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Comparison of the influence of egg yolk of different avian species on cryopreservation of canine semen

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Abstract

Egg yolk is one of the most widely used as a constant 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 egg yolk from different species, namely the domestic chicken, goose, duck and ostrich on sperm quality following cryopreservation of dog semen. Ejaculates were collected using digital manipulation from five fertile dog. Semen samples were extended with a Tris-citric acid-fructose extender containing the different avian egg yolk (20%) and glycerol (7%). Straws were equilibrated at 4 °C for 2 h, frozen in liquid nitrogen vapor for 10 min and stored in liquid nitrogen (−196 °C). After thawing (37 °C for 30 s), sperm motility, viability, abnormal acrosome and membrane integrity (HOST) were evaluated. Results showed that the duck egg yolk have the best cryoprotective effect in terms of the highest ($p < 0.05$) post thaw motility and viability percentage (60.00±0.30% and 161.00±5.05, respectively) compared to the other avian egg yolks. The percentage of acrosomal abnormalities after thawing was significantly ($p < 0.05$) the lowest (19.20±0.41%) and sperm membrane integrity and alkaline phosphatase enzyme concentration were the highest (78.00±0.89% and 23.33±1.18IU/cm^3, respectively) in the duck egg yolk compared to other species. Results suggested that duck egg yolk could be used as an alternative for chicken egg yolk, in a semen extender for the cryopreservation of dog semen, but it requires further evaluation in fertility trials

Keywords:dog semen- Avian- Egg yolk- Cryopreservation

Introduction

Egg yolk (EY) is frequently used as a cryoprotectant in mammalian semen diluents, and showed to be highly effective for the maintenance of sperm fertility in different species **(Sansoneet al., 2000)**. It was believed that the beneficial role of egg yolk in sperm cryopreservation can be attributed to phospholipids, cholesterol (**Combes et al., 2000)**, and low density
lipoproteins (LDL) content which afford successful protection to the sperm against cold shock and the lipid-phase transition effect during the freeze–thaw process (**Moussa et al., 2002)**.
Among many different species of birds throughout the world, domestic chickens (Gallus gallus domesticus) egg yolk is a common component as a cryoprotectant agent for sperm storage in different animals (**Salamon and Maxwell, 1995; Sansone et al., 2000)**. Chicken egg yolk was commonly used in extenders for deep freezing, probably because it was easily available and cheap (**Amirat et al., 2004**).

Bathgate et al. (2006) showed thatthe media containing yolk from eggs of avian species other than domestic chickens resulted in significantly higher motilities and longevities of frozen–thawed boar, Jackass or stallion sperm. There had also been numerous reports that egg yolk from avian species such as the duck, quail, pigeon or chicken had different combinations of fatty acids, phospholipids and cholesterol, which could result in different cryopreservation effects on the sperm (**Humes and Webb, 2006; Clulow et al.,2007; Moreno et al ., 2008; Su et al., 2008**).

The following experiments were designed to compare the cryoprotective effects of 20% egg yolk of different avian species (chicken, duck, goose and ostrich) in Tris–citrate–fructose buffer extender on canine sperm cryopreservation.

Materials and Methods

Experimental animals

Semen from five, 3-6 years-old Rottweiler athletic healthy dogs belonging to Vangaurd farm –El mansourya- Giza, Egypt were used. Clinical examination of their genital organs showed no obvious abnormality and revealed their soundness. The dogs were housed in individual boxes of concrete floor with outdoor and covered shelter to avoid direct sun light and to facilitate their training for semen collection that lasted for one month, fed with commercial maintenance diet and water ad libitum. The current study was conducted in the Artificial Insemination Department, Animal Reproduction Research Institute, Agricultural Research Centre at Giza

Preparation of extenders

Tris buffer was prepared as described by **Rota et al. (1995).** This was composed of Tris (3.025 g), monohydrated citric acid (1.7 g) and D-fructose (1.25g) in 100 ml of ultrapure distilled water.The pH of the solution was adjusted to 6.74, supplemented with egg yolk 20% (chicken-duck-goose-ostrich), glycerol (7%), antibiotics (Na-benzyl-penicillin (100,000 IU) and dihydrostreptomycin sulphate (100 mg) were added to the so composed extender.

