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Review of Livestock Semen Extension and Cryopreservation of Spermatozoa

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ABSTRACT

The need for livestock sustainability and conservation of genetic resources, artificial dissemination seeing to be a viable tool/means in achieving this whole paying much attention to effective semen extension and cryopreservation techniques. The study reviewed the challenges associated with semen extension and cryopreservation and solutions were proffered to the identified challenges. The research community have observed that in the process of extension and cryopreservation of semen, the specimen is predisposed to detrimental effects of ice crystal formation, hyper-osmosis, alterations in the integrity of sperm cells and denaturation, important to the problem associated with cold stock during cooling, and relative in the availability of commercial extenders and powered especially in the developing countries. The solutions proffered include the embrace of using phytogenic and natural products in semen extension and research stations where alternative power sources could be implored. It is concluded that for effective conservation of genetic resources and breeding, assisted reproductive technologies with vital emphasis on quality semen extension and cryopreservation in livestock should be embraced.

Keywords: Assisted reproductive technologies, Cryopreservation, Extension, Genetic resources, Semen,

INTRODUCTION

The need to improve the efficiency and sustainability of producing animals for food in the face of a fast increasing world population cannot be overemphasized. Increasing the fertility and liveability of livestock around the world is pertinent for overcoming the problem of food insecurity. Besides, improving the knowledge of mechanisms and challenges of reproductive technologies are vital for refining the viability of the livestock industry (Matemilola and Elegbede, 2017; Akintunde *et al.,* 2020, 2021).

The artificial insemination process is a procedure in which sperm is obtained from males/bulls, processed, deposited, and manually inserted into the female reproductive tract at the appropriate time for reproduction. AI has become one of the most important techniques for the genetic improvement of farm animals because sperm from genetically superior sires/males is preferentially used to artificially inseminate female animals (Gibbons *et al.*, 2019).

Gibbons *et al.* (2019) reported the artificial insemination has changed the small ruminant industry because it promotes increased genetic improvement, better control of reproduction and sexually transmitted diseases, the dissemination of valuable genetics, and the preservation of the genetics of endangered breeds.

Fertility preservation of animal semen may be done through freezing or chilling equipment for specified period of time. In most cases, samples of blood, semen and others materials are collected and checked under a microscope in the laboratory to find out how healthy they are. The process of preserving sperm or other related materials is called sperm cryopreservation or sperm banking (Olurode and Ajala, 2016).

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Semen may be successfully used for a very long time after cryopreservation. For human cryopreserved sperm, the longest reported successful storage is 24 years (Zhang, 2017). A cryopreservation spermatozoon is a sequential process of reduction in temperature, dehydration of the cell, freezing, storage then thawing. Unlike other cells in the body, sperm cells could be less sensitive to their cryopreserving damage due to their low water content and high fluidity of the membranes. Hence, the method chosen for preserving sperm is important to ensure its fertility when required for use (Olurode and Ajala, 2016).

Need for Semen Extension and Cryopreservation in Livestock Industry

Semen extension and sperm cryopreservation are important methods in the development of biotechnological reproduction of livestock globally. The techniques have been associated with challenges arising from detrimental sperm integrity due to alterations to the membrane structure-function and cell metabolism, metabolic decoupling, ionic imbalance, activation of proteases, cellular acidosis, deprivation of energy, membrane phase transition, destabilization of the cytoskeleton, and production of free radicals or reactive oxygen species (ROS) (Ndubuisi-Ogbonna *et al.*, 2021).These occur during the process of freezing, as sperms are predisposed to detrimental effects of ice crystal formation, hyper-osmolality, alterations in the cell volume, and protein denaturation. This situation has arguably led to challenges in productive reproduction of targeted livestock. For example, the West African dwarf breed of goat which is the most popular genotype in Nigeria (supplying excellent quality meat, milk, skin and other products), and adjudged one of the most prolific in the world, and with a remarkably high reproductive potential, has been preserved over the years through the natural mating method (Olurode and Ajala, 2016). Nevertheless, widespread of diseases, decrease offspring generation interval, and low income generation, is part of the problems associated with this method.

Previous studies have focused on the methods of conducting semen cryopreservation, its benefits and growth, among other issues but there is need to explore more methods, materials and their applications in a view to solving the associated problems (Daramola *et al.*, 2016; Ndubuisi-Ogbonna *et al*., 2021). The situation could be worst if efforts are not intensified to proffering lasting solutions. A situation where few semen extenders are known and used for a long time would result to several challenges. Also, problem with electricity has been a bane in assisted reproductive techniques in Nigeria.

