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REVIEWS

Effects of different extenders, storage temperatures, and antioxidant supplementation on chilled semen quality: a review

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Abstract
The successful preservation of ram semen is essential to promote genetic variability, ensure semen transportation, and inseminate multiple ewes. Currently, either animal or plant-based lipoprotein-based extenders are used for semen preservation. Animal product-based extenders include milk and egg yolk, while soybean lecithin is a plant-based extender. Although extenders containing products of animal origin better preserve the quality of chilled semen, the in vivo efficacy after 24 h of storage is still of great concern. Storage temperature is another important and effective factor in preserving sperm quality, whereby different storage temperatures are adopted to enhance the storage life of sperm. Low temperatures (4–5 °C) better preserve sperm quality for a longer duration than high temperatures (15, 20, and 25 °C). Moreover, antioxidant supplementation has a positive impact on sperm quality during liquid storage. The current review summarizes the outcomes of various extenders, different storage temperatures, and antioxidant supplementation on the liquid storage of ram sperm.

Keywords Ram semen · Extenders · Soybean lecithin · Egg yolk · Artificial insemination

Introduction

Artificial insemination (AI) is considered a major tool for genetic improvement (De et al. 2015). In sheep, AI started in the late 1930s in Russia and is still considered a major avenue for the genetic propagation of elite rams (Foote 2010). However, AI is not as widely spread in sheep as in other livestock species (Kumar Patel et al. 2017) due to limitations such as poor post-thaw sperm quality and pregnancy rates (Anel et al. 2006), among others. Ewe fertility is typically lower when frozen-thawed rather than fresh/chilled ram semen is used for cervical AI, which is probably due to an impairment in sperm transit after cryopreservation-induced alterations (Souza-Fabjan et al. 2023). As studies that focus on semen proteomics and transcriptomics (Warr et al. 2023) have not yet developed tools for overcoming this bottleneck, the use of fresh/chilled/cooled semen remains the only option for achieving high pregnancy rates in sheep (Souza-Fabjan et al. 2023).

To achieve high pregnancy rates, the recommended time for AI with chilled semen is short, ranging from 6 to 12 h after storage (Kulaksiz et al. 2012; Paulenz et al. 2002). Interestingly, it is well documented that extenders do not preserve sperm quality similarly in different sheep breeds (Benmoula et al. 2018; Kasimanickam et al. 2007) and cattle (Sukirman et al. 2020). Mostly, milk, sodium citrate, and Tris-based extenders are used for the liquid storage of ram semen. At present, there is some disagreement on the protective effects of Tris and milk-based extenders. Earlier reports suggest that Tris-based extenders better preserve sperm quality as compared to sodium citrate and skim milk-based extenders when stored at 4 and 5 °C (Paulenz et al. 2002; Quan et al. 2016). On the other hand, for the Akkaraman ram breed it

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has been documented that skim milk-based extenders offer improved in vitro semen storage at 4 °C than Tris-based extenders (Kulaksiz et al. 2012). Meanwhile, no difference was found between sodium citrate, Tris, and milk-based extenders when subjected to liquid storage at 4 °C (Lopez et al. 1999). Accordingly, different temperatures ranging from 4 to 20 °C (Kasimanickam et al. 2011; Menchaca et al. 2018; Paulenz et al. 2002) and breed-specific extenders (Kulaksiz et al. 2012; Rather et al. 2017) are being used to preserve the sperm quality for longer durations.

Keeping in mind the disagreements on the protective effect of different extenders in various sheep breeds and the unavailability of literature summarizing the recent approaches to preserving sperm quality during the liquid storage of semen, the current review summarizes the outcomes of various extenders, different storage temperatures, and antioxidant supplementation on chilled semen quality in rams.

