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The Effects of Water-Based Coconut Extenders on Semen Preservation : A review

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ABSTRACT

For animal reproduction, artificial insemination has become a need. Semen preservation has been crucial in artificial insemination as it can determine the success of fertilization and pregnancy. Semen extenders have been critical in prolonging the usability of semen while also retaining its viability, motility, and integrity so that it can be used to fertilize female animals. Due to its nutritional and chemical qualities that can create an environment for spermatozoa to survive and thrive before insemination, coconut water has been the focus of various research addressing its effect on semen preservation. These studies have shown that coconut water has the potential to be an effective semen extender due to its ability to protect the semen during cooling and cryopreservation. Coconut water also includes nutrients (water, sugars, proteins, salts, and vitamins) to help spermatozoa thrive in a healthy environment. However, its performance lags that of commercial extenders, but it can be a less expensive alternative or replacement when commercial extenders are unavailable.

Keywords: coconut water, semen extender, semen preservation

INTRODUCTION

Artificial insemination is the least invasive and essential artificial breeding technique that can be performed on companion, non-domestic, and endangered species because it evades the physiological and behavioural obstacles but provides the same quality of genetic exchange between animals without transferring them physically (Durrant, 2009). This process can be critical in terms of reproduction and production advancements, such as increasing the level of genetic development and production percentage, as well as conservation of rare breeds or endangered species and a means for reproductive biotechnology like sperm cryopreservation (Morell, 2011). The success of artificial inseminations largely depends on the quality of semen being used because high-quality semen results in better conception rates and lower production fees. Furthermore, semen quality is greatly affected by the semen handling or thawing protocol (Zoccolaro et al., 2013).

More than ever, artificial insemination can be very significant right now for owners of all kinds of animals, whether for food or breeding purposes, due to the COVID-19 pandemic restrictions that limit the movement of people and animals. In seminal preservation, diluents used can be of chemical, vegetable or animal origin, the latter being the most frequently used (dos Reis et al., 2023). The exposure of sperm cells to the low temperatures that are necessary for the semen preservation process may hinder its viability and use and also affects its integrity and morphology (Gangwar et al., 2020). It is important to find cheaper alternatives to expensive chemicals and equipment for storing animal semen for a long time. In tropical countries, coconut water (*Cocus nucifera* L.) is a locally available abundant product. Coconut water contains sugars, vitamins, minerals, amino acids, and phytohormones and is conventionally used for plant tissue culture and micropropagation (Yong et al., 2009).

New studies have emerged seeking a diluent of vegetable origin capable of guaranteeing sperm quality and eliminating the risk of contamination. Coconut water preserves sperm cells alive in a liquid state until the second preservation day (dos Reis et al., 2023). Salts, sugars, vitamins, proteins, and neutral fats in coconut water are essential nutrients for sperm viability, fertility and preservation (Moreira et al., 2021). This nutrient helps in cell dehydration; these compounds act in the stabilization of the plasma membrane as cellular protectors and provide a good environment for the survival of these gametes (dos Reis et al., 2023). However, there are some disadvantages, such as the inability to store coconut water for an extended period and its limited availability in some regions of the world (Brasileiro et al., 2019). Therefore, this study aims to evaluate the effectiveness of water-based coconut extenders on semen preservation of

different farms and small animals as well as their effects on the various sperm parameters.

DISCUSSION

The purpose of sperm extenders is to keep sperm alive long enough for conception, to retain the sperm's metabolic processes, to maintain the pH during and after thawing, to avoid bacterial transmission or contamination, and to lessen the damage caused by cryogenics (Bustani and Baiee, 2021). A coconut-water-based extender is often prepared with other natural and chemical elements. Esguerra *et al.* (2020) combined coconut water with either tomato juice or garlic extract to serve as sperm extenders of Paraokan native chickens. The combination of coconut water and tomato juice increased sperm cell metabolism, pH regulation, osmotic pressure, and microbial growth suppression, according to the findings. Tomato juice can enrich semen extenders due to the lycopene in tomatoes that can reduce the sperm's susceptibility to lipid peroxidation, improving its quality (Al-Daraji, 2014). A diluent containing coconut water, fructose, and egg yolk has been proven to increase rooster spermatozoa quality and viability for up to 7 days when stored at 5°C (Rochmi and Sofyan, 2019). Powdered coconut water with the addition of glycerol and egg yolk has also been proven effective for the cryopreservation of collared peccaries (*Tayassu tajacu*) (Silva *et al.,* 2012).

