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Substitution of Egg Yolk with Arabic Gum in Extenders Used for Ram Semen Cold Storage or Cryopreservation

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Abstract: The current research was designed to compare the effects of substitution of egg yolk (EY) in ram extender with Arabic gum (AG) in different concentration in cooled or frozen semen collected by artificial vagina (AV) or electro-ejaculator (EE) in Noemi rams. Semen was collected 2 times per week for 6 weeks from 6 rams by EE method and after a rest period of 2 weeks, semen samples were collected by AV method. Tris extender either contained 15% EY (Control) or 3%, 5% or 7% AG (AG3, AG5 and AG7, respectively) were used as semen extenders. Spermatozoa were evaluated after cold storage (4 °C) or freezing/thawing in liquid nitrogen for sperm motility parameters: total motile spermatozoa (% TMS), rapid progressively motile sperm (% PRS), curvilinear velocity (VCL) µm/s, rectilinear velocity (VSL) µm/s, the average path velocity (VAP) µm/s, straightness index (% STR), linearity coefficient (% LIN), morphological defects and acrosome membrane integrity. Results showed that AV-collected sperm and diluted with AG or EY were more efficient in motility than EE. The values of VCL, VSL and VAP were higher in AG3 and AG5 and EY extenders for semen collected by AV compared to EE. After cold storage, AG3 gave better ($P<0.01$) results of LIN and STR either in spermatozoa collected by AV or EE. Significant differences in morphology defects were noted in semen collected by EE regardless of extenders used, however, AG supplementation enhance morphological defects in spermatozoa collected by AV ($P<0.05$). Post-freezing, Tris extender containing EY and AG5 in AV-collected spermatozoa had higher TMS, PRS, VCL, VSL and VAP compared to others. Sperm morphological defects were higher in AG7 extender in EE and EY extender in AV group. Plasma membrane integrity was significantly ($P<0.05$) higher in AG3 and AG5 extenders in AV-group compared to control and other groups. The acrosome membrane integrity in AV-semen groups was greater ($P<0.05$) than that of EE-semen group. It can be concluded that, Arabic gum is a good candidate to replace egg yolk in ram semen extender when added at a concentration of 3 or 5%. Although method of artificial vagina semen collection gave better results than electro-ejaculator. However, Arabic gum could be added to ram semen extender to enhance the electro-ejaculator-collected semen characteristics when added at a concentration of 5%.

Keywords: ram semen storage, semen collection method, egg yolk, Arabic gum.

INTRODUCTION

Artificial insemination in sheep has long been used worldwide to propagate the genetically superior rams. Ram semen cryopreservation is the key factor for artificial insemination success. During semen cryopreservation, sperm cells suffer from dangerous thermal changes. To protect spermatozoa from these fatal thermal shocks, certain substrates must be added to semen during cooling process to avoid harmful effects of chilling on spermatozoa. Numerous buffers and cryoprotectants have been used for ram semen to increase post-thaw sperm quality (Coyan *et al.*, 2010; Amini Pour *et al.*, 2013). Ram spermatozoa have been reported to be more cryosensitive than humans, rabbits, cats and dogs (Grötter *et al.*, 2019), this has been linked to elevated levels of polyunsaturated fatty acids in plasma membrane of ram spermatozoa (Quinn *et al.*, 1980; White, 1993). Moreover, elevated levels of polyunsaturated fatty acids make sperm more susceptible to lipid peroxidation in the presence of reactive oxygen species (Watson, 2000; Samadian *et al.*, 2010) which is produced inside spermatozoa as a result of active metabolism (Griveau and Lannou, 1997; Baumber *et al.*, 2000). Egg yolk is among cryoprotectants used in ram semen cryopreservation. Egg yolk keeps plasma membrane and acrosome integrity in association with the other components (Futino *et al.*, 2010). However, egg yolk has some biosecurity risks of microbial contamination. Moreover, egg yolk may interact with its coagulating enzyme presenting in seminal plasma (Purdy, 2006) which ultimately can reduce the quality and fertilizability of spermatozoa (Leboeuf *et al.*, 2000; Bittencourt *et al.*, 2008). Accordingly, efforts have been made toward finding suitable substitutes of egg yolk to be used in ram semen cryopreservation. Arabic gum is one of the recently used plant origin cryoprotectants and has been included in extenders for cryopreservation of semen of stallion, ram, and buck (Ali *et al.*, 2017; Ali and Zeitoun, 2017). Arabic gum is a dried resin exudate from stems and branches of some trees of Acacia genus. Chemically, AG is a water-soluble polysaccharide with sugars including rhamnose, arabinose, and galactose and contains highly branched complex arabinogalactan proteins. Furthermore, it contains glucuronic acid and minerals such as calcium, magnesium, and potassium (Akiyama *et al.*, 1984; Islam *et al.*, 1997; Lopez-Torrez *et al.*, 2015). Ali and Zeitoun (2017) supposed that AG is a suitable substitute for macromolecules contained in the egg yolk and it can protect the spermatozoa plasma membrane from chilling injury during cryopreservation. In addition, AG is well known in food industry as it has been long used as food additive as an emulsifier