Mechanisms of sperm preservation using animal or plant-based extenders

Various extenders have been developed to protect sperm from the harmful effects of pH and osmolarity changes during liquid storage. At present, semen extenders contain either animal-based (egg yolk (EY) or skimmed milk) or plant-based (soybean lecithin) constituents to preserve sperm quality during storage (Bustani and Baiee 2021). EY is an important component of the animal-based products used during the liquid storage of sperm. The effective components of EY are low-density lipoprotein (LDL) and phospholipids, such as phosphatidylcholine, which maintain the sperm membrane and protect it against cold shock during semen preservation (Bergeron and Manjunath 2006). The LDL generally binds to the seminal plasma proteins, preventing interaction with the sperm membrane and the outflow of phospholipids and cholesterol from the membrane (Bergeron et al. 2004). The LDL of EY interacts with sperm binder proteins (BSP), which prevents cholesterol and phospholipid extraction from the sperm membrane, thereby protecting the sperm during preservation (Bergeron and Manjunath 2006) (Fig. 1). Several molecules have already been tested as supplements to extenders to maintain membrane integrity, prevent oxidative stress, and preserve the motility of ram sperm (Martı et al. 2003). Soybean lecithin (SL) is one of the important components used for semen preservation, and it has been reported that skim milk and EY-based extenders can be replaced by SL-based extenders (Forouzanfar et al. 2010; Sharafi et al. 2009). SL contains a mixture of phospholipids, fatty acids, and low-density

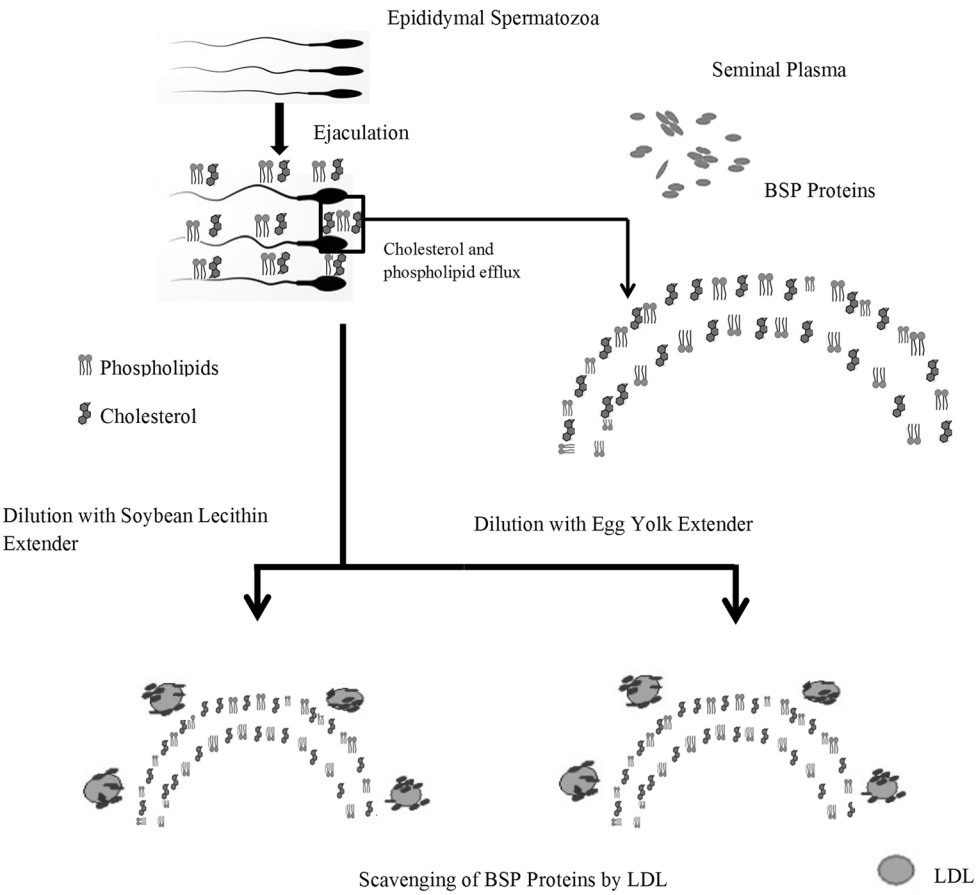

Fig. 1 Mechanism of sperm protection using egg yolk (EY) and milk. BSP: Binder of Sperm Proteins; LDL: Low-Density Lipoprotein. Modified from (Bergeron and Manjunath 2006)

lipoproteins, which protect the sperm cell membranes by restoring the phospholipids lost during heat shock (Forouzanfar et al. 2010). The lecithin component in soy can bind to the spermatozoa membrane to provide extracellular protection. The protection mechanism of sperm membranes is thought to act in two ways. The first mechanism involves exogenous phospholipids that can replace membrane phospholipids to maintain the membrane structure (Zhang et al. 2009), while the other involves the attachment of lecithin to phospholipids without entering the cell membrane, thereby forming a protective coating outside the cell during the dilution or storage process at low temperatures (Ducha et al. 2020) (Fig. 1).

