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PERSPECTIVE

RECENT ADVANCES IN SPERM CRYOBIOLOGY: NANOMATERIAL - ASSISTED PRESERVATION, AI-BASED QUALITY ASSESSMENT AND CELLULAR PROTECTION STRATEGIES

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Abstract

Modern biological and medical technologies require low-temperature storage of cells, tissues, and tissue-engineered constructs to facilitate their transfer and to prolong their usability prior to application. Many industries, including biological research, medicine, and agriculture, depends on low-temperature preservation to preserve the integrity and viability of cells, tissues, and organs for long periods of time. Nanotechnology has had an influence on low-temperature preservation in recent years, providing advanced solutions that greatly enhance biological sample storage. Development of metal oxide and inorganic metal nanoparticles (MNPs) has drawn interest in several biotechnological and medicinal domains, including cryopreservation. The integration of MNPs into reproductive biology protocols represents a novel and emerging research area. Although AI has long been used in medicine, the recent rise of deep learning is a major factor in the growth of this field. Sperm morphology categorization has been automated using deep learning frameworks that focused on different sperm cell components. ROS generation is significantly elevated during sperm cryopreservation. Low, medium, and high ROS levels in cells cause apoptosis, auto-phagocytosis, and necrosis, respectively. Antioxidants can be added to semen extenders to reduce the elevated ROS levels during cryopreservation.

Keywords: antioxidants; artificial intelligence; automated cryopreservation; deep learning; metal oxide nanoparticles; nanomaterials.

INTRODUCTION

Mammalian sperm cryopreservation is a complex process that is suitable for exploration through artificial intelligence, as it depends on multiple factors to maintain high-quality semen, including the type of cryoprotectants or extenders used and the rates at which the sperm cells cooled and thawed (1). The need for in vitro preservation of human spermatozoa is rising. However, due to various environmental stressors, human spermatozoa kept in vitro eventually degrade irreversibly, usually around an hour after ejaculation (2). Fresh semen samples are therefore required for most semen exams in the andrological laboratory. When they have to be preserved for prolonged periods, ranging from a few hours to years, then protective measures must be taken to prevent spermatozoa from prematurely aging, particularly in oligoasthenzoospermic or cancer patients. Sperm cryopreservation is by far the most used approach for this purpose. Human semen cryopreservation is essential for medical and research applications (3). However, difficulties arise throughout the cryopreservation process, including the control of

ice inhibition during freezing, rapid heating during rewarming, and loading and unloading of cryoprotective agents (CPAs).

Long-term cryostorage of spermatozoa is required for assisted reproductive technology (ART), in vitro culture of germline cells, reproductive biology and biomedical research (4). Analogous to the principles and methodologies of sperm cryopreservation, certain parts or all the seminal plasma is replaced with a formulated medium known as an extender, that contains protective ingredients to help the sperm survive and function for longer in vitro. The use of nanomaterials in cryopreservation has surfaced as an alternative to tackle these issues at every stage due to their unique properties (5).

Genetics, health, diet, season, stress, and semen cryopreservation are some of the factors that influence and lower semen quality and fertilizing potential. Recent advances in nanotechnology support innovative developments in sperm manipulation. Biological materials may be stored at low temperatures using a range of methods, such as vitrification, gradual freezing, hypothermia, and freeze-drying preservation (6). However, the benefits and limitations of each approach dictate its own range of applications. Thus, there is no common procedure for biobanking human semen (7). Optimizing low-temperature preservation is crucial to obtaining the highest possible yield and uniformity of sperm function. Furthermore, unlike the cryopreservation procedure for human sperm, current research on nanoparticle storage is restricted, and progress in this sector has been lagging. Artificial Intelligence (AI) is mostly applied to sperm analysis via the use of computer vision and deep learning algorithms on pictures taken using digital video systems or traditional microscopes (8). When AI is programmed with thousands or millions of expert-annotated samples, it may mimic and, in many situations, outperform human diagnostic skills in sperm evaluations.

PRINCIPLES OF SPERM CRYOPRESERVATION

Sperm cryopreservation is a reproductive biotechnology, allowing the long-term storage of genetic material and supporting ART. Despite its pervasive application, the procedure is associated with significant limitations due to the cryo-induced cellular injury that occurs during cooling and thawing (9). This mostly affects sperm nuclear DNA, motility, and plasma membrane, which reduces the ability to fertilize. Sperm cell cryopreservation, including by vitrification, is not a safe procedure, with cryoinjury know to impair many sperm parameters. For example, cryopreservation can cause structural disruption and metabolic dysfunction in spermatozoa, reducing not only fertilization competence but also affecting embryo development. Potential molecular and cellular alterations affecting sperm RNAs and proteins can be identified by combining recent developments in omics with bioinformatic techniques (10). The existing cryopreservation protocols may be improved by identifying additional potential indicators of sperm resistance to freezing and thawing.

Mechanisms of cryoinjury and ice formation

Maintaining sperm cell membrane function is crucial to cell survival throughout the cryo processes, particularly during the temperature drop and thawing to restore to normal temperature (11). Cryopreservation efficiency is strongly influenced by the freezing rate, because rapid cooling from 37 °C to 0 °C generates thermal shock. The transition of membrane phospholipids from fluid to gel phases is facilitate by an initial drop in temperature. The subsequent temperature reduction below 0 °C stimulates intracellular ice crystal production (Fig. 1). As ice crystal formation and growth physically damage cellular organelles and membranes, it is essential to promote sufficient cellular dehydration to minimize intracellular water content during cryopreservation. Thus, supra-optimal freezing rates stimulate ice crystal formation due to inadequate cytoplasmic dehydration. In contrast, excessively slow (sub-optimal) freezing rates are associated with osmotic injury resulting from elevated solute concentrations in the extracellular medium (12). Therefore, osmotic rupture by extra- or intra-cellular ice production can occur. Additionally, the cellular physicochemical and biophysical responses are influenced by the cooling velocities, which change survivability. Also, cryopreservation modifies the metabolism and mitochondrial bioenergetic activities of the cell (13). Moreover, there have been reports of phosphorylation and protein breakdown during thawing. The advent of proteomics and transcriptomic technologies could provide insights into cryoinjury processes.

Conventional cryoprotectants and limitations

Both permeable and non-permeable CPAs is are used for sperm freezing. Through their lipophilic characteristics, permeable CPAs may readily pass through the sperm cell membrane, which is linked to higher cell toxicity. Such hypertonic conditions during the freezing process can trigger osmotic shock in mammalian sperm. It is recognized that regulating the osmolarity during cryopreservation is of tremendous significance (14). The osmolarity, which usually varies between 600 and 1000 mOsm/L, is increased when permeable cryoprotectants are used in the cryopreservation medium. Current research is increasingly focused on permeable-cryoprotectant-free vitrification as an approach to preserve sperm potential, including acrosome reaction, capacitation ability, and the structural stability of both mitochondrial and cytoplasmic membranes.

Cooling rates and the choice of CPAs for use in the cryopreservation procedure are the main factors that affect optimal sperm survival rates (15). Non-permeable CPAs are useful for accelerating cellular dehydration because of their large molecular weight and inability to pass across membranes. Consequently, fastger cooling rates might be used. In contrast, permeable CPAs are used in conventional slow freezing, which is still the most widely used method for freezing sperm. Although a significant amount of sperm ejaculate are preserved with slow freezing, about half of the spermatozoa are lost due to cell lysis, caused by the production of ice crystals. After thawing, this lowers the quality of the semen. Determining the optimal cooling kinetics is essential, as improper rates can severely impact the morpho-functional viability of sperm (16). There are particular, additional risks to sperm quality during the administration and elution of CPAs. Therefore, many steps in the cryopreseration procedure can trigger various forms of cellular damage, including thermal stress, osmotic imbalance, dehydration, and the nucleation of ice crystals both inside and outside the cell membrane.

EMERGING NANOMATERIALS IN SPERM CRYOPRESERVATION

The degree of intracellular ice crystallization that takes place during the freezing process is the primary determinant of cell survival after cryopreservation. Since this procedure causes toxicity and cellular damage, ideal cryopreservation extenders like permeable additive agents, have been developed (17). Nevertheless, these substances lead to lipid peroxidation (LPO), which negatively impacts sperm as they thaw.

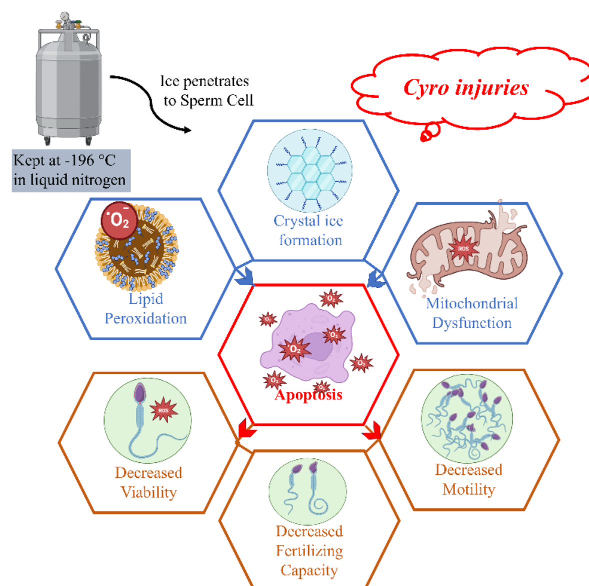

Figure 1. Sperm cell cryoinjury and ice-induced damage. This image shows how sperm cells experience oxidative stress, ice crystal formation, and mitochondrial malfunction as a result of cooliIng to and rewarming from –196 °C. These cryoinjuries cause apoptosis, which eventually lowers the viability, motility, and fertilizing potential of sperm.

