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Current Semen Extenders for Bulls

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Abstract
Artificial insemination is the most widely used biotechnological application for animal breeding in cattle breeding. It is crucial to properly store the sperm obtained from the breeding bulls while maintaining their spermatological characteristic using suitable methods. During both long or short-term storage of spermatozoa, an ideal storage medium must be employed. For this purpose, diluents have been developed to meet the needs of spermatozoa. An ideal semen diluent contains ingredients that spermatozoa need, such as energy substances, protective agents against cold shock, buffering solutions that protect against pH changes, cryoprotectants to reduce damage to spermatozoa during freezing and antibiotics against microbial contamination. Semen dilution also allows for increasing the available semen volume to obtain more straws. Maintaining spermatological parameters at the best possible level during semen storage has important economic implications in this industry. For this reason, scientists continue to develop new diluents to achieve the optimum benefits from semen diluents. This review is aims to provide information about semen diluents used in bulls.
Keywords: Bull, extender, semen, storage.

Boğalarda Kullanılan Mevcut Sperma Sulandırıcıları

Öz
Suni tohumlama, sığır yetiştiriciliğinde hayvan ıslahı için en yaygın kullanılan biyoteknolojik uygulamadır. Damızlık boğalardan elde edilen spermlerin uygun yöntemler kullanılarak spermatolojik özelliklerini koruyarak uygun şekilde saklanması çok önemlidir. Spermatozoanın gerek uzun gerekse kısa süreli saklanması sırasında ideal bir saklama ortamı kullanılmalıdır. Bu amaçla, spermatozoanın ihtiyaçlarını karşılamak için seyrelticiler geliştirilmiştir. İdeal bir semen seyreltici; enerji maddeleri, soğuk şokuna karşı koruyucu ajanlar, pH değişikliklerine karşı koruma sağlayan tampon çözeltiler, dondurma sırasında spermatozoanın zarar görmesini azaltan kriyoprotektanlar ve mikrobiyal kontaminasyona karşı antibiyotikler gibi spermatozoanın ihtiyaç duyduğu bileşenleri içerir. Semen dilüsyonu ayrıca daha fazla payet elde etmek için mevcut semen hacminin artırılmasına da olanak tanır. Spermanın saklanması sırasında spermatolojik parametrelerin mümkün olan en iyi seviyede tutulması, bu sektörde önemli ekonomik etkilere sahiptir. Bu nedenle, bilim insanları sperma sulandırıcılarından optimum faydayı elde etmek için yeni sulandırıcılar geliştirmeye devam etmektedir. Bu derleme, boğalarda kullanılan sperma sulandırıcıları hakkında bilgi vermeyi amaçlamaktadır.
Anahtar kelimeler: Boğa, sulandırıcı, sperma, saklama.

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Introduction

The bull holds the important economic value in the cattle breeding (Foote, 2003). A single ejaculate from a bull typically contains much more spermatozoa than is required for impregnantion. Therefore, it is important to dilute the semen use in insemination of multiple cattle is possible (Arthur et al., 1996; Hinsch et al., 1997). For example, 6 ml of semen contains a sufficient number of spermatozoon cells to inseminate 200-300 cows. However such a volume of semen cannot be actually divided into 200-300 parts. In addition, it has been determined that spermatozoa in undiluted sperm live for a short time and many spermatozoa die even if they are cooled down to 5 °C very slowly. Consequently, scientists started to work on how to store spermatozoa for a long time without losing their life span. They tried to dilute the semen using various diluents. They used natural fruit juices such as tomato juice and coconut milk to dilute the semen. Later, they started to develop semen diluents prepared with various organic and non-organic chemicals that not to harm the spermatozoa (Sönmez, 2015).

