

## EFFECT OF COCONUT WATER ON FREEZABILITY AND FERTILITY OF BUFFALO BULLS' SPERMATOZOA

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### SUMMARY

The aim of the current study was to instead of egg yolk with various levels of coconut water as a cryoprotectant in Tris-extenders and to investigate its effect on quality and fertility of frozen buffalo bulls' semen. Semen was collected twice a week from five mature buffalo bulls for 12 weeks and ejaculates with 70% progressive motility prior were pooled to have sufficient semen volume for a replicate and to eliminate the bull effect. After evaluation semen quality, the pooled fresh semen was divided into five equal fractions; one fraction was diluted with Tris-egg yolk extender (control) and the other four replicates, whole egg yolk was replaced by different concentrations of five; 10; 15 and 20% coconut water extracted. Semen was diluted to eighty million sperm/ml packaged into 0.25ml straws, cooled held at 5°C for 4 h, and then frozen in liquid nitrogen and stored at -196°C until for artificial insemination. The percentages of sperm progressive motility, live sperm, sperm abnormality, intact sperm acrosome and plasma membrane integrity were evaluated post dilution, post-equilibration and post frozen-thawed processes. The results revealed that 20% coconut water extender was more effective on cryopreservation of semen characteristics (progressive motility, live sperm, sperm abnormality, intact sperm acrosome and plasma membrane integrity) of buffalo spermatozoa than whole egg yolk extender and other coconut milk extenders. Also, conception rates were higher in 20% coconut water extender compared with control egg yolk extender (66.7% vs 58.3%, respectively). In conclusion, added 20% coconut water to extender improves the freezability and fertility of buffalo spermatozoa and can be used as an alternate to egg yolk in cryopreservation of buffalo bulls semen.

**Keywords:** cryopreservation, coconut water, extender, fertility, buffalo bull

### INTRODUCTION

Artificial insemination (AI) is the most important technique for rapid genetic improvement of farm animals. Egg yolk (EY) was considered an essential additive to maintain viability of cooled and frozen spermatozoa (Hafez, 2000). The use of EY in higher concentration has several drawbacks besides being a source of bacterial contamination and some of its constituents could have detrimental effects on spermatozoa (Amirat *et al.*, 2004). Semen extender is an important aspect of semen processing for AI. Extenders free of animal protein have been tested in the study of Bousseau *et al.* (1998), because the use of egg yolk associated with sanitary risks may contribute in lower fertility of cryopreserved semen directly through deteriorating the semen quality by producing harmful metabolites and toxins or indirectly through local infection leading to abortion (Akhter *et al.*, 2008; Althouse, 2008). Cardoso *et al.*, (2005) indicated that using coconut water-based extender has been proposed as an alternative semen extender that is non-toxic, buffering, low cost, practical, and effective. Fertility of extended semen gradually deteriorates with the increase in storage time, related to oxidative stress during storage (Johnson *et al.*, 2000; Kumaresan *et al.*, 2009). Coconut water is a non-pathological fluid, which is free of contamination, toxins and infection. Preparation of new culture medium with coconut water as supplement for human semen preparation, for oocyte fertilization, for embryo growth and development has been reported by Velmurugan (2014). DebMandal and Mandal (2011) indicated that coconut water and milk have antiviral, antibacterial, anti-fungal, antiparasitic, and antioxidant properties among other medicinal and health benefits. Coconut water has positive effects on semen quality due to its high content of free sugars, antioxidants, and minerals (Vasconcelos *et al.*, 2009). Coconut water has been successfully used for culture and bovine embryo freezing (Martins *et al.*, 2005; Cordeiro *et al.*, 2006). Coconut water is easy to prepare, readily available and cheap compared to egg yolk and milk. The aim of

this study was to evaluate the effect of instead of Egg yolk (EY) by different concentrations of coconut water on freezability and fertility of Egyptian buffalo bulls' semen.

## **MATERIALS AND METHODS**

This study was conducted at the International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt, in participation with Department of Animal Production, Faculty of Agriculture, Tanta University, during the period from January to September, 2020.

### ***Experimental animals:***

Five sexually mature healthy buffalo bulls with average of 600 kg body weight and aged 4.5 years old at the beginning of experiment were used in the present study as semen donors. They were housed individually under semi-open sheds. All bulls were healthy and clinically free from external and internal parasites. Palpation of the external genital showed that they were typically normal. The testicular tone was glandular; all epididymal regions were present in both testes, almost equal in size and moved freely up and down within the scrotal pouches.