Sperm membranes and cellular organelles suffer severe physicochemical damage as a consequence of elevated LPO. When cryopreserving reproductive cells, Nanoparticles (NPs), i.e., molecules having a diameter of less than 100 nm, can be helpful. Without interfering with regular biological processes, NPs have been shown to improve the integration of diverse variables in cellular processes and physiological activities (Fig. 2). A recent study reported that, human acrosome protein integrity and cryopreserved sperm quality were enhanced by nano-micelles (18). Metalloid, metallic, and/or organic components are often the basis for nanoparticles, which may be tailored to suit the intended use. High specificity, cellular uptake, intracellular release, and the incorporation of biocompatibility, biodegradability, and cost-effectiveness are all considered during the nanoparticle design process.

Metal and metal oxide nanoparticles

Core materials such as metals and metal oxides, have been used to generate nanometric colloidal particles. An unique combination of magnetic, thermal, and optical behaviours are found in inorganic MNPs and this makes them preferable to organic NPs and more usable in veterinary and biomedical fields (19). In semen biology, the co-precipitation method is widely used to produce colloidal metal oxide NPs, producing homogenous NPs.

The quality of the semen may decline throughout the post-thaw process if fresh ejaculate samples include damaged or poor quality spermatozoa. As a result, a number of methods have been used to carefully select and gather viable spermatozoa by removing damaged spermatozoa from insemination dosages without intrusive procedures prior to cryopreservation. In comparison to conventional methods, MNPs can improve the selection of sperm cells by rejecting dead or damaged cells within an ejaculate (20, 21).

Biocompatibility, bioconjugation, and magnetism of magnetic Fe3O4 NPs make them an option for sperm purification. Fe_3O_4 nanoparticles can be functionalized with PNA or PSA, which binds to specific sugars on the sperm surface, or with anti-ubiquitin antibodies (22). This allows for the targeted removal of dead or acrosome-reacted spermatozoa. The nano-purification method is comparable to the magnetic-activated cell sorting (MACS) technique, a recognized tool in assisted reproductive technologies for treating male infertility. For instance, spermatozoa for improved fertility have been separated using this nano-purification technique.

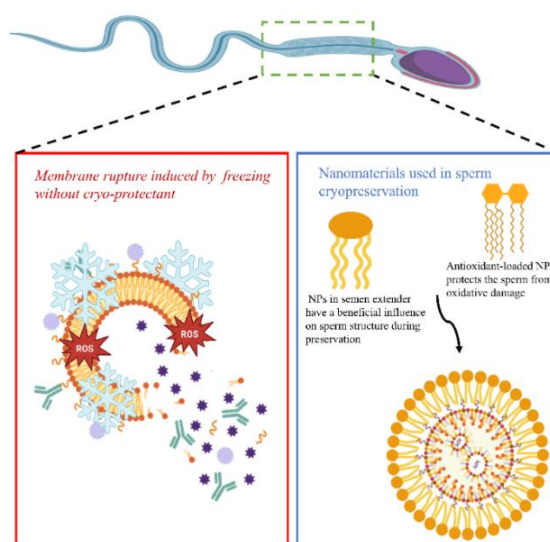

Figure 2. Comparing nanomaterial protection with cryopreservation damage in sperm membranes. It illustrates how freezing sperm cells without cryoprotectants causes membrane rupture and oxidative damage. On the other hand, during cryopreservation, antioxidant-loaded nanomaterials protect the lipid membrane and lessen damage caused by ROS.

The antioxidant potential of MNPs might significantly prevent the peroxidative injury to the sperm plasma membrane (23). Multiple in vitro studies highlighted the ZnO nanoparticles in sperm preservation. Their application is associated with better chromatin stability and improved overall sperm quality, antioxidant activity in seminal plasma, cell viability, acrosome function, and plasma membrane integrity (Table 1). One action of ZnO NP on sperm is to provide a protective coating around the spermatozoa, thereby limiting lipid peroxidation at the plasma membrane (24). Metal nanoparticles (MNPs) have shown considerable potential in the livestock industry, including cryopreservation. However, MNP-induced reproductive toxicity affects sexual functions in adult men, such as low sperm parameters and testicular injury. Therefore, further study is needed to determine the proper dose and functionality of MNPs to ensure repro-safe uses.

Table 1. Applications of metal and metal oxide nanoparticles in sperm cryopreservation across species.

Nanomaterial & characteristics	Species	Dosage	Experimental context	Major effects on sperm & reproductive physiology	Ref
Fe_3O_4 NPs (coated with lectins or annexin V)	Pigs	0, 87.5, 175 μg/mL	Semen purification	Improved motility and viability; structural and mitochondrial integrity maintained	(25)
Fe_3O_4 NPs (coated with avidin + DNA aptamers)	Bulls	NA	Semen purification	Quality of both unsorted and sex-sorted sperm cells were increased.	(26)
Fe_3O_4 NPs (coated with lectin or ubiquitin antibodies)	Bulls	0.1 mg/mL	Semen purification	Reduced semen volume required for effective artificial insemination (AI).	(27)
Se NPs (1%) + Vitamin E	Rooster	5 μg/mL	Cryopreservation	Improved total motility (TM), progressive motility (PM), viability, membrane integrity; reduced LPO/MDA.	(28)
Se NPs	Bull	1.0 μg/mL	Cryopreservation	Better post-thaw motility and structure; decreased apoptosis	(29)
Se NPs (Cysteamine coated)	Sanjabi ram	1 μg/mL	Cryopreservation	Improved motility, viability, Plasma Membrane Integrity (PMI), SOD; reduced MDA and abnormalities.	(30)
SeO NPs (~47 nm)	Holstein bull	2 μg/mL	Cryopreservation	Increased motility, viability, mitochondrial activity, PMI, acrosome integrity (ACI).	(31)
Se NPs	Bucks	0.3 mg/kg	Cryopreservation	Reduced sperm abnormality rate and mitochondrial abnormalities; enhanced testis Se content.	(32)
Se NPs	Ram	0.5 and 1 μg/mL	Storage (4°C)	Improved motility, viability index, acrosome protection, and membrane integrity.	(33)
CeO_2 NPs	Sarda ram	220 μg/mL	Storage (4°C)	Resulted in higher TM, PM while preserving membrane integrity.	(34)

Table 1 (continued). Applications of metal and metal oxide nanoparticles in sperm cryopreservation across species.

Nanomaterial & characteristics	Species	Dosage	Experimental context	Major effects on sperm & reproductive physiology	Ref
CeO_2 NPs (<30 nm)	Human	0.1 mg/mL	Cryopreservation	Samples had superior membrane integrity, viability, and motility parameters; reduced DNA damage and MDA.	(35)
CeO_2 NPs (~17 nm)	Buck (*Capra hircus*)	50 µg/mL	Cryopreservation	Increased SOD, CAT, GSH, TM, PM, PMI; decreased MDA and ROS.	(36)
ZnO NPs (30–50 nm)	Human	100 µg/mL	Cryopreservation	Reduced DNA damage and MDA.	(37)
ZnO NPs (~25 nm)	Albino Wistar rat	1 µg/mL	Cryopreservation	Plasma membrane and acrosomal integrity preserved	(38)
ZnO NPs	Goat	1 µg/mL	Cryopreservation	Optimized epididymal sperm and plasma quality	(39)
ZnO NPs	Buffalo	50 µg/mL	Cryopreservation	Improved sperm membrane integrity.	(40)
Zn NPs (10–30 nm)	Turkey	25 µmol/L	Storage (4°C)	Improved sperm motility, viability, membrane integrity, NO levels.	(41)
Mn NPs (40–60 nm)	Turkey	25 µmol/L	Storage (4°C)	Upregulated mitochondrial membrane potential and SOD activity, while improving PMI and NO levels.	(42)
Au NPs (3–15 nm)	Buffalo bull	10 µg/mL	Cryopreservation	Reduced apoptosis, MDA, H_2O_2, NO.	(43)
Au-Ag-AFT NPs	Human	100 U/mL	Cryopreservation	Improved TM, PM, viability; reduced apoptosis, superoxide ($O_2^{\cdot-}$), H_2O_2.	(44)

Carbon-based nanostructures (graphene, CNTs)

The ultrahigh thermal conductivity of graphene and carbon nanotubes (CNTs) promotes consistent heat transfer during freezing and thawing. There are fewer intracellular ice crystals as a result of a reduction in temperature gradients that often contribute to ice crystal formation. For instance, in cryomedia graphene oxide (GO) nanosheets function as a nano-heater, allowing regulated cooling rates and avoiding localized freezing damage. The surface oxygen groups of graphene oxide could form hydrogen bonds with proteins and phospholipids in sperm membranes, assisting in the preservation of structural integrity at low temperatures (45). When a CNT is functionalized with biocompatible coatings (such as PEG or chitosan), it forms a protective nano-layer around the sperm and reduces the level of osmotic shock and cryo-induced apoptosis. In the meantime, a substantial amount of research indicates that there are numerous intracellular mechanisms for the toxicity of nanostructured materials (NSMs) during in vitro studies. These mechanisms include the production of ROS, DNA damage, and cytokine induction through oxidative stress, endoplasmic reticulum stress, and subsequent mitochondrial impairment in different tissues. Therefore, it has been well established that cytotoxicity, inflammation, and increased oxidative stress due to ROS production are important considerations for the safety of NPs, or NSMs (46). For instance, the cytotoxicity of nanohydroxyapatite (NHAPs) may be partially due to their generation of cellular oxidative stress via ROS and free radicals. Nonetheless, a body of research has shown that the diverse sizes and shapes of nanoparticles—such as NHAPs, CNTs,

graphene—play a significant role in their interactions with a variety of biomolecules, such as DNA, cytochrome, mitochondria, and haemoglobin. Studies have also shown that cytotoxicity of graphene is highly dependent on conditions of different trials, including the duration of exposure (acute or chronic), dosage, cell type, size, administration routes, and methods of monitoring cell viability (47). These factors can affect cell viability and morphology, membrane integrity, ROS biosynthesis, gene and protein expression, DNA damage. Higher concentrations of graphene ($X > 60$ ug/mL) have been shown to dose-dependently reduce cell viability and superoxide dismutase (SOD) levels, as well as increase TBARS content and ROS production, which in turn caused membrane damage and LDH leakage in a variety of cell lines.