Various diluents are used both for short-term storage of semen at +4˚C and when frozen at -196˚C. These diluents can be made in vitro environments by adding substances such as egg yolk, milk, and milk powder (Filho et al., 2018). Since sperm extenders contain energy-providing substances such as simple sugars, they help spermatozoa maintain their vitality for a long time (Sevinç & Hafs, 1961). In addition, antioxidants added to semen during dilution protect the proteins in the plasma membrane of spermatozoa, allowing spermatozoa to survive longer (Stout et al., 2009). Since the spermatozoon membrane in mammals is rich in polyunsaturated fatty acids, membrane structures can be damaged due to lipid peroxidation, protein denaturation and loss of function in cases where reactive oxygen species increase. As a result, DNA damage, apoptotic changes in spermatozoa and deprotamination may occur. For the aforementioned reasons, the addition of various antioxidants to sperm diluents in order to protect the semen from damage caused by reactive oxygen species during long or short-term storage leads to the prevention or reduction of this damage (Gupta et al., 2022).

Table. Characteristics of an ideal semen extender (Merdan, 2017).

1	Semen extenders should be isotonic
2	Semen extenders must contain the essential mineral substances required to maintain the viability of spermatozoa
3	Semen extenders should contain energy substances such as glucose and fructose
4	Semen extenders should contain cyroprotective agents [such as glycerol, dimethyl sulfoxide (DMSO), dimethyl acetamit (DMA) ethylene glycol]
5	Semen extenders should contain incorporate substances that can eliminate metabolic residues of spermatozoa (buffer, tris, etc.)
6	Semen extenders should be able to preserve both the plasma membrane integrity and the acrosome membrane integrity and structure of spermatozoa
7	Semen extenders should include antibiotics to control of bacterial contamination (penicillin, streptomycin, etc)

Substances Added to the Semen Extenders

Buffer Solutions

The main role of buffer agents added to semen extender is to prevent pH changes resulting from the metabolic activities of sperm cells. These agents also provide isotonic pressure needed for sperm cell. On the other hand this agents should not be toxic to the sperm cells (Sönmez, 2015).

Phosphate Buffer Solutions: Phosphate buffer solution is prepared by adding 2 g of Na_2PHO_4 and 0.2 g of K_2PHO_4 to 100 ml of distilled water. Although this solution is sufficient to buffer the pH, when combined with egg yolk, it clouds the environment, making it difficult to observe spermatozoa (Sönmez, 2015).

Sodium Citrate Buffer Solutions: Sodium citrate buffer solution is prepared by adding 2.9 g of $Na_3C_6H_5O_7.2H_2O$ to 100 ml of distilled water. The other modified solution is prepared by adding 2.12 g of $Na_3C_6H_5O_7.2H_2O$ and 0.183 g of citric acid to 100 ml of distilled water (Sönmez, 2015).

Tris Buffer Solutions: It is reported that the concentration of Tris in semen diluent, ranging between 10-50 mM, does not adversely affect spermatozoon motility and metabolism. The desired pH level and osmotic pressure of the Tris solution are achieved by adding citric acid to the extender (Sönmez, 2015).

Egg Yolk

Cold shock during the cooling of semen adversely affects sperm motility. Egg yolk, which contains phospholipids help protect the sperm cell membrane and acrosome against cryogenic injury. The protective effect of egg yolk against cold shock is due to its low concentration of substances such as lecithin and B lipovitellin during the cooling storage and freezing process. On the other hand, the high antioxidant content in egg yolks has been reported to reduce the rate of lipid peroxidation in the spermatozoon membrane. In the extenders prepared for bull semen, egg yolk is added at a rate of 20% to various diluents prepared in general (Sönmez, 2015; Bustani et al., 2021).

Soybean Lecithin

Soybean lecithin can be used as an alternative to egg yolk. Like egg yolk, soy lecithin contains palmitic acid, oleic acid, stearic acid, and phosphotidylcholine. Also, it has advantages over egg yolk by avoiding contamination from animal sources. Soy lecithin has been successfully used to freezing bull semen as a replacement for an egg yolk (Kumar et al., 2015; Gamal et al., 2016; Migule-Jimenez et al., 2020).

