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Privilege of Gum Arabic inclusion in semen extender compared with egg yolk on the Herri ram's subsequent fertility outcomes

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ABSTRACT

This experiment was designed to scrutinize effects of inclusion of Gum Arabic versus egg yolk in either fresh or frozen extender for Herri ram' semen on subsequent pregnancy rate. Semen ejaculates were collected from two rams. Two trials were attempted. Trial I, semen ejaculates were diluted with freshly prepared extenders either contained 10% EY (Control) or 6% GA (GA). Ewes were randomly selected and subdivided into 2 groups and inseminated. Trial II, extenders were frozen (-20°C) for 30 days before using, thawed and semen ejaculates from same rams were diluted and used in inseminations. Ewes were subdivided into 2 groups and inseminated with semen diluted in either control or GA6 extender. Results revealed that using fresh extender surpassed frozen one in achieving better pregnancy of the artificial insemination. Pregnancy rate derived of inseminations prepared by GA6 extender was significantly higher (P<0.0) than control extender. Therefore, using 6% of Gum Arabic in fresh extender can ultimately improve the fertility rate in Herri sheep.

Keywords: Gum Arabic inclusion, Semen extender, Egg yolk, Herri ram's subsequent fertility outcomes

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Introduction

The common diluents used for dilution of ram semen for cervical or vaginal artificial insemination contain either Tris or Citrate as buffer, glucose or fructose as an energy source and egg yolk to protect the sperm cell membrane against accidental cold shock (Evans and Maxwell, 1987). The main reason of the use of egg yolk in the semen extenders is mainly due to its content of the low-density lipoprotein (LDL) fraction (Moussa et al., 2002). However, the use of egg yolk has been associated with sanitary risk issues, including the production of harmful metabolites and toxins and the risk of transmitting infection resulting in a reduced semen quality (Akhter et al., 2008). One major harmful product inherited to egg yolk is the hydrogen peroxide, which is formed from egg yolk in presence of oxygen. This substance can cause inhibition of motility and metabolism. In fact, the risk of introducing exotic diseases (i.e., avian influenza) through the transportation of egg-based products is a widespread concern (Bousseau et al., 1998). Therefore, a well-defined and pathogen-free substitute of non-animal origin cryoprotectant, i.e. GA was recently tested in stallion' semen extender (Ali et al., 2017). It has been suggested that replacing egg yolk as the non-permeable cryoprotectant in extenders with a new substance like Gum Arabic (GA) may improve the physical traits of semen and subsequent fertility of ram semen used in artificial insemination in ewes. Gum Arabic is a polysaccharide of natural origin that is exuded from the stems or branches of the tree *Acacia* genus. The exudate is a complex resin mixture of a high molecular weight, being composed of six carbohydrate moieties and a protein fraction. It exhibits good dissolving capacity and a remarkably low viscosity (McNamee et al., 2001) and is extensively used in microencapsulation procedures, film formation, emulsion stabilization and is widely used as coating of stored food to extend their shelf-life.

In addition, studies were based on the assumption that GA has strong anti-oxidant properties which impedes the toxicities generated by the free radicals (Ali and Al Moundhri, 2006; Hinson et al., 2004). Therefore, the main goal of this study was to determine effects of supplementing semen extender (i.e. fresh or frozen) with an optimum percentage of GA on subsequent pregnancy rate in Herri sheep.

Materials and Methods

Animals and location

Two Herri rams and 44 adult ewes were lodged in semi-shaded yards located at the University of Qassim, College of Agriculture and Veterinary Medicine experimental farm. Animals were offered 300 g barley/head/day in addition to alfalfa hay, salt block licks and clean water *ad lib.* A permission was approved from the Committee of the University of Qassim for Animal Use and Ethics.

Trials

Two trials were conducted;

Trial I (Fresh extenders): Two extenders were freshly prepared, one contained 10% EY (Control) and the other contained 6% GA (GA6). Extenders composition are shown in Table 1. Twenty two adult multiparous ewes were utilized to be inseminated in trial I. Ten ewes were inseminated with control extended semen and 12 ewes were inseminated with GA6 extended semen. Extenders in this trial were freshly prepared at the time of semen collection.

Trial II, same two extenders as in trial 1 (Table 1) were prepared and frozen (-20°C) for 30 days before semen collection and insemination. In this trial a set of 22 adult ewes were randomly and equally allotted into the two treatment (n=11 ewes/ extender).

Table 1. Composition of extenders used in the experiment

Ingredient	Control (EY 10%)	GA6%
Tris (hydroxyl methyl) amino methane (g)	3.63	3.63
Fructose (g)	0.50	0.50
Citric acid (monohydrate) (g)	1.99	1.99
Egg yolk (ml)	10	-
Gum Arabic (g)	-	6
Glass distilled water to:	100	100
*p*H	7.0	7.2
Viscosity (cp)	2.255	1.035

Ewe's estrus synchronization

Estrus was synchronized with progestagen sponges (sponges impregnated with 40 mg of flourogestone acetate (FGA)) being left in situ for 12 days, with an i.m. injection of 250 IU PMSG (Pregnant Mare Serum Gonadotrophin) given at sponge withdrawal. AI was conducted at approximately 54 h after sponge withdrawal for all ewes (i.e. fixed time AI).