ROS scavenging and antioxidant activity

Oxidative damage, a process that is necessary for normal physiological function contributes to poor post-thaw semen quality following sperm cryopreservation. It leads to harmful changes in the spermatozoon, particularly affecting the plasma membrane and the nucleus (48). Numerous efforts have been made in the past to lessen oxidative damage in semen, for example, neutralizing ROS using plant-based antioxidants or reductants that are enzymatic or non-enzymatic. It has shown that the metallic oxides of NPs have antioxidant qualities that may make them more efficient in preventing oxidative stress-induced spermatozoa damage. Zinc oxide nanoparticle (ZnO NP) supplementation resulted in elevated Cu–Zn SOD expression, seminal plasma anti-oxidase activity, and semen quality in ram epididymal spermatozoa (49). When ZnO NPs were added to normozoospermic semen in the cryopreservation medium, it resulted in reduced cold shock-related sperm injury and sperm function was not affected. ZnO NPs were thought to have a protective impact on the chromatin of spermatozoa because they prevented destabilization of the lipid matrix and formed a protective coating on the spermatozoa. In a different human study, adding CeO_2 NPs to semen at a concentration of 0.1 μg/mL enhanced DNA integrity, membrane functioning, progressive and total motility, and viability (50).

Semen can be nano-purified to separate healthy, undamaged spermatozoa from moribund cells and also for spermatozoa with surface modifications. Magnetic-activated cell sorting (MACS), which separates living and dead spermatozoa, is currently the most efficient method for nano-purification of semen. Ferric oxide nanoparticles (Fe_3O_4 NPs/IONPs) have been used to separate dead spermatozoa with damaged membranes based on their magnetic, biocompatible, and bio-functionalization properties (51). Meanwhile, iron oxide nanoparticles (IONPs) bind to antiubiquitin antibodies or other phytolectins interact selectively with specific carbohydrate residues (glycans) on the sperm surface.

Reprotoxicity and biocompatibility considerations

Toxicological and biocompatibility of metal-based NPs, such as gold (GNPs), silver (SNPs), iron oxide (IONPs), cerium oxide (CeO_2 NPs), zinc oxide (ZnO NPs), and titanium oxide (TiO_2 NPs), have been studied in relation to their effects on spermatozoa. ROS, chromatin fragmentation, acrosomal disruption, and decreased motility have been shown to be induced by GNPs and SNPs (52). However, there are differences in their cellular internalization, indicating variation in sperm membrane permeability. IONPs are regarded as biocompatible and have no effect on sperm motility and acrosomal integrity, except for oxidative stress caused by long-term exposure. CeO_2 NPs exhibit both pro-oxidant and antioxidant action, protecting sperm quality at low concentrations while incurring DNA damage and decreased fertilization potential at higher concentrations. Similarly, ZnO and TiO_2 NPs shows concentration-dependent effects: lower doses enhance sperm preservation and antioxidant capacity, whereas higher concentrations affect motility, reduce viability, and compromise genomic integrity (53). This suggests particle size, surface chemistry, concentration, and exposure length affect the reproductive toxicity of metal-based NPs.

CNT induced toxicity in the reproductive system appeared from oxidative stress and mitochondrial malfunction (54). CNTs may disrupt the operation of blood-testis barrier (BTB), blood-brain barrier (BBB), and hemato-testicular barrier (HTB). CNTs could cross these barriers and directly affect spermatogenesis, neuroendocrine pathways, and reproductive-related organs. Recent evidence suggests that CNTs may attenuate the adverse biological effects associated with endocrine disruptors like

triclosan and phthalates (55). The current effects of CNTs may be due to decreased bioavailability of endocrine disruptors. Therefore, further studies are required to determine the effects of prenatal exposure to single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) on progeny development. According to graphene research using atomic force microscopy to monitor male gamete membrane structure, hydrophilic derivatives of this atom-thick molecule, specifically graphene oxide (0.5, 1, 5, 10, and 50 mg/mL), can alter reproductive-related indices in an in vitro system (56). Higher concentrations (5, 10, and 50 mg/mL) have a toxic effect on boar male gametes, while lower concentrations (0.5 and 1 mg/mL) enhanced the ability of spermatozoa in an in vitro fertilization (IVF) study. Overall, CNTs have improved sperm capacitation across all graphene-exposed cell densities.

AI-DRIVEN ASSESSMENT AND OPTIMIZATION OF SPERM CRYOPRESERVATION

The application of AI into sperm analysis represents an emerging development within this area of research. Sperm motility is a factor that AI-based Computer-Assisted Sperm Analysis (CASA) systems use when assessing male fertility potential (57). In recent years ARTs and CASA have increasingly used AI and machine learning (ML). CASA systems offer greater accuracy and consistency by using advanced ML algorithms to evaluate motility, morphology, and DNA integrity of cells. So, it is effectively replacing standard manual semen evaluation, which is often subject to human error. Additionally, ML models are being trained to identify small genetic abnormalities and to do DNA integrity analysis, that is essential for assessing genetic viability. CASA has greatly benefited from the use of traditional machine learning techniques particularly in sperm motility and morphology analysis (58). Support vector machines (SVM), RFs, and k-Nearest Neighbors (kNN) are examples of conventional techniques that have been successfully used to assess and forecast CASA system results. However, these conventional ML techniques often require considerable feature engineering, a process that can be time-consuming and not always readily accessible.

Deep learning and image-based predictive modelling

In CASA, DL offers superior performance compared to traditional ML due to its capacity to automatically extract complex patterns from datasets (images and videos). Convolutional neural networks (CNNs) and other advanced models are used by DL frameworks to assess and categorize sperm pictures, offering a more consistent and effective method than human evaluation (59). In order to improve the efficacy of these systems, VGG16 models have been modified for sperm morphology classification from massive datasets. A primary strength of integrating big data into CASA is its ability to strengthen predictive modelling. However, despite its potential, big data applications in CASA are still at an early stage of development. The VISEM-Tracking dataset is an important advancement, making it possible to train complex models (60).

Sperm concentration and motility may be predicted using artificial neural networks (ANNs), particularly multilayer perceptron (MLP) networks, based on lifestyle and environmental variables collected via surveys. Other AI approaches in assessing semen quality include SVMs, particle swarm optimization, and fuzzy radial basis function neural networks (61). Apart from machine learning techniques, hybrid methods have also been developed to improve the quality of collected data. For example, a compact, interpretable network termed LSPARCOM was developed by integrating SPARCOM with model-based DL via algorithm unfolding. It allows super-resolution reconstruction from high-emitter-density frames without prior optical system parameters (62). These can achieve less than 10% inaccuracy in morphology measurement and 95.6% accuracy in motility using enhancing multi-sperm tracking and image processing. A DL algorithm is used by the LensHookeVR X12 semen analysis equipment to automatically examine sperm morphology which may offer a consistent and accurate diagnosis of male infertility (63). A high and repeatable success rate in terms of pregnancy and birth rates, the ability to identify high-quality sperm, and a non-invasive and economical technique of sperm selection are all necessary for ART. Similarly, ML can improve the data that doctors have at their disposal to help them choose the optimal spermatozoon. The opportunity to link sperm quality measurements is provided by ML algorithms' capacity to process huge

amounts of data (Table 2). High-resolution pictures of moving spermatozoa may be obtained using an automated magnification switching approach or a high-speed off-axis holographic technology.

Automation and IoT integration for sperm quality control

By using graphics processing units (GPUs) to integrate big data from many modalities, such as sensor and participant data, automation of human semen analysis has paved a new path in ART quality control (81).

Table 2. Reproductive prediction of various AI models in sperm analysis.