Techniques for Semen Extension and Cryopreservation of Livestock Spermatozoa

Semen extender is a liquid diluent that could be added to semen to preserve its fertilizing ability. These extenders may be categorized as conventional and non-conventional. Conventional or traditional semen extenders contain protein especially from animal source. While skimmed milk is extracted from certain animals, egg york extracts are sourced from poultry birds. These are the two commonly used conventional semen extenders (Khalifa and Khalil, 2016; Ugur *et al*., 2019). Kulaksiz *et al.* (2011) reported that, semen is commonly diluted with conventional extenders such as Tris plus egg yolk, glucose phosphate solution, egg yolk citrate solution, homogenized whole milk, fresh and dried skim milk, lactose solution and commercial diluents.

Khalifa and Khalil (2016) further observed that sodium citrate, Tris or milk-based extenders produced maximal result when ovine liquid semen was stored at 5°C during a short period (2 days). Moreover, Quan et al. (2016) and Gundogan *et al.* (2010)

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reported better sperm motility and integrity of sperm membrane in a Tris-based extender and in sodium citrate and skimmed milk extenders. On the other hand, non-conventional semen extenders are the modern day extenders such as sugar cane, honey bee, soya milk, salt of sodium chloride, and saline solution, among others which have been proven to facilitate sperm motility (Okukpe *et al.,* 2012; Khalifa and Khalil, 2016; El-Sheshtawy *et al.,* 2016).

Semen presentation techniques

In the past, the natural method of keeping a preferred breed of animal was the only practice. However, as technology improved, two popular techniques for semen storage – chilling, also known as freezing, and cryopreservation emerged. Chilling or freezing is divided into slow and rapid freezing. The slow freezing technique as proposed by Behrman and Sawada (1966) consists of progressive sperm cooling over a period of 2–4 h in two or three steps, either manually or automatically using a semi-programmable freezer. The manual method is performed by simultaneously decreasing the temperature of the semen while adding a cryoprotectant in a stepwise manner and after plunging the samples into liquid nitrogen. In spite of the success recorded through slow freezing, there were some challenges with its manual techniques which resorted to programmable freezers (Holt, 2000).

The rapid freezing was introduced by Sherman (1990). This technique requires direct contact between the straws and the nitrogen vapours for 8–10min and immersion in liquid nitrogen at −196°C. Inside nitrogen vapors, there is a thermal gradient, as a function of the distance and the volume of the liquid below. The sample is initially mixed in dropwise manner with equal volume of cold cryoprotectant; the mixture is loaded into the straws and left to incubate at 4◦C for 10 minutes. The straws are then placed at a distance of 15–20 cm above the level of liquid nitrogen (−80◦C) for 15min; after this stage, the straws are immersed in liquid nitrogen. During cooling it is preferable to place the straws in horizontal position to minimize the heat difference between the two end ends. The semen chilling technique is usually stored at 4-5°C for 3 days for maximum and best results, whereas during the cryopreservation technique, semen is exposed to freezing for 3 h at 4°C (Bustani and Baiee, 2021). Meanwhile, it is filled into 0.25-mL straws and eventually preserved and stored in liquid nitrogen for years (Baiee *et al.,* 2017). Therefore, the crucial factors for long-term semen preservation to retain its quality include cooling for 2-3h, adding a cryoprotectant and freezing in liquid nitrogen (Baiee *et al.,* 2018).

Benefits of semen cryopreservation

Sperm cryopreservation is an important procedure in the development of biotechnological reproduction with positive impact on livestock production. Cryopreserved semen from the best breeding animals can be used for artificial insemination to improve livestock production. This technique has also been used for prevention of genital infections transmittable via natural mating as well as breeding progress and success by rapid spreading of valuable genes. Relating to non-food (pet) animals, the avoidance of travel and quarantine restrictions and the conservation of genetic resources (Yanez-Ortiz *et al.*, 2021) are possible through cryopreservation of semen.

Additionally, cryopreservation of semen has made long-term storage of semen virtually available for over 60 years (Yanez-Ortiz *et al.*, 2021). Besides, the introduction of cryopreservation of semen has enabled large scale provision of highly valuable genetic material became possible as evident in Germany (Ugur *et al.*, 2019). Gandini *et al.* (2017) considered cryopreservation as the freezing of sperm, which is a technique used

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to keep cells and tissues in a vital state at −196°C in liquid nitrogen. The use of liquid nitrogen started in the modern cryobiology era. This method is further advantageous considering long-distance transportation of valuable genetic materials and preventing the spread of pathogens (Gupta *et al.*, 2011).

