EFFICACY OF EGG YOLK FROM THREE AVIAN SPECIES ON SEMEN FREEZABILITY OF THARPARKAR BULL

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ABSTRACT

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Egg yolk is one of the most widely used cryoprotective components for sperm preservation and a wide range of factors affect its action on sperm motility, viability and fertilizing ability. The aim of this experiment was to determine the effect of different species egg yolk, namely the domestic chicken *(Gallus domesticus),* duck *(Anas platyrhynchos)* and Japanase quail *(Coturmix japonica)* on sperm quality following cryopreservation of Tharparkar bull semen. A total of 30 ejaculates from three bulls (10 from each bull) were used in the experiment. Each ejaculate was divided into three equal parts and diluted with three different extenders with 20 per cent egg yolk from domestic chicken *(Gallus domesticus)* (CEY), domestic duck *(Anas platyrhynches)* (DEY) and Japanase quail *(Coturnix japonica)* (QEY). The semen straws were equilibrated at 4°C for 4 hrs and freezing was done using biological cell freezer (-140°C) and stored in liquid nitrogen (-196 °C). After thawing (37°C for 30 s), sperm motility, viability, acrosomal integrity and membrane integrity (HOST) were evaluated. The post extension sperm motility did not differ among three extenders. Result of present experiment showed that QEY based tris extender had better post-thaw motility, viability, acrosomal integrity and membrane integrity (HOST) ($p<0.01$) in comparison to CEY and DEY based tris extender.

Keywords: Tharparkar bull, Semen, Cryopreservation, Egg yolk

INTRODUCTION

Cryopreservation has been applied as a routine technique for processing bovine sperm in Artificial Insemination (Watson, 1995). Fertility with frozen thawed bull semen was generally acceptable in spite of the fact that cryopreservation techniques resulted in the loss of 40 to 50 per cent of viable sperm during the freezing-thawing process. Howeve, a little improvement over the last several decades has been reported (Holt, 2000: Watson, 2000; Prathalingam *et al.*, 2006). Cold shock, osmotic stress, ice crystal formation and oxidative damage were the main sources of sperm cryoinjury finally caused the loss of sperm viability and fertility (Amirat *et al.*, 2004; Li *et al.*, 2005). High survival and fertility rates of bull sperm in extender with 20 per cent egg yolk have been reported ^Corresponding author-Principal Scientist, Division of AR, I.V.R.I. by various workers (Steinbach *et al.*, 1964; Wall and Foote, 1999). A widely used domestic chicken's *(Gallus domesticus)* egg yolk can help in resisting cold shock in association with other components (Amirat *et al.*, 2004). Protective action of egg yolk to sperm against cold shock during the freeze-thaw process is due to phospholipids, cholesterol and low density lipoproteins (Bergeron and Manjunath, 2006).

The egg yolk from the other avian species like duck, quail and chicken has different components of fatty acid, phospholipids and cholesterol, which resulted in different cryopreservative effects on sperm. The media containing yolk from eggs of avian species other than domestic chickens resulted in significantly higher

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motilities and longevities of frozen-thawed boar, jackass or stallion sperm (Trimeche ef a/., 1997; Bathgate *et a/* ., 2006). However, there have been no reports comparing the effects of egg yolk from domestic chicken *(Gallus domesticus),* domestic duck *(Anas platyrhynches)* and Japanese quail *(Coturnix japonica)* in extenders on semen cryopreservation especially from Tharparkar bull.

The objective of this study was thus to investigate the effect of egg yolk from different avian species (chicken, duck, quail) on the freezability and post-thaw sperm quality of Tharparkar bull semen.

MATERIALS AND METHODS

Three Tharparkar bulls (aged 4-7 years) maintained at the Germ Plasm Centre (GPC), Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P.) were used as a semen source. The bulls were kept under natural light and maintained under a uniform and constant nutritional regime, with each bull being fed a daily diet of concentrate fodder, dried grass, salt lick and water *adlibidum.* Semen was collected during morning hour using artificial vagina as per the standard practice during the month of April and May. A total of 10 ejaculates from each bull were used in the experiment.

The each ejaculate was divided into three equal parts and each part was diluted with three different tris based extenders with 20 per cent egg yolk from domestic chicken *(Gallus domesticus),* domestic duck *(Anas platyrhynches)* and Japanase quail *(Coturnix japonica).* as Group I (chicken egg yolk, CEY), Group II (duck egg yolk, DEY) and Group III (quail egg yolk, QEY), in glass tubes at 37°C. Following dilution, the diluted semen was drawn into 0.5 ml straws (IMV, France) and sealed with polyvinyl alcohol (PVA) powder. Straws were equilibrated at 4°C for 4 h in refrigerator and freezing was done with biological cell freezer (-140°C) (IMV, France). After Freezing, straws were directly plunged into the liquid nitrogen (-196°C). Semen straws were thawed in a water bath (37°C for 30 seconds) for post-thaw semen evaluation after storage for a period of 24 h.

