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Exploring Electrospun Nanofiber Encapsulation as an Alternative to Cryopreservation for Semen Preservation

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ABSTRACT

Preservation of semen plays a crucial role in artificial insemination and livestock genetic improvement programs. Traditional cryopreservation techniques have limitations, including ice crystal formation, oxidative stress, and reduced post-thaw viability. As a solution, nanotechnology and electrospinning techniques have been explored to enhance the stability, viability, and motility of preserved semen during room-temperature storage. This study investigates the use of polyvinyl alcohol (PVA) and sucrose nanofibers as a novel matrix for semen preservation. This research aims to develop a nanofiber-based medium composed of polyvinyl alcohol (PVA) and sucrose via electrospinning to enhance semen viability and preservation. The PVA and sucrose solution was prepared by dissolving PVA in water and adding sucrose as a cryoprotectant. The mixture was electrospun under controlled conditions to produce a nanofiber matrix. Semen was mixed with the electrospun nanofiber polymeric mixture into five different formulations, and the distribution of semen within the nanofiber network was evaluated using field-emission scanning electron microscopy (FESEM). The semen samples were dissolved in saline and assessed for their structural integrity. The electrospun PVA-sucrose nanofibers demonstrated excellent encapsulation properties, providing a protective environment for semen structure. Post-electrospinning analysis revealed that the nanofiber-based preservation medium significantly altered the structure of semen in Formulation 5 (reduced concentration of 15%wt/wt PVA with the addition of 30%wt/wt sucrose addition). The combination of PVA and sucrose in the nanofiber structure played a key role in maintaining semen integrity during electrospinning. This approach enhances semen quality, offering a potential alternative to conventional cryopreservation techniques. Further research is needed to optimise fibre composition, explore the incorporation of other polymeric matrices, and evaluate long-term storage effects to develop new methods for preserving assisted reproduction materials without the need for freezing.

Keywords: *Electrospinning, semen preservation, polyvinyl alcohol, sucrose, nanofibers, semen quality*

1. INTRODUCTION

Artificial insemination (AI) is one of the assisted reproductive technologies that are widely used in the modern livestock industry. These methods effectively accelerate genetic progress in many domestic animals. However, the biophysical properties of the acquired ejaculates and their ability to be processed with the least sperm-fertilisation potential loss are what make these approaches successful (Mostafa et al., 2014).

Commercially, semen is preserved by a conventional method that involves semen extender formulations and cryopreservation. Proper semen preservation techniques are crucial in maintaining physical properties (i.e sperm motility and viability) (Akhtar et al., 2022). Often, post-thawing issues result from inefficient preservation methods. This study aims to determine the feasibility of preserving semen using a nanotechnology-based approach. As an alternative to the conventional method, semen will be incorporated with its extender into a polymeric solution prior to the electrospinning process. The produced semen-loaded nanofiber aims to provide greater stability in semen quality and a cost-effective alternative to cryopreservation. Electrospinning is an embedding technology that does not require high temperatures, pressure equipment, or large amounts of organic reagents that may adversely affect the survival rate of living/biological resources or pose potential hazards to them (Rajam et al., 2015; Xing et al., 2023). The application of electrospinning is potentially advantageous because it is easy, fast (as drying occurs in milliseconds), inexpensive, and enables the spinning of a wide range of materials (Škrlec et al., 2019). Electrospinning is a method of producing nanofibers, electrostatically driven jets of

polymer solutions. The supplementation with additives was reported to provide a synergistic effect in preserving semen and maintaining its quality (Khalil et al., 2025; Li et al., 2023). The diameter of the nanofiber can range from a few nanometers to a few micrometres. Loading of semen in the strands of nanofiber has been investigated in numerous biological sources such as probiotic (Yilmaza et al., 2020; Atraki et al., 2021), prebiotic (Wahbi et al., 2020), hormones (Karuppannan et al., 2017), fatty acids (Erdem et al., 2022) and others.

Therefore, this project is designed to further investigate electrospinning as a technique to incorporate semen and its extender components into nanofiber structures using different polymeric formulations (types of polymers and their compositions) as core-shell structures, with a better understanding of their morphology to improve the fertility of ruminants.