Study	AI model	Task/input	Dataset	Performance	Ref
Sukhikh, G. T. (2021)	ANN-MLP	Normal vs pathology (semen)	345 samples	Acc: 100%	(64)
Liu (2022)	Deep NN / YOLOv3-tiny	Sperm head detection	Sperm head	F1: 0.951	(65)
Ghasemian et al. (2022)	CNN	Sperm detection	309 specimens	Acc: 94.65%	(66)
Barnett-Itzhaki et al. (2022)	Ensemble DL	Sperm detection	2,254 images	Acc: 94%	(67)
You et al. (2021)	SVM, CNN, LSTM	Sperm selection	Sperm images	Acc: 88%	(68)
Sato et al., 2022	YOLOv3 (CNN)	Sperm selection	4,625 images	Sen: 88%	(69)
Butola et al. (2020)	Feedforward DNN	Sperm classification (stress)	Head, tail images	Acc: 85.6%	(70)
Cao et al. (2024)	XGBoost, SVM	Clinical pregnancy (surgical sperm)	420 couples	ROC: 0.85	(71)
Gunderson (2021)	Gradient Boosted	IVF (Sperm pH)	76 patients	AUC: 0.81	(72)
Zeadna (2020)	GBT, RF, LogReg	Sperm prediction	119 patients	AUC: 0.807	(73)
Mehrjerd et al. (2024)	Bagging, RF	Sperm quality evaluation	599 couples	ROC: 0.79	(74)
Javadi et al. (2019)	Deep NN	Sperm assessment	Head, acrosome	Acc: 77% (Head)	(75)
Jiang (2023)	LASSO, XGBoost	Seminal plasma/sperm characteristics	19,539 cycles	AUC: 0.75	(76)
Peng (2023)	K-means (Unsupervised)	Sperm DNA damage	1,258 couples	Odds ratio: 0.73	(77)
Ory (2022)	Random Forest	Varicocele repair	240 men	AUC: 0.72	(78)
Hook et al. (2021)	Linear SVM	Sperm ultrastructure	Sperm head	-	(79)
Kandel et al. (2020)	Deep CNN (U-Net)	Reproductive outcomes	Head, tail	-	(80)

Acc (Accuracy) - percentage of correct predictions.
F1 (F1 Score) - balancing precision and recall.
Sen (Sensitivity) - measures true positive rate.
ROC (Receiver Operating Characteristic) and AUC (Area Under the Curve) - model's ability to distinguish between classes (0.5 = random, 1.0 = perfect).
Odds Ratio - strength of association between predictor and outcome.

On the other hand, a manual sperm analysis is an infertility inquiry that relies on trained lab technicians to provide precise and repeatable findings in accordance with WHO standards. To reduce differences in results across and within laboratories, modern IVF labs use computer-aided sperm analyzer systems. Agarwal et al. compared the manual approach with AIOM (automated artificial intelligence optical microscopic technology) (82). They discovered that quantitative measurements of primary sperm parameters were positively correlated with AIOM. Over time, advances in AI have made it possible to standardize the evaluation of sperm morphology. For instance, FERTECH is an image-based operating system that complies with the stringent WHO standards for sperm analysis. When compared to the manual method, it had a 95% sensitivity in identifying any aberration in sperm morphology (83). Abnormal spermatozoa were grouped according to their specific morphological defects using SVM, decision trees, and naïve Bayes algorithms, with achieved prediction accuracies of 49%, 40%, and 44%, respectively, which exceed classical statistical methods (84). Compared to manual analysis, this might provide clinicians with more accurate understanding of the frequency and severity of male infertility. Another research utilizing a two-stage classification algorithm classified sperm using a data set where two out of three experts agreed on the classification, resulting in a prediction accuracy of 58% (85).

The management of cryogenic systems has changed after IoT-based smart sensors. The system ensures optimum nitrogen levels and sample safety with continuous monitoring and real-time alarms. Cryogenic storage conditions (temperature, liquid-nitrogen levels, humidity) continuously monitored by IoT-enabled sensors, allows automatic detection of environmental fluctuations and enables remote oversight. In this approach, typical cryobank procedures may include machine-vision-based AI algorithms to assist standardized pre-freeze assessment to enable consistent assessment of sample quality prior to storage. Facilities that used this technology have been able to increase the interval between refills and had less nitrogen shortages. This results in cost savings, improved operating efficiency with ideal storage conditions. Predictive models might help with precise date of the next refill, hence reducing downtime and maintaining continuous functioning (86). IoT-based sensors have improved both the efficiency and the safety of liquid nitrogen storage systems. With fewer human inspections this technology reduced nitrogen loss by around 25%. The frequency of human inspections was cut by 40% using automated alarms and data tracking, it also lowered labour expenses and staff responsibilities.

CELLULAR AND MOLECULAR PROTECTION MECHANISMS

Oxidative stress refers to a condition in which the generation of oxygen and oxygen-derived reactive species exceeds the capacity of cellular antioxidant defenses, resulting in increased rates of cellular damage. Oxidative stress damage in spermatozoa occurs when uncontrolled ROS generation surpasses antioxidant capacity of seminal plasma. Sperm naturally produce ROS, however an imbalance can harm the sperm and has been linked to male infertility. Sperm cells have a strong antioxidant defence mechanism against ROS assault, protecting cells from free radicals (87). ROS are extremely reactive molecules that are derived from diatomic O_2, H_2O, and H_2O_2. They are essentially oxygen-containing molecules that are inherently unstable and include byproducts of aerobic metabolism like H_2O_2, the hydroxyl radical (OH), and the superoxide anion ($O_2^{\cdot}$−). Freeze-thaw stress induces ROS, which catalyzes the peroxidation of membrane lipids. This results in the formation of several toxic byproducts, including conjugated dienes, malondialdehyde (MDA), and lipid hydroperoxides (4-hydroxynonenal, 4-HNE, and various 2-alkenals), which have adverse effects on the membrane system (88). An error in spermiogenesis that results in the discharge of spermatozoa from the germinal epithelium with abnormally high levels of cytoplasmic retention is often linked to an excess of free radical production. Because of the high concentration of polyunsaturated fatty acids (PUFAs) in their plasma membrane and their poor cytoplasmic antioxidant capacity, spermatozoa are especially vulnerable to oxidative damage via free radicals. PUFAs provide the fluidity required for sperm movement and membrane fusion processes (such as the acrosome reaction and sperm-egg fusion) (89). Membrane PUFA peroxidation kicks off a chain reaction of lipid peroxidation on the sperm surface, impairing sperm-oocyte fusion and

causing permanent loss of motility by disrupting membrane permeability and fluidity.

Oxidative stress and antioxidant defence systems

Antioxidants are essential for counteracting the excess ROS produced during sperm cryopreservation. Vitamin E is a prominent chain-breaking antioxidant because it can directly quench free radicals like peroxyl and alkoxyl (ROO•) produced during ferrous ascorbate-induced LPO (90). Vitamin E addition in extenders may reduce ROS throughout the cryopreservation and thawing procedures of semen (Table 3). Often, around 10 mmol/L is the ideal dose for such preventive supplements. By reducing oxidative stress, Mn_2+ improves sperm response. When Ca_2+ or Mg_2+ ATPase are boosted, calcium channel opening is activated and the extracellular addition of Mn_2+ ions also raises the amount of cAMP, stimulating the acrosome reaction (91).

Table 3. Antioxidant efficacy on post-thaw sperm quality.

Antioxidants	Classification/ solubility	Outcomes	Reference
Vitamin C	Water soluble	Increased viability and progressive motility; reduces ROS and apoptotic cells.	(95)
Vitamin E	Lipid soluble	Protects against oxidative stress; improves motility and decreases DNA fragmentation.	(96)
Zinc (zinc sulphate)	Cofactor	Significantly reduces freeze-thaw DNA damage; preserves mitochondrial integrity and capacitation.	(97)
L-Carnitine	Scavenger	Increases total antioxidant capacity and membrane integrity; improves sperm viability and motility.	(98)
Melatonin	Water and lipid soluble	Increases mitochondrial membrane potential (MMP) and antioxidant enzyme activity; reduces ROS and lipid peroxidation.	(99)
Quercetin	Water and lipid soluble	Reduces DNA fragmentation; improves sperm motility recovery rate after thawing.	(100)
MitoTEMPO	Scavenger	Mitochondria-targeted: Improves sperm vitality, MMP, and antioxidant enzyme activity (SOD, CAT, GPx).	(101)
Iodixanol	Stabilizer	Expressed levels of protamine and BCL2 (anti-apoptotic gene); reduces cell death and MDA levels.	(102)
Catalase	Enzymatic antioxidant	Reduces apoptotic-like changes and DNA damage.	(103)
Hypotaurine	Water soluble	Significantly decreases DNA fragmentation in cryopreserved samples.	(104)
Lactoferrin	Iron chelator	Increases functional plasma membrane integrity; reduces free iron concentration.	(105)
BHT (butylated hydroxytoluene)	Lipid soluble	Improves motility and viability; prevents loss of highly unsaturated fatty acids (HUFAs).	(106)
Trehalose	Osmotic stabilizer	Improves cell viability, proliferation, and colony recovery; reduces lipid peroxidation.	(107)
Glutathione	Water soluble	Increases fertilization rate and motility recovery; decreases mitochondrial ROS and lipid peroxidation.	(108)
Resveratrol	Water and lipid soluble	Decreases DNA damage and MDA levels; increases SOD activity.	(109)

Sperm cells use thiol groups as defence mechanisms to combat oxidative damage. Recent research indicates that adding antioxidants to cryopreservation extenders may improve semen parameters by having a cryoprotective impact on sperm quality. Excess ROS are neutralized and kept from harming cellular structure by enzymatic antioxidants. It was recently shown that adding gamma-tocopherol to cryopreservation medium increased the vitality and motility of human sperm after thawing compared to alpha-tocopherol (92). The pineal gland secretes melatonin, a potent antioxidant. Seminal plasma contain melatonin and melatonin receptors are on the sperm plasma membrane. Adding 0.01 mM melatonin to the freezing medium increases sperm motility and viability while reducing intracellular ROS and membrane malondialdehyde (93). Antioxidant functions may be weakened by selenium deficit. Selenium supplementation raises plasma GSH-Px concentration, having better cellular peroxidase control and increased selenium levels. PRDX6, an important line of defence which may react with H_2O_2 in spermatozoa at low concentrations. During sperm maturation, it modulates redox signalling, and plays a fundamental role in supporting fertility and protecting mammalian spermatozoa from oxidative damage (94). A common process in sperm preservation methods is diluting semen with extenders, which may lower the sample's antioxidant content. Thus, antioxidant supplementation during sperm cryopreservation, even at low concentrations, is a viable method to improve these processes.