### ***Feeding system:***

The feeding regime was applied according to the live body weight as recommended by Animal Production Research Institute (1975), every bull was fed on daily ration composed of 8 kg concentrate fed mixture (CFM) (14.2 % crude protein, 7.66 % crude fiber, 1.6 % ether extract and 12.8% moisture), 6 kg rice straw and 40 kg green berseem (*Trifolium alexandrium*) during the green feeding period (winter season). The CFM was composed of 32% un decorticated cotton seed cake, 26% wheat bran, 22% yellow maize, 12% rice bran, 5% linseed meal, 0.5% limestone, 2% vines and 0.5% salt. The daily ration was given individually to all buffalo bulls at 8.0 a.m., while drink clean fresh water and mineral blocks were available for all bulls at all day times.

### ***Experimental procedures:***

#### ***Semen collection:***

The semen was collected from five experimental buffalo bulls twice a week for 3 months using the conventional artificial vaginal method. Semen ejaculates were collected before feeding from 7.0 am to 8.0 am. A bull was used as a teaser animal for semen collection and sexual preparation. Collected semen immediately was held in a water bath at 37°C for examination.

#### ***Semen processing:***

Ejaculates having good mass motility ( $\geq 70\%$ ) were pooled for each collection day. On each collection day, semen was pooled and divided into five portions; the first was diluted with tris-20% egg yolk extender (control) and in others four extenders the whole egg yolk was replaced by different concentrations of five, 10, 15 and 20% coconut water. Thereafter, each portion of semen was placed into a refrigerator at 5°C for 4 hours for gradual cooling as an equilibration period of spermatozoa, then at the end of the equilibration period the extended semen was filled by automatic semen filling machine in French straw of 0.25ml capacity and put it on liquid nitrogen vapor (-90 – 100°C) for ten minutes after that it was immersed in liquid Nitrogen (-196°C) for freezing. The frozen straws were thawed in water bath at 37°C for 30 seconds for examination.

#### ***Preparation of experimental extender:***

Tris-citric acid extender was used as a buffer, consists of 3.025g tris-(hydroxymethyl-amino methane), 1.675g citric acid, 0.75g glucose and 7.0% glycerol, 20% fresh egg yolk (EY), 0.25g linco-spectin and 0.005g streptomycin and completed with bi-distilled water up to 100 ml. Preparation of Coconut water extender: Fresh coconut fruits were purchased from the market, they were punctured, and water was collected from them into a beaker. The water was decanted, filtered, and collected in a sterile glass bottle. The whole 20% EY in Tris-based extender was replaced by different levels of 5, 10, 15 and 20% coconut water extraction.

#### ***Semen quality assessment:***

Semen characteristics (the percentages of progressive motility, live sperm, sperm abnormality,

plasma membrane integrity and acrosome integrity) were estimated during different stages of preparation (after dilution, post equilibration and post-thawing).

**Progressive motility (%):** It was estimated according to Amman and Hammerstedt (1980).

**Live sperm (%):** Live sperm percentage was assessed according to (Hackett and Macpherson, 1965).

**Sperm abnormality (%):**

Sperm abnormalities percentage was determined during the examination of live/dead sperm percentage at a high -power magnification (400x), according to the classification adopted by Blom (1983).

**Plasma membrane integrity (%):**

The plasma membrane integrity of spermatozoa was assessed using the hypo-osmotic swelling test (HOST) as described by Jeyendran *et al.* (1984).

**Acrosome integrity (%):**

Acrosome integrity was determined by using a Giemsa stain procedure as described by Watson, (1975).