Semen collection and insemination

Two fertile rams were used to supply fresh semen for ewes. Three ejaculates per a ram were collected, checked for progressive motility, pooled and diluted with Tris extender to achieve a final concentration of 200×10^6 motile sperm/0.5ml (i.e. straw). Artificial insemination was achieved 54 h after sponge removal and semen was deposited into the posterior *os* of the cervix (cervical insemination). After 30 days of artificial insemination, pregnancy was diagnosed by ultrasound (Esaote, pie medical, Netherlands).

Viscosity, osmolarity and *p*H measurements of extenders

Viscosity measurements of the extender were determined by vibroviscometer (model SV-10, Tokyo, Japan) at room temperature and at 4°C. Osmolarity measurements of the extender were determined by osmometer (model 800 CLG, SLAMED, Germany). *p*H measurement was adjusted at 7-7.2 (HANNA HI 2211, Italy).

Images of an extender film

An image was taken for each extender before and after freezing (ISAS, Proiser R+D, Spain) using a magnification of X 400.

Statistical analysis

Data were analyzed using SPSS statistical software release, version 16. Proportional data (pregnancy rate) were analyzed by Chi-square (Petrie and Watson, 2002).

Results

As shown in Table 2, viscosity of egg yolk or Gum Arabic didn't change due to freezing, however the viscosity of egg yolk was higher than that of GA. Osmolarity of egg yolk increased by freezing, whereas the opposite was found in GA. Values of *p*H range between 6.9-7.4. Freezing of the extenders tended to decrease pH value by a value of 0.2.

Ewes inseminated with semen extended in GA revealed higher ($P<0.05$) pregnancy rates when inseminated with fresh (58.3%) or frozen (54.5%) cryoprotectant. Contrariwise, control diluent containing egg yolk resulted in lower pregnancy rate either before (30%) or after (18.2%) freezing. The decline in pregnancy rate in case of control diluent after freezing was massive (-11.8%), however the decline in pregnancy rate when GA-diluent was used after freezing was marginal (- 3.8%). Regardless of extender treatment, mean pregnancy rate in case of GA was 59.1%, however pregnancy rate derived of semen extended in EY-diluent was 23.8% ($P<0.05$).

Table 2. Chemical traits of the extenders and pregnancy rates to AI of semen diluted in fresh or frozen extenders

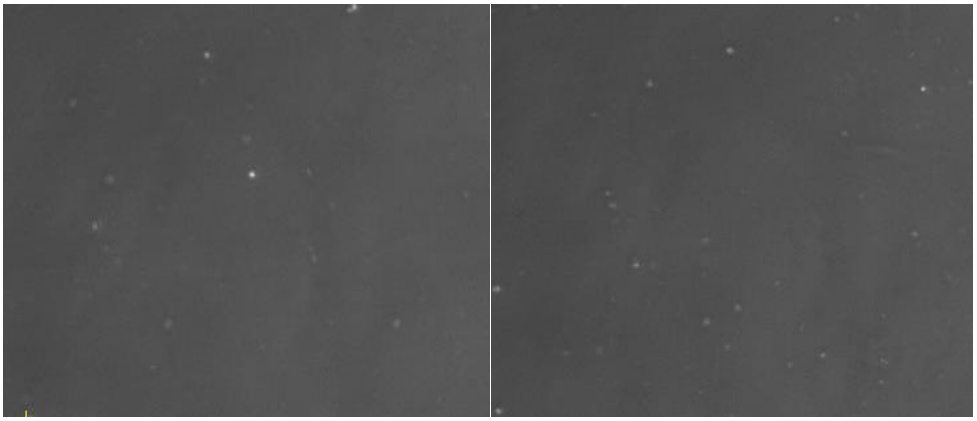
Trial	Number of ewes	Number of Pregnant ewe (%)	Viscosity (cp)	*p*H	osmolarity
Trial I: Fresh extenders					
Control (EY 10%)	10	3 (30.0)[ab]	2.255	7.1	358
GA (6 %)	12	7 (58.3)[a]	1.035	7.4	518
Trial II: Frozen extenders					
Control (EY 10%)	11	2 (18.2)[b]	2.210	6.9	452
GA (6 %)	11	6 (54.5)[a]	1.101	7.2	409

[a ,b] Means in the same column with different superscripts are significantly different (P<0.05).