Mitochondrial integrity and DNA fragmentation control

Sperm quality is affected by increasing the production of mitochondrial ROS and metabolic disruption of the mitochondrial electron transport chain. Damage to the mitochondrial membrane may potentially affect sperm motility (110). In fact, a number of studies have shown that ROS are the primary cause of DNA damage in sperm freezing and thawing, and that caspase-related pathways have no relation to DNA damage (Figure 3). Cryopreservation increases the number of spermatozoa with single-stranded DNA fragmentation by 10%, but it has no effect on the quantity of double-stranded DNA breaks (111). Additionally, improved sperm motility has been linked to a better mitochondrial state when caffeine was combined with melatonin. Besides the ability to inhibit production of reactive oxygen species (ROS), resveratrol also causes the release of calcium into the cytoplasm of cells and promotes the phosphorylation of sperm AMP-activated protein kinase (AMPK). Specifically, trans-resveratrol improves mitochondrial membrane potential, reduces ROS production, and protects against ROS-mediated membrane lipid peroxidation and DNA damage. Despite its antioxidant qualities against oxidative damage to lipids, resveratrol did not protect sperm motility during cryopreservation (112). When GSH, ascorbate, myoinositol, or the mitochondria-targeted antioxidant MitoTEMPO are supplemented to cryopreservation medium, sperm motility and mitochondrial functionality increased. Additionally, the antioxidant enzymes SOD, CAT, and GSH-peroxidase in spermatozoa are potentiated, which reduces the activation of oxidative stress markers and lowers the degree of DNA fragmentation (113).

More research is required on other novel antioxidants. For instance, several investigations using human spermatozoa have shown that the nucleophilic thiol penicillamine protects against oxidative damage. In normozoospermic donor semen samples, penicillamine has been shown to protect against oxidative damage caused by ionomycin and H_2O_2. Penicillamine was able to lower ROS levels in medium containing ionomycin or H_2O_2, which enhanced the preservation of sperm motility, ATP levels, and mitochondrial membrane potential (114). Plasma membrane integrity, mitochondrial membrane potential, and progressive sperm motility can be improved by glutamine and cysteine, which can also act as strong antioxidants by reducing intracellular ROS and sperm DNA damage.

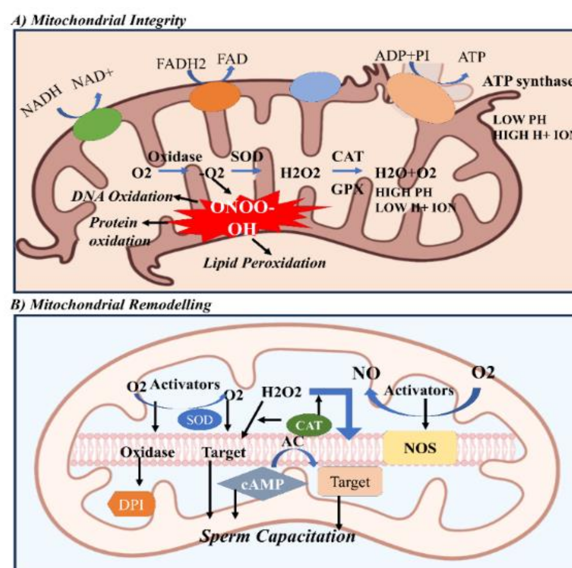

Figure 3. Integrity and remodelling of mitochondria during sperm function. A) Illustration of how high levels of ROS and reactive nitrogen species damage mitochondrial integrity, resulting in oxidation of DNA, proteins, and lipids. B) Mitochondrial remodelling, where sperm capacitation and functional activation are supported by controlled ROS, NO signalling, and cAMP pathways.

Membrane lipid remodelling and protein stabilization

The cryopreservation and thawing of sperm is linked to a drop in antioxidant levels and an increase in ROS. Cell water volume is greatly reduced by both freezing and thawing. During the last stages of development, cells lose cytoplasmic component that contains antioxidants to mitigate ROS and LPO (115). As a result, during cryopreservation and thawing, spermatozoa are highly susceptible to LPO, which puts mechanical stress on the cell membrane. Sperm membrane integrity depends on maintaining the equilibrium of cholesterol. The protein Niemann-Pick C2 (NPC2) has the ability to attach itself to cholesterol molecules. The NPC2 protein has a complicated regulatory function in the integrity of sperm membranes (116). NPC2 may influence membrane fluidity by facilitating cholesterol efflux and its redistribution across membrane domains. But its primary function appears to mediate the balance of systemic cholesterol. Research has shown that the deletion of the NPC2 protein increases the loss of cholesterol from the membranes of mouse sperm (117). Lipid-raft–associated proteins flotillin-1 and flotillin-2 contribute to the maintenance of sperm plasma membrane stability and fluidity by scaffolding specialized cholesterol-rich microdomains (lipid rafts). Maintaining the integrity of membranes requires the calcium-dependent membrane-associated protein annexin V (118). By selectively binding to phosphatidylserine and taking part in the membrane repair process, it stabilizes the membrane structure and stops internal contents from leaking. Coupling annexin V to magnetic beads, apoptotic and dead spermatozoa that display externalized phosphatidylserine can be selectively removed from semen samples. This may enhance the quality of sperm used in assisted reproductive procedures. So its obvious to develop efficient cryomedia and preservation protocols for clinical applications. Meanwhile, antioxidants, like genistein, could be used to shield sperm DNA from oxidative stress throughout cryopreservation (119). Genistein, a phyto-estrogen obtained from soybeans, protects against lipid peroxidation and sperm DNA damage (120). It works by inhibiting ROS-producing enzymes, like xanthine oxidase, preventing lipid peroxidation by scavenging peroxyl radicals, and increasing the activity of antioxidants that are naturally present in spermatozoa, such as SOD, glutathione peroxidase, and glutathione reducase. Various antioxidants, both enzymatic and non-enzymatic, have been shown to support DNA integrity and sperm function. For example, glutathione prevents sperm DNA damage caused by H_2O_2.

CHALLENG ES IN NANOMATERIAL STANDARDIZATION AND AI DATASET DIVERSITY

A varied dataset is required to train precise models for standardized semen analysis. Sonication, which may be done using a cup-type sonicator, an ultrasonic bath, or a probe, is the preferred technique for dispersing NMs. In certain instances, sonication might not be an ideal option for SbD (Safety by Design) hazard testing. For example, sonication may reduce the length of MWCNT, which results in different toxicity profiles than those would not exposed (121). Dissolution acts as a significant factor in the toxicity of certain NMs (for example, release of silver or copper), and reducing the dissolution capacity of NMs may be regarded as an intervention aligned with SbD principles. A model may not reliably predict outcomes for patients of different ethnicities if it is predominantly trained on data from patients of a particular ethnicity (122). Similarly, Vitamin E is particularly helpful in keeping sperm alive but it cannot be blended into Ham's F10 medium to maintain human sperm motility and vitality during liquid storage and thus fails SbD principles (123).

While NPs improve male fertility, it is important to consider the duration of exposure. Existing AI models have limitations and pitfalls. A diverse and representative dataset is required to train precise models for standardized semen analysis. Furthermore, biases present in the data used to train AI algorithms can undermine fairness of IVF treatments (124). Biases can arise from historical biases in medical practice, unequal representation of various populations in training data, or even the methods used to gather and annotate data. Studies employing certain AI-based methods are constrained due to a “black-box” effect, such that how they arrive at their predictions is not clear, which may be a hurdle to clinical adoption. When features are well-defined, these AI models could deliver excellent outcomes, but it is mostly dependent on the ability of the expert selecting the variables and the appropriate data preprocessing.

FUTURE DIRECTIONS

Even with advances in extenders, long-term preservation of spermatozoa always degrades the quality of the sperm and has to be improved. Also, the underlying mechanism of damage sustained during this treatment is yet unknown, which might provide information to enhance the preservation effect. Certain metal NPs, (zinc oxide, silver and selenium) exhibit antioxidative or antibacterial properties that make them effective in extenders. However, it is important to consider the possible hazards associated with these NMs (125). Several in vitro studies confirmed metal oxide NPs harm sperm quality by inducing excessive ROS generation. The survival of human spermatozoa treated with zinc oxide NPs for no more than 3 h was reduced and so NPs in extenders could vary between liquid storage and cryopreservation. Recent studies indicate that extracellular vehicles (EVs), including exosomes, modulate sperm function and enhance sperm preservation. AI technologies may be used in the context of IVF to assess various and complicated data, including time-lapse recordings, clinical records, photos from embryo monitoring, and omics data from embryo culture (126). AI solutions have the ability to improve IVF process and increase the likelihood of child births. In order to provide patient-centered care, GPT-4 might be upgraded with specialized information about reproductive treatments to provide prompt, frequently asked questions. This could improve the patient understanding and lower anxiety. Future research should focus on developing AI models that are trained on large, representative, and varied datasets in order to overcome bias issues (127). AI should be assessed with cross-validation and tested on separate datasets for consistent performance across different conditions. Although AI-assisted IVF may increase success rates, follow-up studies are required to evaluate the health of infants conceived using these techniques.