Cryoprotectants

To prevent damage to spermatozoa by the temperature changes during the freezing and thawing processes, certain chemical substances must be added to the extenders. These substances which reduce the formation of ice crystals during freezing, are called as cryoprotectants. Cryoprotectants can be divided into two classes: those that act by entering the cell (internal cryoprotectants) and those that act from outside the cell (external cryoprotectants). The most commonly used internal cryoprotectant is glycerol. Glycerol penetrates into the cell, allowing some of the water inside the cell to escape and freezing the water inside to form smaller ice crystals. Glycerol is usually added 3-10% to semen diluents. Dimethisulfoxide, DMA, ethylene glycol and propylene glycol are also internal cryoprotectants (Akçay, 2023). However some researchers suggested that of 5-15% cryoprotectant in extender yields better results in protecting against cold shock (Bhattacharya, 2018). Also, disaccharides are considered impermeable substances to cells. These sugars interact with phospholipids of the plasma

membrane, increasing sperm survival after cryopreservation (El-Sheshtawy et al., 2015, Akçay, 2023).

In addition to glycerol, DMSO can also be used for the protection of spermatozoa against cold shock. Also, ethylene glycol and propylene glycol too can be used as a cryoprotectant. Various carbohydartes such as sucros, trehalose, raffinose, and lactose can serve as cryoprotectant. However, glycerol remains the most succcesful cryoprotectant.

Sugars

Sugars, especially fructose, are the main energy source of spermatozoon cells. Sperm cells also can metabolize glucose, galactose, sucrose, maltose, xylose, raffinose, and mannose. The sugars added to the diluent also increase the level of protection spermatozoa against cold shock (Raheja et al., 2018; Bustani et al., 2021).

Antibiotics

The microbial load of semen is highly variable. The bacterial load of semen may increase, especially if the prepuce is not adequately cleaned during semen collection or if equipment hygiene is not maintained. Although the vast majority of the bacteria here are not pathogenic, they consume energy substances that are essential for spermatozoa. Many antibiotic agents like penicillin and streptomycin, ceftiofur, apramycin, and aminoglycosides or linco-spectin + tylosin + gentamycin are used to inhibit the growth of bacteria that can infect the semen (Sönmez, 2015; Raheja et al. 2018).

Other Additives

In semen diluents, many substances of plant and animal origin are also being experimented to achieve better results. In studies conducted by various researchers, plant-based additives such as pomegranate juice, green tea, strawberry, and coconut oil were added to the diluent. In a study, it was found that the addition of 1-5% strawberry juice to the Tris diluent was beneficial for the preservation of spermatological parameters during the cooling of semen and 3-6% during the freezing of semen (El-Sheshtawy et al., 2018). In another study, it was found that the addition of 1% green tea extract to tris-citric acid diluent improved in vivo and in vitro fertilization results and reduced lipid peroxidation (Ahmed et al., 2020). Tarig et al. (2017) reported that 2% coconut oil did not improve spermatological parameters in their study.

Honey is rich in sugar and antioxidants. Malik (2019) reported in his study that the addition of honey to the diluent significantly affected the motility values before the freezing and reduced sperm anomalies after freezing and thawing. In a scientific study, it was reported that the addition of 2.5% honey to tris diluent gave optimum results compared to BioXcell® diluent during the freezing of spermatozoa (Yimer et al., 2015). In another study, it was revealed that the addition of 1% honey to BioXcell® diluent in the storage of bull semen produce more effective results than the group without honey (El-Nattat et al., 2016).

Another substance of animal origin added to semen is fish oil. Malik et al. (2018) reported in their study that the diluent prepared as include 150 mg/100 ml fish oil in the reconstituent improved sperm quality after the freezing/thawing of buffalo sperm.

In a study, it was reported that the addition of nano selenium particles to tris-egg yolk-fructose semen diluent at a dose of 1.0 μg/ml improved sperm quality after freezing and thawing (Khalil et al., 2019).

Hu et al. (2009) reported that the addition of vitamin B_{12} to semen diluent at a dose of 2.5 mg/mL improved semen quality after thawing.

