Journal of Applied Biology & Biotechnology Vol. 12(3), pp. 8-16, May-Jun, 2024
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2024.151890

Antimicrobial peptide and their role in sperm cryopreservation

Manish Kumar[1,2], Ravi Ranjan[1]*, Alok Bhardwaj[2]

[1]Male Reproduction Laboratory, Animal Physiology and Reproduction Division, Central Institute for Research on Goats, Farah, Mathura, Uttar Pradesh, India.
[2]Department of Biotechnology, Institute of Applied Sciences and Humanities, GLA University, Mathura, Uttar Pradesh, India.

ARTICLE INFO

Article history:
Received on: October 03, 2023
Accepted on: March 04, 2024
Available online: April 20, 2024

Key words:
Antimicrobial peptide,
Antibiotic resistance,
Microbial load,
Semen cryopreservation.

ABSTRACT

Antimicrobial peptides (AMPs) are host defense peptides having antimicrobial properties that have been used as an additive in semen diluent of various mammalian species for enhancement of semen quality and prevention of bacterial load. The continuous use of antibiotics reduced the efficacy in checking of bacterial growth as well as semen quality. This review aims to provide an overview of the AMPs used as semen additives in different mammalian species as an alternative to antibiotics. We have discussed systematic development study on AMPs, their structure, classification, mechanism of action, application, future prospective, and challenges in this review. We have also reviewed our research on the use of different AMP as an additive for enhancement of post-thaw fertility of goat semen. Particular focus has been given on AMPs as a potential substitute strategy to deal with bacterial strains that are resistant to antibiotics. Synthetic AMPs may be designed to increase antimicrobial activity against microbes, particularly those resistant to antibiotics. AMPs also aid in the protection of the host by modifying host cellular immunity and improving post-thaw sperm fertility. Due to the growing issue of antibiotic resistance, the development of AMPs has sparked attention as a future-oriented anti-infective and antimicrobial agent to improve cryo-survivability and sperm fertility.

1. INTRODUCTION

Antimicrobial peptides (AMPs) are host defense peptides, originally found in 1980s in insects and frogs, and today, a huge number of AMPs has been used in a variety of clinical and medical applications. AMPs have antibacterial, antimicrobial, antiviral, anticancer, and immunomodulatory properties [1]. The antimicrobial effectiveness of AMPs against microbes is broad spectrum and powerful [2]. AMPs can be used to treat a deep range of bacteria including drug-resistant ones [3]. Thousands of AMPs discovered and proven that are found in various species such as fungi, bacteria, and plants. The majority of them are cationic AMPs which serve critical antibacterial roles. We explored AMPs about current issues, improvement initiatives, and AMPs prospects. External factors can either make them essential or inducible. Amino acids that are cationic and hydrophobic are commonly seen in AMPs [1]. According to reports, AMPs are classified into two antimicrobial categories according to their amino acid content and shape [4]. AMPs were initially employed using boar semen ejaculate in semen extender and it was discovered that AMPs had enzymatic stability, high thermal stability, microbial specificity, and sensitivity, making them the suitable alternative for drugs in semen diluter for protected semen preservation [5].

AMPs are macromolecule inclusive of amino acids linear chains that can be found in practically all living things and produced either as endogenous molecules for endocrine/neural signaling as a result of protein breakdown. AMPs have important roles in the metabolic operations of animals and can have antibacterial, antitumor, antidiabetic, antioxidative, immunomodulatory, and other actions based on their sequence. Isolated bioactive peptides are employed in the development and manufacturing of preventive dietary sources, medications, cosmetics, and nutritional supplements. Acid and alkali degradation or enzymatic breakage is the two most frequent techniques for generating peptides from proteins [6]. AMPs appear to offer a novel way to fight infections. These proteins were previously discovered in a variety of species from microbes to human innate immunity. Natural AMPs are part of the first line of immunological biological protection and are the result of millions of years of coevolution between superior beings and bacteria. Because those are not as potent as certain traditional antibiotics, their effectiveness against multidrug-resistant bacteria is the most important attribute of such peptides as antibiotics. Another remarkable feature of these molecules is the speed with which they destroy bacteria when compared to chemical antibiotics [7].

Resistance to AMPs is thought to be developing in this direction; however, it is less likely to occur than with traditional drugs due to

*Corresponding Author:
Dr. Ravi Ranjan,
Male Reproduction Laboratory,
Animal Physiology and Reproduction Division,
Central Institute for Research on Goats, Makhdoom, Farah,
Mathura - 281 122, Uttar Pradesh, India.
E-mail: dr_raviranjan @ yahoo.co.in

the principal goal of these macromolecules, the bacterial cytoplasmic membrane, and the consequent requirement to rearrange it [8]. Researchers are making an effort to develop a suitable alternative for antibiotics in semen extenders for preservation to solve this particular difficulty during cryopreservation. Some researchers feel that using AMPs as a semen extender is a beneficial alternative to a wide spectrum of antibiotics [9]. AMPs were extensively examined by numerous researchers, particularly at the time of cryopreservation of boar sperm samples for artificial insemination (AI). Many researchers have looked into using AMPs in semen extenders to substitute normal antibiotics and prevent antibiotic resistance from developing. In this paper, we looked at a variety of AMPs from various origins and their application in semen diluter as a good alternative to regular antibiotics in place of semen cryopreservation [10].

2. AMPS

AMPs are present in practically all living species from fungi to plants and organisms as part of their innate immune systems, which serve as a primary defense system. As a result, AMPs are produced naturally by both eukaryotic and prokaryotic cells partially their immunity [11]. The main function of AMP is to destroy invading microorganisms by altering the host's innate immune response. Bacteria were one of the first sources of AMPs also known as bacteriocins, which can be a novel treatment option for human disorders. Bacterial AMPs cannot protect bacteria from disease by another microorganism; instead, they dispense with different microscopic organisms as a wellspring of assets or the decrement of contest for supplements. Although few AMPs have a parochial slice, the majority of them have an expansive range and target a variety of bacterial enzyme processes or structures. Most AMPs are distinguished by their tiny size, which contains almost 30 amino acids [11]. Finally, bacteria and biofilm sites prefer positively charged peptides to negatively charged peptides on cell membranes. Antimicrobial activity is enhanced in microorganisms that are dynamic and sluggish in biofilms and they are easily killed by AMPs [6]. On the other hand, AMPs can be bacteriostatic in low-intensity conditions. In liquid medium, AMPs are classified based on secondary structure [12]. Some may be essentially alpha-helical, whereas others are primarily beta-pleated sheet structures. Cysteines produce an intramolecular disulfide bridge in circumstances, stabilizing the shape and allowing AMPs to pass the cell membrane [13]. The hydrophobic interactions of AMPs enhance cell adhesion. The antibacterial action is determined by the balance of density charge, aquaphobic property, and length of polypeptide chain. The secondary structure of AMPs and their antibacterial property can be impacted by expanding the quantity of fundamental amino acids [14].