CONCLUSION

Cryopreservation increases ROS production, which compromises the fertilizing capacity of sperm. They specifically rely on extracellular antioxidant systems found in seminal plasma to counteract oxidative reactions. However, this plasma is isolated and discarded during sperm processing. Antioxidants are effective and have been shown to enhance the quality of post-thawed semen after supplementation. But biological functions of many antioxidants and its mechanisms of action remain unclear. Research on robotic intracytoplasmic sperm injection (ICSI) has progressed considerably, with emerging technologies allowing automation of the procedure and enabling remote ICSI, where trained personnel conduct the micromanipulation process from a distant location. During ICSI, the micromanipulation performed by embryologists, including cytoplasmic suction, may affect the results. In biotechnology and pharmaceutical sectors, IoT-based cryogenic monitoring system have opportunities to improve oversight and efficiency. These industries also rely on accurate cryogenic storage for pharmaceuticals, research materials, and biological samples. The system supports effective compliance with stringent regulatory standards by continuously recording data and enabling remote monitoring of storage conditions. The next generation of biotechniques that may be utilized to enhance animal reproduction and address human reproductive issues can benefit from the precision, practice, and sensitivity that NMs can provide. It is crucial to conduct further in vitro tests to evaluate the efficacy and safety of these novel approaches, that can develop ART and AI in IVF and provide patients and medical professionals cutting-edge resources to manage the next wave of reproductive care. As AI technology advances sperm analysis, cryopreservation, and genetic screening procedures could become more accurate and efficient.

REFERENCES

1. Arif A, Zahoor N, Tang J, Tang M, Dong L, Khan SZ & Dai G (2025) *Vet Sci* **12**, 145. https://doi.org/10.3390/vetsci12020145
2. Kumar N & Singh AK (2022) *Environ Sci Eur* **34**, 6. https://doi.org/10.1186/s12302-021-00585-w
3. Tamburrino L, Traini G, Marcellini A, Vignozzi L, Baldi E & Marchiani S (2023) *Int J Mol Sci* **24**, 4656. https://doi.org/10.3390/ijms24054656
4. Reyes Palomares A & Rodriguez-Wallberg KA (2022) *Front Cell Dev Biol* **10**, 881550. https://doi.org/10.3389/fcell.2022.881550
5. Huang M, Hu M, Cai G, Wei H, Huang S, Zheng E & Wu Z *J* (2025) *Nanobiotechnology* **23**, 187. https://doi.org/10.1186/s12951-025-03265-6
6. Fu Y, Dang W, He X, Xu F & Huang H (2022) *Bioengineering* **9**, 545. https://doi.org/10.3390/bioengineering9100545
7. Björndahl L, Esteves SC, Ferlin A, Jørgensen N & O'Flaherty C (2023) *Andrology* **11**, 1225–31. https://doi.org/10.1111/andr.13504
8. Louis CM, Erwin A, Handayani N, Polim AA, Boediono A & Sini I *J* (2021) *Assist Reprod Genet* **38**, 1627–39. https://doi.org/10.1007/s10815-021-02123-2
9. Whaley David, Damyar Kimia, Witek Rafal P, Mendoza Alan, Alexander Michael & Lakey Jonathan R T (2021) *Cell Transplant* **30**, 0963689721999617. https://doi.org/10.1177/09636897219617
10. Liu K, Zhang Y, Martin C, Ma X & Shen B (2023) *Int J Mol Sci* **24**, 4. https://doi.org/10.3390/ijms24010004
11. Lin M, Cao H, Meng Q, Li J & Jiang P (2022) *Cryobiology* **106**, 13–23. https://doi.org/10.1016/j.cryobiol.2022.04.008

12 Magyar Tábori K, Mendler Drienyovszki N, Hanász A, Zsombik L & Dobránszki J (2021) *Plants* **10**, 670. https://doi.org/10.3390/plants10040670

13 Gualtieri R, Kalthur G, Barbato V, Di Nardo M, Adiga SK & Talevi R (2021) *Antioxidants* **10**, 337. https://doi.org/10.3390/antiox10030337

14 Bai B, Xue C, Wen Y, Lim J, Le Z, Shou Y, Shin S & Tay A (2023) *Adv Funct Mater* **33**, 2303373. https://doi.org/10.1002/adfm.202303373

15 Sharafi M, Borghei-Rad SM, Hezavehei M, Shahverdi A & Benson JD (2022) *Animals* **12**, 3271. https://doi.org/10.3390/ani12233271

16 Garcia-Oliveros LN, Arruda RP de, Batissaco L, Gonzaga VHG, Nogueira VJM, Florez-Rodriguez SA, Almeida F dos S, Alves MBR, Pinto SCC, Nichi M, Losano JD de A, Kawai GKV & Celeghini ECC (2022) *J Therm Biol* **106**, 103237. https://doi.org/10.1016/j.jtherbio.2022.103237
17 Lomba L, García CB, Benito L, Sangüesa E, Santander S & Zuriaga E (2024) *ACS Biomater Sci Eng* **10**, 178–90. https://doi.org/10.1021/acsbiomaterials.3c00859
18 Farrokhi Z, Sharafi M, Hezavehei M, Torabi A, Shahverdi M & Rahimi S (2023) *Biopreserv Biobank* **21**, 593–8. https://doi.org/10.1089/bio.2022.0135
19 Verma C, Verma DK, Berdimurodov E, Barsoum I, Alfantazi A & Hussain CM (2024) *J Mater Sci* **59**, 325–58. https://doi.org/10.1007/s10853-023-08914-5
20 Paul N, Kumaresan A, Talluri TR, Raval K, Elango K, Pradeep Nag BS, Duraisamy R & Manimaran A (2024) *Reprod Domest Anim* **59**, e14733. https://doi.org/10.1111/rda.14733
21 Manimaran K, Rojviroon T, Rojviroon O, Rajendran R, Yuli Yanto DH, Venkatesh G, Segaran G & Niamlang S (2025) *Int J Pharm* **685**, 126232. https://doi.org/10.1016/j.ijpharm.2025.126232
22 Kumar A, Andonissamy J, Selokar NL, Thakur NS, KK K, Aderao GN, Mehta H, KC N, Sarkar S, Naskar S, Sarkar B, Goli RC, Kaith S, Bhadana VP & Rakshit S (2025) *Bionanoscience* **15**, 385. https://doi.org/10.1007/s12668-025-02001-6
23 Samrot A V & Noel Richard Prakash LX (2023) *Life* **13**, 767. https://doi.org/10.3390/life13030767
24 Abedin SN, Baruah A, Baruah KK, Bora A, Dutta DJ, Kadirvel G, Katiyar R & Doley S (2023) *J Trace Elem Med Biol* **80**, 127296. https://doi.org/10.1016/j.jtemb.2023.127296
25 Abedin SN, Deori S, Baruah KK, Baruah A & Kadirvel G *Indian* (2022) *J Hill Farming* **35**, 115–22.
26 Bisla A, Honparkhe M, Srivastava N (2022) *Andrologia* **54**, e14589. https://doi.org/10.1111/and.14589
27 Rasool A, Sakthivel J, Veerappa VG, Abhonkar RM & Misal S *Indian* (2025) *J Anim Reprod* **46**, 15–23. https://doi.org/10.48165/ijar.2025.46.03.2
28 Akar M, Kaya C & Çevik M (2021) *Livest Stud* **61**, 37–45. https://doi.org/10.46897/livestockstudies.610106
29 Li S, Ren J, Zhang W, Wang B, Ma Y, Su L, Dai Y & Liu G (2023) *Front Vet Sci* **10**, 1093274. https://doi.org/10.3389/fvets.2023.1093274
30 Salimi T, Hajarian H, Karamishabankareh H & Soltani L (2024) *Sci Rep* **14**, 852. https://doi.org/10.1038/s41598-023-50221-1
31 Mukherjee S, Togla O & Mukherjee A (2022) *Recent Adv Appl Nanotechnol Livest Prod Manag* 142.
32. Horky P, Urbankova L, Bano I, Kopec T, Nevrkla P, Pribilova M, Baholet D, Chilala P, Slama P & Skalickova S (2023) *Animals* **13**, 2460. https://doi.org/10.3390/ani13152460
33. Piri M, Mahdavi AH, Hajian M, Nasr-Esfahani MH, Soltani L & Vash NT (2024) *Sci Rep* **14**, 24171. https://doi.org/10.1038/s41598-024-75792-5
34. Hosseinmardi M, Siadat F, Sharafi M, Roodbari NH & Hezavehei M (2021) *Biopreserv Biobank* **20**, 24–30. https://doi.org/10.1089/bio.2021.0020
35. Hosseinalipour E, Karimipour M & Ahmadi A (2021) *Int J Reprod Biomed* **19**, 801–10. https://doi.org/10.18502/ijrm.v19i9.9712
36. Khalique MA, Andrabi SMH, Majeed KA, Yousaf MS, Ahmad N, Tahir SK, Fayyaz MH, Haider MS, Naz SS, Qureshi IZ, Sulaiman S, Zaneb H & Rehman H (2024) *Theriogenology* **214**, 166–72. https://doi.org/10.1016/j.theriogenology.2023.10.022
37. Soltani L, Ghaneialvar H & Mahdavi AH (2023) *Nucleosides Nucleotides Nucleic Acids* **42**, 262–79. https://doi.org/10.1080/15257770.2022.2124269
38. Saleh NT, Hosseinzadeh Z, Banadkuki NG, Novin MS, Zaman SS & Gardeshi TM (2023) *Clin Exp Reprod Med* **51**, 20. https://doi.org/10.5653/cerm.2023.06079
39. Bodu M, Hitit M, Greenwood OC, Murray RD & Memili E (2025) *Front Vet Sci* **12**, 1554771. https://doi.org/10.3389/fvets.2025.1554771
40. Fadl A, Abdelnaby E, El-seadawy I, Kotp M, Abo El-Maaty AM & El-Sherbiny H (2022) *J Adv Vet Res* **12**, 259–64.