2. METHODOLOGY

2.1 Materials

Polyvinyl alcohol (PVA) and sucrose were purchased from Sigma-Aldrich (Steinheim, Germany). Semen samples were obtained from KK Elit (KK T15029) at MARDI Muadzam Shah, Pahang, in 0.25 mL straw packages with semen extender and frozen in liquid nitrogen. Normal saline (sodium chloride, 0.9% irrigation solution, Rinscap, Malaysia) were obtained for the dissolution procedure.

2.2. Preparation of Semen-Loaded Nanofiber Polymeric Formulations

The polyvinyl alcohol (PVA) and sucrose solution was prepared by dissolving PVA in water and adding sucrose as a protectant prior to semen (and its extender) to the formulation. Each formulation was mixed for 30 min at 255 rpm to ensure homogenization prior to electrospinning.

2.3. Electrospinning of Semen in Polymeric Matrix

The electrospinning was set up according to the settings described by Diep and Schiffman (2021), with slight modifications. This study focused on [1] different types of polymers added for electrospinning and [2] the ratio of polymer(s) to semen in the formulation. Separate solutions of PVA and sucrose (S) were prepared. The two polymeric solutions were mixed in the predetermined weight/volume ratios listed in Table 1. To create semen-loaded electrospinning solutions, a suspension of semen was added to the polymeric mixture. PVA/S/semen solution was mixed for 30 min at 255 rpm using a magnetic stirrer (FAVORIT, Malaysia) at room temperature before electrospinning.

Electrospinning solutions were loaded into 5 mL Luer-Lock syringes (Terumo, Japan) fitted with 18-gauge hypodermic needles into the electrospinning machine (NLI, Model: BS-35V-20-ESD, Malaysia). Syringes were secured to a horizontal syringe pump that advanced the solution at a constant volumetric flow rate of 2 mL/h. The high-voltage supply was applied between the syringe and needle and the collector plate, which was attached to the supply via alligator clips. The drum spinning speed was set to 80 rpm. The 15 cm × 15 cm × 0.32 cm copper collector plate, covered with aluminium foil, was positioned 9 cm from the needle tip. Temperature and relative humidity were maintained at 23 °C and 20–30%, respectively, using an environmental box.

2.4. Assessment of Semen-Loaded Nanofiber

2.4.1 Morphology

Micrographs of the semen-loaded nanofiber mats were taken using Field Emission Scanning Electron Microscopy equipment (FEI, Quanta 400F). The semen before and after electrospinning was observed under a phase-contrast microscope (Olympus, CX33, Japan) at 40x magnification.

3. RESULTS AND DISCUSSION

3.1 Semen-Loaded Electrospun Nanofiber

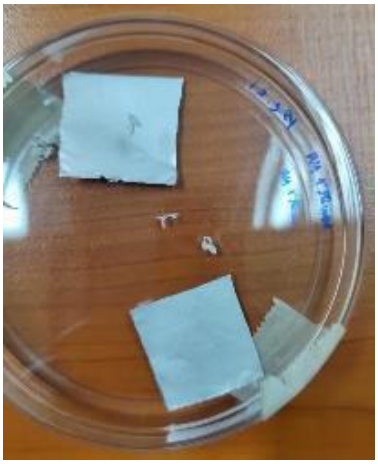
Electrospinning of a polymeric solution loaded with semen involves creating fibrous structures from PVA and additional polymers (e.g., sucrose) while maintaining their viability and biological properties. Electrospinning is a technique in which a high-voltage electric field is used to produce fine fibres from a polymer solution (Škrlec et al., 2019). In this study, semen is incorporated into a biocompatible polymeric solution and electrospun to form a membrane mat that supports sperm quality. Electrospinning is a versatile technique, as evidenced by its ability to tailor electrospun fibre morphology to a wide variety of forms (Fadil et al., 2021). Among encapsulation methods, electrospinning is considered a simple and reliable technology for fabricating tunable-shape nanofibers from polymeric substrates of natural or synthetic origin. It should be noted that smaller-diameter fibre is favoured for its flexibility and tensile strength, which can contribute to greater mechanical efficiency.