Semen samples were evaluated for semen quality parameters (SQP), viz., progressive motility (%), viability (%), acrosomal integrity (%) and membrane integrity (HOST) (%) at fresh, pre-freeze and post-thaw stages using microscope (Motic) with a biotherm stage maintained at 37°C.

The viability of sperm in the sample was assessed by means of eosin-nigrosin stain (Evans and Maxwell, 1987). Acrosomal Integrity was assessed by Giemsa staining as described by Watson (1975). The hypo-osmotic swelling test (HOST) was used to evaluate the functional integrity of the sperm membrane based on curled and swollen tails (Jeyendran *et a/.*, 1984; Prasad *et a/.*, 1999).

RESULTS AND DISCUSSION

Results of the present experiment showed that quail egg yolk based tris extender had better pre-freeze and post-thaw motility, viability, acrosomal integrity and membrane integrity (HOST) ($p<0.01$) in comparison to CEY and DEY based tris extenders (Table).

Egg yolk from different avian species have different composition as duck egg yolk have more monounsaturated fatty acids than chicken egg yolk followed by quail egg yoik (Bathgate *et a/.*, 2006), whereas quail egg yolk contains significantly more phosphatidylcholine, less phosphatidylethanolamine and a smaller ratio of polyunsaturated to saturated fatty acids than chicken egg yolk (Trimeche *et a/.*, 1997).

These chemical differences may explain the differences in frozen-thawed motility and integrity of sperm when frozen in extender containing the different avian egg yolks (Bathgate *et a/.*, 2006), and low density lipoproteins (LDL) have been implicated during sperm cryopreservation (Amirat *et a/.*, 2004). Trimeche *et a/.*, (1997) showed a beneficial effect of quail yolk compared to chicken yolk in the jackass, using 10 per cent in extender.

The present study revealed that quail egg yolk had the best cryoprotective effect in terms of different physico-morphological seminal attributes as compared to chicken egg yolk and duck egg yolk at pre-freeze

and post-thaw stage. *In-vitro* sperm function test (HOST) also indicated better membrane integrity of spermatozoa cryopreserved in QEY based tris extender indicating its superiority over other avian egg yolks used in the study.

It was concluded that bull semen could be cryopreserved using a tris-based extender containing quail egg yolk, instead of chicken egg yolk and duck egg yolk depending upon availability of respective avian eggs in the geographical areas. Since, the present findings are based on sperm characteristics and laboratory based semen evaluation tests, a large number of *in vitro* fertility trials are needed to understand the beneficial effects of quail egg yolk based extender on conception rate.

Table : Percent (mean ± SE) sperm motility, live-dead sperm count, acrosomal integrity and HOST of Tharparkar bulls semen ejaculates diluted with chicken, duck and quail egg yolk.

		Groups of Extenders		
Parameter		Chicken Egg Yolk (CEY)	Duck Egg Yolk (DEY)	Quail Egg Yolk (QEY)
Motility (%)	Fresh	87.00±1.33	87.00±1.33	87.00±1.33
	Pre-freeze	74.00±2.33^{b}	75.50±1.89^{b}	81.50±1.98^{a}
	Post-thaw	42.50±1.34^{b}	45.50±3.02^{b}	53.50±1.50^{a}
Live Count (%)	Fresh	91.00±1.16	91.00±1.16	91.00±1.16
	Pre-freeze	80.00±1.90^{b}	84.00±1.29^{b}	88.00±1.07^{a}
	Post-thaw	44.70±4.58^{b}	55.70±3.15^{b}	64.60±2.08^{a}
Acrosomal Integrity (%)	Fresh	86.20±1.68	86.20±1.68	86.20±1.68
	Pre-freeze	75.60±1.95^{b}	75.20±1.50^{b}	82.50±1.79^{a}
	Post-thaw	47.20±1.50^{C}	51.60±3.39^{b}	61.60±2.37^{a}
HOST (%)	Fresh	84.20±2.08	84.20±2.08	84.20±2.08
	Pre-freeze	72.50±1.75^{b}	71.80±1.87^{b}	78.00±1.94^{a}
	Post-thaw	45.20±1.39^{C}	51.70±3.10^{b}	60.00±2.08^{a}

Means bearing different superscripts in a row differ significantly ($p<0.01$).

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