Discussion

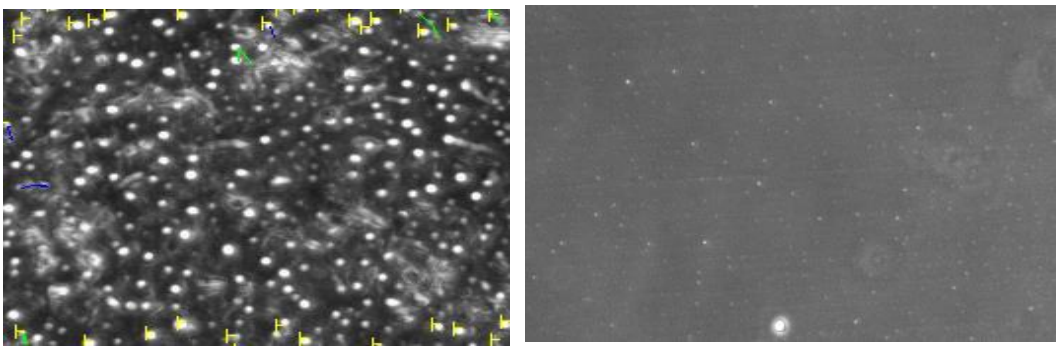
Pregnancy rate derived of AI in small ruminants still beyond the acceptable values. Egg yolk has been extensively used for long time as a cryoprotectant in the semen extenders. Several risk factors are inherited with the use of egg yolk as a biological material.

The increase of pregnancy rates derived of inseminations with GA-diluted semen was comparatively higher (54.5 to 58.3%) than that obtained of the use of EY- diluents (18.2-30%). Cervical artificial insemination (AI) using fresh ram semen has been widely used around the world (Gordon, 1983; Evans and Maxwell, 1987). Pregnancy rate was found to be lower in ewes inseminated artificially compared to natural mating (Ali, 2014). This may be due to the failure of artificial insemination to provide a sufficient number of active sperm in the oviduct at the time of fertilization or some other factors. Pregnancy rates with fresh or chilled semen are satisfactory (65 to 75%) but miserably low (10 to 30%) if frozen-thawed semen is used (Olesen, 1993). Ali (2014) found 35 to 52 % conception rate by using fresh semen of rams in cervix. Also, Pervage et al. (2009) reported a 63.61 % pregnancy rate using fresh semen diluted in a commercial egg yolk-citrate extender. The optimum motile sperm number required to maximize pregnancy rate following cervical insemination is around 200 x 10^6 in synchronized ewes. Culham and Hill (2009) found approximately 75 % conception rate from laparoscopic-guided AI, which is very acceptable for this technology in the sheep industry but its application is limited by its expenses, the required skills and the ethics of animal welfare. Successful artificial insemination program involve multidimensional management including the detection of heat, proper time of insemination, semen processing and storage method, handling and quality of semen, the efficiency of AI technician, ewes health, breed, feeding and others. In the current study, the similarity of the high percentage of pregnancy rate in GA-diluent either before or after freezing is probably due to the homogeneity of the GA solution which might easily permeate the sperm cellular membrane to protect against chilling temperature (Fig 1 a & b).



(a) (b)

Fig 1. Image of an extender containing GA before (a) and after (b) freezing.



(a) (b)

Fig 2. Image of an extender containing EY before (a) and after (b) freezing.

As it is well known that egg yolk contains a high percent of fat globules which in terms of usage in fresh condition might not had the chance to impede the vigor of sperm motility, however after freezing these fat globules disappeared of the image (Fig 2 a& b). The possible explanation for such an observation is possibly due to the disruptive effects of ice crystals on such globules. This disruption might exposed these globules to the lipolysis resulting in elevated levels of free fatty acids in the media. These high levels of fatty acids in the extender could deteriorate the sperm metabolism, integrity and viability. Also, the physical characteristics of the solution like its viscosity and osmolarity might have an impact on the sperm motility and ability for fertilization. The viscosity of GA was about 50% less than that of EY, being the most effective trait ruling sperm motility (Ye et al., 2012). The motion of the spermatozoa through the cervix of ewes requires less viscosity and high speed to overcome this obstacle.

It has been established that the viscosity of the extender affects the pattern of sperm motion (Amann and Hammerstedt, 1980; Hirai et al., 1997). In previous studies, different levels of viscosity were achieved by the addition of Ficoll, carboxy methyl cellulose (CMC), methylcellulose or egg yolk. Hirai et al., (1997) demonstrated that the elevated viscosity primarily affect fast swimming sperm cells and impede spermatozoal velocity slightly above 30 µm/sec. Thus, the percentage of immotile spermatozoa (<10 µm/sec) increased with the increase of viscosity. This phenomenon might confirm the low fertility rates resulted of the use of egg-yolk extenders in the current study. The ultimate explanation for this impediment of fertility is the high viscosity with low *p*H

negatively affected the sperm motility during its journey in the cervical mucus. Freezing of both extenders resulted in reductions in the fertility rates with acute decline in case of egg yolk inclusion.

Conclusion

Inclusion of GA as a cryoprotectant at 6% in the ram' semen extender resulted in a relatively high percentage of pregnancy rates in ewes and surpassed the egg yolk in this trait. This study is probably the first to use GA as an alternative to egg yolk in the mammalian semen extenders and open a new avenue for the study of cryobiology.

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