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(Mortensen *et al.*, 2017). Also, Calame *et al.*, (2008) reported that AG possess a potential prebiotic effect. Moreover, Clark *et al.* (1993) showed that AG is traditionally used as an oral hygiene substance that has anti-bacterial effects against periodontal pathogens.
Therefore, the main goal of the present study is to find the suitable concentrations of AG to substitute egg yolk in ram semen extenders and the subsequent effects on semen quality and freezability in Noemi rams.

MATERIALS AND METHODS

The present study was performed in Sheep and Goats Unit, Agricultural and Veterinary Research Station, Qassim University, Al-Qassim region, Kingdom of Saudi Arabia.

Animals:

Six 2–4-year-old fertile Noemi rams free of health problems were used in the experiment. Rams were kept in semi-open stable with free access to clean water and fed pellets and alfalfa hay. Integrated mineral licks were available as well.

Semen collection:

Semen was collected by using two different techniques: either an electro-ejaculator or artificial vagina. Rams were subjected to semen collection by using electro-ejaculator (ElectroJac® 5, Neogen Animal Safety, KY, USA). Semen was collected from each ram twice a week for 6 successive weeks. After a rest period of 2 weeks, the same rams were prepared for semen collection by using artificial vagina. The semen was collected via artificial vagina from the 6 rams twice a week for 6 successive weeks. The collected semen was immediately kept in water bath at 37°C. Semen evaluation parameters such as color, volume and gross motility were assessed. Sperm motility and concentration were estimated with the computer assisted semen analysis system (CASA; ISAS® program, Proiser R+D, Valencia, Spain).

Extenders:

Semen samples were cryopreserved by using ordinary Tris based extender. Egg yolk (EY) was added at a concentration of 15% (control group) or replaced with Arabic gum (AG) (Sigma–Aldrich Company, USA) at different concentrations (3, 5 and 7%) AG3, AG5 and AG7, respectively. Before using, AG was heated at 80°C for 60 minutes to inactivate the enzymes. Tris extender was prepared using 0.5 g glucose, buffering agents (3.643 g Tris and 1.99 g citric acid), 5 mL glycerol and non-pyrogenic water (added to a volume of 100 mL).

Cold storage and cryopreservation:

The semen from each ram ejaculate was re-suspended with Tris extender containing EY (15%) or AG3, AG5 and AG7; the final volume after dilution was one mL semen to 4 mL extender and then cooled to 4 °C for 120 min. After cooling, diluted semen was evaluated using ISAS® and then loaded into straws (0.5 mL). Freezing processes were performed by subjecting the straws horizontally at 3-4 cm height from the surface of liquid nitrogen for 10 minutes for sensitization with liquid nitrogen vapor and then plunged directly into liquid nitrogen.

Thawing of frozen semen: at day 7 post freezing in liquid nitrogen, straws were thawed immediately in a water bath at 37 °C for 40 seconds and then analyzed by ISAS system.