3. STRUCTURE AND CHARACTERISTIC

AMPs are found in bacteria, fungi, animals, and plants. According to their structure, AMPs are categorized into 4 main groups: β-sheet, α –helical, extended AMPs, and β-hairpin [15].

3.1. β-sheet AMPs

AMPs have an antiparallel β-sheet structure that stabilized by disulfide bonds. Minor helical segments may be seen in larger peptides in this family. Gomesin, tachyplesins, and polyphemusins are examples of this class of AMPs [16]. The tiny 17–18 residues (gomesin) are well characterized class of β-sheet AMPs [Figure 1a]. Tachyplesin 1 is an antiparallel β-sheet that has 3-16 residues joined by type I turn which is stabilized by two disulfide links and has an amidated C-terminus. The necessity of disulfide bonds for the antibacterial action of these compounds has been the subject of several structure-activity relationship studies [17]. Studies using linear tachyplesins chemically coated with acetamidomethyl groups (T-Acm) show that the linear molecule had lower antibacterial and antiviral action as well as less calcein release from membrane models [18].

3.2. α-helical AMPs

α-helical AMPs form α-helical structures due to their amphipathic properties [4]. α-helical AMPs are normally unorganized in a fluid solvent [Figure 1b]. When a peptide comes into touch with a membrane (negatively charged), it could intrude into it and produce transmembrane holes causing membrane instability, depolarization, and cell death [19]. LL-37 and magainins are examples of this class. Magainins were confined from the epidermis of the African tined frog. Xenopus laevis are characteristic of this structural class. Magainin 1 and 2 are 23 residues long and have mild antibacterial activity [20].

3.3. Extended AMPs

Extended peptides lack traditional secondary structures due to their high proline/glycine concentration. The final structure of these peptides is formed through hydrogen bonds and van der Waals interactions with membrane lipids. Tryptophan and proline-rich indolicidin are the most popular individual from the more distant family of cationic peptides [Figure 1c] [4]. Indolicidin has the largest known proportion of tryptophans, with 5 of its 13 residues being tryptophan. H-NMR experiments in anionic SDS and zwitterionic DPC micelles revealed that the proof of indolicidin is depending on its environment [21]. Extended AMPs are peptides that are rich in histidine, glycine, and arginine but lack secondary structural components [19].

3.4. β-hairpin (Loops) AMPs

Peptides belonging to this family exhibit a distinctive loop structure due to the presence of a single bond. β-hairpin AMPs have a hairpin structure interconnected by a type II-turn and stabilized by disulfide

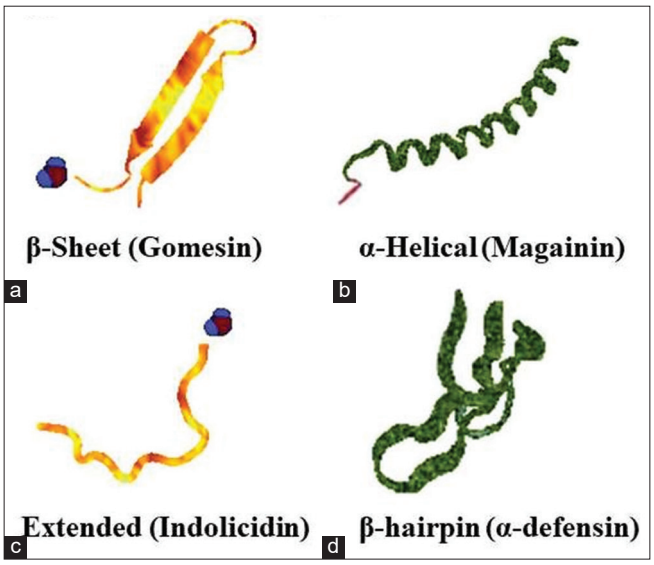

Figure 1: AMPs representation of structural classes: (a) Gomesin, β-sheet, (b) Magainin, α-helical, (c) Indolicidin, extended form, (d) α-defensin, β-hairpin [23].

bonds created between the β-strands [19]. α-defensin is the oldest representative of this class possessing a magnified structure [Figure 1d]. Thanatin is β-hairpin peptide of 21 residues that were derived from the spined soldier beetle. The antiparallel B sheet produced by residues 11 and 18 in solution, as established by H-NMR, is the structure of Thanatin. Thanatin has antimicrobial property apposed Gram-ve and Gram+ve bacteria and fungi [22].

4. CLASSIFICATION

Natural AMPs are challenging to classify due to their diversity. AMPs are divided into three categories: (1) source, (2) activity, and (3) amino acid-rich species [Figure 2].

4.1. Source-based Classification of AMPs

As per the statistical information by AMP database, AMPs sources are animals, amphibians, microbes, and invertebrates. AMPs discovered in oceans have also gotten a lot of interest. Humans, sheep, cattle, and other animals all have AMPs. Cathelicidins and defensin are the two most common AMPs family of Mammalian. Defensins are classified as α, β, and γ-defensin, based on where the disulfide bonds are located [4].

Amphibian AMPs are vital in protecting amphibians from infections that have caused a global drop in amphibian populations [23]. The most well-known amphibian AMP is magainin, which is found in frog skin secretion from the genera Xenopus, Silurana, Hymenochirus, and Pseudhymenochirus, all of which belong to the Pipidae family [24]. AMPs are mostly generated in insect's overweight bodies and blood cells which are one of the primary reasons for their high adaptation to life. Cecropin is a well-known family of insect AMPs and is present in guppy silkworms, drosophila, and bees. Cecropin has anti-inflammatory and anti-cancer properties [25]. Some AMPs such as nisin and gramicidin are derived from bacteria and fungi [26].

4.2. Activity-Based Classification

AMP's activity can be split into 18 categories as per ADP3 database statistics. The inhibitory effect of AMPs against pathogenic bacteria spans a broad spectrum.

