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CRYOPRESERVATION OF DOMESTIC CHICKEN'S (*Gallus gallus domesticus*) SPERMATOZOA USING DIFFERENT CRYOPROTECTANTS AND EXTENDERS FOR ARTIFICIAL INSEMINATION

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ABSTRACT

The study investigated the most efficient cryoprotective agent (CPA) for artificial insemination of domestic chicken Gallus gallus domesticus from: Quail Egg Yolk and Phosphate Buffer Saline (QEY+ PBS), Quail Egg Yolk and Hank's Balance Salt Solution (QEY+HBSS), Quail Egg Yolk and Ginzburg Fish Ringer (QEY+GFR), Dimethyl suphoxide and Phosphate Buffer Saline (DMSO+PBS) with the G. gallus domesticus semen and were cryopreserved in triplicates for 3 days before insemination. Results of the percent fertility revealed significant difference ($p<0.05$) in the control (74.44± 12.62%) compared to all other treatments. The QEY+ HBSS (30.76± 8.09%), QEY+ GFR (42.86± 14.29%), EY+ GFR (29.77±9.00%) and EY+ HBSS (35.24±6.84%) were not significantly different from one another($p>0.05$) but with others ($p<0.05$); however QEY+ PBS (14.18± 5.67%), EY+PBS (9.25±5.84%) and DMSO+ PBS (13.65± 0.63%) were not significantly different from each other ($p>0.05$) but also with the rest of the cryoprotectants ($p<0.05$). Therefore, CPA without PBS as extender gave significant result, hence could be employed as reliable cryoprotectants, QEY+ HBSS, QEY+ GFR and EY+GFR, for G. gallus domesticus.

Keywords: cryoprotective agent; *Gallus gallus domesticus;* Egg Yolk; artificial insemination

INTRODUCTION

Cryopreservation is one of the techniques of biotechnology that helps in the protection of biodiversity of organisms. It is the process whereby organelles, cells, tissues, extracellular matrix, organs or any other biological constructs that are vulnerable to damage caused by unregulated chemical kinetics are being preserved by cooling to very low temperatures (Pegg and David, 2007). The storage at cold temperature is presumed to provide extended longevity to cells, however, the cryopreservation process weakens the fertility of spermatozoa. While valuable genes can be preserved over many years by cryopreservation, it assists in the artificial insemination of poultry. Cryoprotectant is a substance being used to protect biological tissues from freezing damage. The cold semen storage is used to reduce the metabolism of the sperm cell and to maintain sperm viability over an extended period of time (Karunaharan *et al*., 2007). The extender is a solution mixed with cryoprotectants and used in the dilution of semen for artificial insemination. These semen extenders and holding temperature play a significant role in maintaining cock sperm motility (Dumpala *et al*., 2006). Diluted semen stored at 2–5^{0}C retained its fertilizing capacity, even after 24 h (Van Wambeke, 1972).The objective of the present study was to determine the most efficient cryoprotectant for the artificial insemination of *G. gallus domesticus*'s sperm and its effects on the on fertility and hatchability of inseminated eggs.

MATERIALS AND METHODS

***Samples and Location*:** Sexually matured cocks of indigenous ecotype and exotic hens were used for this study. The cryopreservation of the semen was done at the Biotechnology Laboratory, Department of Animal Sciences, Obafemi Awolowo University (O.A.U) Ile-Ife, Nigeria; and the artificial insemination was carried out at the First-Agro Farms situated at Odeda, Abeokuta, Odeda Local Government Area

(LGA) of Ogun State, Nigeria. The hatching of the eggs was done at the Obasanjo Farm Nigeria Ltd, Oluyole Ibadan.

Sample collection: Semen was collected from the cock using reproductive-vent massage method into a sterilized 5ml graduated collecting tube and placed uprightly in a cooler containing ice flakes. The semen was viewed under a biological light microscope to check for its quality using method described by Olaniyi and Sule (2019).

Preparation of Cryoprotectant Agent (CPA): CPA was prepared following the procedures of Olaniyi and Sule (2019) and Omitogun *et al.* (2012). The quail egg and chicken egg were prepared such that the yolk was separated from the albumen. 4ml of the yolks are scrapped gently into different test-tubes and diluted 1:1 with 4ml of distilled water and centrifuged for 10minutes at 2,500rev/min. The centrifuged chicken egg yolk and quail egg yolk were mixed in new collecting tubes with the following extenders to form cryoprotectants at ratio yolk 1:9 extenders. The following are the CPA prepared; i) Dimethyl Sulphoxide and Phosphate Buffer Saline (DMSO+PBS); ii) Chicken Egg Yolk and Phosphate Buffer Saline (EY+PBS); iii) Chicken Egg Yolk and Hank's Balance Salt Solution (EY+HBSS); iv) Chicken Egg Yolk and Ginzburg Fish Ringer (EY+GFR); and all were stored in the refrigerator.