41. Chakraborty SB (2021) *Proc Zool Soc* **74**, 417–431. https://doi.org/10.1007/s12595-021-00399-x
42. Aschner M, Skalny A V, Lu R, Santamaria A, Paoliello MMB, Tsatsakis A, Kirichuk AA, Li Y-F, Domingo JL & Tinkov AA (2025) *Nanotoxicology* **19**, 413–52. https://doi.org/10.1080/17435390.2025.2511694
43. Khalil WA, El-Rais MS, Hegazy MM, Hassan MAE, El-Raghi AA & El-Moghazy MM (2025) *Biol Trace Elem Res* **203**, 2636–53. https://doi.org/10.1007/s12011-024-04348-5
44. Le G, Ma X & Muhmood A (2025) *Langmuir* **41**, 18369–87. https://doi.org/10.1021/acs.langmuir.5c01641
45. Chen Q, Li L, Zhao J, Zhang Y & Xue X (2023) *Toxicol Vitr* **92**, 105653. https://doi.org/10.1016/j.tiv.2023.105653
46. Min Y, Suminda GG, Heo Y, Kim M, Ghosh M & Son Y-O (2023) *Antioxidants* **12**, 703. https://doi.org/10.3390/antiox12030703
47. Rhazouani A, Gamrani H, El Achaby M, Aziz K, Gebrati L, Uddin MS & AZIZ F (2021) *Biomed Res Int* **2021**, 5518999. https://doi.org/10.1155/2021/5518999
48. Zhang B, Wang Y, Wu C, Qiu S, Chen X, Cai B & Xie H *BMC* (2021) *Vet Res* **17**, 127. https://doi.org/10.1186/s12917-021-02804-1
49. Bakhtiary Z, Eyvazi Y, Finelli R, Baskaran S, Sikka SC & Selvam MKP (2025) *Front Biosci* **30**, 39945. https://doi.org/10.31083/FBL39945
50. Lazareva PI, Stupin VA, Lazarev KA, Litvitskiy PF, Manturova NE & Silina E V (2025) *Pharmaceutics* **17**, 1475. https://doi.org/10.3390/pharmaceutics17111475
51. Mahna A (2025) *J Nanoparticle Res* **27**, 40. https://doi.org/10.1007/s11051-025-06238-w
52. Sysak S, Czarczynska-Goslinska B, Szyk P, Koczorowski T, Mlynarczyk DT, Szczolko W, Lesyk R & Goslinski T (2023) *Nanomaterials* **13**, 1531. https://doi.org/10.3390/nano13091531
53. Xuan L, Ju Z, Skonieczna M, Zhou P-K & Huang R (2023) *MedComm* **4**, e327. https://doi.org/10.1002/mco2.327
54. Prajapati SK, Malaiya A, Kesharwani P, Soni D & Jain A (2022) *Drug Chem Toxicol* **45**, 435–50. https://doi.org/10.1080/01480545.2019.1709492
55. Gacem A, Khan Z, Alreshidi MA, Yadav KK, Muzammil K, Almulla N, Kavitha V, Assad H, Grewal T, Kumar A, Almalawi JF, Dar MA & Wani AK (2025) *Mar Pollut Bull* **221**, 118491. https://doi.org/10.1016/j.marpolbul.2025.118491
56. Singh R, Rawat H, Kumar A, Gandhi Y, Kumar V, Mishra SK & Narasimhaji CV (2024) *Heliyon* **10**. https://doi.org/10.1016/j.heliyon.2024.e33542.
57. Qaderi K, Sharifipour F, Dabir M, Shams R & Behmanesh A (2025) *Eur J Med Res* **30**, 246. https://doi.org/10.1186/s40001-025-02479-6
58. Belala R, Bourahmoune D & Mimoune N (2024) *Kafkas Univ Vet Fak Derg* **30**, 741–51. https://doi.org/0.9775/kvfd.2024.32819
59. Liang B & Wang M (2025) *BMC Urol* **25**, 261. https://doi.org/10.1186/s12894-025-01946-w
60. Hammer HL, Thambawita V & Riegler MA (2025) *Hum Reprod* **40**, 1595–604. https://doi.org/10.1093/humrep/deaf136
61. Amini Mahabadi J, Enderami SE, Nikzad H & Hassani Bafrani H (2024) *Andrologia* **2024**, 8165541. https://doi.org/10.1155/and/8165541
62. Astratov VN, Sahel Y Ben, Eldar YC, Huang L, Ozcan A, Zheludev N, Zhao J & Burns Z (2023) *Laser Photon Rev* **17**, 2200029. https://doi.org/10.1002/lpor.202200029
63. Aktas A, Serbes G, Yigit MH, Aydin N, Uzun H & Ilhan HO (2024) *IEEE Access* **12**, 196070–91. https://doi.org/10.1109/ACCESS.2024.3521643
64. Sukhikh GT, Skibina JS, Zanishevskaya AA, Shuvalov AA, Yanchuk NO, Gryaznov AY, Sysoeva AP & Makarova NP (2021) *Obstet Gyneco* **7**, 74-80. https://doi.org/10.18565/aig.2021.7.74-80
65. Liu G, Hao S, Zhang H, Zhou Y, Sun Y, Li W, Huang X, Jiang Y, Fang Y & Yang G (2022) *Microsc Microanal* **28**, 1–13. https://doi.org/10.1017/S1431927622012132
66. Ghasemian F, Bahadori MH, Hosseini Kolkooh SZ & Esmaeili M (2022) *Cell J* **24**, 7–14. https://doi.org/10.22074/cellj.2022.7448
67. Barnett-Itzhaki Z, Elbaz M, Butterman R,

Amar D, Amitay M, Racowsky C, Orvieto R, Hauser R, Baccarelli AA & Machtinger R (2020) *J Assist Reprod Genet* **37**, 2405–12. https://doi.org/10.1007/s10815-020-01908-1
68. You JB, McCallum C, Wang Y, Riordon J, Nosrati R & Sinton D (2021) *Nat Rev Urol* **18**, 387–403. https://doi.org/10.1038/s41585-021-00465-1
69. Sato T, Kishi H, Murakata S, Hayashi Y, Hattori T, Nakazawa S, Mori Y, Hidaka M, Kasahara Y, Kusuhara A, Hosoya K, Hayashi H & Okamoto A (2022) *Reprod Med Biol* **21**, e12454. https://doi.org/10.1002/rmb2.12454
70. Butola A, Popova D, Prasad DK, Ahmad A, Habib A, Tinguely JC, Basnet P, Acharya G, Senthilkumaran P, Mehta DS & Ahluwalia BS (2020) *Sci Rep* **10**, 13118. https://doi.org/10.1038/s41598-020-69857-4
71. Shun C, Liu X, Song B & Hu Y (2024) *BMC Urol* **24**, 156. https://doi.org/10.1186/s12894-024-01537-1
72. Gunderson SJ, Puga Molina LC, Spies N, Balestrini PA, Buffone MG, Jungheim ES, Riley J & Santi CM (2021) *Fertil Steril* **115**, 930–9. https://doi.org/10.1016/j.fertnstert.2020.10.038
73. Zeadna A, Khateeb N, Rokach L, Lior Y, Har-Vardi I, Harlev A, Huleihel M, Lunenfeld E & Levitas E (2020) *Hum Reprod* **35**, 1505–14. https://doi.org/10.1093/humrep/deaa109
74. Mehrjerd A, Dehghani T, Jajroudi M, Eslami S, Rezaei H & Ghaebi NK (2024) *Sci Rep* **14**, 24283. https://doi.org/10.1038/s41598-024-73326-7
75. Javadi S & Mirroshandel SA (2019) *Comput Biol Med* **109**, 182–94. https://doi.org/10.1016/j.compbiomed.2019.04.030
76. Jiang X, Cai J, Liu L, Liu Z, Chen J, Yang C, Chen K, Yang X, Geng J, Ma C, Lian S, Xu L & Ren J (2023) *Front Cell Dev Biol* **11**, 1133512. https://doi.org/10.3389/fcell.2023.1133512
77. Peng T, Liao C, Ye X, Chen Z, Li X, Lan Y, Fu X & An G (2023) *Reprod Biol Endocrinol* **21**, 26. https://doi.org/10.1186/s12958-023-01080-y
78. Ory J, Tradewell MB, Blankstein U, Lima TF, Nackeeran S, Gonzalez DC, Nwefo E, Moryousef J, Madhusoodanan V, Lau S, Jarvi K & Ramasamy R (2022) *World J Mens Heal* **40**, 618–26. https://doi.org/10.5534/wjmh.210159
79. Hook KA, Yang Q, Campanello L, Losert W & Fisher HS (2020) *BioRxiv* **2020**, 12. https://doi.org/10.1101/2020.12.05.413120
80 Kandel ME, Rubessa M, He YR, Schreiber S, Meyers S, Matter Naves L, Sermersheim MK, Sell GS, Szewczyk MJ, Sobh N, Wheeler MB & Popescu G (2020) *Proc Natl Acad Sci* **117**, 18302–9. https://doi.org/10.1073/pnas.2001754117
81 Mahmud M, Kaiser MS, McGinnity TM & Hussain A (2021) *Cognit Comput* **13**, 1–33. https://doi.org/10.1007/s12559-020-09773-x
82 Agarwal A, Sharma R, Gupta S, Finelli R, Parekh N, Selvam MKP, Pompeu CP, Madani S, Belo A & Darbandi M (2021) *World J Mens Health* **40**, 52. https://doi.org/10.5534/wjmh.210022
83 Khan HL, Khan S, Bhatti S & Abbas S (2022) *Fertil Reprod* **5**, 1–7. https://doi.org/10.1142/S2661318223300015
84 Revollo Sarmiento N, Revollo Sarmiento G, Delrieux C, Herrera M & Gonzalez-Jose R (2021) *Springer* pp 177–91. https://doi.org/10.1007/978-3-030-75945-2_9
85 Dar AU, Singh M, Assad A, Macha MA & Bhat MR (2025) *Int J Syst Assur Eng Manag* pp 1-16. https://doi.org/10.1007/s13198-025-03070-2
86 Hoffmann MA & Lasch R (2023) *ACM Comput Surv* **56**, 1–33. https://doi.org/10.1145/3623378
87 Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D & Sharifi-Rad J (2023) *Front Chem* **11**, 1158198. https://doi.org/10.3389/fchem.2023.1158198
88 Wahjuningsih S, Khaeruddin K, Pratiwi H, Sari APZNL, Tribudi YA, Arif AA & Kostaman T (2025) *Ital J Anim Sci* **24**, 754–62. https://doi.org/10.1080/1828051X.2025.2470269
89 Yuan C, Wang J & Lu W (2023) *Front Vet Sci* **10**, 1119153. https://doi.org/10.3389/fvets.2023.1119153
90 Baschieri A & Amorati R (2021) *Antioxidants* **10**, 1551. https://doi.org/10.3390/antiox10101551
91 Sánchez-Cárdenas C, De la Vega-Beltrán JL, Weber WD, Orta G, Sánchez-Guevara