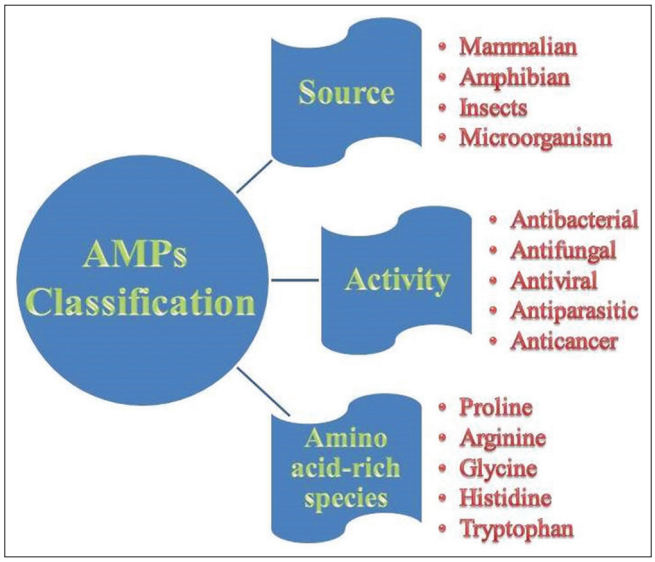

Figure 2: Classification of antimicrobial peptides.

Antifungal peptides (AFPs) target drug-resistant fungal infections. Several AFPs have shown potent antifungal action against common fungal pathogens in clinical medicine. COVID-19, foot and mouth disease, avian influenza virus, and HIV are everlasting risks to human beings [4]. Antiviral peptides kill viruses by blocking virus attachment and fusing of virus-cell membranes, destroying the virus envelope, and slowing virus reproduction [27]. Antiparasitic peptides eliminate parasitic organisms responsible for malaria and leishmaniasis, and AMPs (cathelicidin and temporins-SHd) have high inhibitory activity against parasites [28]. Anticancer peptides have the mechanism of anticancer that seeks immune cells to kill tumor cells and inhibit tumor nutrition and prevent metastasis. *In vitro*, tritrpticin, indolicidin, and puroindoline function as anticancer peptides [29].

4.3. Amino Acid-Rich Species Based Classification

Proline-rich AMPs pass into the bacterial cytoplasm through the inner membrane transporter SbmA and bind to ribosomes [4].

Tryptophan significantly effects on the interface of the lipid bilayer, while arginine is relevant to combine with the bacterial membrane's copious anionic element. In addition, tryptophan residues operate as arginine-rich AMPs' natural aromatic catalysts through ion-pair interactions, enhancing the connections between peptides and membranes [30]. AMPs that are high in histidine have good membrane permeability activity. This peptide breaks down cell membranes by increasing the permeability of bacterial membranes [31]. Naturally found attacins is an example of glycine-rich AMPs [4].

5. MECHANISM OF ACTION

Natural AMPs are often unstable and have a short half-life. Modifying and synthesizing long-acting peptide analogs for possible clinical applications is so vital. The bilayer lipid membranes and how to disrupt the permeability barriers must be studied while designing novel antibacterial peptides. Identifying AMPs and their methods of action can help researchers develop a strategy for developing novel synthetic and effective AMPs [32,33]. The interfacial activity of AMPs, rather than their unique amino acid components or three-dimensional architectures, is responsible for several of their primary activities. The biological functions of AMPs with membrane-permeabilizing capacities are determined by their interfacial characteristics and physical-chemical interactions [Figure 3] [34].

5.1. Membrane Targeting Mechanism

The processes by which AMPs target the membrane can be described using models such as the pole and carpet models.

5.1.1. The pole model

The wormhole model is another name for the toroidal pore model. AMP is inserted vertically in the outer layer of cell to produce a cellular gap with 1–2-nm diameters in this model. The pole model is even separated into the toroidal pore and barrel-stave models [4]. Magainin 2, lacticin Q, and nicin are common instances of this paradigm [35].

AMPs clump together and penetrate the layer of the cell membrane, forming pathways that enable intracellular leakage. AMPs break down cell membrane and cause cell death in severe circumstances. Alamethicin uses this mechanism to execute its pore-forming action [36].

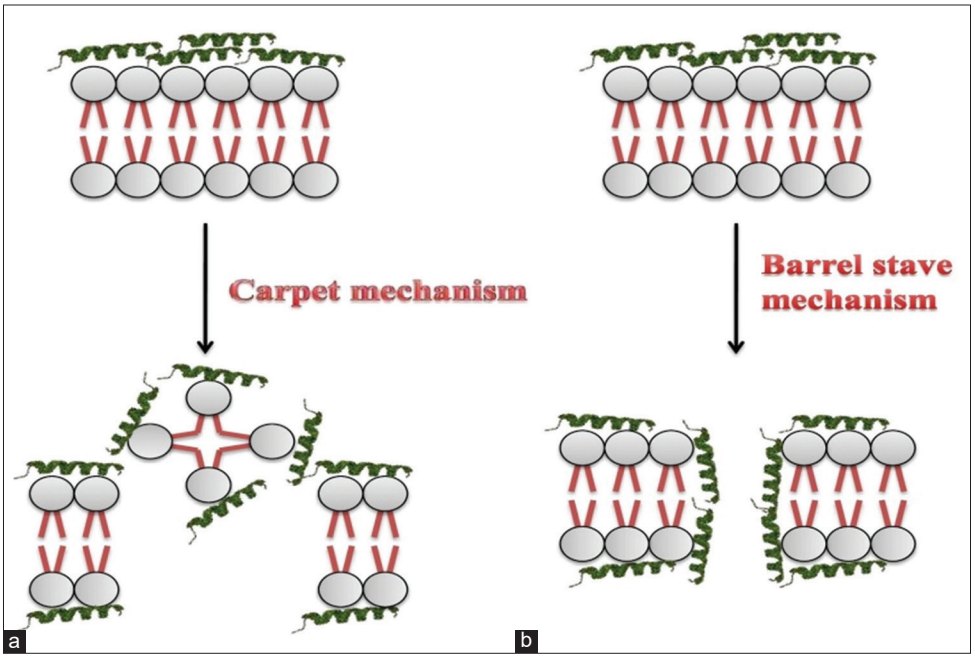

Figure 3: AMPs mechanism of action (a) Carpet mechanism: Peptide forms a layer in the manner of carpet, (b) Barrel staves mechanism: Peptide enters into the membrane in the manner of multimers [53].

5.1.2. Carpet-like model

Similar to the cell membrane, AMPs hydrophilic portion is interacted to the fluid, while the hydrophobic portion is interacted to the phospholipid layer. AMPs have the potential to cover the membrane surface like carpeting and disrupt the cell membrane similarly to how detergent might [4]. Human cathelicidin (LL-37) and AMPs having β-sheet structure utilized this mechanism also contribute in this paradigm [37].

Mechanisms for targeting membranes can be modified further to considerable discrepancies in the lipid composition of bacteria, fungi, and mammal cell membranes. Glycerophospholipids, lysolipids, sphingolipids, and sterols are the most common lipids found in cell membranes [8]. Moreover, the cell membranes of fungi are too anionic and have a greater phosphatidylcholine concentration than mammalian cell membranes [38].