Assessment of sperm motility:

Semen samples whether cold stored at 4 °C or frozen in liquid nitrogen were examined for motility patterns by using the ISAS® program. Seven consecutive digitalized images obtained from several fields using a 10X negative-phase contrast objective were examined for sperm motility analysis. At least 300 spermatozoa per sample were analyzed. Subsequently sperm motility parameters were recorded: total motile spermatozoa (% TMS), rapid progressively motile sperm (% PRS), curvilinear velocity (VCL) μm/s, rectilinear velocity (VSL) μm/s, the average path velocity (VAP) μm/s, straightness index (% STR), linearity coefficient (% LIN), Spermatozoa with a swimming speed or VAP values below 10 μm/s were considered immotile spermatozoa.

Evaluation of sperm morphological defects, and acrosome membranes integrity:

All samples of cooled diluted semen and frozen-thawed semen were evaluated for plasma membrane integrity, acrosome integrity, and morphological defects. The functionality of the plasma membrane assessed by the Hypo-osmotic swelling test (HOST). A solution of 100 mOsmol fructose-base was used and placed in tubes (2 mL) at 37 °C followed by addition of 20 μL of semen to each tube and then incubated in a water bath at 37 °C for 50 minutes. Subsequently, sperms were analyzed for the presence or absence of a coiled tail. One hundred sperm cells were counted by phase contrast microscopy (400×) (Fonseca *et al.*, 2005).

Giemsa staining procedure was used to examine the defected acrosome (Hafez, 1993). At least 200 spermatozoa were examined to determine the percentages of spermatozoa with altered acrosomes. The morphologically normal spermatozoa were examined by the nigrosine-eosin stain (Evans *et al.*, 1987). Defects of sperm and acrosome were determined under a light microscope (1,000X).

Statistical analysis:

Descriptive analyses were performed for variables evaluation: TMS, PRS, VCL, VSL, VAP, STR, LIN, acrosome integrity, HOST and morphological defect. One-way analysis of variance (ANOVA) was performed for statistical comparisons between groups. Analysis of the normal distribution of data was examined with Kolmogorov-Smirnov test (SPSS, version 22). The data were considered statistically different if $P<0.05$. Data were expressed as the means± SEM.

RESULTS

Results of the studied sperm evaluation parameters are represented in Table (1) and Figure (1). Tris containing 15% EY was used as a control extender.

Spermatozoa collected by artificial vagina and diluted with extenders supplemented with AG or EY were more efficient in sperm motility than spermatozoa collected by electro-ejaculator (EE) and diluted with the same extenders. The values of VCL, VSL and VAP were higher in AG3 and AG5 and EY extenders in semen collected by AV compared to EE. After cold storage, AG3 gave better (P<0.01) results for LIN and STR either in spermatozoa collected by AV or EE. Significant (P<0.05) differences in sperm defective morphology were noted in semen collected by EE regardless of extenders used. However, AG supplementation decreased (P<0.05) morphological defects in spermatozoa collected by AV (Figure1). Similarly, AV gave better (P<0.05) results of acrosome integrity and HOST regardless of extender used compared to EE (Figure 1).

Table (1): Spermatozoa motility and viability parameters of ram semen collected by electro-ejaculator or artificial insemination and stored at 4°C for 120 min (mean±SEM)