Coconut water's effectiveness as a sperm extender is improved by combining the nutritional components of the natural substances. Using organic or raw materials reduce the cost of producing animal sperm extenders while maintaining the same level of quality as chemical sperm extenders. Researchers set out to develop a conservation media based on powder coconut water (PCW), characterized by the standardization and stabilization of natural coconut water through a dehydration process, followed by the formulation of specific media for cells and tissues. This is based on the excellent results obtained in the initial studies with natural coconut water. In its processed form, the base product (coconut endosperm liquid) outperforms any other conservation method in terms of shelf life and durability, as well as the potential to be stored indefinitely (Nunes and Salgueiro, 2011). Freeze-drying or spray-drying is done to produce powdered coconut water, and PCW still contains the same nutritional components as fresh, natural coconut water (Boonnumma *et al.,* 2014; Cardoso *et al.,*2005).

Nutritional and chemical properties

Coconut water is made up of both organic and inorganic molecules, with the most significant component being soluble carbohydrates. Still, it also contains protein, salts, minerals, amino acids, and a minor amount of oil. Sugars are the main elements of soluble solids in coconut water, such as sucrose, sorbitol, glucose, and fructose (Prades *et al*., 2012). Ponglowhapan *et al*. (2004) found that glucose and fructose can strongly influence the sperm motility and movement patterns of chilled canine semen for long-term preservation. Both sugars are significant components of coconut water. Coconut water is also rich in minerals such as sodium, calcium, magnesium, iron, zinc, and potassium, as the most prominent component (Santoso *et al.,* 1996; Wynn, 2017). It's also comprised of vitamin B, namely, nicotinic acid B3, pantothenic acid B5, biotin, riboflavin B2, folic acid, and a trace amount of thiamin B1 and pyridoxine B6, and has a low caloric value (DebMandal and Mandal, 2011). The other chemical constituents are phytohormones (auxin, cytokinin, and gibberellin), naturally occurring organic compounds involved in plant growth regulations (Yong *et al*., 2009). Other active components include methionine, L-arginine, cytokines, selenium, and vitamin C (Zulaikhah, 2019). L-arginine, together with Tris-yolk extender, was supplemented to poorly motile semen of sub-fertile buffalo bulls and was proven to have improved sperm motility, velocity vitality, and decreased tail and neck abnormalities after 4 hours of incubation at 4°C (Hegazy *et al*., 2021). Coconut water has phenolic compounds and flavonoids that can induce antioxidant activity, generate free radicals, and decrease lipid peroxidation (Lima *et al.,* 2015). These can help protect the sperm from any morphological defects and prevent low motility brought on by reactive oxygen species (Ahmadip *et al*., 2016; Banday *et al.,* 2017).

Parameters for evaluating the effects of coconut water on semen preservation

Sperm evaluation is done on fresh and stored semen to determine its morphology and viability. Sperm morphology traits establish the physiological or pathological status of the spermatozoa, and sperm viability is to assess its ability to fertilize. Semen viability is an essential basis for developing semen preservation to know the relationship between the biological method or

product used and the spermatozoa (Saacke, 1983). There is no single test that can conclude this that's why there are sets of tests that are performed through conventional microscopic methods, computer-assisted sperm analyzers (CASA), and flow cytometric analysis to determine the sperm's morphology and function (Tanga *et al*., 2021). The ability to maintain cellular volume in the face of changing conditions, bind to the oviductal epithelium, and undergo capacitation in a timely and suitable manner should all be investigated to appropriately assess male fertility (Petrunkina *et al.,* 2007). In terms of semen preserved via cryopreservation, the post-thaw function should be evaluated and tested because of cryoinjury. Watson (1995) said that the cold and warm shocks that can happen during and after cryopreservation could be potential stressors damaging the spermatozoa. In his study, he also wrote that only a proportion of the original semen sample survived during cryopreservation due to the "unphysiological" conditions set by the diluents, but this could be solved by examining different solutions that can stabilize the pH and osmolality of the samples while in storage.