**Recovery rate:**

Recovery rate for the percentages of sperm motility, live sperm, acrosome and plasma membrane integrity was calculated according to the following formula: Recovery rate (%) = (post-equilibrium % / post-dilution %) Recovery rate (%) = (post-thawing% / post-dilution %)

**Fertility trial:**

A total of 49 Egyptian buffalo owned by small and medium scale breeding holder in different village in Kafr Elsheikh Governorate were artificially inseminated with random frozen doses from control and 10, 15 and 20% of coconut water extenders. At observed estrus buffalos were rectal examined to clarify the occurrence of heat. Each female buffalo was inseminated with a single insemination dose (0.25ml French straw  $20 \times 10^6$  motile spermatozoa) 8-14h after estrus behavior had begun. At the time of insemination, the frozen semen was thawed at 37°C for 30 seconds. Using recto-vaginal technique and the universal insemination gun, the semen was deposited just next to the anterior end of the cervix. Conception rate was calculated based on pregnancies confirmed by rectal palpation 45-50 days after insemination as following:

$$\text{Conception rate (\%)} = (\text{No. of conceived cows} / \text{No. of inseminated cows}) \times 100$$

**Statistical analysis:**

Data were statistically analyzed using general model program (SAS, 2004). Duncan multiple range tests were used to test the differences among means (Duncan, 1955). The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

## **RESULTS AND DISCUSSION**

**Progressive sperm motility%:**

The effect of different concentrations of coconut water in Tris-extender and Tris-20%egg yolk on progressive motility of Egyptian buffalo bulls' spermatozoa during different stages of preparation are presented in Table 1. Post-dilution, progressive motility of spermatozoa were superior ( $P < 0.05$ ) in extender containing 15 and 20% of coconut water compared to extenders had low concentration of CW (5 and 10%, respectively), while the differences did not significant among - levels of 15 and 20% and control EY extender. After equilibration and post-thawing process the percentages of sperm progressive motility were superior ( $P < 0.05$ ) in extender containing 20% CW (55.2 and 45.8%) compared with control EY extender (49.8 and 39.1%, respectively). The best sperm progressive motility had been found in the extender containing 20% CW. Also, it was observed that sperm progressive motility percentage was improvement by increasing coconut water level.

**Table (1): Effect of coconut water (CW) on percentage of Egyptian buffalo bulls 'sperm progressive motility during different stages of semen cryopreservation.**

| Stages                       | Control       | Coconut water concentrations % |              |              |              |
|------------------------------|---------------|--------------------------------|--------------|--------------|--------------|
|                              |               | 5                              | 10           | 15           | 20           |
| Post-dilution                | 66.6ab ± 0.75 | 57.8c ± 0.94                   | 63.8b ± 0.77 | 67.6a ± 1.85 | 67.5a ± 0.99 |
| Post-equilibration (at 5 °C) | 49.8b ± 1.01  | 38.0c ± 1.21                   | 49.7b ± .92  | 54.8a ± 1.14 | 55.2a ± 1.32 |
| Post-thawing                 | 39.1b ± 0.74  | 19.3d ± 0.95                   | 33.7c ± 0.79 | 37.8b ± 0.61 | 45.8a ± 1.52 |

Means have different superscripts a, b, c and d in the same row are differ significantly ( $P < 0.05$ )

#### Live sperm percentage:

Live sperm percentage of buffalo bulls' semen during different stages of freezing with different concentrations of coconut water and tris-egg yolk extender (control) are presented in table (2). In post dilution and post equilibration the highest value was obtained in the extender containing 20%, the differences among other concentrations of CW were significant ( $P < 0.05$ ) but the differences were not significant among the extenders containing 10, 15 and 20% CW compared to control group, the lowest value was obtained in 5% CW extender. In post-thawing, the differences between different concentrations of coconut water and tris-egg yolk extenders were ( $P < 0.05$ ) significant. The highest value was found in 20% extender followed by 15% and control extender while the lowest value of live sperm was obtained in 5% CW extender.

**Table (2): Effect of coconut water (CW) on percentage of Egyptian buffalo bulls' live sperm during different stages of semen cryopreservation.**

| Stages                       | Control       | Coconut water concentrations % |              |              |              |
|------------------------------|---------------|--------------------------------|--------------|--------------|--------------|
|                              |               | 5                              | 10           | 15           | 20           |
| Post-dilution                | 74.4ab ± 1.67 | 66.4c ± 1.11                   | 72.9b ± 0.71 | 77.7a ± 0.66 | 74.4a ± 0.76 |
| Post-equilibration (at 5 °C) | 59.1b ± 1.21  | 48.5c ± 1.48                   | 59.1b ± 1.44 | 63.5a ± 1.19 | 60.5a ± 1.52 |
| Post-thawing                 | 41.4bc ± 0.83 | 23.3d ± 0.61                   | 38.6c ± 1.08 | 44.9b ± 1.00 | 50.3a ± 1.93 |

Means have different superscripts a, b, c and d in the same row are differ significantly ( $P < 0.05$ )

#### Sperm abnormalities%:

The data of the effect of different levels of coconut water in extender on percentage of buffalo bull sperm abnormality at different stages of spermatozoa (post-dilution, equilibration and post-thawing) are presented in Table 3. The percentage of sperm abnormalities was lower in semen extended with CW extender during post-dilution, equilibration and post-thawing stages. The highest sperm abnormality was observed in 5% CW extender compared to control extender and 10, 15 and 20% coconut water extenders.