Gupta et al. (2022) reported that addition of curcumin (at the dose of 10 µM) in tris-egg yolk extender heled the protection of spermatological quality in post-thawing period in *Hariana* bull semen.

Currently Used Semen Extenders

Sodium Citrate-Egg Yolk Extender

Sodium citrate-egg yolk extender is prepared by adding 20% egg yolk and glucose (0.5%) to sodium citrate solution. Also, antibiotic such as pencilllin and streptomycin are added to the extender (Sönmez, 2015). This extender prepared by adding egg yolk to a 2.9% Sodium citrate solution. Nebel et al. (1985) reported that sodium citrate diluents containing 10% and 15% egg yolk provided much better preservation than sodium citrate diluents containing 5% and 0% egg yolk used in the freezing process.

Tris-Egg Yolk Extender

Researchers have developed a Tris yolk diluent that has better buffering capacity than phosphate buffer and sodium citrate buffer solutions and has less toxic effects on sperm cells. This extender yields significantly better results in the freezing compared to other extenders (Sönmez, 2015). In a study conducted by Prastiya et al. (2023), sodium citrate–egg yolk extender was prepared as Tris aminomethane 1.6%, citric acid 0.9%, lactose 1.4%, distilled water 80%, raffinose 2.5%, egg yolk 20%, penicillin 100000 IU/100 mL, streptomycin 0.1 g/100 mL, and 13% glycerin. They also add greeen tea extract to the tris- egg yolk extender according to their results green tea extract added at 0.15 mg/mL provided better preservation of cell membrane integrity, sperm motility and viability during cryopreservation of semen from Bali bulls.

Homogenized Milk And Skim Milk Extenders

Fat or skim milk alone fulfills many of the characteristics of a good semen diluent. However, if milk is to be used as a diluent, it must initially neutralize a substance called lactenin, which is harmful to sperm cells. For this purpose, the milk should be heated to 95ºC and kept at this temperature for 10 minutes. Fat and lipoproteins in the milk diluent help protect sperm cells from pH changes and cold shock. Glycerol and antibiotics are also added when preparing the milk diluent to be used in the freezing process (Sönmez, 2015; Bustani et al., 2021).

Commercial Extenders

There are ready-made diluents prepared by commercial companies to be used for long and short-term storage of semen in bulls. Egg yolk and glycerol are also added to these diluents. These powdered diluents can be dissolved in distilled water and made ready for use. Laiciphos®, Biociphos-plus®, BioXcell®, and OptiXcell® (IMV, LAigle, France), Andromed® and Triladyl® (Minitube, Tiefenbach, Germany), Optidyl® and Triladyl® (Biovet, France) are commercial semen extenders prepared for bull semen storage.

Many researchers have conducted comparative studies on which commercial diluent works best for long or short-term storage of bull semen. In a comparative study, OptiXcell® extender preserved sperm motility much better than Andromed® and BioXcell® extender during short-term storage in the refrigerator (at the +5ºC degree) (Fernandez- Novo et al., 2021). In another study conducted by Muiño et al. (2007), they reported that the use of Biladyl diluent containing egg yolk resulted in a significantly higher rate and longer survival of spermatozoa compared to Andromed® and Biociphos-plus® diluents without egg yolk in post-thawing period. Pieper et al. (2023) stated that Triladyl®, OptiXcell® and

BioXcell® diluents may show different results in the preservation of sperm motility, membrane integrity and acrosomal degradation according to differences in the duration of the equilibration process during freezing of bull semen.

Conclusion

In conclusion, studies are ongoing on the development of semen diluents to eliminate or minimize the damage that may occur during short or long term storage of bull semen.

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Ethical Statement

This study does not present any ethical concerns.

Author Contributions

Investigation: E.H.A.; Supervision: E.H.A.; Visualization: E.H.A.; Writing-Original Draft: E.H.A.; Writing- review & Editing: E.H.A.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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