S. K. Adiga, and G. Kalthur. 2021. 'Quinoline Derivative Enhances Human Sperm Motility and Improves the Functional Competence', *Reprod Sci*, 28: 1316-32.

Li, Y. X., L. Zhou, M. Q. Lv, P. Ge, Y. C. Liu, and D. X. Zhou. 2019. 'Vitrification and conventional freezing methods in sperm cryopreservation: A systematic review and meta-analysis', *Eur J Obstet Gynecol Reprod Biol*, 233: 84-92.

Lierman, S., A. Tolpe, I. De Croo, S. De Gheselle, J. Defreyne, M. Baetens, A. Dheedene, R. Colman, B. Menten, G. T'Sjoen, P. De Sutter, and K. Tilleman. 2021. 'Low feasibility of in vitro matured oocytes originating from cumulus complexes found during ovarian tissue preparation at the moment of gender confirmation surgery and during testosterone treatment for fertility preservation in transgender men', *Fertil Steril*, 116: 1068-76.

Lusignan, M. F., X. Li, B. Herrero, G. Delbes, and P. T. K. Chan. 2018. 'Effects of different cryopreservation methods on DNA integrity and sperm chromatin quality in men', *Andrology*, 6: 829-35.

Mahadevan, M., and A. O. Trounson. 1983. 'Effect of cryoprotective media and dilution methods on the preservation of human spermatozoa', *Andrologia*, 15: 355-66.

Menon, S., N. Rives, N. Mousset-Simeon, L. Sibert, J. P. Vannier, S. Mazurier, L. Masse, V. Duchesne, and B. Mace. 2009. 'Fertility preservation in adolescent males: experience over 22 years at Rouen University Hospital', *Hum Reprod*, 24: 37-44.

Montagut, M., N. Gatimel, S. Bourdet-Loubere, M. Daudin, L. Bujan, R. Mieusset, F. Isus, J. Parinaud, and R. Leandri. 2015. 'Sperm freezing to address the risk of azoospermia on the day of ICSI', *Hum Reprod*, 30: 2486-92.

Moubasher, A. E., A. M. El Din, M. E. Ali, W. T. El-sherif, and H. D. Gaber. 2013. 'Catalase improves motility, vitality and DNA integrity of cryopreserved human spermatozoa', *Andrologia*, 45: 135-9.

O'Connell, M., N. McClure, and S. E. Lewis. 2002. 'The effects of cryopreservation on sperm morphology, motility and mitochondrial function', *Hum Reprod*, 17: 704-9.

O'Neill, H. C., M. Nikoloska, H. Ho, A. Doshi, and W. Maalouf. 2019. 'Improved cryopreservation of spermatozoa using vitrification: comparison of cryoprotectants and a novel device for long-term storage', *J Assist Reprod Genet*, 36: 1713-20.

Philippon, M., G. Karsenty, B. Bernuz, B. Courbiere, T. Brue, J. Saias-Magnan, and J. Perrin. 2015. 'Successful pregnancies and healthy live births using frozen-thawed sperm retrieved by a new modified Hotchkiss procedure in males with retrograde ejaculation: first case series', *Basic Clin Androl*, 25: 5.

Prisant, N., R. Tubiana, G. Lefebvre, P. Lebray, A. G. Marcelin, V. Thibault, O. Rosenblum, M. Bonmarchand, D. Vauthier-Brouzes, J. L. Golmard, C. Katlama, and C. Poirot. 2010. 'HIV-1 or hepatitis C chronic infection in serodiscordant infertile couples has no impact on infertility treatment outcome', *Fertil Steril*, 93: 1020-3.

Punyatanasakchai, P., A. Sophonsritsuk, S. Weerakiet, S. Wansumrit, and D. Chompurat. 2008. 'Comparison of cryopreserved human sperm in vapor and liquid phases of liquid nitrogen: effect on motility parameters, morphology, and sperm function', *Fertil Steril*, 90: 1978-82.

Rahana, A. R., S. P. Ng, C. F. Leong, and M. D. Rahimah. 2011. 'Comparison between mechanical freezer and conventional freezing using liquid nitrogen in normozoospermia', *Singapore Med J*, 52: 734-7.

Reignier, A., J. Lammers, C. Splingart, D. Redhead, J. J. Labat, S. Mirallie, P. Barriere, and T. Freour. 2018. 'Sperm cryopreservation and assisted reproductive technology outcome in patients with spinal cord injury', *Andrologia*, 50.

Santonastaso, M., F. Mottola, C. Iovine, N. Colacurci, and L. Rocco. 2021. 'Protective Effects of Curcumin on the Outcome of Cryopreservation in Human Sperm', *Reprod Sci*, 28: 2895-905.