5.2. Non-membrane Targeting Mechanism

Direct penetration and endocytosis are the two methods through which AMPs enter cells. After entering the cytoplasm, AMPs will detect their target and take action. AMPs are categorized as follows based on their target [36].

5.2.1. Protein biosynthesis inhibition

AMPs modulate transcription, translation, and peptide assembly by interacting with molecular chaperones, thereby influencing associated enzymes and effector molecules involved in these processes. Bac7 inhibits protein translation by targeting ribosomes. Bac7 inhibits protein translation by targeting ribosomes while Tur1A prevents protein synthesis in organisms (*Thermus thermophilus and Escherichia coli*) by impeding the shift from the initial to the elongation stage [36].

5.2.2. Nucleic acid biosynthesis inhibition

AMPs can impede nucleic acid synthesis by interfering with critical enzymes. Indolicidin is a Trp-rich AMP comprising 13 amino acids with a C-terminal amidation. It demonstrates specific binding to the basic region of DNA, resulting in the crosslinking of both single-stranded and double-stranded DNA. Moreover, it possesses the capability to inhibit the activity of DNA topoisomerase I [4]. TC24 is an AMP derived from the tongue. It gains entry into the cytoplasm of target cells following the disruption of the cell membrane, where it acts on DNA and RNA strands, causing their fragmentation [39].

5.2.3. Protease activity inhibition

Several AMPs can block a variety of metabolic functions by reducing protease action. Histatin 5 exhibits a strong inhibitory effect on the proteases secreted by both the host and bacteria, displaying potent activity. Microbial serine proteases, elastase, and chymotrypsin are inhibited by the AMPs. Cathelicidin BF, derived from Bungarus fasciatus venom, possesses the ability to inhibit thrombin-induced platelet aggregation and effectively disrupts the function of protease-activated receptor 4 [40].

5.2.4. Cell division inhibition

AMPs prevent cell division by preventing DNA replication and the SOS reaction (DNA damage response), halting the cell cycle or causing chromosomal separation failure [4]. MciZ is a 40 amino acid residue protein that inhibits bacterial cell division, development of Z-rings, and localization [41]. Furthermore, some AMPs have been shown to have negative effects on fungal organelles. Histatin 5 has the capacity to engage with mitochondria, resulting in the production of reactive oxygen species (ROS) and subsequent induction of cell death [36].

6. APPLICATION OF AMPS IN SEMEN FREEZING/ CRYOPRESERVATION

AMPs were utilized for 1st time in boar semen ejaculate [42]. Many researchers looked at the usage of cyclic hexapeptides and synthetic magainin compounds as semen extenders for preserving boar and other species' ejaculates [43]. There was little/no activity on eukaryotic cells while using AMPs, which is a crucial requirement for their use in semen extenders. Hexapeptides exhibit favorable characteristics for their use

as antibiotics in semen extenders during the cryopreservation of human semen samples. These characteristics include proteolytic stability, thermodynamic stability, bacterial selectivity, and sensitivity [5].

Following that a huge number of AMPs were discovered and are now used in a variety of therapeutic and medicinal settings. Researchers initially predicted that AMPs would only have bactericidal effects but later discovered that they also have antifungal, antiviral, anticancer, and immunomodulatory properties. Surprisingly, many studies suggest that a particular AMP can perform all of these functions [4].

Many higher animals, including humans, have inherent immune defense mechanisms and AMPs have been discovered to be essential components. The antimicrobial properties of each AMP differ due to changes in the structure (primary and secondary) of the peptides, which impact their functions [5]. It is the most important way to consider when choosing AMP to replace antibiotics in semen extenders. AMPs were shown to have preserved cationic charge and amphipathicity, which explains their selective action on bacterial membranes. Alexander Fleming extracted lysozyme (130 amino acids) from human saliva in 1929 and it remains the most extensively utilized human-origin antibacterial protein [44]. Several peptides have been discovered with high levels of specific amino acids (phenylalanine, tryptophan, and arginine). As a result, natural protein antibacterial patterns are promising aspirants for developing particular AMPs in clinical and medical uses [4]. There were several alternative approaches in place of antibiotics in semen extenders such as doing single-layer centrifugation (SLC) under more stringent aseptic settings, which are very useful in minimizing bacterial load, but SLC has its own set of restrictions [44]. Numerous research studies have explored different AMPs as potential substitutes for conventional antibiotics in semen diluter. Tables 1 and 2 summarize important studies on AMPs in human semen and different animal and their consequences. Some AMPs are used specifically in the field of reproduction.

AMPs are cationic and have direct antibacterial and antifungal activity. AMPs have biological characteristics including anti-inflammatory, immunomodulatory, and immunosuppressive activity associated with lipopolysaccharide [45]. Due to the increasing prevalence of antibiotic resistance in conventional antibiotics, cationic AMPs have emerged as a more advantageous alternative for use in semen cryopreservation [46]. This study investigated the cryopreservation of two specific synthetic cationic AMPs (c-WWW and c-WFW), which fall under the category of cyclic hexapeptides, utilizing a boar semen extender [47]. Speck utilized magainin II (MK5E), which is a synthetic helical amide analog for boar semen sample preservation *in vitro*. The findings demonstrated the enhanced efficacy of the tested approach against both Gram-positive and Gram-negative bacteria commonly found in boar sperm.

Human parotid secretory protein could be employed as a semen extender by sharing structural similarities with bactericidal as well as two other proteins permeability-increasing protein and lipopolysaccharide-binding protein [48]. Researchers have reported that GL13K exhibits activity against bacteria within both biofilm communities and monospecies environments [10]. GL13K was observed to significantly reduce the cell count in biofilms formed under both aerobic and anaerobic conditions. As a result of these findings, GL13K may be a source of AMPs that can be used to replace antibiotics in the semen extender [49].