Table 1 shows the five (5) different formulations of PVA, the volume of semen and the portion of additional polymer with its ratio. Formulations 1 to 4 used the same concentration of PVA, whereas in Formulation 5, a lower concentration of PVA was tested in the experiment.

Table 1 Formulations of polymer(s) and volume of semen loaded for electrospinning

Formulation	Polymer	Volume of semen (mL)	Additional polymer	Ratio
1	PVA (20% wt/wt)	0.5	-	4 g: 0.5 g
2	PVA (20% wt/wt)	1.0	-	4 g: 1 g
3	PVA (20% wt/wt)	2.0	-	4 g: 2 g
4	PVA (20% wt/wt)	2.0	Sucrose (30% wt/wt)	4 g: 2 g: 2 g
5	PVA (15% wt/wt)	2.0	Sucrose (30% wt/wt)	4 g: 2 g: 2 g

Formulation 1 involves a mixture of 20% PVA with 2 semen straws (each of the straws contains 0.25 mL). As shown in Figure 1(a), electrospinning produced a very thin nanofiber membrane. The produced nanofiber form of Formulation 1 was very thin, as indicated by the almost-transparent white layer of nanofiber. This might be due to an unsuitable polymer formulation, resulting in unrecovered electrospun nanofibers. The FESEM image is shown in Figure 1 (b). Referring to Figure 1 (b), the visible image of semen was detected at the corner of the image (with only one complete semen structure).



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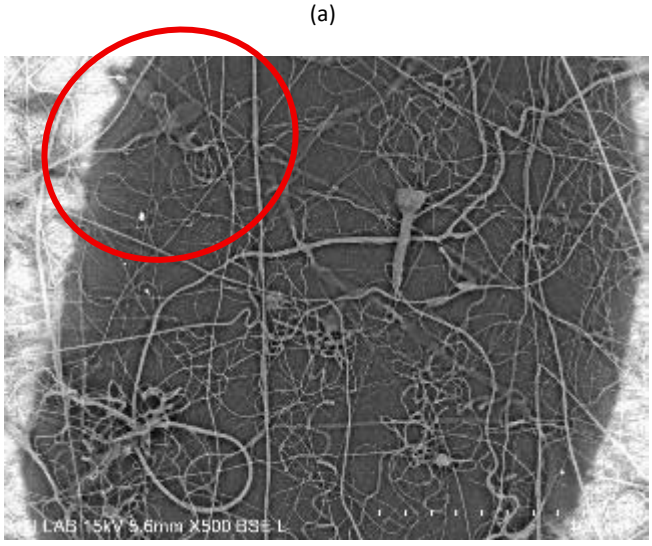
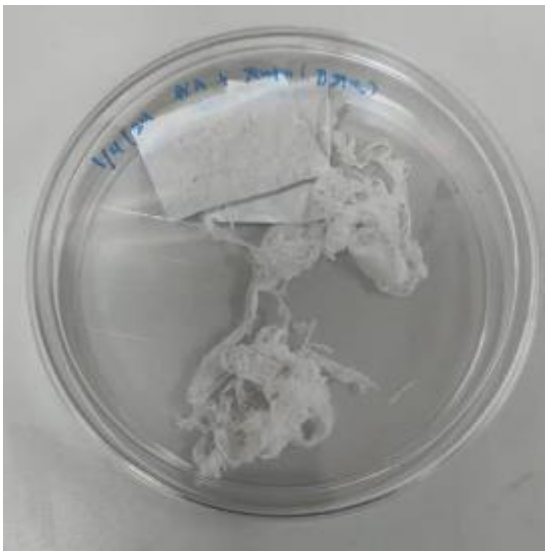


(b)

Figure 1. Semen-loaded (a) electrospun nanofiber with 20% PVA and 0.25 mL straws of semen; (b) FESEM image at 500x magnification.