	Electro-ejaculator				Artificial vagina			
	EY	**AG3**	**AG5**	**AG7**	**EY**	**AG3**	**AG5**	**AG7**
TMS	75.50±10.9^{b}	52.66±5.9^{b}	80.70±10.5^{b}	78.80±11.1^{b}	98.66±0.2^{a}	95.43±0.1^{a}	95.80±0.3^{a}	91.70±0.3^{a}
PRS	46.63±12.6^{b}	22.16±3.9^{b}	57.70±17.5^{b}	45.33±11.2^{b}	90.46±0.9^{a}	72.70±1.2^{ab}	82.26±0.5^{a}	65.03±0.9^{b}
VCL	77.80±9.5^{b}	64.66±4.2^{b}	88.23±15.0^{b}	86.73±12.9^{b}	113.43±0.8^{a}	91.16±0.5^{ab}	101.76±0.3^{a}	90.76±0.6^{ab}
VSL	22.66±1.2^{b}	22.93±3.0^{b}	23.76±2.3^{b}	25.13±1.5^{b}	31.76±0.2^{a}	33.20±0.4^{a}	27.43±0.1^{ab}	24.33±0.1^{b}
VAP	42.63±4.4^{b}	37.63±4.0^{b}	47.00±7.1^{b}	46.80±6.1^{b}	60.20±0.1^{a}	54.73±0.4^{ab}	53.83±0.2^{ab}	49.00±1.2^{b}
LIN	29.60±1.8^{b}	35.16±2.5^{a}	27.66±2.1^{b}	29.76±2.7^{b}	28.00±0.1^{b}	36.43±0.1^{a}	26.96±0.0^{b}	26.83±0.0^{b}
STR	53.66±2.3^{b}	62.63±3.4^{a}	51.46±2.9^{b}	54.70±3.8^{b}	52.73±0.1^{b}	60.66±0.2^{a}	51.03±0.0^{b}	48.73±0.0^{b}

EY: Egg yolk; AG: Arabic gum (3, 5 and 7%); TMS: Total motile spermatozoa (%); PRS: Rapid progressively motile spermatozoa (%); VCL: Curvilinear velocity (μm/s); VSL: rectilinear velocity (μm/s); VAP: average path velocity (μm/s); LIN: linearity coefficient (%); STR: straightness index (%).
^{a,b} Means in the same row with different superscript are significantly different (P<0.05)

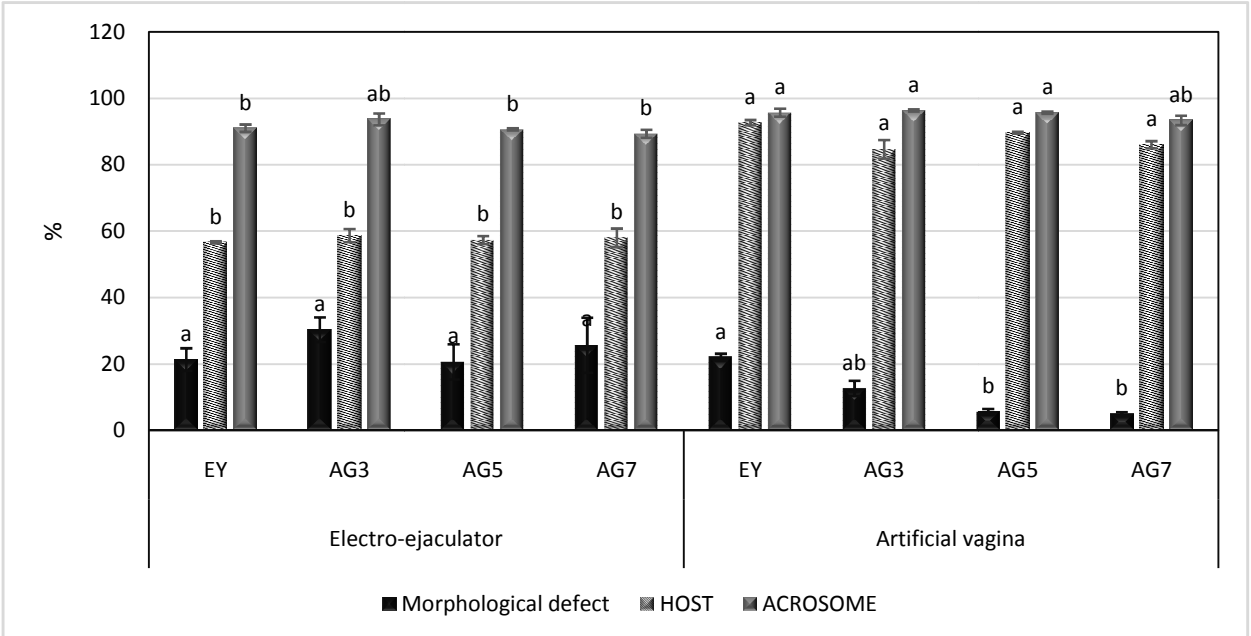

Figure (1): Mean± SEM of morphological defects, HOST and acrosome integrity of spermatozoa collected by electro-ejaculator or artificial vagina and stored at 4°C for 120 min.
EY: Egg yolk; AG: Arabic gum (3, 5 and 7%); HOST: Hypo-osmotic swelling test.
^{a,b} bars bearing different superscripts are significantly different (P<0.05)