Effect of extender type on sperm quality in various breeds

The short storage life of extended ram sperm is of great concern as it hinders the widespread application of AI in sheep. Various extenders, such as Tris, skim milk, and sodium citrate-based extenders, have been used for liquid storage. It has been documented that Tris-based extenders supplemented with 20% EY better preserve the semen quality than skim milk and sodium citrate-based extenders (Lopez Saez et al. 2000; Paulenz et al. 2002). Compared to milk-based extenders, Tris and sodium citrate-based extenders better sustain the in vitro semen quality (sperm motility, morphology, and osmotic resistance characteristics) of Daglic x Kivircik rams when kept at 4 °C (Gündoğan 2009; Gundogan et al. 2011). Similarly, Tris-based extenders with the addition of 10% EY have a greater protective impact on the chilled semen quality of Yunnan semi-fine wool rams than skim milk-based extenders (Quan et al. 2016). Recently, it was demonstrated that Tris-based extenders better protect sperm quality than skim milk and sodium citrate-based extenders during the liquid storage of Kail ram semen at 5 °C (Hameed et al. 2023).

In contrast, Kulaksiz et al. (2012) reported that skim milk-based extenders ensure higher quality in vitro semen stored at 4 °C compared to Tris-based extenders for Akkaraman rams. Milk-based extenders, as compared to Bioxcell®, sodium citrate, and Tris-citric acid EY-based extenders, lead to higher sperm motility (Kulaksiz et al. 2012). Similarly, a study documented that the protective effect of a skim milk-based extender was higher than that of a Tris-based extender for Boujaad rams (Badi et al. 2018) (Table 1). Moreover, a Tris citric acid-fructose extender (TCFEY) has been reported to better preserve sperm motility and membrane integrity for 72 h at 4 °C than Tris citric acid glucose egg yolk (TCGEY), EY citrate fructose, and EY citrate glucose (Rather et al. 2016) (Table 1). It can be concluded that the capacity of extenders to maintain sperm quality during liquid storage varies with breed due to differences in the biochemical composition of the seminal plasma.

The success of AI in sheep depends upon several factors, such as the season, type of extender, storage temperature, timing, and site of insemination (Palacín et al. 2012). It has been demonstrated that insemination with fresh semen,

Table 1 Effect of different extenders on the quality of ram chilled semen. Data were expressed as mean ± SEM

Extenders	Parameters			ST	References
	Motility %	PMI %	Viability %		
TR	60.6 ± 4.6^{a}	57.4 ± 3.5^{a}	63.2 ± 3.1^{a}	48 h	Hameed et al. 2023
SM	49.9 ± 4.3^{b}	42.2 ± 2.3^{b}	53.1 ± 3.2^{b}		
SC	46.7 ± 3.7^{b}	47.8 ± 2.1^{b}	55.1 ± 2.4^{b}		
Skim milk	63.5 ± 0.4^{a}	68.2 ± 0.7^{a}	86.2 ± 1.1^{b}	24 h	Badi et al. 2018
Tris EY	44.1 ± 1.6^{b}	66.1 ± 0.7^{b}	91.9 ± 0.5^{a}		
TCFEY	61.2 ± 2.1^{b}	53.9 ± 1.9^{b}	74.9 ± 4.5	72 h	Rather et al. 2016
TCGEY	55.6 ± 2.4^{b}	43.9 ± 2.7^{a}	72.1 ± 2.9		
EYCF	46.9 ± 2.8^{a}	44.9 ± 2.3^{a}	73.4 ± 4.0		
EYCG	40.0 ± 2.5^{a}	44.5 ± 1.7^{a}	70.3 ± 4.3		
Tris-citric acid EY	48.7 ± 0.3^{a}			24 h	Kulaksiz et al. 2012
Milk extender	68.5 ± 0.3^{b}	NR	NR		
SC extender	51.2 ± 0.5^{a}				
Bioxcell®	55.0 ± 0.6^{a}				
Tris-based extender	56.0 ± 2.0^{a}	61.1 ± 1.2^{a}	9.3 ± 0.5^{c}	96 h	Gündoğan 2009
SC-based extender	56.0 ± 5.1^{a}	60.1 ± 2.5^{a}	9.4 ± 1.5^{c}		
SM extender	46.0 ± 5.1^{b}	33.9 ± 3.6^{b}	14.8 ± 1.4^{a}		
GP extender	38.0 ± 2.0^{b}	13.8 ± 6.9^{c}	15.0 ± 1.1^{a}		