7. EFFECT OF AMP BETA DEFENSIN 1 ON CRYOPRESERVED SEMEN OF GOAT

In our laboratory, we have used β-defensin, an AMP that possesses both innate and adaptive immune response and has antimicrobial activity and motility enhancer as an additive in goat semen diluent. AMP was designed using tool NCBI/uniprot/ORF finder/kolaskar and Tongaonkar B-cell epitope prediction and Bepipred 2.0. Microbial growth was observed nil with antibiotic plus AMP and AMP-fortified semen alone but there was microbial growth in control group (without antibiotic and AMP) and also little microbial growth was in the antibiotic group. Thirty ejaculates were collected from Barbari bucks and were extended with tris-citric acid-fructose diluent and beta-defensin 1 in different concentrations (0, 10, 15 and 20 μM/mL) were added in sperm preparation medium. Sperm concentrations were adjusted to 4×10^8 mL^{-1} and diluted semen samples were equilibrated at 5°C for 4 h before being cryopreserved in liquid nitrogen. Post-thaw samples were evaluated for sperm motility, live sperm count, acrosomes integrity, hypo osmotic swelling (HOS) positive spermatozoa, malondialdehyde production, DNA integrity by Tunel assay, and transmembrane mitochondria membrane potential (MMP). Semen sample with 15 (μM/mL) beta-defensin 1 was found significantly higher ($P < 0.05$) percentage of progressive motility, viability, HOS-positive spermatozoa, acrosome integrity, DNA integrity, MMP, and antioxidant potential (MDA) than control group. Beta-defensin 1 protects sperm membrane lipid peroxidation by lowering MDA production by reducing the harmful effect of ROS. It also protects DNA and mitochondrial damage from oxidative and freezing stress during cryopreservation when specific

Table 1: Different types of AMPs used in human semen.

AMPs	Derived species	Structure	Suitable concentration	Result	References
Human β-defensin 118 (DEFB118)	Human	β-sheet	10 μg/mL	It provides protection to sperm cells, safeguarding them from potential attacks by microorganisms present in both the male and female reproductive tracts	[54]
Nisin	*L. lactis*	Polycyclic	200 μg/mL	Considering its antimicrobial properties and ability to affect sperm viability, nisin holds potential for future development as a powerful vaginal contraceptive in human populations	[55]
Subtilosin	*B. subtilis*	Cyclic	64.5 μg/mL	In a dose-dependent manner, it inhibits the motility and forward progression of human spermatozoa, indicating its potential as a broad-spectrum spermicidal agent	[56]
LL-37	Human cathelicidin	α-helical	0.36 μM	It inhibits human sperm fertilizing ability	[57]
17BIPHE2	Human engineered cathelicidin	α-helical	32.4 μM	It is an effective as spermicidal and microbicide against Neisseria gonorrhoeae	[58]

B. subtilis: Bacillus subtilis, L. lactis: Lactococcus lactis. AMPs: Antimicrobial peptides.

Table 2: AMPs used in different animals species.

AMPs	Derived species	Animal species	Structure	Suitable concentration	Result	References
Nisin	*L. lactis*	Rat	Polycyclic	50 µg/mL	Effective as antimicrobial agent and also enhanced sperm viability	[55]
LL-37	Human cathelicidin	Mouse	α-helical	0.36 µM	It inhibits mouse sperm fertilizing ability	[57]
Hexapeptides (c-WFW, c-WWW)	Synthetic	Boar	Cyclic	10 µM c-WFW, 20 µM/c-WWW	Throughout the preservation period, the quality of sperm remained similar to that of the standard extender	[53]
Magainin II amide derivative (MK5E)	Synthetic (frog)	Boar	Helical	20 µM	It led to a reduction in the proportion of spermatozoa exhibiting progressive motility	[53]
PG1	Porcine (leukocytes)	Boar	β-hairpin	2.5 µg/mL	Protegrin 1 exhibited a significant (P<0.05) efficacy in controlling the bacterial load, albeit at the expense of reduced sperm viability due to cytotoxic effects	[59]
PMAP-37	Pig (bone marrow)	Boar	α-helical	3 µM	No significant impact on boar sperm quality was observed, while it demonstrated effectiveness in controlling the bacterial load	[60]
Proline-arginine-rich antimicrobial peptide (PR-39)	Porcine (small intestine)	Boar	-	10 µM	The concentration of PR-39 (10 µM) significantly improved sperm viability but not effective to check bacterial growth. On the other hand, at a concentration of 20 µM, PR-39 successfully prevented bacterial overgrowth but exhibited significant cytotoxicity toward spermatozoa	[60]
PBD1 and PBD2	Porcine	Boar	β-sheet	3 µM	It has inhibitory effect on bacterial growth	[61]
Bacteriocin	*L. salivarius*	Boar	-	1%	Bacteriocin showed no impact on sperm quality. The addition of bacteriocin resulted in a 50% reduction in the growth of *E. coli* in BTS-extended semen	[62]
Beta defensin 1	*C. hircus*	Goat	β-hairpin	15 µM	Effective to get higher post-thaw quality of goat semen	[50]
LL-37	*C. hircus*	Goat	α-helical	10 µM	Improved cryopreserved semen quality and fertility	[51]

C. hircus: Capra hircus, L. salivarius: Lactobacillus salivarius, L. lactis: Lactococcus lactis, E. coli: Escherichia coli. PG1: Protegrin 1, PBD1 and PBD2: Porcine beta defensins-1 and 2, PMAP: Porcine myeloid antimicrobial peptide, AMPs: Antimicrobial peptides.

concentration of beta-defensin 1 was added in goat semen diluent. In conclusion, supplementation of beta-defensin 1 in freezing medium was effective to get higher post-thaw quality of goat semen [50].

8. EFFECT OF AMP LL-37 FORTIFICATION ON POST-THAW QUALITY OF BUCK SEMEN

LL-37 is a cationic AMP, member of cathelicidin family of AMPs functions as the immune system's initial line of defense in epithelial cells and is also essential for healing infected sperm cells. A wide variety of bacteria (Gram +ve and –ve), some fungi, and also viruses are susceptible to LL-37 AMPs activity. Semen cryopreservation is crucial for AI technique and typically requires diluents with additives that exhibit immunomodulatory actions and show antimicrobial activity. The objective of present study was to assess the effect of LL-37 AMP in different concentrations on seminal parameters, oxidative status, and antimicrobial activity after cryopreservation of Barbari buck sperm. LL-37AMP of 19 bp sequence of *Capra hircus* was designed and synthesized using the bioinformatic tools. Ejaculates (N30) were collected from Barbari buck using an artificial vagina and were extended using a diluent of tris-citric acid-fructose with egg yolk (10%) and glycerol (6%). Different concentrations (0, 10, 20, 30 µM/mL) of LL-37 were tested in sperm preparation medium. The sperm motility, live sperm count, acrosomes integrity, HOS-positive spermatozoa malondialdehyde (MDA), DNA integrity MMP, and calcium assay differed significantly ($P < 0.05$) at different concentrations of LL-37 and were significantly ($P < 0.05$) highest in 10 µM of LL-37. Microbial growth was observed nil with antibiotic plus LL-37 and LL-37 fortified semen alone but there was microbial growth in control group (without antibiotic and LL-37) and also little microbial growth was in antibiotic group. LL-37 aids in keeping spermatozoa motile during cryopreservation and is crucial for improving live count, undamaged acrosomes, and HOS-positive spermatozoa. LL-37 also protects against microbes. It also protects DNA and mitochondrial damage from oxidative and freezing stress during cryopreservation when specific concentration of LL-37 was added in goat semen diluent. In conclusion, LL-37 can be used to check microbial load and improved cryopreserved semen quality and fertility [51].