**Table (3): Effect of coconut water (CW) on Egyptian buffalo bulls' abnormal spermatozoa during different stages of cryopreservation.**

| Stages                       | Control      | Coconut water concentrations % |              |             |              |
|------------------------------|--------------|--------------------------------|--------------|-------------|--------------|
|                              |              | 5                              | 10           | 15          | 20           |
| Post-dilution                | 7.4bc ± 0.12 | 8.2a ± 0.19                    | 7.4bc ± 0.22 | 7.3c ± 0.22 | 8.0ab ± 0.24 |
| Post-equilibration (at 5 °C) | 8.4ab ± 0.19 | 8.8a ± 0.24                    | 7.9b ± 0.21  | 7.8b ± 0.28 | 8.1ab ± 0.26 |
| Post-thawing                 | 8.9b ± 0.20  | 9.7a ± 0.28                    | 8.9b ± 0.25  | 8.2b ± 0.26 | 8.5b ± 0.23  |

Means have different superscripts a, b, c and d in the same row are differ significantly ( $P < 0.05$ )

#### Acrosome integrity%:

The effect of CW concentrations in extender on acrosome integrity of buffalo bulls' spermatozoa at all three stages of semen preparation are presented in Table 4. In post-dilution and post-equilibration, acrosome integrity percentages did not significantly differ among 10, 15, 20% extenders and control, while extender containing 5% significantly ( $P < 0.05$ ) decreased the acrosome integrity

percentage compared to control extender or the other concentrations of cw extenders.

At the post-thawing stage, the percentages of acrosome integrity was significantly ( $P<0.05$ ) higher in extender containing 15, 20% compared with control and other CW extenders. The lowest value was obtained in 5% cw extender during three stages of preparation.

**Table (4): Effect of coconut water (CW) on sperm acrosome integrity percentage on Egyptian buffalo bulls' spermatozoa during different stages of cryopreservation.**

| Stages                       | Control       | Coconut water concentrations % |                |               |               |
|------------------------------|---------------|--------------------------------|----------------|---------------|---------------|
|                              |               | 5                              | 10             | 15            | 20            |
| Post-dilution                | 83.7 a ± 1.08 | 77.0 b ± 1.54                  | 84.9 a ± 0.72  | 85.9 a ± 0.71 | 85.0 a ± 0.66 |
| Post-equilibration (at 5 °C) | 74.6a ± 1.22  | 67.9b ± 1.90                   | 77.0a ± 1.06   | 76.3a ± 0.91  | 76.7a ± 1.00  |
| Post-thawing                 | 62.1 b ± 1.45 | 48.8 c ± 2.21                  | 65.1 ab ± 1.33 | 67.6a ± 0.99  | 69.1a ± 1.27  |

Means have different superscripts a, b, c and d in the same row are differ significantly ( $P<0.05$ )

**Plasma membrane integrity%:**

The effect of CW concentrations on plasma membrane integrity percentages during different stages of freezing of Egyptian buffalo bulls' spermatozoa are presented in Table 5. At post-dilution, the percentages of plasma membrane integrity were significantly ( $P<0.05$ ) higher in extender containing 15 and 20% compared with control and 5% cw extender.

In post-equilibration and post-thawing, the differences were not significant in plasma membrane integrity percentages among the extenders containing 10, 15 and 20% CW compared to control, while the highest value was found in 20% cw extender. The extender containing 5% cw was significantly ( $P<0.05$ ) decreased plasma membrane integrity percentage when compared to control or other cw extenders.