Saritha, K. R., and A. Bongso. 2001. 'Comparative evaluation of fresh and washed human sperm cryopreserved in vapor and liquid phases of liquid nitrogen', *J Androl*, 22: 857-62.

Satirapod, C., C. Treetampinich, S. Weerakiet, A. Wongkularb, S. Rattanasiri, and W. Choktanasiri. 2012. 'Comparison of cryopreserved human sperm from solid surface vitrification and standard vapor freezing method: on motility, morphology, vitality and DNA integrity', *Andrologia*, 44 Suppl 1: 786-90.

Seifi, S., A. Shahverdi, T. Rezaei Topraggaleh, M. Sharafi, V. Esmaeili, H. Choobineh, M. J. Zamiri, M. Habibi, and A. Alizadeh. 2020. 'Inclusion of ovine enriched serum with vitamin E and polyunsaturated fatty acids in the freezing medium: a new strategy to improve human frozen-thawed sperm parameters', *Andrologia*, 52: e13541.

Sharma, R., A. J. Kattoor, J. Ghulmiyyah, and A. Agarwal. 2015. 'Effect of sperm storage and selection techniques on sperm parameters', *Syst Biol Reprod Med*, 61: 1-12.

Taher-Mofrad, S. M. J., T. R. Topraggaleh, N. Ziarati, M. N. Bucak, M. Nouri, S. Seifi, V. Esmaeili, P. Rahimizadeh, and A. Shahverdi. 2020. 'Knockout serum replacement is an efficient serum substitute for cryopreservation of human spermatozoa', *Cryobiology*, 92: 208-14.

Thomson, L. K., S. D. Fleming, L. Schulke, K. Barone, J. A. Zieschang, and A. M. Clark. 2009. 'The DNA integrity of cryopreserved spermatozoa separated for use in assisted reproductive technology is unaffected by the type of cryoprotectant used but is related to the DNA integrity of the fresh separated preparation', *Fertil Steril*, 92: 991-1001.

Tongdee, P., M. Sukprasert, C. Satirapod, A. Wongkularb, and W. Choktanasiri. 2015. 'Comparison of Cryopreserved Human Sperm between Ultra Rapid Freezing and Slow Programmable Freezing: Effect on Motility, Morphology and DNA Integrity', *J Med Assoc Thai*, 98 Suppl 4: S33-42.

Tvrda, E., J. Gosalvez, F. Arroyo, P. Sanchez, R. de Jesus Risco Delgado, and R. Sanchez. 2020. 'Dynamic assessment of human sperm DNA damage III: the effect of sperm freezing techniques', *Cell Tissue Bank*.

———. 2021. 'Dynamic assessment of human sperm DNA damage III: the effect of sperm freezing techniques', *Cell Tissue Bank*, 22: 379-87.

Valipour, J., M. S. Nashtaei, Z. Khosravizadeh, F. Mahdavinezhad, S. Nekoonam, S. Esfandyari, and F. Amidi. 2020. 'Effect of sulforaphane on apoptosis, reactive oxygen species and lipids peroxidation of human sperm during cryopreservation', *Cryobiology*.

———. 2021. 'Effect of sulforaphane on apoptosis, reactive oxygen species and lipids peroxidation of human sperm during cryopreservation', *Cryobiology*, 99: 122-30.

Vutyavanich, T., W. Piromlertamorn, and S. Nunta. 2010. 'Rapid freezing versus slow programmable freezing of human spermatozoa', *Fertil Steril*, 93: 1921-8.

Wyns, C., C. De Geyter, C. Calhaz-Jorge, M. S. Kupka, T. Motrenko, J. Smeenk, C. Bergh, A. Tandler-Schneider, I. A. Rugescu, S. Vidakovic, and V. Goossens. 2021. 'ART in Europe,

2017: results generated from European registries by ESHRE', *Hum Reprod Open*, 2021: hoab026.

Zribi, N., N. F. Chakroun, F. Ben Abdallah, H. Elleuch, A. Sellami, J. Gargouri, T. Rebai, F. Fakhfakh, and L. A. Keskes. 2012. 'Effect of freezing-thawing process and quercetin on human sperm survival and DNA integrity', *Cryobiology*, 65: 326-31.

Zribi, N., N. Feki Chakroun, H. El Euch, J. Gargouri, A. Bahloul, and L. Ammar Keskes. 2010. 'Effects of cryopreservation on human sperm deoxyribonucleic acid integrity', *Fertil Steril*, 93: 159-66.