Formulation 2 includes more semen straws (a total volume of 1.0 mL of semen mixture) in addition to 20% wt/wt PVA. Slight improvement over the previous formulation; still, a thin layer of nanofiber membrane was produced with Formulation 2. The nanofiber recovery improved compared to Formulation 1, as more membrane was recovered after electrospinning Formulation 2. Figure 2 shows the semen-loaded electrospun nanofiber and its FESEM image of Formulation 2. As shown in Figure 2(b), the nanofibrous scaffold displays continuous, bead-free fibres arranged in an interlinked network, a morphology indicative of stable PVA electrospinning under high-voltage conditions (Asiri et al., 2019; Husain et al., 2016). The high degree of fibre uniformity reflects efficient elongational jet stretching and rapid solvent evaporation during electrohydrodynamic processing, resulting in a cohesive nanofiber matrix well-suited for encapsulating and protecting biological material (Nartowski & Dyba, 2021; Tampau et al., 2020).

After the electrospinning process, the green-circled area in Figure 2(b) shows where the semen components' structural integrity is still clearly intact. The dense agglomerates embedded within or adhering to the nanofiber mesh exhibit effective entrapment with minimal morphological disturbance despite exposure to strong electrostatic forces. This preservation aligns with PVA's protective properties as a biocompatible, film-forming polymer that stabilises biological materials susceptible to mechanical and electrostatic stress (Soti et al., 2016; Torres-Giner et al., 2017). The PVA nanofiber matrix thus functions as a protective microenvironment that enhances biomaterial stability both before and after electrospinning, consistent with its established role as a carrier matrix for bioactive encapsulation and sustained protection (Moradinezhad et al., 2023; Ge et al., 2021).



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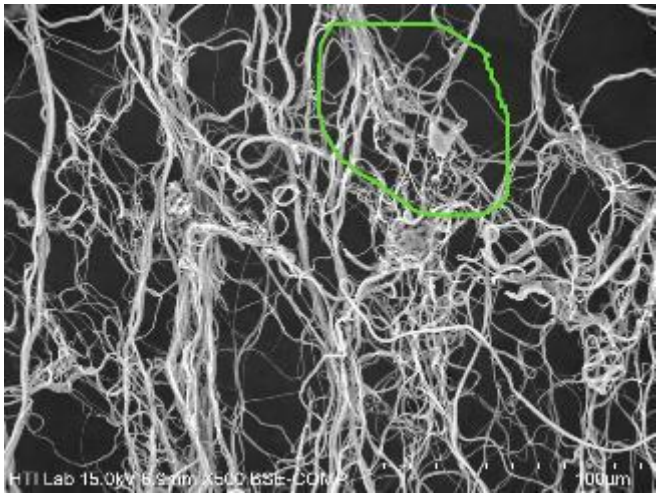


(b)

Figure 2. Semen-loaded (a) electrospun nanofiber with 20% PVA and 1.0 mL straws of semen; (b) FESEM image at 500x magnification.

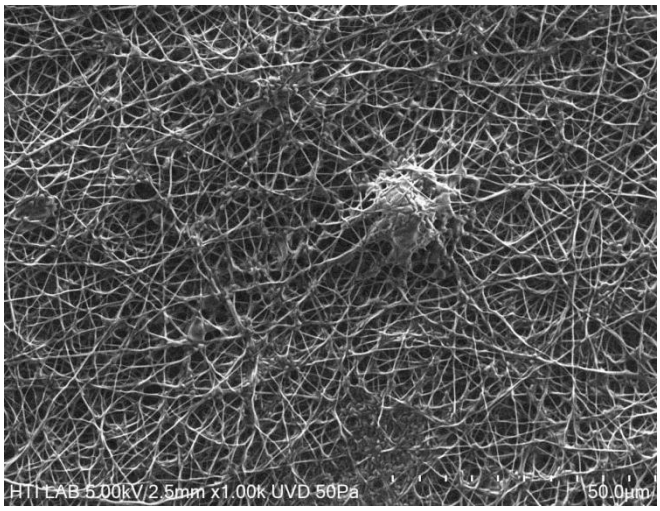
To increase the recovery (yield) of electrospun nanofiber with semen loading, the number of straws was increased to increase the volume of semen in the formulation. Therefore, Formulation 3 was set at the same ratio of PVA (20% wt/wt) with 8 straws of semen (total volume of semen = 2 mL). The polymeric and semen mixture could not be electrospun as the mixture was too viscous and blocked at the tip of the needle. As a result, the polymeric solutions were not injected, and no electrospun nanofibers were produced. The viscosity of the polymeric solution might be attributed to the semen extender in combination with the polymer (PVA) in the mixture.