9. INFLUENCE OF AMP ON *IN VIVO* FERTILITY POTENTIAL (AI)

Intra-cervical AI was used to get maximum benefits. For intra-cervical AI, the estrus goat is lifted from the back for clear visualization of genitalia. A lubricated glass vaginal speculum was inserted through the vagina for visualization of cervical opening under sunlight. Then frozen thawed semen straw was inserted through vaginal speculum

which passed through cervical opening and semen was deposited there and waited for 2–3 min. The insemination was carried out 12 h after the detection of estrus and repeated after 12 h of first insemination. The conception rate was estimated on an actual kidding basis. The kidding percentage is significantly more in the treatment group (50%) compared to control group (35%). Hence, we can conclude that specific concentrations of different types of additives used in goat semen diluent had significantly higher post-thaw *in vitro* and *in vivo* qualities.

AMPs with several functions were produced in mammalian organs as well as the entire digestive, respiratory, and reproductive process are the most targeted [4]. Numerous AMPs are generated by sperm cells and seminal plasma, which are subsequently transmitted to the female reproductive tract. In addition to this, the female reproductive tract itself has the capability to produce AMPs as a protective measure against infections and to fulfill immunomodulatory functions. The AMP (lipophilin) produced from the female reproductive tract was found to have wide antibacterial action. Hence, it can also be utilized as a cryopreservation semen extender [4].

10. FUTURE PROSPECTIVE

Researchers must gain insight into the molecular mechanism and structure-activity relationship of these AMPs to develop novel cationic peptide medications. When designing synthetic cationic AMPs, it is crucial to take into account the unique properties of these peptides, specifically their cationic nature and amphipathicity, despite the variability in sequence and structure observed among them [52]. The cationic nature of AMPs plays a vital role in their ability to selectively bind to the negatively charged outer surface of bacterial cell membranes while minimizing interaction with the neutral outer surface of eukaryotic cell membranes. The amphipathicity of AMPs influences whether it may enter into the membranes of bacterial cells to generate hydrophobic channels or pores. AMPs affect the integrity of bacterial cell membranes. The varying susceptibilities of different microbial membranes to membrane permeability induced by individual peptides present a significant challenge. AMPs may also have hemolytic properties. Designing unique and perfect AMPs with significant antibacterial efficacy and no hemolytic activity is a difficult task [52]. Natural AMPs have a limited half-life in circulation and are often non-stable. Long-term utilization of AMPs may result in cell growth suppression, cytotoxicity of host cells, and other unspecified adverse effects. The search for the ideal AMP is a complex, resource-intensive, and challenging pursuit; however, AMPs present a remarkable opportunity for a promising and favorable future.

11. CHALLENGES OF AMPS

According to a growing body of evidence, long-term usage of antibiotics causes bacteria to develop drug resistance. Numerous drug-resistant pathogenic strains correlate to each of these standard antibiotics [34]. As a result, finding a new antibiotic is getting increasingly challenging. AMPs exhibit a broad antibacterial spectrum as well as excellent bactericidal activity. The development of AMPs presents us with a unique chance to develop AMP medication candidates in place of standard antibiotics [2]. The fact is that the Food and Drug Administration has authorization for various AMPs. Typically, the majority of these peptides are considered too small to be suitable for clinical use as drugs.

12. CONCLUSION

Living organisms face a continuous threat from numerous microorganisms that strive to occupy the same environmental niche. To counter the significant microbial threat, the majority of cells produce naturally occurring antibiotic-like molecules that directly eliminate or impede the growth of foreign microorganisms. Over 2,500 AMPs have been discovered in diverse organisms such as single-celled organisms, plants, insects, and animals. These molecules exhibit selective antimicrobial activity against a wide range of organisms, primarily due to their potent electronic interaction with negatively charged membranes. In vertebrates, certain AMPs not only contribute to host defense by modulating cellular immunity but also enhance post-thaw sperm fertility. The escalating concern of antibiotic resistance has sparked interest in utilizing AMPs as next-generation anti-infective, offering a more targeted approach to combating pathogens in semen diluents to improve cryosurvivability and sperm fertility.

13. ACKNOWLEDGMENT

The authors are thankful to the Department of Biotechnology, Ministry of Science, and Technology, New Delhi and the Director, ICAR-CIRG, Makhdoom.

14. AUTHORS' CONTRIBUTIONS

The manuscript was designed and written by MK and RR, while AB prepared the figures. The manuscript was reviewed by all authors.

15. FUNDING

There is no funding to report.

16. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

17. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

18. DATA AVAILABILITY

All data generated and analyzed are included in this review article.

19. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Lei J, Sun L, Huang S, Zhu C, Li P, He J, *et al*. The antimicrobial peptides and their potential clinical applications. Am J Transl Res 2019;11:3919-31.
2. Starr CG, Maderdrut JL, He J, Coy DH, Wimley WC. Pituitary adenylate cyclase-activating polypeptide is a potent broad-spectrum antimicrobial peptide: Structure-activity relationships. Peptides 2018;104:35-40.
3. Pontes JT, Toledo Borges AB, Roque-Borda CA, Pavan FR. Antimicrobial peptides as an alternative for the eradication of bacterial biofilms of multi-drug resistant bacteria. Pharmaceutics 2022;14:642.