Parameters of motility and viability of ram sperm after freezing/thawing are shown in Table (2) and Figure (2). Tris extender containing EY and AG5 in AV-collected samples showed a significantly (P<0.05) higher TMS, PRS, VCL, VSL and VAP compared to others. Sperm defects were higher in AG7 extender in EE and EY extender in AV group. Plasma membrane integrity (HOST) was significantly (P<0.05) higher in AG3 and AG5 extenders in AV-group compared to other groups. The acrosome integrity in AV-semen collected groups was greater (P<0.05) than that of EE-semen collected group.

Table (2): Spermatozoa motility and viability parameters of ram semen collected by electro-ejaculator or artificial vagina and stored in deep freezing (-196°C) (mean±SEM)

	Electro-ejaculator				Artificial vagina			
	EY	**AG3**	**AG5**	**AG7**	**EY**	**AG3**	**AG5**	**AG7**
TMS	13.96±3.6^{b}	19.78±4.8^{b}	21.12±0.5^{b}	11.93±3.5^{b}	40.13±3.8^{a}	14.00±3.4^{b}	34.20±0.8^{a}	8.43±0.1^{b}
PRS	4.23±1.4^{b}	6.74±2.8^{b}	5.62±1.6^{b}	3.93±1.2^{b}	19.65±8.2^{a}	5.56±1.7^{b}	13.97±0.4^{a}	2.23±0.1^{b}
VCL	44.23±3.1^{b}	52.70±4.6^{b}	50.02±1.8^{b}	42.36±1.5^{b}	61,40±4.3^{a}	48.93±1.4^{b}	61.52±0.5^{a}	41.84±0.4^{b}
VSL	14.80±0.6^{b}	15.84±0.8^{b}	15.40±0.8^{b}	14.16±0.6^{b}	19.83±1.8^{a}	13.96±0.5^{b}	18.52±0.1^{a}	13.70±0.1^{b}
VAP	26.03±1.5^{b}	28.64±2.0^{b}	27.80±1.1^{b}	24.36±0.1^{b}	35.40±3.0^{a}	26.36±0.8^{b}	34.65±0.2^{a}	25.10±0.2^{b}
LIN	33.56±0.8	29.96±0.5	30.75±0.6	33.60±2.5	32.30±0.7	28.56±0.9	33.35±0.7	32.70±0.1
STR	50.86±0.8^{b}	54.94±1.0^{ab}	55.32±0.9^{ab}	58.30±2.7^{a}	55.82±0.4^{ab}	52.96±0.8^{b}	53.47±0.5^{b}	54.46±0.1^{ab}

EY: Egg yolk; AG: Arabic gum (3, 5 and 7%); TMS: Total motile spermatozoa (%); PRS: Rapid progressively motile spermatozoa (%); VCL: Curvilinear velocity (µm/s); VSL: rectilinear velocity (µm/s); VAP: average path velocity (µm/s); LIN: linearity coefficient (%); STR: straightness index (%).
^{a,b} Means in the same row with different superscript are significantly different (P<0.05)