TR = Tris-based extender; SM = Skim milk-based extender; SC = Sodium citrate-based extender; TCFEY = Tris citric acid fructose egg yolk; TCGEY = Tris citric acid glucose egg yolk; EYCF = Egg yolk citrate fructose; EYCG = Egg yolk citrate glucose; EY = Egg yolk; NR = Not reported; ST = Storage time; GP = Glucose phosphate. ST indicates the time of significant difference. Different superscripts ^{abc} within the column indicate a significant difference ($P < 0.05$) between different types of extenders

diluted either with EY citrate or AndroMed®, results in a similar pregnancy rate (Madrigali et al. 2021). Similarly, the dilution of semen with Triladyl® and Tris citric acid fructose EY (TCFEY) extender results in similar sperm quality and pregnancy rates after up to 24 h of storage. However, semen diluted in Triladyl® has better sperm quality in vitro and in vivo than a TCFEY extender after 48 h of storage (Rekha et al. 2018). The effect of various concentrations of trehalose on in vitro and in vivo sperm quality using a TCFEY extender was evaluated during the liquid storage of semen at 0 °C. It has been reported that the addition of trehalose has no influence on the pregnancy rate in the Duolang sheep breed (Zhao et al. 2020). Similarly, the effect of storage temperatures and TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) supplementation to a sodium citrate-based extender was evaluated. The findings revealed that storing semen at 15 °C improves the in vitro fertilization ability compared to at 22 °C. Moreover, TEMPOL supplementation has a more positive impact on sperm quality than a skim milk-based extender during storage at 15 °C (Mara et al. 2005). The effect of storage time during liquid storage at 5 °C on the pregnancy rate was also evaluated. For instance, it was documented that insemination with stored semen for 12 h results in a similar pregnancy rate to that of fresh semen (Menchaca et al. 2018). Similarly, the addition of ram seminal plasma to a Tris EY-based extender improves the pregnancy rate during liquid storage at 5 °C for 24 h (López-Pérez and Pérez-Clariget 2012). Similarly, the storage of semen at 5 °C for 12 and 24 h results in a similar pregnancy rate, while a milk-based extender supplemented with gelatin has no positive impact on the pregnancy rate after vaginal insemination (Paulenz et al. 2010). However, the supplementation of extenders with various additives improves sperm quality in vitro, although it does not influence the pregnancy rate in sheep.

Commercially available extenders of animal and plant origin

To date, various commercial extenders containing products of animal (Triladyl®, and Ovipro®) or plant origin (AndroMed®, Bioxcell®, and Optidyl®) are being marketed (Hegedusova et al. 2012; Rehman et al. 2013), with a claim to better preserve ram semen (Gil et al. 2003; O'Hara et al. 2010). Based on the review, a comparison of these extenders suggests that one or the other is best for the short-term liquid storage of ram semen. For the short-term storage of ram semen, Ovipro® seems to be the best extender as it offers higher sperm motility during the first 48 h of liquid storage compared to the Optidyl®, Triladyl®, and Andromed® extenders (Hegedusova et al. 2012). Similarly, the authors of another study concluded that commercially available extenders with an animal-origin product (Optidyl®) better preserve chilled semen quality compared to ultra-heat-treated UHT milk-based extenders and Andromed® (Maksimovic et al. 2018) (Table 2). Other researchers found that both animal (INRA 96®; skimmed milk-based) and plant (Ovixcell®; SL-based) product-based extenders are equally effective in maintaining the in vitro and in vivo quality of chilled semen stored at 5 °C (Arando et al. 2019).