Techniques for Semen Extension and Cryopreservation of Livestock Spermatozoa

Numerous studies have been carried out on semen extension and cryopreservation of livestock sperm. The most important factors influencing sperm function include nutrition (Akintunde *et al.,* 2020, 2023), genotype (Akintunde *et al.*, 2021), collection temperature, storage temperature, and the suspension medium (Ugur *et al.*, 2019). When spermatozoa are cooled too abruptly from body temperature to less than 15 degrees C, cold shock can occur, which might reduce sperm viability (Gupta *et al.*, 2011). Semen is expected to be extended as soon as possible after collection from the bulls. Pre-extension is also thought to provide a more gradual osmotic change using a small amount (100 ml) of extender and following up with full extension. Pre-extending semen also quickly exposes the raw ejaculate to antibiotics. Bacteria are a normal component of the bull ejaculate and have been reported to range in concentrations of up to 109 cfu/ml.3 (Morrow, 2005).

Mustofa *et al.* (2022) reported that supplementation of 0.001 mg/mL of nanoparticle green tea extract in skim milk-egg yolk extender and thawing temperature of 39°C resulted in a better quality of frozen-thawed Kacang goat semen. Also, in a study by Susilowati *et al.* (2024), it was observed that higher quality post-thawing Boer buck semen was achieved by adding 1 µg/mL of chitosan nanoparticles of green tea extract to the skimmed egg yolk diluent and thawing at 39°C.

Also in a study by Mustofa *et al.* (2023) on the impact of epigallocatechin-3-gallate chitosan nanoparticles (EGCG CNPs) in an extender on the antioxidant capacity and post-thawed quality of Kacang goat semen, it was observed that post-thawed semen that was previously frozen without EGCG CNPs in the extender (control group) exhibited the lowest levels of catalase, DPPH, sperm viability, sperm motility, IPM, and the highest levels of MDA. However, the addition of EGCG CNPs at doses of 1.5 µg/mL extender increased post-thawed catalase, DPPH, sperm IPM, viability, and sperm motility and decreased MDA levels than those of control group. They however concluded that the addition of only 1.0 µg/mL of EGCG CNPs in extender increased the antioxidant capacity and post-thawed quality of Kacang buck semen (Mustofa *et al.,* 2023).

Although the various techniques of semen extension and cryopreservation have been associated with problems of sperm integrity, metabolic decoupling, ionic imbalance, activation of proteases, cellular acidosis, deprivation of energy, membrane phase transition, destabilization of the cytoskeleton, and production of free radicals or reactive oxygen species (ROS), these challenges vary from place to place and also depend on the level of advancement of the laboratory. Also, the quality of semen differs from one animal to another. Hence, it is suggested that proper and adequate treatment be continuously administered to the livestock whose sperm would be preserved (Ugur *et al*., 2019).

Further Table 1 provides the evidences in the growth of studies on sperm extension of livestock production.

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Table 1. Summary of Major Findings on Semen Extension and Cryopreservation

S/N	Reference	Study	Results
1	Kulaksiz *et al*. (2011).	The effects of different extenders and myo-Inositol on post-thaw quality of ram semen	Extenders of T {T-5I, T-10I, T (control)} and M {M-5I, M-10I, M (control)} resulted in higher sperm motility (50.00±2.24% and 55.00±0.42%) and HOST (49.00 3.32% and 48.17±2.97%) rates, compared to NaC {Na-10I, NaC (control)} (37.00±3.74% and 31.80±2.96%, P< 0.01), following the freeze/thawing process. Extenders supplemented with myo-inositol not significantly affect malondialdehyde (MDA) levels and activities of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GSH-PX) in comparison to the control groups (P>0.05), except for MDA level of T extender containing 10 mM inositol. MDA level was found lower (1.22±0.07 nmol/ml) in T than those of the M and NaC (P<0.01).
2	El-Sheshtawy *et al*. (2016)	Natural honey as a cryoprotectant to improve Arab stallion post-thawing sperm parameters	Relative to the control group, supplementation with honey (2%, 3% and 4% significantly improved (P < 0.01 at least) post-thaw sperm motility, viability index (P < 0.001 at least) and had a positive effect on membrane integrity and intact acrosome percentage (P < 0.001 at least) at 0, 1, 2 and 3 h post-thawing. For all semen parameters, the lower concentration of honey (1%) and higher concentration (5%) did not show significant differences (P > 0.05) compared with the control.
3	Khalifa and Khalil (2016).	Impact of Conventional and Non-Conventional Extenders on Rams Semen Quality During Storage at 5ºC	The results indicated that cooling extended ram semen with each of sodium citrate (E1), Tris (E2) or saline solution 0.9% intravenous infusion (E5) extenders was significantly (P<0.05) higher the percentage of sperm motility (SPM), recovery sperm motility (RSM) and positive osmotic resistant (POR)than those extended with E3 and E4 extenders during storage at 5ºC for up to 6 days.However, the cooling ram semen with E2 and E5 showed non-significantly parameters during cooled at 5ºC till 6 days.
4	Olurode and Ajala (2016).	Effects of storage temperature and extension media on motility of caprine spermatozoa	Results showed that goat milk in part can replace egg-yolk as a medium for semen extension
5	Bamanga *et al*. (2021).	Effects of Coconut, Groundnut and Tigernut Milk-Based Extenders on Fresh and Chilleduda Ram Semen in Maiduguri, Nigeria	It was found that coconut, ground nut and tiger nut milk-based extenders maintained good semen quality of Uda rams till 48 hours post extension when