Semen collection and initial evaluation

For obtaining semen, a collection equipment consisting of a plastic graduated centrifuge tube connected to a rubber cone was used. Digital manipulation of the penis in absence of teaser was employed **(Johnston et al., 2001; Freshman, 2002 and Kutzler, 2005).**

Semen evaluation

For initial evaluation,a completespermiogram had been performed for each ejaculate. Ejaculate volume, color, pH, motility and Percentage of live spermatozoa **(Campbell et al., 1956)** were assessed using conventional techniques for semen evaluation.

Semen processin

As for dilution procedure, semen was extended 1:1 immediately after collection and evaluation with each of the extenders according to **Silva et al. (2005).**

The extended semen was cooled to 4 °C gradually and maintained at this temperature for 2 hr **(Songsasen et al., 2002)**, packaged in 0.25 ml French straws and frozen horizontally for 10 min in liquid nitrogen vapour in a foam box filled with the liquid nitrogen according to **Silva et al. (2005).** Straws were then rapidly plunged in the liquid nitrogen, transferred to a storage tank and left there for at least one week before thawing. Thawing was performed at 37 °C for 30 sec.

Evaluation Procedures

Sperm motility Estimation.

The percentages of progressively motile spermatozoa were estimated after cold equilibration (immediately before freezing)and after thawing.

Viability assay:

Post-thaw samples were incubated in a water bath at 37 °C for three hours during which the percentage of progressive motile sperm was recorded at 0, 1, 2, and 3 hours of incubation. The post-thaw viability index was calculated according to **Milovanov (1962).**

Morphological Assays:

Sperm acrosomal integrity:

Acrosome integrity was assessed using silver nitrate stain in a procedure slightly modified from the method described by **Chinoy et al. (1992).**

Samples of thawed semen were spread on microscope slides and left to dry at room temperature. The preparations were fixed firstly in 70% ethyl alcohol for 2 minutes followed by 95% ethyl alcohol for a similar period. The slides were stained with silver nitrate solution for 2 hours in an incubator at 65°C in a completely humid atmosphere. After the preparations turned

gold in colour, the chemical reaction was interrupted and the preparation rinsed several times with distilled water and dried at room temperature. The stained preparations were examined for acrosomal integrity using the Olympus BX50 light microscope under a 100X objective. The percentage of spermatozoa with deteriorated or lost acrosomes was counted in at least 300 sperm cells per each slide.

Plasma membrane integrity:

Plasma membrane integrity (PMI) of canine spermatozoa was assessed by hyposmotic swelling (HOS) assay **(Jeyendran et al., 1984)**. The solution of HOS consisted of sodium citrate 0.73 g and fructose 1.35 g, dissolved in 100 ml distilled water. The assay was performed by mixing 50 µl of frozen-thawed semen sample to 500 µl of HOS solution and incubated at 37 ◦C for 40 min. After incubation, drop of semen sample was examined under microscope. Two hundred spermatozoa were counted for their swelling characterized by coiled tail indicating intact plasma membrane **(Ahmad et al., 2003; Pinto and Kozink, 2008).**

Alkaline Phosphatase enzyme concentration:

Alkaline Phosphatase enzyme concentration was estimated in the post-thawed dog semen according to **Kosiniak-Kamysz et al. (2007)**

Table (1) Effect of egg yolk type on the studied canine semen parameters (Means ± SEM)