Experimental Design: Semen were collected from four male chickens, while the inseminated domestic chickens (*G. gallus domesticus*) with cryopreserved and fresh semen (control) were in triplicates. The semen collected was measured for cryopreservation at ratio 1:12 CPA and stored in the refrigerator ($4\pm1^{o}C$) for 72hrs and thereafter used for insemination. Percent fertility and hatchability of the eggs were calculated. The data were subjected to statistical analysis using the General Linear Models (GLM) procedure of software SAS, 2002 (SAS Institute, 2002).

***Percent Fertility and hatch*:** The percent fertility was calculated by dividing the total number of fertile eggs by the total number of eggs collected from each treatment and multiplied by 100. While the percent hatch was calculated by dividing the total number of hatched eggs by the total number of fertile eggs, and multiplied by 100.

RESULTS AND DISCUSSION

***Cryoprotectant effects*:** Table 1 shows the fertility and hatchability of eggs inseminated with cryopreserved semen of *G. gallus domesticus.* The chicken inseminated with cryopreserved semen using EY+PBS (9.25±5.84%), QEY+PBS (14.18± 2.67%) and DMSO+PBS (13.65± 0.63%) had least fertility potential and no significant difference among them while they showed significant difference with QEY+HBSS (30.76± 8.09%), QEY+GFR (32.86± 11.29), EY+GFR (29.77±9.00%) and EY+HBSS (35.24±6.84%) that in turn revealed no significant difference with each other. However, the control treatment (74.44± 12.62%) was significantly different from all the cryoprotectants at their fertility rates. This showed that cryproservation process weakens the fertilizing capacity of spermatozoa (Kowalczyk, 2008; Alexander, 1993). The CPA with the PBS revealed the least fertility and suggested the effect of chemical unfavourable composition of PBS. The hatchability was not statistically different from one another ($p>0.05$) except for EY+PBS that recorded no hatch ($p<0.05$). However, the hatchability depended on the fertility recorded.

Table 1: Fertility and hatchability of eggs inseminated with cryopreserved semen of *G. gallus domesticus.*

Treatment	Fertility (%)	Hatchability (%)
CONTROL	74.44 ± 12.62^{a}	100.00 ± 0.00^{a}
QEY+HBSS	30.76 ± 8.09^{b}	77.78 ± 18.49^{a}
QEY+GFR	32.86 ± 11.29^{b}	100 ± 0.00^{a}

QEY+PBS	14.18± 2.67[c]	83.33± 28.87[a]
DMSO+PBS	13.65± 0.63[c]	100.00± 0.00[a]
EY+PBS	9.25±5.84[c]	0.00±0.00[b]
EY+GFR	29.77±9.00[b]	100±0.00[a]
EY+HBSS	35.24±6.84[b]	87.5±17.68 [a]

[abc]Mean values with the same alphabet along the same column are not significantly different (P>0.05) from each other., EY = Chicken Egg Yolk; QEY = Quail Egg yolk; PBS = Phosphate Buffer Saline; GFR = Ginzburg reagent; HBSS = Hanks Balance Salt Solution; DMSO = Dimethyl Sulphoxide

Cryoprotectants have effects on fertility and hatchability potentials however it improves the life span of the semen for further utilisation. Egg yolk is the most effective cryoprotective agent of freezing extender that protects sperm against cold shock (Bergeron and Manjunath, 2006); and has been reported to increase sperm fertilizing ability when present in extenders for semen storage at ambient temperature (Dunn *et al.*, 1950; Shannon and Curson, 1983; Barak *et al.*, 1992; Manjunath, 2012) whereby it prevents sperm cell damage during cooling and freezing (Phillips and Lardy, 1940; De Leeuw *et al.*, 1993).

CONCLUSION

The present study has revealed that the CPA without PBS as extender gave significant result. Therefore, QEY+ HBSS, QEY+ GFR and EY+GFR, were the most efficient cryoprotectants and could be recommended for *G. gallus domesticus* cryoprotection and insemination.

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