Y, Hernández-Cruz A, Darszon A & Visconti PE (2024) *FASEB J* **38**, e70129. https://doi.org/10.1096/fj.202401243R
92 Moretti E, Signorini C, Corsaro R, Giamalidi M & Collodel G (2023) *Antioxidants* **12**, 1098. https://doi.org/10.3390/antiox12051098
93 Alevra AI, Exadactylos A, Mente E & Papadopoulos S (2022) *Animals* **12**, 791. https://doi.org/10.3390/ani12060791
94 O'Flaherty C (2025) *Asian J Androl* pp 10-4103. https://doi.org/10.4103/aja2024126
95 Kowalczyk A (2022) *Reprod Sci* **29**, 1387–94. https://doi.org/10.1007/s43032-021-00795-w
96 Ebrahimi B, Matavos-Aramyan H & Keshtgar S (2022) *Cell Tissue Bank* **23**, 213–25. https://doi.org/10.1007/s10561-021-09953-5
97 Mahmoudi A, Razi M, Jalilpour M & Shalizar Jalali A (2025) *F&S Sci*. https://doi.org/10.1016/j.xfss.2025.10.005
98 Al-Ebady AS (2025) *Open Vet J* **15**, 4199. https://doi.org/10.5455/OVJ.2025.v15.i9.25
99 Ofosu J, Qazi IH, Fang Y & Zhou G (2021) *Anim Reprod Sci* **233**, 106850. https://doi.org/10.1016/j.anireprosci.2021.106850
100 Shokri-Asl V, Khodavirdilou R, Hajipour H, Pashaiasl M & Fattahi A (2025) *Biopreserv Biobank*. https://doi.org/10.1177/19475535251359868
101 Partyka A & Niżański W (2021) *Antioxidants* **10**, 1927. https://doi.org/10.3390/antiox10121927
102 Mahiddine FY & Kim M-J (2021) *Animals* **11**, 1930. https://doi.org/10.3390/ani11071930
103 Rizkallah N, Chambers CG, de Graaf SP & Rickard JP (2022) *Animals* **12**, 244. https://doi.org/10.3390/ani12030244
104 Mangoli V, Evgeni E & Wyns C (2025) *Asian J Androl* **27**, 392-8. https://doi.org/10.4103/aja202466
105 Cao X, Ren Y, Lu Q, Wang K, Wu Y, Wang Y, Zhang Y, Cui X, Yang Z & Chen Z (2023) *Front Nutr* **9**, 1018336. https://doi.org/10.3389/fnut.2022.1018336
106 Figueroa Villalobos E, Pereira WA, Pérez-Atehortúa M, Sandoval-Vargas L, Romero J, Oliveira RPS, Valdebenito I & Villasante A (2025) *Rev Aquac* **17**, e12968. https://doi.org/10.1111/raq.12968
107 Gostage J, Domingo-Lopez DA, Tarpey R, Duffy GP & Levey RE (2025) *Mol Ther Methods Clin Dev* 33. https://doi.org/10.1016/j.omtm.2025.101613
108 De Luca MN, Colone M, Gambioli R, Stringaro A & Unfer V (2021) *Antioxidants* **10**, 1283. https://doi.org/10.3390/antiox10081283
109 Chimento A, De Luca A, Venditti M, De Amicis F & Pezzi V (2025) *Cells* **14**, 1122. https://doi.org/10.3390/cells14141122
110 Abilash D & Sridharan TB (2025) *Afr J Reprod Health* **29**, 30–9. https://doi.org/10.29063/ajrh2025/v29i10.3
111 Esteves SC, Coimbra I & Hallak J (2023) *Andrology* **11**, 1613–34. https://doi.org/10.1111/andr.13405
112 Tiwari S, Mohanty TK, Bhakat M, Kumar N, Baithalu RK, Nath S, Yadav HP & Dewry RK (2021) *Cryobiology* **101**, 125–34. https://doi.org/10.1016/j.cryobiol.2021.04.007
113 Marimuthu MM, Balamurugan BS, Sundaram VA, Anbalagan S & Chopra H (2025) *Explor. target. anti-tumor the* **6** 1002312. https://doi.org/10.37349/etat.2025.1002312
114 Uribe P, Meriño J, Matus CE, Schulz M, Zambrano F, Villegas J V, Conejeros I, Taubert A, Hermosilla C & Sánchez R (2022) *Hum Reprod* **37**, 680–95. https://doi.org/10.1093/humrep/deac021
115 Abilash D, Parameswari R, Madhan Kumar P & Sridharan TB (2025) *Indian J Clin Biochem* **10**, 1-1. https://doi.org/10.1007/s12291-025-01364-7
116 Lee D & Hong JH (2023) *Antioxidants* **12**, 2021. https://doi.org/10.3390/antiox12122021
117 Serafini S & O'Flaherty C (2025) *Hum Reprod* **40**, 1440–51. https://doi.org/10.1093/humrep/deaf085
118 Leclère J-C & Dulon D (2023) *Front Cell Neurosci* **17**, 1197611. https://doi.org/10.3389/fncel.2023.1197611
119 Collin MS, Venkatraman SK, Vijayakumar N, Kanimozhi V, Arbaaz SM, Stacey RGS, Anusha J, Choudhary R, Lvov V, Tovar GI, Senatov F, Koppala S & Swamiappan S (2022) *J Hazard Mater Adv* **7**, 100094. https://doi.org/10.1016/j.hazadv.2022.100094
120 Katiyar D, Saxena R, Kumar A, Bansal P, Prakash S, Ghosh D & Nagarajan K (2024)

Toxin Rev **43**, 329–57. https://doi.org/10.1080/15569543.2024.232 9907 Udayakumar G & Veerakumar P (2026) *Mater Res Bull* **196**, 113926. https://doi.org/10.1016/j.materresbull.2025. 113926

122 Huang J, Galal G, Etemadi M & Vaidyanathan M (2022) *JMIR Med Inf* **10**, e36388. https://doi.org/10.2196/36388

123 Ullah A, Chen W, Shi L, Wang M, Geng M, Na J, Akhtar MF, Khan MZ & Wang C (2025) *Vet Sci* **12**, 807. https://doi.org/10.3390/vetsci12090807

124 Chen RJ, Wang JJ, Williamson DFK, Chen TY, Lipkova J, Lu MY, Sahai S & Mahmood F (2023) *Nat Biomed Eng* **7**, 719–42. https://doi.org/10.1038/s41551-023-01056-8

125 Vieira IRS, de Carvalho APA de & Conte-Junior CA (2022) *Compr Rev Food Sci Food Saf* **21**, 3673–716. https://doi.org/10.1111/1541-4337.12990

126 Luong T-M-T & Le NQK (2024) *J Assist Reprod Genet* **41**, 239–52. https://doi.org/10.1007/s10815-023-02973-y

127 Sudarmozhi K, Devi VS, Öztop HF, Ahmad H, Abd-Elmonem A, Jamshed W & Siddig NAA (2026) *Int Commun Heat Mass Transf* **171**, 110107. https://doi.org/10.1016/j.icheatmasstransfer. 2025.110107