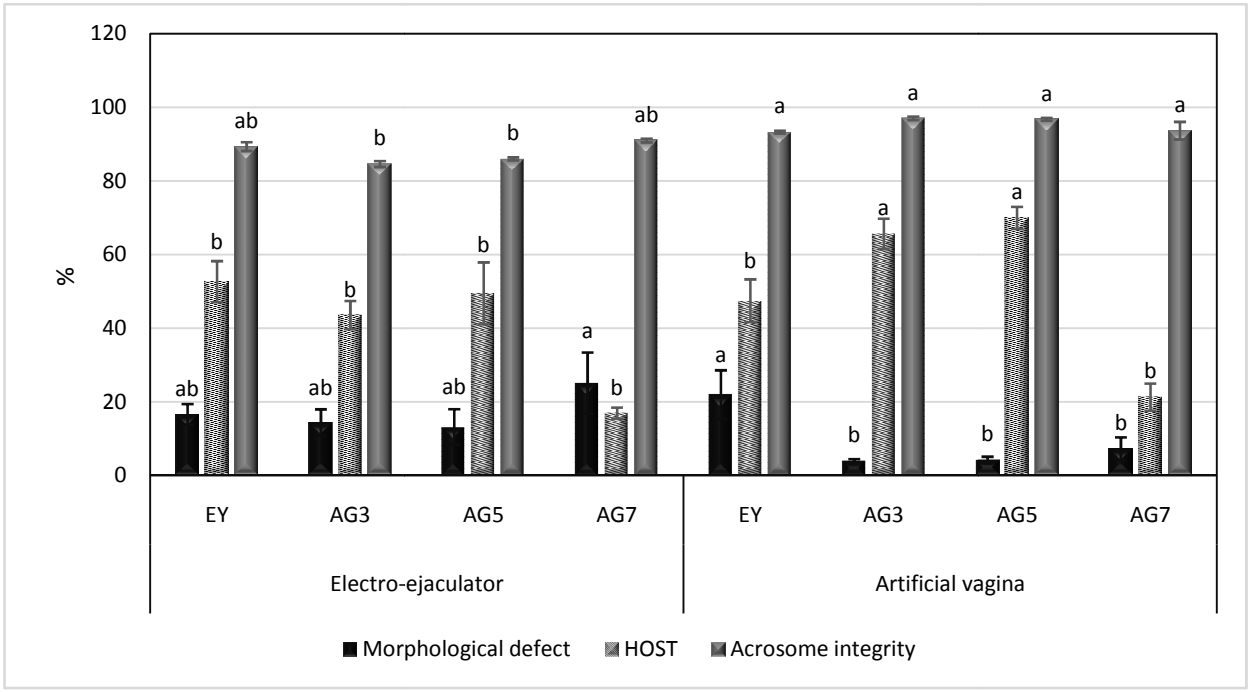

Figure (2): Mean± SEM of morphological defect, HOST and acrosome integrity of spermatozoa collected by electro-ejaculator or artificial vagina and stored in deep freezing at -196°C.
EY: Egg yolk; AG: Arabic gum (3, 5 and 7%); HOST: Hypo-osmotic swelling test.
^{a,b} bars bearing different superscripts are significantly different (P<0.05)

DISCUSSION

The present study showed that Arabic gum successfully replaced egg yolk at different concentrations in ram semen extender. The Noemi ram spermatozoa were evaluated for, motility, acrosome integrity, HOST and morphological defects pre- and post-cryopreservation. Noemi rams are known for difficult semen collection by artificial vagina. The time required for ram training on AV semen collection makes it difficult to use AV at large scale. Moreover, the AV semen collection can be risky for practitioner (Wulster-Radcliffe *et al.*, 2001) especially for large breeds like Noemi. Therefore, Electro-ejaculation is a good option for semen collection in animals whose training for AV is difficult (Jiménez-Rabadán *et al.*, 2012). Our results showed that ejaculates obtained by EE are inferior either in volume or in concentration than those collected by AV. However, Arabic gum maintained the STR in ejaculates collected by EE at 3% concentration during cold storage and at 7% after deep freezing ($P<0.05$).In all other motility traits, AV was significantly ($P<0.05$) better than EE (Tables 1 and 2).

During cold storage, acrosome and plasma membranes integrity were better ($P<0.05$) in ejaculates collected by AV than those collected by EE. However, there were no differences in morphological defects (Figure 1). Arabic gum added at concentrations of 5 or 7% significantly decreased morphological defects in AV-ejaculates ($P<0.05$) with no apparent effects on EE-ejaculates. Arabic gum-extenders in different concentrations were similar to egg yolk-extenders in acrosome and plasma membranes integrity either in EE or in AV ejaculates.