After analyzing various published reports, we suggest that a direct relationship exists between storage temperature, extender type, breed, and ram semen quality. For example, at 15 °C, skim milk, Duragen®, and INRA 96®, while at 5 °C, skim milk, INRA 96®, Duragen®, Andromed®, and

Table 2 Effect of different commercially available extenders on chilled semen quality of rams. Data were expressed as mean ± SEM

Extenders	Parameters			ST	References
	Motility %	PMI %	Viability %		
INRA82®	81.0 ± 5.2^{a}	77.5 ± 6.4^{a}	80.4 ± 4.9^{a}	24 h	Fadl et al. 2022
Tris-citrate glucose	66.4 ± 5.4^{b}	59.3 ± 5.9^{b}	61.54 ± 5.0^{b}		
Tris-citrate fructose	72.5 ± 5.2^{c}	68.4 ± 5.7^{c}	72.6 ± 5.1^{c}		
AndroMed®	70.7 ± 4.9^{b}		37.2 ± 5.4^{bc}	24 h	Maksimovic et al. 2018
Optidyl®	79.4 ± 4.3^{a}	NR	53.6 ± 5.2^{a}		
Skim milk	62.4 ± 5.1^{b}		29.5 ± 5.9^{c}		
Andromed®	79.2 ± 2.9^{ab}	NR	NR	8 h	Benmoula et al. 2018
Duragen®	63.8 ± 2.6^{c}				
INRA96®	70.7 ± 2.4^{bc}				
SM	76.8 ± 1.7^{ab}				
Optixcel®	76.8 ± 2.6^{ab}				
Ovipro®	82.1 ± 1.7^{a}				
TEY	80.5 ± 1.8^{a}				
Triladyl®	80.7 ± 1.4^{ab}				

PMI = Plasma membrane integrity; SM = Skim milk-based extender; TEY = Tris egg yolk-based extender; ST = Storage time; NR = Not reported. ST indicates the time of significant difference. Different superscripts ^{abc} within the column indicate a significant difference ($P < 0.05$) between different types of extenders

Ovipro®, better preserve semen quality for INRA180 rams up to 24 h. However, for Boujaad ram semen, Ovipro®, Triladyl®, Duragen®, skim milk, Tris EY, INRA 96®, and Triladyl are the best extenders for maintaining the motility parameters at both 5 and 15 °C (Benmoula et al. 2018) (Table 2). For longer storage durations, Triladyl® has been suggested to better preserve chilled semen quality, both in vitro and in vivo, compared to a Tris fructose EY-based (TFE) extender; however, the pregnancy rate after 24 h of storage was comparable between Triladyl® and TFE (Rekha et al. 2018). Another report furthered the notion that breed is a pertinent factor to be considered before selecting an extender, as it found that INRA82® protects the chilled semen quality of Awassi rams better than Tris-citrate glucose and Tris-citrate fructose extenders (Fadl et al. 2022) (Table 2).