Sperm motility is the capability of the spermatozoa to swim towards the ovum. In an article by Love (2016), it was suggested thaan experienced clinician should evaluate stallion sperm motility by determining the total motility (TMOT) and progressive motility (PMOT) using a phase-contrast microscope with both x20 and x40 objectives such that the final magnification should be x200 and x400 because using a light microscope might result in unclear visualization. Love added that computer-assisted sperm analyzers are also available to provide a more objective result with the same parameters as the subjective examination via microscope. In sperm morphology, two different techniques can be used to produce slides for analysis by using the eosin-nigrosin stain and Wright-Giemsa stain. The numbers and types of abnormal structures are recorded alongside the normal spermatozoa, and abnormalities are classified as primary and secondary (Freshman, 2002).

Coconut-water-based extenders on the semen of farm animals

Effect on cattle

Regarding sperm motility, coconut water with other diluents can significantly affect spermatozoa. Based on the study of El-Sheshtawy *et al*. (2017), the spermatozoa diluted with coconut water and 4% glycerol even performed better than the control mixture (tris-citric acid-fructose-egg yolk) in both cooled and frozen states. However, Aji *et al.* (2019) observed that coconut water-diluted semen couldn't outperform a commercial semen extender based on motility. The authors also noted that semen diluted with coconut water and 20% egg yolk should be used within the day for artificial insemination as its quality decreases over time due to damage in the plasma membrane of the spermatozoa, yet Muhammad *et al.* (2019) suggested that coconut water diluted semen can still be used after five days of dilution; both studies have stored semen at 5◦C.

All the trials found that coconut water when combined with another diluent (glycerol or egg yolk) provided to add nutrition or prevent cold shock, may be utilized as a semen extender for cow sperm, however, the viability of the sperm to be used for artificial insemination varies. According to the studies, spermatozoa diluted with coconut water performed as expected and can be utilized for artificial insemination. However, limited data may be available on the efficacy of coconut water-based semen extenders in fertilizing and impregnating cows. Coconut water-based extenders produce comparable outcomes when compared to other additives and diluents. Commercial extenders, on the other hand, can still produce better and higher results. Coconut water can be used as a substitute if you're seeking a less expensive way to stretch cow sperm or if there aren't any commercial extenders accessible at present. Still, it won't perform as well as commercial extenders.

Effect on goat

Powdered coconut water (ACP-101®) diluted buck semen has been compared against TRIS-diluted semen in terms of kinetic and morphological characteristics and evaluation and the TRIS-diluted semen still managed to outdo the PCW diluted semen post-thaw (Oliveira *et al.,* 2011; Oliveira *et al.,* 2009). On the other hand, Aragao *et al.*, (2013) stated that semen diluted with PCW (ACP-101c) was able to preserve sperm viability and morphology after cryopreservation, very similar to the performance of semen diluted with TRIS. The version of powdered coconut water on buck spermatozoa is at par with the semen extended with TRIS. It can be used as an alternative extender when a cheaper replacement is needed.

Coconut water-based extenders affect goat semen the same way as commercial extenders

(Eg. TRIS) but are often in need of additional products (e.g., egg yolk, glycerol, etc.) to serve as cryoprotectants to avoid cryoinjuries while in storage. Coconut water can be used instead when commercial extenders are too expensive or unavailable. However, the authors cautioned that the optimum ratio of coconut water to cryoprotectant should be determined since too much coconut water can damage sperm motility and quality. Coconut water has antioxidant enzymes that act to prevent the formation of Reactive oxygen species (ROS), one of the main causes of plasma membrane damage in sperm (dos Reis et al., 2023). It can be suggested that the concentration of this agent in the diluent was insufficient to protect the plasma membrane when the sperms were refrigerated. The efficiency of coconut water in preserving sperm motility in small ruminants has been previously reported, with results above 60% for this variable in cooled semen while using coconut water as a seminal diluent (Salim et al., 2018).

Effect on pig

The effects of a water-based coconut extender on pig semen have also been studied by Rodriguez (2016) and compared to a commercial extender. Although the motility performance of the spermatozoa diluted with coconut water was slow compared to the semen mixed with a commercial extender, the performance was still enough to consider coconut water as an alternative diluent for boar sperm preservation. Similarly, Toniolli *et al.,* (2010) also learned that even if boar semen diluted with PCW (ACP-103) can produce good sperm parameters results, it can't perform better in terms of in vitro fertilization rate. Beltsville Thawing Solution (BTS) is a commercial boar semen extender used as the control in the experiment. The authors wrote that the powdered coconut water extender could be enough to preserve semen for routine laboratory work. Compared to other extenders, Duroc pig semen diluted with Tris-egg yolk-young coconut water was also tested by Berek *et al.,* (2021) based on viability, abnormality, and pH according to their shelf life. The results revealed that this combination of ingredients had a significant effect on the parameters and can maintain those numbers up until 48 hours of shelf life.