4. Huan Y, Kong Q, Mou H, Yi H. Antimicrobial peptides: Classification, design, application and research progress in multiple fields. Front Microbiol 2020;11:582779.
5. Hall-Stoodley L, Stoodley P, Kathju S, Høiby N, Moser C, Costerton JW, *et al.* Towards diagnostic guidelines for biofilm-associated infections. FEMS Immunol Med Microbiol 2012;65: 127-45.
6. Sahoo A, Swain SS, Behera A, Sahoo G, Mahapatra PK, Panda SK. Antimicrobial peptides derived from insects offer a novel therapeutic option to combat biofilm: A review. Front Microbiol 2021;12:661195.
7. Hollmann A, Martinez M, Maturana P, Semorile LC, Maffia PC. Antimicrobial peptides: Interaction with model and biological membranes and synergism with chemical antibiotics. Front Chem 2018;6:204.
8. Li J, Koh JJ, Liu S, Lakshminarayanan R, Verma CS, Beuerman RW. Membrane active antimicrobial peptides: Translating mechanistic insights to design. Front Neurosci 2017;11:73.
9. Vickram AS, Kuldeep D, Archana K, Parameswari R, Pathy MR, Iqbal HM, *et al*. Antimicrobial peptides in semen extenders: A valuable replacement option for antibiotics in cryopreservation-a prospective review. J Exp Biol Agric Sci 2017;5:578-88.
10. Bussalleu E, Sancho S, Briz MD, Yeste M, Bonet S. Do antimicrobial peptides PR-39, PMAP-36 and PMAP-37 have any effect on bacterial growth and quality of liquid-stored boar semen? Theriogenology 2017;89:235-43.
11. Moretta A, Scieuzo C, Petrone AM, Salvia R, Manniello MD, FrancoA, *et al*. Antimicrobial peptides: A new hope in biomedical and pharmaceutical fields. Front Cell Infect Microbiol 2021;11:668632.
12. Stoica P, Chifiriuc MC, Rapa M, Lazăr V. Overview of biofilm-related problems in medical devices. In: Biofilms and Implantable Medical Devices. United Kingdom: Woodhead Publishing; 2017. p. 3-23.
13. Chen GF, Xu TH, Yan Y, Zhou YR, Jiang Y, Melcher K, *et al.* Amyloid beta: Structure, biology and structure-based therapeutic development. Acta Pharmacol Sin 2017;38:1205-35.
14. De La Fuente-Núñez C, Cardoso MH, de Souza Cândido E, Franco OL, Hancock RE. Synthetic antibiofilm peptides. Biochim Biophys Acta 2016;1858:1061-9.
15. Gomes B, Augusto MT, Felício MR, Hollmann A, Franco OL, Gonçalves S, *et al*. Designing improved active peptides for therapeutic approaches against infectious diseases. Biotechnol Adv 2018;36:415-29.
16. Kountouras J, Deretzi G, Gavalas E, Zavos C, Polyzos SA, Kazakos E, *et al*. A proposed role of human defensins in helicobacter pylori-related neurodegenerative disorders. Med Hypotheses 2014;82:368-73.
17. Vernen F, Harvey PJ, Dias SA, Veiga AS, Huang YH, Craik DJ, *et al*. Characterization of tachyplesin peptides and their cyclized analogues to improve antimicrobial and anticancer properties. Int J Mol Sci 2019;20:4184.
18. Imura Y, Nishida M, Ogawa Y, Takakura Y, Matsuzaki K. Action mechanism of tachyplesin I and effects of PEGylation. Biochim Biophys Acta 2007;1768:1160-9.
19. Hollmann A, Martínez M, Noguera ME, Augusto MT, Disalvo A, Santos NC, *et al*. Role of amphipathicity and hydrophobicity in the balance between hemolysis and peptide-membrane interactions of three related antimicrobial peptides. Colloids Surf B Biointerfaces 2016;141:528-36.
20. Bechinger B, Juhl DW, Glattard E, Aisenbrey C. Revealing the mechanisms of synergistic action of two magainin antimicrobial peptides. Front Med Technol 2020;2:615494.
21. Rozek A, Friedrich CL, Hancock RE. Structure of the bovine antimicrobial peptide indolicidin bound to dodecylphosphocholine and sodium dodecyl sulfate micelles. Biochemistry 2000;39:15765-74.
22. Dash R, Bhattacharjya S. Thanatin: An emerging host defense antimicrobial peptide with multiple modes of action. Int J Mol Sci 2021;22:1522.
23. McMillan KA, Coombs MR. Review: Examining the natural role of amphibian antimicrobial peptide magainin. Molecules 2020;25:5436.
24. Conlon JM, Mechkarska M. Host-defense peptides with therapeutic potential from skin secretions of frogs from the family *Pipidae*. Pharmaceuticals (Basel) 2014;7:58-77.
25. Dutta P, Sahu RK, Dey T, Lahkar MD, Manna P, Kalita J. Beneficial role of insect-derived bioactive components against inflammation and its associated complications (colitis and arthritis) and cancer. Chem Biol Interact 2019;313:108824.
26. Cao J, de la Fuente-Nunez C, Ou RW, Torres MT, Pande SG, Sinskey AJ, *et al*. Yeast-based synthetic biology platform for antimicrobial peptide production. ACS Synth Biol 2018;7:896-902.
27. Jung Y, Kong B, Moon S, Yu SH, Chung J, Ban C, *et al.* Envelope-deforming antiviral peptide derived from influenza virus M2 protein. Biochem Biophys Res Commun 2019;517:507-12.
28. El-Dirany R, Shahrour H, Dirany Z, Abdel-Sater F, Gonzalez-Gaitano G, Brandenburg K, *et al*. Activity of anti-microbial peptides (AMPs) against *Leishmania* and other parasites: An overview. Biomolecules 2021;11:984.
29. Arias M, Haney EF, Hilchie AL, Corcoran JA, Hyndman ME, Hancock RE, *et al*. Selective anticancer activity of synthetic peptides derived from the host defence peptide tritrpticin. Biochim Biophys Acta Biomembr 2020;1862:183228.
30. Walrant A, Bauzá A, Girardet C, Alves ID, Lecomte S, Illien F, *et al*. Ionpair-π interactions favor cell penetration of arginine/tryptophan-rich cell-penetrating peptides. Biochim Biophys Acta Biomembr 2020;1862:183098.
31. Dong N, Wang C, Zhang T, Zhang L, Xue C, Feng X, *et al*. Bioactivity and bactericidal mechanism of histidine-rich β-hairpin peptide against Gram-negative bacteria. Int J Mol Sci 2019;20:3954.
32. Graf M, Wilson DN. Intracellular antimicrobial peptides targeting the protein synthesis machinery. Adv Exp Med Biol 2019;1117:73-89.
33. Aisenbrey C, Marquette A, Bechinger B. The mechanisms of action of cationic antimicrobial peptides refined by novel concepts from biophysical investigations. Adv Exp Med Biol 2019;1117:33-64.
34. Amso Z, Hayouka Z. Antimicrobial random peptide cocktails: A new approach to fight pathogenic bacteria. Chem Commun 2019;55:2007-14.
35. Omardien S, Drijfhout JW, Vaz FM, Wenzel M, Hamoen LW, Zaat SA, *et al*. Bactericidal activity of amphipathic cationic antimicrobial peptides involves altering the membrane fluidity when interacting with the phospholipid bilayer. Biochim Biophys Acta Biomembr 2018;1860:2404-15.
36. Singh T, Choudhary P, Singh S. Antimicrobial peptides: Mechanism of action. In: Insights on Antimicrobial Peptides. London: IntechOpen; 2022. p. 23.
37. Corrêa JA, Evangelista AG, de Melo Nazareth T, Luciano FB. Fundamentals on the molecular mechanism of action of antimicrobial peptides. Materialia 2019;8:100494.
38. Faruck MO, Yusof F, Chowdhury S. An overview of antifungal peptides derived from insect. Peptides 2016;80:80-8.
39. He SW, Zhang J, Li NQ, Zhou S, Yue B, Zhang M. A TFPI-1 peptide that induces degradation of bacterial nucleic acids, and inhibits bacterial and viral infection in half-smooth tongue sole, *Cynoglossus semilaevis*. Fish Shellfish Immunol 2017;60:466-73.
40. Shu G, Chen Y, Liu T, Ren S, Kong Y. Antimicrobial peptide cathelicidin-BF inhibits platelet aggregation by blocking protease-activated receptor 4. Int J Pept Res Ther 2019;25:349-58.
41. Cruz GF, de Araujo I, Torres MD, de la Fuente-Nunez C, Oliveira VX Jr., Ambrosio FN, *et al*. Photochemically-generated silver chloride nanoparticles stabilized by a peptide inhibitor of cell division and its antimicrobial properties. J Inorg Organomet Polym Mater 2020;30:2464-74.