Effect of storage temperature on chilled semen quality

The basic principle of sperm storage is to reduce the sperm's metabolism, thus extending its storage life. For this purpose, sperm is stored at low temperatures ranging from 4 to 22 °C, in liquid storage, or is frozen at − 196 °C (Salamon and Maxwell 2000). Short- and long-term storage is used for sperm transport, genetic storage, and medical purposes (Barbas and Mascarenhas 2009). The success of short- and long-term storage methods is overall dependent on the storage temperature, cooling rate, chemical composition of the extender, reactive oxygen species (ROS), and seminal plasma composition (Barbas and Mascarenhas 2009; Batellier et al. 2001). The cooling rate to the storage temperature is important for the successful storage of semen. Various cooling regimens (rapid, medium, and slow) are adopted to preserve sperm quality. An inappropriate cooling rate can lead to temperature shock, resulting in membrane disruption due to protein structural disorganization, ion channel disruption, ROS generation, and mitochondrial membrane potential loss (Martorana et al. 2014). It has been demonstrated that slow cooling to the storage temperature better preserves sperm quality (Ashrafi et al. 2012; Santymire et al. 2007; Zhang et al. 2023). Ram semen has been successfully stored at different temperatures, including 4 °C (Kasimanickam et al. 2011), 5 °C (Purdy et al. 2010), and 15 °C (Yániz et al. 2011). However, it has been reported that reduced temperatures (4 and 5 °C) cause permanent damage to sperm (Gheller et al. 2018). The low temperature affects the plasma membrane integrity (Aitken and Nixon 2013), thus decreasing the motility and fertilizing ability after 24 h of storage (O'Hara et al. 2010). Contrarily, storage at 15 °C results in a high metabolic activity of sperm, reducing the storage lifespan of the sperm to 24 h (Purdy et al. 2010). A previous study documented that a Tris-based extender is suitable for the short-term storage (30 h) of ram semen at 5 and 20 °C as compared to sodium citrate and skim milk-based extenders (Paulenz et al. 2002). Similarly, it has been demonstrated that storing ram semen at 15 °C improves the survival and in vitro penetration capacity of the sperm (Yániz et al. 2005). It is well documented that semen storage at high temperatures (12, 15, and 20 °C) decreases the storage life as it promotes the metabolism of sperm (O'Hara et al. 2010). Another study documented that sperm motility parameters can be better maintained when stored at a low temperature (4 °C) for a short period (2 to 3 days) (Acharya et al. 2017). Similarly, the storage of Hu ram semen at 15 °C better preserves the chilled semen quality as compared to 20 and 25 °C (Zhang et al. 2022). Based on the fact that sperm motility (Paulenz et al. 2002) and kinematics parameters are affected differently depending upon the storage temperature (Benmoula et al. 2018), it is concluded that low temperatures (4–5 °C) better preserve the chilled semen quality for longer durations in comparison to the storage of semen at high temperatures (15, 20 and 25 °C) (Akporhuaho et al. 2017).

Effect of antioxidant supplementation on chilled semen quality

Ram semen is extremely sensitive to ROS, potentially producing large amounts of ROS during liquid storage, leading to a decline in semen quality. Several experiments have been conducted to evaluate the effect of EY and SL supplementation on sperm preservation. EY supplementation protects ram semen quality, similar to a skim milk-based extender at 4 °C for 8 days (Kasimanickam et al. 2011), and supplementing the extender with 10% EY has a significant protective effect on sperm quality compared to other EY concentrations (5, 15, and 20%) when kept at 5 °C for up to 48 h (Azizunnesa et al. 2013). EY supplementation has been shown to enhance short-term semen storage in Awassi rams in a Tris-based extension (Varisli et al. 2018). Similarly, in another study, EY supplementation of a Tris-based extender improved sperm motility and viability when kept at 4 °C (Tămâianu et al. 2021). Although EY supplementation improves the shelf life and sperm quality during liquid storage, microbial contamination is of great concern when using animal product-based extenders.

To overcome the microbial contamination concern, various plant-origin ingredients have been tested to improve sperm quality. Among these, SL is commonly used for sperm preservation. The effects of different concentrations (0.5, 2.0, and 3.5%) of SL on chilled semen quality stored at 15 and 5 °C reveal that an EY-based extender supplemented

with 2% SL has higher motility and viability rates compared to other concentrations (Paulenz et al. 2010). Likewise, a study on Duolang ram semen evaluated the effect of different concentrations of SL and found that supplementation of the Tris-based extender with 0.5% SL improves progressive motility compared to other SL concentrations (0.25, 0.75, 1.0, and 1.25%; Table 3) (Zhao et al. 2021). The addition of SL to a semen extender has been shown to improve sperm motility, viability, acrosome integrity, and sperm membrane structure in rams (Emamverdi et al. 2013). In contrast, other researchers did not find Tris SL-based extenders as promising as milk-EY, milk-SL, and Tris EY-based extenders and reported a decline in sperm motility. In a milk-SL extender, curvilinear velocity has been found to be better than in other extenders, although sperm viability was similar between different extenders (Gogol et al. 2019). In conclusion, the SL-based extender appears to be an appropriate substitute for extenders prepared from animal sources, especially due to the concerns regarding the microbial contamination of products (milk and EY) of animal origin (Kasimanickam et al. 2011).