**Table (5): Effect of coconut water on (CW) plasma membrane integrity percentage on Egyptian buffalo bulls' spermatozoa during different stages of cryopreservation.**

| Stages                       | Control      | Coconut water concentrations % |               |              |              |
|------------------------------|--------------|--------------------------------|---------------|--------------|--------------|
|                              |              | 5                              | 10            | 15           | 20           |
| Post-dilution                | 73.8b ± 0.76 | 68.9c ± 1.19                   | 76.0ab ± 0.74 | 78.0a ± 0.85 | 76.8a ± 0.99 |
| Post-equilibration (at 5 °C) | 65.2a ± 1.16 | 58.8b ± 2.24                   | 65.8a ± 1.48  | 69.0a ± 1.04 | 66.9a ± 1.62 |
| Post-thawing                 | 54.6a ± 1.53 | 42.8b ± 2.84                   | 55.6a ± 1.63  | 58.8a ± 1.45 | 60.2a ± 1.72 |

Means have different superscripts a, b, c and d in the same row are differ significantly ( $P<0.05$ )

**Conception rate:**

The effect of different coconut water extenders and Tris-egg yolk extender on conception rate are presented in Table 6. Semen extended with 20% coconut extender improved conception rate 66.7% compared to control-Tris egg yolk extender and 10 and 15% CW extender (58.3% vs 40% and 50%, respectively).

**Table (6): Effect of and different CW and Tris-EY extenders on conception rate of buffalo bulls' spermatozoa.**

| Items               | Tris- EY (control) | Coconut water concentrations % |     |       |
|---------------------|--------------------|--------------------------------|-----|-------|
|                     |                    | 10                             | 15  | 20    |
| Inseminated animals | 12                 | 10                             | 12  | 15    |
| Conceived animals   | 7                  | 4                              | 6   | 10    |
| Conception rate %   | 58.3%              | 40%                            | 50% | 66.7% |

Results indicated that inclusion coconut water in semen extender improved cryopreserved spermatozoa of buffalo bulls' semen. The present results indicated that coconut water extender (20%) provided a more adequate medium to sustain the percentages of progressive motility, live sperm, sperm abnormality, plasma membrane integrity and intact acrosome of buffalo spermatozoa as evidenced from

its ability to maintain the sperm parameters better than the control egg yolk extender. The improvement observed on sperm parameters indicated that the extender contain 20% coconut water contained essential components such as sugars, vitamins, minerals and amino acids (Yong *et al.*, 2009; USDA National Nutrient Database, 2015) required for cryo survival of spermatozoa. Many literatures confirmed that sugar as a source of energy, an osmolyte and a cryo protectant play an important role for sperm survival following cryopreservation (Yancey, 2005; Purdy, 2006; Naing *et al.*, 2010 and Daramola *et al.*, 2016). Purdy (2006) observed that sugar increase the osmotic potential of cells and protect plasma membrane from chilling-induced injury. Koshimoto and Mazur (2002); Aboagla and Terada (2003) suggested that the goat spermatozoa readily utilize sugars for respiration, and these sugars also provide osmotic balance and protection. In addition, the protective effect of coconut water could also be linked to its major essential amino acids which play an important role in cell membrane integrity (Sakanaba *et al.*, 2004; Yong *et al.* ( 2009). In mammalian spermatozoa, Kundu *et al.* (2001) and Atessahin *et al.* (2008), suggested that the cryoprotective potential of amino acids during freezing stemmed from their ability to form a layer on the spermatozoa surface, and positively charged molecules combined with the phosphate groups of sperm plasma membrane phospholipids has been observed. Furthermore, the improved sperm characteristics with coconut water indicated the ability of coconut water extenders to efficiently harness potassium contained in coconut water and spermatozoa for survival of the spermatozoa during cryopreservation. Yong *et al.* (2009) observed that coconut water is rich in potassium. Also, Mansour *et al.* (2002) demonstrated that addition of potassium levels to storage medium have beneficial effect on the viability of diluted spermatozoa. In the present study, the improvement observed in the percentages of sperm motility, live sperm, sperm abnormalities, intact acrosome and plasma membrane integrity could be linked to essential antioxidants in coconut water. Many authors confirmed that in previous studies low toxicity and good water solubility of antioxidants such as pyridoxine and vitamin C derived from the addition of coconut water may be attributed to the improved semen characteristics, (Kannan and Jain, 2004; Arabi and Seidaie, 2008). The protective effect of coconut water compounds against oxidative damage due to increased production of reactive oxygen species (or free radicals) associated with *in vitro* storage at low temperatures, especially the polyunsaturated fatty acids in the cell membrane, or to the nucleic acids in the cell nucleus (Evans and Halliwell, 2001). In the present study, extender contain 20% improved sperm parameters specially during post-thawed stage. These improvement in cryopreserved spermatozoa was agreement with that reported by many authors (Daramola *et al.* (2016) in buck semen; El-Sheshtawy *et al.* (2017) in cattle semen and El-Shamaa *et al.* (2018) in Holstein bull spermatozoa). Conception rate in buffaloes inseminated with semen cryopreserved semen containing 20% coconut water was higher than control and other 10% or 15% coconut water extenders (66.7% vs 58.3% and 40% or 50%, respectively). Higher frozen–semen quality and conception rate was achieved with the use of 20% coconut water extender as compared to 20% tris-egg yolk extender.