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			chilled at 5°C and that the tiger nut milk based extenders have better semen preservative ability than the coconut and the ground nut milk based extenders
6	Simonik *et al.* (2022).	Boar Sperm Cryopreservation Improvement Using Semen Extender Modification by Dextran and Pentaisomaltose	Results show a lower impact of cryopreservation on sperm qualitative parameters when the extender is modified by pentaisomaltose.

The above studies have espoused various methods and materials adaptable to sperm extension practices which included both conventional and non-conventional materials. The challenges posed by conventional methods gave rise to the interest in non-conventional methods. Non – conventional methods include the use of honey, coconut, groundnut, tiger nut, milk, and extracts from sugarcane, vegetables, fruits, and minerals.

Semen Extension and Cryopreservation of Livestock Spermatozoa in Nigeria

The theory of semen extension and cryopreservation is not new in Nigeria's agro-allied industry but the practice is still a challenge. The knowledge of a semen collection tool– Electroejaculator (EE) is common with commercial livestock farmers especially those in the northern part of Nigeria (Olurode and Ajala, 2016). Electro-ejaculators were known to be used for collection of semen from special breed of rams, goats and cows in order to preserve and produce same quality of the livestock over a period of time (Sekoni, 1993; Daramola *et al.*, 2010). Olurode and Ajala (2016) added that the essence of this semen extension and cryopreservation is to ascertain the individual motility, livability and morphologic abnormalities of the ruminants. However, these exercises have not yielded desired results as the farmers have over the years lacked necessary information needed for improvement of their business. Agricultural institutes suffer the problem of ill-equipped laboratories, lack important preservative equipment such as chillers among others. Thus, the process of selectively breeding a special type of livestock for developing and improving preferred livestock has been difficult.

Also, in a study on Sperm viability of FUNAAB-alpha chicken at refrigeration and freezing by Ndubuisi-Ogbonna *et al.* (2022) observed that refrigeration decreased sperm abnormality when compared to freezing, it was also observed that freezing resulted in lower leukocyte and malondialdehyde (MDA) levels. They however concluded that refrigerated spermatozoa had higher sperm viability than those frozen. Also, in a study on the influence of different cryoprotocols and strains on the sperm viability of FUNAAB alpha chickens by Ndubuisi-Ogbonna *et al,* (2021). The study concluded that slow and rapid freezing cryoprotocols had a deleterious effect on the spermatozoa of different strains of FUNAAB alpha chickens and removal of seminal plasma through centrifugation did not improve the viability of the spermatozoa.

The work of Bamanga *et al.* (2021) on the effects of coconut, groundnut and tigernut milk-based extenders on fresh and chilled uda ram semen in Maiduguri, Nigeria further showed that semen extension practices can improve agricultural production in the country.However, the use of modern reproductive biotechnology such as Artificial Insemination (AI) for assisted reproductive technology significant for contributing to genetic improvement in the country is still at the rudimentary stage owing to, underdeveloped nature of agriculture, lack of important equipment, unstable electricity, illiteracy, poverty, among others. Therefore livestock farmers have resorted to depending on the natural

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method of preserving a preferred breed of animals (Bitto and Egbunike, 2012).

CONCLUSION

Taking into account the gaps in the literature, the study discovered that during the process of sperm extension and cryopreservation, the specimen is predisposed to detrimental effects such as ice crystal formation, hyper-osmosis, alterations in the integrity of sperm cells, and denaturation, which are important to the problem associated with cold stock during cooling, and relative in the availability of commercial extenders and powered, particularly in developing countries. The proposed solutions include the use of photogenic and natural products in sperm extension and research stations where alternative power sources could be explored.

The practice of semen extension and cryopreservation of spermatozoa as methods for keeping preferred breeds of livestock has tremendous importance in food production across the world. It has become a bedrock for the progress of livestock business in some advanced countries like Germany, etc. This success could be replicated in many other countries, including Nigeria, if deliberate efforts are made to develop the practice.

Although there might not be complete absence of challenges associated with semen extension and cryopreservation of spermatozoa, a closer study and advanced research carried out could lead to solving some of the issues. It has been demonstrated that the longer the semen last, the weaker they become. Therefore, the research suggests that cryopreservation of sperm should not exceed five years.

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