42. Li J, Zhao W, Zhu J, Ju H, Liang M, Wang S, *et al*. Antioxidants and oxidants in boar spermatozoa and their surrounding environment are associated with AMPK activation during liquid storage. Vet Sci 2023;10:214.
43. Varella Coelho ML, de Souza Duarte AF, do Carmo de Freire Bastos M. Bacterial labionin-containing peptides and sactibiotics: Unusual types of antimicrobial peptides with potential use in clinical settings (a Review). Curr Top Med Chem 2017;17:1177-98.
44. Morrell JM, Wallgren M. Alternatives to antibiotics in semen extenders: A review. Pathogens 2014;3:934-46.
45. Mulder KC, Lima LA, Miranda VJ, Dias SC, Franco OL. Current scenario of peptide-based drugs: The key roles of cationic antitumor and antiviral peptides. Front Microbiol 2013;4:321.
46. Zasloff M. Antimicrobial peptides: Do they have a future as therapeutics? In: Antimicrobial Peptides: Role in Human Health and Disease. Cham: Springer; 2016. p. 147-54.
47. Speck S, Courtiol A, Junkes C, Dathe M, Müller K, Schulze M. Cationic synthetic peptides: Assessment of their antimicrobial potency in liquid preserved boar semen. PLoS One 2014;9:e105949.
48. Li Z, Zhang D, He Y, Ding Z, Mao F, Luo T, *et al.* Lipopolysaccharide compromises human sperm function by reducing intracellular cAMP. Tohoku J Exp Med 2016;238:105-12.
49. Gorr SU, Brigman HV, Anderson JC, Hirsch EB. The antimicrobial peptide DGL13K is active against drug-resistant gram-negative bacteria and sub-inhibitory concentrations stimulate bacterial growth without causing resistance. PLoS One 2022;17:e0273504.
50. Hirt H, Gorr SU. Antimicrobial peptide GL13K is effective in reducing biofilms of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2013;57:4903-10.
51. Ranjan R, Kumar M, Swain DK, Singh SP, Kharche SD. Effect of Antimicrobial Peptide on Cryopreserved Semen of Goat. XXXI Annual Conference and Symposium, Society of Animal Physiologists of India; 2023. p. 2.
52. Kumar M, Ranjan R, Bharadwaj A. Effect of Antimicrobial Peptide LL-37 Fortification on Post thaw Quality of Buck Semen. XXXI Annual Conference and symposium, Society of Animal Physiologists of India; 2023. p. 3.
53. Moravej H, Moravej Z, Yazdanparast M, Heiat M, Mirhosseini A, Moghaddam MM, *et al.* Antimicrobial peptides: Features, action, and their resistance mechanisms in bacteria. Microb Drug Resist 2018;24:747-67.
54. Schulze M, Junkes C, Mueller P, Speck S, Ruediger K, Dathe M, *et al.* Effects of cationic antimicrobial peptides on liquid-preserved boar spermatozoa. PLoS One 2014;9:e100490.
55. Yenugu S, Hamil KG, Radhakrishnan Y, French FS, Hall SH. The androgen-regulated epididymal sperm-binding protein, human beta-defensin 118 (DEFB118) (formerly ESC42), is an antimicrobial beta-defensin. Endocrinology 2004;145:3165-73.
56. Aranha C, Gupta S, Reddy KV. Contraceptive efficacy of antimicrobial peptide Nisin: *In vitro* and *in vivo* studies. Contraception 2004;69:333-8.
57. Sutyak KE, Anderson RA, Dover SE, Feathergill KA, Aroutcheva AA, Faro S, *et al.* Spermicidal activity of the safe natural antimicrobial peptide subtilosin. Infect Dis Obstet Gynecol 2008;2008:540758.
58. Srakaew N, Young CD, Sae-wu A, Xu H, Quesnel KL, di Brisco R, *et al.* Antimicrobial host defence peptide, LL-37, as a potential vaginal contraceptive. Hum Reprod 2014;29:683-96.
59. Lee SG, Kiattiburut W, Khongkha T, Schinkel SC, Lunn Y, Decker AP, *et al.* 17BIPHE2, an engineered cathelicidin antimicrobial peptide with low susceptibility to proteases, is an effective spermicide and microbicide against *Neisseria gonorrhoeae*. Hum Reprod 2022;37:2503-17.
60. Sancho S, Briz M, Yeste M, Bonet S, Bussalleu E. Effects of the antimicrobial peptide protegrine 1 on sperm viability and bacterial load of boar seminal doses. Reprod Domest Anim 2017;52 Suppl 4:69-71.
61. Puig-Timonet A, Castillo-Martín M, Pereira BA, Pinart E, Bonet S, Yeste M. Evaluation of porcine beta defensins-1 and-2 as antimicrobial peptides for liquid-stored boar semen: Effects on bacterial growth and sperm quality. Theriogenology 2018;111:9-18.
62. Jakop U, Hensel B, Orquera S, Rößner A, Alter T, Schröter F, *et al.* Development of a new antimicrobial concept for boar semen preservation based on bacteriocins. Theriogenology 2021;173:163-72.

How to cite this article:
Kumar M, Ranjan R, Bhardwaj A. Antimicrobial peptide and their role in sperm cryopreservation. J App Biol Biotech. 2024;12(3):8-16.
DOI: 10.7324/JABB.2024.151890