## CONCLUSION

The results indicated that the inclusion of 20% coconut water in tris-extender markedly improved semen quality of cryopreserved spermatozoa (the percentages of progressive motility, live sperm, sperm abnormality, acrosome integrity and plasma membrane integrity) and fertility of buffalo bulls' spermatozoa.

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## تأثير استبدال صفار البيض بماء ولبن جوز الهند في تحسين السائل المنوي المجمد والخصوبة لطلائق الجاموس

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اجريت هذه الدراسة بقسم الانتاج الحيوانى- كلية الزراعة – جامعة طنطا بينما اجريت التجربة العملية بالمركز الدولى للتدريب على رعاية الحيوان بسخا التابع لمعهد بحوث الانتاج الحيوانى – وزارة الزراعة. تهدف الدراسة الحالية الى استبدال صفار البيض بمستويات مختلفة من ماء جوز الهند فى مخفف السائل المنوى وتأثيره على القدرة التجميدية والخصوبة لطلائق الجاموس. تم جمع السائل المنوى مرتين أسبوعيا من خمس طلائق جاموس ناضجة جنسيا لمدة 12 اسبوع يتم عمل خلط للقفذات التي تزيد حيويتها عن 70% قبل الحفظ وذلك لإيجاد كمية مناسبة من السائل المنوى وايضا لازاله تأثير الطلوقه بعد تقيم جوده السائل المنوى تم تقسيم القفذات المخلوطه الى 5 أجزاءمتساويه. الجزء الأول يخفف باستخدام مخفف الترس + 15% صفار بيض كامل وباقي الأجزاء يتم استبدال صفار البيض بماء جوز الهند بنسب 5%، 10%، 15%، 20% علي التوالي. كل 1مل من السائل المنوى المخفف يحتوى على 80 مليون حيوان منوى قبل التجميع. تم تعبئة السائل المنوى المخفف فى قصبيات سعتها 0.25 مل توضع فى الثلجة على درجة حرارة 5 م لمدة 4 ساعات كفترة موازنة ثم تجمد فى النيتروجين السائل على درجة حرارة -196 م ثم تخزن لحين استخدامها فى التلقيح الاصطناعى. تم تقدير النسب المنوية للحركة التقدمية للحيوانات المنوية والحيوانات المنوية الحية والميتة والشاذة وسلامة الاكروسوم وسلامة الغشاء البلازمي بعد التخفيف مباشره ثم بعد الموازنة ثم بعد التجميد والاسالة. اظهرت النتائج ان المخفف المحتوى على 20% ماء جوز الهند كان اكثر تأثير على حفظ خصائص السائل المنوى(الحركة التقدمية للحيوانات المنوية والحيوانات المنوية الحية والميتة والشاذة وسلامة الاكروسوم وسلامة الغشاء البلازمي) للجاموس عن مخفف السائل المنوى المحتوى على صفار البيض و كذلك مخففات ماء جوز الهند الاخرى وايضا كانت نسبة الاخصاب عاليه فى المخفف المحتوى على 20% ماء جوز اللهند مقارنة بمجموعه المقارنه والتي تحتوى على صفار البيض الكامل (66.7% مقابل 58.3%) على التوالي

الخلاصة:- ان المخفف المحتوى على 20% ماء جوز الهند ادى الى تحسين قدره التجميديه وخصوبة السائل المنوى لطلائق الجاموس ويمكن استخدامه بديلا عن صفار البيض فى حفظ السائل المنوى الجاموسى .