Effect on horse

Unfortunately, there are not so many studies regarding the effects of coconut water on stallion semen. The two studies had opposite results and concluded differently. Although, Brasileiro *et al.*, (2019) only studied the effects of coconut water at refrigerated temperature while London *et al.,* (2017) worked on cryopreserved semen. So far, water-based coconut extenders have only been shown to work on cryopreserved sperm. Coconut water hasn't been thoroughly investigated in terms of its effects on stallion spermatozoa or even in comparison to other diluents and additions.

Coconut water-based extenders on the semen of small animals

Effect on cat and dog

In a study conducted by Cardoso *et al.,* (2003), coconut water extenders at three different glycerol concentrations (4, 6, 8%) were compared using semen from six adult dogs. Results showed no significant difference in motility and vigour was observed among the three groups. Cardoso *et al.,* (2007) examined the PCW (ACP-106) diluted semen using an in vitro semen-oocyte interaction assay (SOIA) to determine its fertilizing capacity and were able to accomplish good results as there was 75% successful sperm-oocyte interaction despite a low percentage of the intact plasma membrane. Therefore, powdered coconut extender diluted semen can maintain the fertility potential of canine spermatozoa. Moreover, Cardoso *et al.*, (2005) suggested that powdered coconut water (ACP-106) can be used as an alternative to natural coconut water if it is not present or cannot be obtained. Based on their study, the performance of NCW and PCW diluted semen had no significant differences and both diluents could maintain amounts of spermatozoa higher than 60% (ACP-106: 71.4% & NCW: 70.7%). In general, natural or powdered coconut water can be used as a canine semen extender.

Meanwhile, powdered coconut water (ACP-117c) was compared to TRIS extenders for evaluation of sperm viability, functionality, mitochondrial activity, and morphology using 18 cats, and results show that ACP-117c can be a substitute for the recovery of cat epididymal spermatozoa but was not as efficient for frozen samples (Barbosa *et al.,* 2020). A study by Lima *et al.* (2016) also tested spermatozoa diluted with powdered coconut water collected from cat epididymides. The authors proved that ACP-117c was able to maintain a higher number of living sperms compared to using the Tris extender; therefore, powdered coconut water may be used to recover feline spermatozoa that have been cooled for up to 4 hours.

Coconut water has been shown in numerous studies to have the ability to serve as a semen extender. The coconut water components successfully generated an environment where canine and feline spermatozoa could survive and sustain viability during cooling, freezing, and post-thawing with the inclusion of other substances like egg yolk and glycerol. Regarding canine and feline semen, coconut water is a proven semen extender. Artificial inseminators still prefer commercial extenders, although coconut water can be a cheap option if extenders become too pricey.

Effect on rabbit

A study by Jimoh (2020) showed high conception rates, litter size, and productivity index at the birth of rabbits inseminated with semen diluents prepared with 60% and 80% coconut water, and acceptable values were acquired for spermatozoa motility, structural membrane integrity and acrosome integrity of the fresh semen stored with coconut water diluent. Another study showed an 8% increase in fertility of rabbits artificially inseminated with semen dilated with coconut water compared to those diluted with Brackett-Oliphant Medium. Still, there were no significant differences in sperm motility and viability (Trejo *et al.,* 2013). The authors found that the number of rabbits born using the semen diluted with PCW was more remarkable than those inseminated with MBO-diluted semen.

Coconut water-based extenders are not yet well studied in terms of their effects on rabbit spermatozoa. The studies published so far have proven promising results, and coconut water is successful in producing high conception rates and suitable sperm parameters.

CONCLUSION

Extenders made from coconut water offer much potential for preserving sperm viability, morphology, motility, and integrity in both cooled and frozen sperm. As a result, coconut water can be utilized as a low-cost and natural semen extender. Coconut water preserves sperm cells alive in a liquid state until the second day of preservation and the 30-day cryopreservation period.

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