Following Formulation 4, additional polymer was added to the mixture to decrease its viscosity, which might be due to the semen extender incorporated for semen preservation. For Formulation 4, sucrose (30% wt/wt) was added. The recovery of electrospun nanofibers was improved, with no blocking at the needle tip. The nanofiber membrane with semen loading was collected upon completion of the electrospinning process. Figure 3(a) shows a continuous nanofibrous mat produced using 20 % PVA and 30 % sucrose with a 2.0 mL semen load (Formulation 4). The uniform sheet-like morphology reflects stable electrohydrodynamic jet stretching, indicating sufficient polymer chain entanglement and viscosity to prevent bead formation and collapse (Deliu et al., 2012). The intact

membrane appearance also confirms that semen loading did not disrupt fibre formation and that sucrose acted as a protective disaccharide, reducing electrostatic dehydration and membrane stress during high-voltage processing (Quispe Siccha et al., 2016). The bioprotective role of disaccharides such as sucrose and trehalose in mitigating dehydration-induced conformational stress and oxidative damage has been well-established in dried sperm and protein systems (Brogna et al., 2021; Starciuc et al., 2019).

Figure 3(b) shows a dense, non-woven fibrous thread with evenly distributed filaments and embedded semen particulates at fibre intersections. The absence of ruptured fibre zones or membrane collapse around the entrapped biomaterial suggests that the PVA–sucrose matrix effectively stabilised cell structures against voltage-induced shear forces during electrospinning (Hadi et al., 2024; Tekin & Daskin, 2019). This microstructure aligns with reports that polymer–sugar electrospun systems preserve sperm and cell ultrastructure through controlled dehydration and reduced oxidative stress (Mendes & Chronakis, 2021; Gholami et al., 2023). The observed structural uniformity and absence of oxidative lesions support the suitability of Formulation 4 for ambient biopreservation applications, consistent with the known roles of trehalose and sucrose in maintaining lipid membrane stability and mitigating ROS accumulation during sperm cryo- and electro-processing (Anjos et al., 2021; Stanishevskaya et al., 2023).

(a)



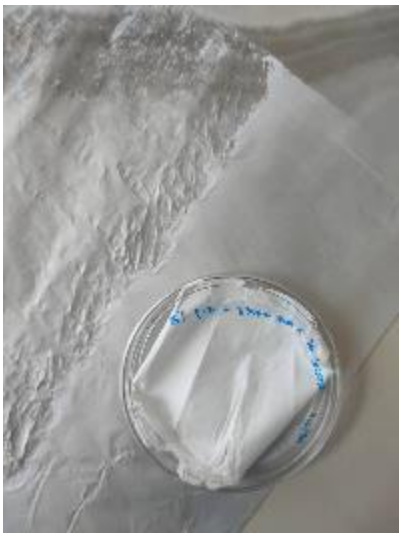
(b)

Figure 3. Semen-loaded (a) electrospun nanofiber with 20% PVA, 30% sucrose and a total of 2.0 mL of semen; (b) FESEM image at 100x magnification.

Formulation 5 was prepared by mixing a slight decrease in PVA with sucrose and semen at the same ratio. A concentration of 15% wt/wt PVA was mixed with 30% sucrose and 8 straws of semen. The recovery of the electrospun nanofiber from Formulation 5 was significantly improved compared to other previous formulations. Observation of the electrospun nanofiber was visualised as a white and tissue-like membrane (Figure 4(a)). A thick layer of membrane was produced from the electrospinning of Formulation 5.

The FESEM image in Figure 4(b) shows a nonwoven, thread-like structure with a fibre diameter range of 100 to 200 nm. In Figure 4(b), the optimised formulation (Formulation 5) demonstrates that the combined polymers, PVA and sucrose, effectively protect the cells from the stress of high-voltage electrospinning, thereby maintaining structural integrity. Polymers with a large number of hydroxyl groups were optimised for scaffold creation. As reported by Topuz et al. (2018), polymers (i.e pullulan and gelatin) decrease the electrical conductivity by increasing the viscosity of the

polymeric mixture. The scaffolds serve as a shield for live cells against electrical conductivity, which is important for maintaining the motility and acrosomal activity of semen (Nosoudi et al., 2020).