Remarkably, Post-thawing results showed that there were no significant differences in morphological defects, acrosome and plasma membranes integrity between EE and AV ejaculates. When Arabic gum was added to extenders, it did not affect morphological defects, acrosome and plasma membranes integrity in EE-ejaculates. However, Arabic gum added to semen extenders collected by AV-ejaculates gave better ($P<0.05$) results in morphological defects than egg yolk extenders (Figure 2). However, Arabic gum concentrations of 3 or 5% were better ($P<0.05$) in plasma membrane integrity in AV-ejaculates compared to egg yolk (Figure 2). However, there were no significant differences between Arabic gum extenders and egg yolk extender in morphological defects, acrosome and plasma membranes integrity in EE-ejaculates (Figure 2).

This study reported successful freezing of ram semen with extenders supplemented with different concentrations of Arabic gum. Finding a suitable plant origin substitute of egg yolk to be used in extenders has attracted attention of researchers because of biosecurity risks related to EY. Recently, Arabic gum has been introduced as a good substitution of egg yolk in extenders because it has the ability to protect sperm during freezing and cryoprotect it during passing the sub-zero phase of cryopreservation (Ali *et al.*, 2017; Ali and Zeitoun, 2017). The results of the current study demonstrated that Arabic gum replaced egg yolk in ram semen extender during either cold storage or cryopreservation. It is clear that inclusion of Arabic gum in extender of ram sperm did not affect sperm motility. Moreover, it is obvious that AG lower post-thaw morphological defects of sperm at all studied concentrations than in EY-extender may be due to the homogeneity of the AG solution which might easily permeate the plasma membrane to protect sperm cell during freezing/thawing stress (Ali and Zeitoun, 2017).

Cryoprotectant of animal origin such as EY, in semen diluters, has been drawn the attention of the biosecurity risks, recent studies declared that AG, which is used in traditional medicine, possess antibacterial effect against some *S. aureus* and *E. coli* strains and increases intracellular ROS production (Baien *et al.*, 2020). Furthermore, AG has recently reported to have a powerful anti-oxidative and anti-inflammatory properties (Ali *et al.*, 2020).

It has been hypothesized that the high lipid content of EY may resulted in high free fatty acids due to lipolysis of this lipid fracture as a result of damage effects of ice crystal formed during freezing. These high levels of free fatty acids are responsible for sperm plasma membrane fluidity, flexibility, and receptor function (reviewed by Collodel *el al.,* 2020). The same authors added that the lipid component of sperm cell membrane is considered as a part of the membrane microdomains which ultimately involved and affected sperm motility. Accordingly, extenders contained high levels of fatty acids may negatively affect sperm cell metabolism and viability, plasma and acrosome membranes integrity. Moreover, the sperm motility is affected by the extender viscosity, Ye *et al.* (2012) reported that AG solution has half viscosity compared to egg yolk. In order to keep motility, the sperm need low resistance in its solution which mean less viscous medium to keep high velocity. It is well known that the viscosity of the diluter is the main factor that affect motion pattern of the spermatozoon (Amann and Hammerstedt, 1980; Hirai *et al.,* 1997). It has been proven that the viscous the extender the slower the sperm motion. Hirai *et al.* (1997) found that viscosity hamper the sperm velocity and increase the percentage of immotile sperm.

CONCLUSIONS

It can be concluded that, Arabic gum is a good candidate to substitute egg yolk in ram semen extender when added at a concentration of 3%. Artificial vagina used for semen collection gave better results than electro-ejaculator, and Arabic gum can be used in ram semen extender to improve the electro-ejaculator-collected semen characteristics when added at a concentration of 5%.