Type of egg yolk	Motility before freezing (%)	Post-thaw motility (%)	Viability index	Acrosomal abnormalities (%)	Host (%)	Alkaline phosphatase enzyme (IU/cm^3)
Chicken	62.0 ± 0.86 ^{b}	36.6 ± 3.20 ^{c}	91.4 ± 0.70 ^{c}	29.4 ± 0.72 ^{a}	57.0 ± 2.73 ^{b}	15.65 ± 0.67 ^{b}
Duck	73.0 ± 0.27 ^{a}	60.0 ± 0.03 ^{a}	161.0 ± 5.05 ^{a}	19.2 ± 0.41 ^{c}	78.0 ± 0.89 ^{a}	23.33 ± 1.18 ^{a}
Goose	62.0 ± 0.27 ^{b}	49.0 ± 1.73 ^{b}	114.4 ± 5.09 ^{b}	25.0 ± 0.92 ^{b}	58.6 ± 2.97 ^{b}	16.78 ± 0.76 ^{b}
Ostrich	73.0 ± 0.86 ^{a}	23.0 ± 1.41 ^{d}	84.0 ± 2.82 ^{c}	21.8 ± 0.60 ^{c}	58.2 ± 1.64 ^{b}	16.6 ± 0.80 ^{b}
Overall mean	67.5 ± 1.95	42.15 ± 4.82	120.22 ±14.20	13.85 ± 1.38	62.95 ± 3.48	17.983 ± 1.29

-Values with different superscripts in the same column are significantly different at least at $P < 0.05$
-Data were obtained from 5 ejaculate (one ejaculate /dog)

Table (2) ANOVA for the differences between egg yolks from the studied avian species

Semen Parameters	Source of variance	Df	Mean Squares	F
Sperm motility before freezing	Between	3	240.000	38.400***
	Within	16	6.250	
Post-thaw motility	Between	3	1161.250	33.179***
	Within	16	35.000	
Viability Index	Between	3	11847.846	87.068***
	Within	16	136.075	
Acrosomal abnormalities	Between	3	114.983	24.996***
	Within	16	4.600	
HOST	Between	3	541.383	18.963***
	Within	16	28.550	
AP	Between	3	62.983	8.569***
	Within	16	7.350	

*** $P < 0.001$.

Statistical analysis

All experiments were repeated five times. All statistical analysis were calculated with commercial software (SPSS, (ver. 15.0; SPSS Inc., Chicago, IL). Appropriate statistical analysis was according to **Snedecore and Cochran (1976)**. Analysis of variance (ANOVA) was used to check the statistical significance at $p < 0.05$ level.

Results

The results of the current experiment are presented in Tables1, 2.
Motility percentage before freezing was significantly higher (P<0.05) in extenders containing duck (73.0 ± 0.27) and ostrich (73.0 ± 0.86) egg yolk than in those containing chicken (62.0 ± 0.86) and goose (62.0 ± 0.27) egg yolk.

Upon freezing and thawing, duck egg yolk extender maintained significantly higher (P<0.05) post-thaw motility (60.0 ± 0.03 %) than with goose (49.0 ± 1.73 %), chicken (36.6 ± 3.20) and particularly ostrich egg yolk (23.0 ± 1.41%).

Regarding viability index, results were parallel to post-thaw motility, where the effect of duck egg yolk extender (161.0 ± 5.05) was significantly higher (P<0.05) than goose (114.4 ± 5.09), chicken (91.4 ± 0.70) and ostrich (84.0± 2.82) egg yolk extenders.

Discussion

Recently, several studies have been conducted in which the domestic chicken egg yolk component of freezing extenders for mammalian spermatozoa has been replaced by yolk from alternative avian species in an attempt to improve post-thaw motility or acrosomal status of cryopreserved spermatozoa. The main finding from this study suggested that the replacement of chicken egg yolk with duck egg yolk as cryoprotective in canine semen diluents improve the sperm progressive motility, viability, acrosomal and membrane integrity among the four egg yolks in extenders

Chicken and duck egg yolk have a different ratio of the fatty acids comprising the total yolk lipids, **Surai et al. (1999) ; Amirat et al. (2004) andBathgate et al.(2006)** showed that, the basic components of the yolks from chicken and duck eggs did not differ, but the ratio of fatty acids and phospholipid classes were different. Yolk from duck eggs had more monounsaturated fatty acids than yolk from chicken eggs. Moreover, yolk from

On the other hand, duck and ostrich egg yolk extenders maintained significantly (P<0.05) lower percentages of acrosomal abnormalities (19.2 ± 0.41 and 21.8 ± 0.60, respectively) compared to goose (25.0 ± 0.92) and chicken (29.4 ± 0.72) egg yolk extenders.