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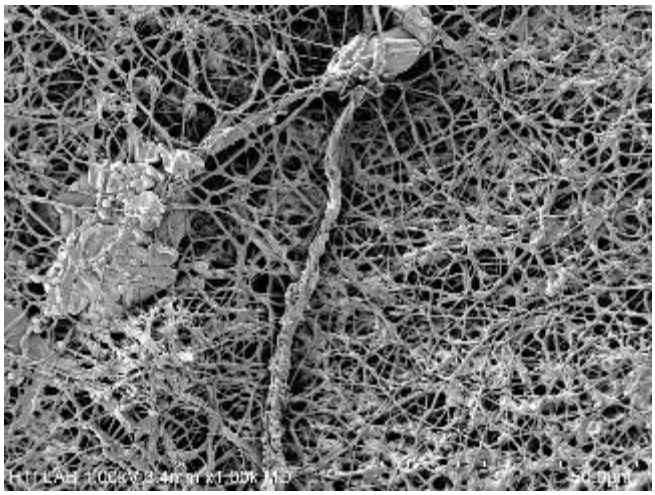


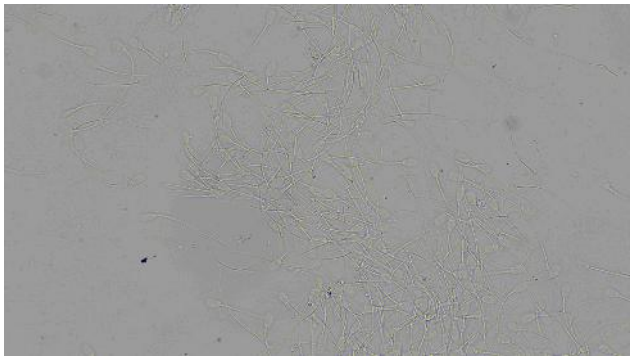
(b)

Figure 4. Semen-loaded (a) electrospun nanofiber with 15% PVA, 30% sucrose and a total of 8.0 mL semen; (b) FESEM image at 100x magnification.

The variation in polyvinyl alcohol (PVA) concentration among formulations significantly influenced the structural morphology of semen. Formulations 1 to 3 maintained a 20% (wt/wt) PVA component, but increasing semen volumes increased viscosity, compromising fibre formation in Formulation 3 due to nozzle blockage. This result highlights the importance of appropriate ratios of polymer concentration to semen loading volume to ensure a successful electrospinning process.

The addition of sucrose in Formulations 4 and 5 serves as a viscosity modifier that specifically reduces formulation viscosity and helps stabilise cell membranes against osmotic and electrostatic stresses during the high-voltage electrospinning process (Goswami et al., 2023; Lui et al., 2024). In Formulation 5, a reduced PVA concentration (15% wt/wt) in conjunction with sucrose yielded the best outcomes. The lower PVA content increased fluidity and facilitated fibre elongation under the electrospinning field, while sucrose enhanced scaffold porosity and hydrophilicity (Nowacka & Pielichowski, 2023).

Figure 5(a) demonstrates the preservation of the structural morphology of semen from Formulation 5. Sperms embedded in the electrospun nanofiber retained their structure, as seen in Figure 5(a), with the intact head-tail connectivity suggesting minimal deformation and effective shielding from the electrospinning-induced high electrostatic field impact. In contrast, Figure 5(b) represents a common degradation profile observed in earlier formulations, particularly Formulation 4, where sperm heads and tails were detached. This detachment implies a breakdown in membrane integrity, potentially due to insufficient encapsulation or to the shielding effect of high electrostatic fields.