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استبدال صفار البيض بالصمغ العربي في المخففات المستخدمة لحفظ السائل المنوي للكباش مبرداً أو مجمداً

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تم تصميم البحث الحالي لمقارنة آثار الاستبدال الكامل لصفار البيض في مخففات السائل المنوي للكباش بالصمغ العربي بتركيزات مختلفة لحفظ السائل المنوي المجمع بالمهبل الاصطناعي أو بالقذف الكهربائي مبرداً أو مجمداً في كباش الأغنام النعيمى. تم جمع السائل المنوي مرتين أسبوعياً ولمدة ٦ أسابيع متتالية من ٦ كباش باستخدام القذف الكهربائي وبعد فترة راحة لمدة أسبوعين تم إتباع نفس الإجراء باستخدام المهبل الاصطناعي. أستخدم مخفف التريس Tris المضاف إليه صفار البيض بتركيز ١٥٪ (الكنترول) أو مضاف إليه صمغ عربي بالتركيزات التالية ٣٪ و ٥٪ و ٧٪ على الترتيب. تم تقييم الحيوانات المنوية بعد حفظها مبردة (٤°م) أو مجمدة في النيتروجين السائل لبعض صفات الحركة في الحيوانات المنوية. العيوب المورفولوجية وسلامة أغشية البلازما والأكروسوم. أظهرت النتائج أن الحيوانات المنوية التي تم جمعها بواسطة المهبل الاصطناعي والمخففة باستخدام صفار البيض أو الصمغ العربي كانت أكثر كفاءة في كل الصفات المدروسة مقارنة بالقذف الكهربائي. كانت قيم الحركة المنحنية (ميكرومتر/الثانية)، الحركة المستقيمة (ميكرومتر/الثانية)، ومتوسط سرعة المسار (ميكرومتر/الثانية) أعلى في السائل المنوي الذي خفف بمخففات احتوت على ٣% أو ٥% صمغ عربي، على الترتيب أو صفار البيض والذي تم جمعه بواسطة المهبل الاصطناعي. بعد الحفظ بالتبريد، أعطى تركيز ٣% صمغ عربي نتائج أفضل من حيث مؤشر الاستقامة ومعامل الخطية سواء في الحيوانات المنوية التي تم تجميعها بواسطة المهبل الاصطناعي أو القذف الكهربائي ($P<0.01$). شوهدت اختلافات كبيرة في العيوب المورفولوجية في السائل المنوي الذي تم جمعه بواسطة(القاذف الكهربي)بغض النظر عن المخفف المستخدم، ومع ذلك، فإن إضافة الصمغ العربي للمخفف قلل العيوب المورفولوجية في الحيوانات المنوية التي تم جمعها بواسطة المهبل الاصطناعي ($P<0.05$). بعد التجميد، كان المخفف المحتوي على صفار البيض أو ٥% صمغ عربي والمتحصل عليه بواسطة المهبل الاصطناعي أعلى معنوياً في قيم الحركة الإجمالية، الحركة التقدمية للأمام، الحركة المنحنية (ميكرومتر/الثانية)، الحركة المستقيمة (ميكرومتر/الثانية) ومتوسط سرعة المسار (ميكرومتر/الثانية). كانت العيوب المورفولوجية للحيوانات المنوية أعلى في المخفف المحتوي على ٧% صمغ عربي في حالة القذف الكهربائي وفي مجموعة صفار البيض في حالة المهبل الاصطناعي. كانت سلامة غشاء البلازما أعلى بشكل ملحوظ في مخففي ٣% و ٥% صمغ عربي في حالة المهبل الاصطناعي مقارنة بالمجموعات الأخرى ($P<0.05$) .كانت سلامة غشاء الأكروسوم أعلى في حالة المهبل الاصطناعي مقارنة بالقاذف الكهربي ($P<0.05$). يمكن أن نستخلص من النتائج أن الصمغ العربي يمكن أن يستخدم بتركيز ٣ أو ٥% في مخففات السائل المنوي للكباش النعيمي عوضاً عن صفار البيض. على الرغم من أن المهبل الاصطناعي أعطى نتائج أفضل من القاذف الكهربي، إلا أنه يمكن إضافة الصمغ العربي بتركيز ٥% إلى مخفف السائل المنوي لتعزيز جودة الحيوانات المنوية في السائل المنوي الذي يتم جمعه بواسطة القاذف الكهربي.

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