Sperm membrane integrity shown by HOST reactive cells in duck egg yolk extender was (78.0 ± 0.89%) significantly higher (P<0.05) than in chicken, goose, or ostrich egg yolk (57.0 ± 2.73%, 58.6 ± 2.97 %and. 58.20 ± 1.64%, respectively) extenders

In duck egg yolk extender, the alkaline phosphatase enzyme activity (23.3 ± 1.81 IU/cm^3) was significantly higher (P<0.05) than in chicken, goose or ostrich (15.65 ± 0.67 IU/cm^3, 16.78 ± 0.76 IU/cm^3 and16.60 ± 0.80 IU/cm^3 , respectively) egg yolk extenders.
Analysis of variance revealed that the differences in all investigated parameters between various types of yolk were statistically highly significant (P<0.001).
duck eggs contained more phosphotidyl-inositol than chicken egg yolk. The improvement or decline in post-thaw quality of mammalian spermatozoa with EY of different avian species in freezing extender was attributed to the differences in the biochemical composition of the yolks. 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, and LDL have been implicated during sperm cryopreservation.

The present study revealed that, the motility before freezing was highest in duck and ostrich egg yolk, in agreement with the present study, **Krawczyk (2009)** and **Sinanoglou et al. (2011)** observed that the total egg yolk lipids was decreased in the following order, ostrich, duck, turkey, and fat from ostrich egg yolk was characterized by the highest content of α-linolenic and low level of linoleic acid and palmitoleic acid compared with other sources of yolk. Similarly, the highest amount of cholesterol

was observed in ostrich compared to partridge, duck and chicken. **Speake et al. (2002)** added also egg yolk from commercially raised ducks, which are normally fed a corn-based diet, can give high amount of arachidonic acid as docosahexaenoic acid.

In agreement with the present results, nearly similar findings were reported by **Clulow et al. (2007) and Andrabi et al. (2008) Waheed et al. (2012)** as theyfound that, the duck egg yolk compare favorably with other avian egg yolks in extenders used to improve the frozen–thawed quality of buffalo bull and stallion sperm. Similarly, duck egg yolk provided better sperm quality in terms of motility, viability, abnormal sperm and membrane integrity than other avian egg yolk. This may be attributed to the higher levels of protein, lipid and cholesterol present in the duck, compared to chicken egg yolk.Moreover **Ali et al. (2013)** added that ostrich, turkey and duck egg yolk offered comparable fertilization and blastocysts yield compared with semen diluents with 20% chicken egg yolk.

In contrary, **Su et al. (2008) and Kulaksiz et al. (2010)** showed that the different avian egg yolks (chicken, goose, and duck) do not have different cryoprotective actions on ram and bull sperm cryopreservation. In conclusion, duck egg yolk compared to other avian yolks in extender improves the frozen-thawed quality of canine spermatozoa.

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الملخص العربي

مقارنه تأثيرصفارالبيض المستخلص من الطيورالمتخلفه علي تجميدالسائل المنوي للكلاب

اجريت الدراسة علي خمس كلاب روت ويلر باستخدام مخففات للسائل المنوي للكلاب تحتوي علي صفار البيض من الدجاج والاوز والبط والنعام. كان المخفف للسائل المنوي المحتوي علي صفار البيض من البط اعلي معدلات في الحركه الاماميه التقدميه للحيوانات المنويه بعد الاساله ،indexviability ، اختبار HOST وتركيز Alkaline phosphatase enzyme وأقل معدل في التشوهات في الاكروسوم في الحيوان المنوي. لذلك يعتبر صفار البيض المستخلص من البط بديل مناسب بصفار البيض المستخلص من الدجاج في تجميد السائل المنوي للكلاب