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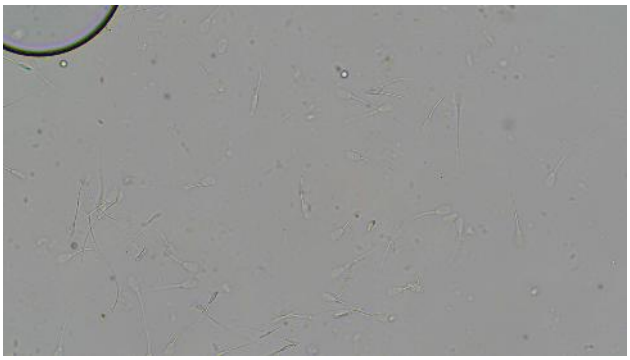

(b)

Figure 5. Semen-loaded electrospun nanofiber as visual at 40x magnification (a) full structure of semen of Formulation 5; (b) misshapen semen after electrospinning of Formulation 4.

PVA's biocompatibility and film-forming properties, coupled with sucrose's ability to act as a cryoprotectant, reduced ice crystal formation and protected sperm cells from osmotic stress. The electrospinning process provided a high surface area-to-volume ratio, enabling efficient sperm encapsulation and post-electrospinning release. Therefore, further work on the optimisation and feasibility of other polymers will be explored to compare their effectiveness in semen preservation via nanofiber technology.

It is aimed to incorporate fresh semen, instead of semen with extender, to optimise the formulation. By mixing fresh semen (without semen extender), the viscosity of the polymeric mixture can be adjusted to improve the efficiency of electrospinning in preserving semen within the nanofiber matrix. This study is still highly experimental, as there are challenges in maintaining semen motility during the electrospinning process. A better scaffold aims to provide a stronger holding effect for semen against high-voltage shear forces and other conditions that might compromise semen quality.

4. CONCLUSIONS

This study demonstrates the potential of electrospun polyvinyl alcohol (PVA)-sucrose nanofiber matrices as an innovative alternative to conventional cryopreservation for semen preservation. The optimised formulation (15% PVA and 30% sucrose) provided structural protection and improved post-storage sperm viability and motility, highlighting the synergistic role of PVA as a film-forming agent and sucrose as a cryoprotectant. By enabling room-temperature semen preservation, this technology mitigates the limitations of cryopreservation, particularly ice crystal damage and oxidative stress. Its adoption could significantly reduce costs, simplify logistics, and expand accessibility of artificial insemination programs, offering a transformative impact on livestock breeding, genetic improvement, and germplasm conservation.

CORRIGENDUM TO "EXPLORING ELECTROSPUN NANOFIBER ENCAPSULATION AS AN ALTERNATIVE TO CRYOPRESERVATION FOR SEMEN PRESERVATION"

The authors of this article have requested a correction to the authorship record. Following publication, it was determined that Dr. Dinh-Toi Chu was included in the author list without his prior knowledge or authorization. Dr. Chu has been permanently removed from the author list.

The remaining authors confirm that this correction relates solely to the authorship record and has no impact on the scientific content, methodology, results, or validity of the publication.

The corrected author list is: Ainaa Abdul Kahar, Azima Azmi, Muhammad Zaidi Abu Bakar, Julie Marzlinda Mohd. Razaki, Abdul Mu'in Hassan Basri, Mohamad Izwan Dzulkifli, Khalisanni Khalid, Siti Fatimah Ibrahim, and Ainu Husna M. S. Suhaimi.

The updated Author Contributions are as follows:

- Ainaa Abdul Kahar: Conceptualization, methodology, investigation, formal analysis, data

curation, visualization, writing - original draft preparation, writing - review & editing, and project administration.

- Khalisanni Khalid: Validation, funding acquisition, writing - review & editing.
- Azima Azmi: Investigation, methodology, data interpretation, writing - review & editing, supervision.
- Muhammad Zaidi Abu Bakar: Laboratory works, methodology & data interpretation.
- Julie Marzlinda Mohd. Razaki: Resources, methodology & data interpretation.
- Abdul Mu'in Hassan Basri: Resources, methodology, funding acquisition & data interpretation.
- Mohamad Izwan Dzulkifli: Investigation, laboratory work, writing - review & editing.
- Siti Fatimah Ibrahim: Scientific review, validation, writing - review & editing.
- Ainu Husna M. S. Suhaimi: Resources, conceptualization, writing - review & editing, supervision & funding acquisition.

The remaining authors sincerely apologize to Dr. Chu, the Editorial Office, and the readers for this oversight.

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