Apart from SL, the addition of argan oil to skim milk or Tris EY-based extender has been shown to have a positive impact on chilled semen as sperm motility, viability, and membrane integrity at 5 and 15 °C for up to 48 h of storage are better maintained than with conventional extenders. The optimum concentration of argan oil to maintain sperm quality appears to be variable according to the type of extender. In Tris EY-based extenders, supplementation with 1% argan oil, while in skim milk extenders 5% argan oil supplementation, proved more beneficial (Allai et al. 2015).

Several antioxidants have been supplemented to extenders to better preserve sperm quality. It was found that supplementation of both Tris and SM-based extenders with 1% *Opuntia ficus-indica* cladodes (ACTEX) improves ($P < 0.05$) sperm viability, motility, and membrane integrity

Table 3 Effect of supplementation of plant-origin ingredients on chilled semen quality of rams

Extenders	Parameters			ST	References
	Motility %	PMI %	Viability %		
Tris SL (0.25%)	47.3 ± 3.5^{b}			216 h	Zhao et al. 2021
Tris SL (0.5%)	56.4 ± 2.6^{a}				
Tris SL (0.75%)	54.6 ± 3.0^{ab}				
Tris SL (1.0%)	53.0 ± 3.0^{ab}				
Tris SL (1.25%	53.3 ± 2.7^{ab}				
TEY	74.0 ± 2.2^{c}	43.0 ± 2.4	66.1 ± 1.9	48 h	El-Harairy et al. 2018
TEY + 0.1 mg PEE	76.3 ± 2.2^{bc}	42.131.8	68.7 ± 2.2		
TEY + 0.5 mg PEE	78.3 ± 2.2^{ab}	40.3 ± 1.9	69.5 ± 2.4		
TEY + 1.0 mg PEE	79.3 ± 2.3^{ab}	43.9 ± 2.3	66.7 ± 2.5		
Tris SL	75.6 ± 2.5^{bc}	42.5 ± 1.9	66.4 ± 2.3		
Tris SL + 0.1 mg PEE	79.0 ± 2.4^{ab}	41.7 ± 1.8	68.5 ± 2.7		
Tris SL + 0.5 mg PEE	78.3 ± 2.1^{ab}	39.5 ± 1.7	66.2 ± 2.6		
Tris SL + 1.0 mg PEE	81.0 ± 2.0^{a}	42.1 ± 1.5	67.8 ± 2.9		
SM CSO 1%	71.6 ± 2.1^{b}		78.4 ± 1.6^{bc}	72 h	Allai et al. 2017
SM CSO 2%	75.5 ± 1.5^{ab}		78.8 ± 1.1^{bc}		
SM CSO 5%	65.0 ± 1.3^{c}		73.5 ± 1.3^{d}		
SM CSO 10%	63.1 ± 2.5^{c}	NR	69.0 ± 1.8^{e}		
Tris CSO 1%	79.2 ± 1.2^{a}		83.2 ± 0.8^{a}		
Tris CSO 2%	78.7 ± 1.6^{a}		81.3 ± 1.4^{ab}		
Tris CSO 5%	73.2 ± 1.7^{b}		75.1 ± 1.0^{cd}		
Tris CSO 10%	72.4 ± 1.0^{b}		73.1 ± 1.6^{de}		
SM ACTEX 1%	72.6 ± 1.0^{a}	60.8 ± 0.9^{a}	80.6 ± 0.7^{a}	72 h	Allai et al. 2016
SM ACTEX 2%	69.3 ± 0.9^{a}	43.3 ± 1.8^{c}	74.5 ± 2.3^{bc}		
M ACTEX 4%	58.0 ± 1.7^{b}	41.7 ± 1.1^{c}	69.4 ± 0.8^{d}		
SM ACTEX 8%	58.9 ± 1.6^{c}	35.0 ± 1.5^{d}	66.1 ± 1.4^{d}		
Tris ACTEX 1%	77.8 ± 1.2^{a}	53.6 ± 1.9^{a}	83.2 ± 1.3^{a}		
Tris ACTEX 2%	69.9 ± 1.8^{bc}	44.8 ± 1.5^{b}	75.8 ± 0.8^{b}		
Tris ACTEX 4%	67.0 ± 2.0^{c}	35.5 ± 1.2^{c}	74.0 ± 1.0^{b}		
Tris ACTEX 8%	61.0 ± 1.9^{d}	34.5 ± 1.7^{c}	69.0 ± 0.6^{c}		

SL = Soybean lecithin; NR = Not reported; ST = Storage time; TEY = Tris egg yolk; PEE = Propolis ethanolic extract; SM = Skim milk; CSO = Cactus seed oil; ACTEX = Opuntia ficus indica cladodes. ST indicates the time of significant difference. Different superscripts ^{abc} within the column indicate a significant difference ($P < 0.05$) between different types of extenders

up to 72 h compared to other concentrations of ACTEX (2%. 4%, 8%; Table 3) (Allai et al. 2016). Similarly, supplementation with 1 and 2% cactus seed oil in SM and Tris EY-based extender has been reported to improve ($P < 0.05$) sperm motility and viability and decrease the level of peroxidation and DNA fragmentation during liquid storage at 5 °C for 72 h (Table 3) (Allai et al. 2017). In another attempt, the addition of caffeine (4 mmol) to a commercially available extender (Triladyl) resulted in the improved total and progressive motility of chilled semen at 4 °C (Špaleková et al. 2011). The supplementation of licorice extract to EY-based extender at 1, 5, and 10 µg/ml has been suggested to improve ram sperm's progressive motility during liquid storage at 5 °C for 72 h (Mahdi 2017). Similarly, the addition of lycopene at 0.5 and 2 mM to a Tris-based extender better preserves sperm motility and viability, and reduces the oxidative stress during the liquid storage of semen (Akalin et al. 2016). The results from previous studies show that supplementing the extender with 0.3 mM melatonin efficiently reduces oxidative stress and improves the chilled storage of ram spermatozoa (Khalifa 2017). Moreover, adding an ethanolic extract of propolis to a Tris SL-based extender was found to have a beneficial effect on the progressive motility and chromatin integrity of sperms without having a significant impact on viability, abnormal sperm ratio, and plasma membrane integrity during liquid storage at 5 °C (Table 3) (El-Harairy et al. 2018). The supplementation of ellagic acid 2 mM and 40 µM ebselen in a semen extender has a potential effect on sperm and oxidative stress parameters during the liquid preservation of ram semen (Bucak et al. 2019). The addition of pomegranate peel alcoholic extract at a concentration of 300 mg/ml to a Tris extender enhances ram sperm viability, acrosome integrity, motility, and plasma membrane integrity at 5 °C for up to 96 h of storage (Banana et al. 2020). Other miscellaneous supplements, such as *Tinospora cordifolia*, flaxseed oil, almond oil, olive oil, and *Arctium lappa* roots, have been reported to improve sperm quality during the liquid storage of semen (Bajia et al. 2022; El-Harairy et al. 2016; Suranagi and Kulkarni 2022; Zarei et al. 2018). Chlorogenic acid (CGA) is a plant extract with an antioxidant capacity that can effectively eliminate free radicals and improve the antioxidant capacity of semen. It has been demonstrated that supplementation with CGA improves the semen quality of Hu rams (Zhang et al., 2022). Furthermore, the effect of various concentrations of vitamin D3 (T1 = 0.02, T2 = 0.004, and T3 = 0.002 g/ml) was evaluated during the liquid storage of Awassi ram semen. The findings revealed that a low dose of vitamin D3 has a potential antioxidant capability, introducing a novel method for extending sperm storage (Abed Bresm and Hassan Habeeb 2023). The findings of previous studies indicate that antioxidant supplementation improves sperm quality during liquid storage.

Conclusion

The success of short- and long-term storage methods is overall dependent on the storage temperature, cooling rate, chemical composition of the extender, ROS, and seminal plasma composition. Mostly skim milk and Tris-based extenders are used for the liquid storage of ram semen, but the protective effect of extenders varies with breed. The supplementation of extenders with EY and antioxidants improves the shelf life and sperm quality during liquid storage. SL-based extenders have successfully replaced skim milk and EY-based extenders. The storage of semen at reduced temperatures (4 or 5 °C) leads to a longer shelf life as compared to storage at higher temperatures (15–25 °C). The study offers a road map for the liquid storage of ram semen. As the protective effect of extenders varies among breeds, future research should focus on evaluating the specific factors that alter the protective effect of extenders across breeds.

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Declarations

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