CRYOPRESERVATION OF SPERM CELLS OF LOCAL CHICKEN (*GALLUS GALLUS DOMESTICUS*) IN LIQUID NITROGEN

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Abstract

This study evaluated the most effective cryoprotectant to be used for spermatozoa of local chicken Gallus gallus domesticus in liquid nitrogen. Semen was collected from local chicken by means of abdominal massaging. The sperm motility and concentration of the fresh, and liquid nitrogen cryopreserved semen were evaluated microscopically for maximum of three weeks. The cryoprotectant used were Dimethyl sulfoxide and Phosphate buffered saline (DP); Dimethyl sulfoxide and Ginzburg fish ringer (DF); Glycerol and Phosphate buffered saline (GP); Glycerol and Ginzburg fish ringer (GF); Egg yolk and Phosphate buffered saline (EY+P); Egg yolk and Ginzburg fish ringer (EY+F); Quail Egg yolk and Phosphate buffered saline (QEY+P); Quail Egg yolk and Ginzburg fish ringer (QEY+F). Results obtained showed that the motility and concentration of freshly collected sperm recorded 70% and 1.353 × 10^9 spermatozoa/ml respectively, while there were decrease in sperm motility and concentration over time, but some cryoprotectants such as QEY+P and EY+P performed best. Hence, these cryoprotectants are recommended for long term cryopreservation.

Key words: cryoprotectant, local chicken, sperm motility, sperm concentration

Introduction

The protection of biodiversity and conservation of gene pool are of prime importance globally, and this is highly necessary to protect livestock that are already labelled red/endangered, and to increase the population of improved breed. of better performance. Cryopreservation is the process of storing biological materials at a very low temperature for a long period of time using extenders and cryoprotectant agents to protect the cells from damage during freezing and thawing of the frozen semen (www.cryobiosystem-imv.com/cbs/cryobiology/ionscbs.usp.52k; www.genpro.mag.comwww.vviacellince.com). Sperm freezing has allowed for transporting samples of livestock and aquaculture across many miles to produce offspring with superior genetics in order to improve the species. This cryopreserved biological sample can be used to increase genetic diversity and prevent extinction of species (Leibo and Songasen, 2002). Presently, one of the most practicable methods for long term storage storage of genetic material is semen cryopreservation (Blesbois *et al.*, 2007). The technique of artificial insemination complements cryopreservation of spermatozoa irrespective of location and availability of male animal. In order to protect the biodiversity of this local chicken (*Gallus gallus domesticus*) successfully, an optimized protocol for cryopreservation needs to be established through cryopreservation of a particular strain of local chicken sperm which can be stored in liquid nitrogen. The straw that has been frozen can then be transported to other facilities without the need for shipping live animals would even demand quarantine.

The loss of adaptive local gene pool and pure strain of male stock has necessitated the need to conserve local chicken genetic resources. Cryopreservation has been established to provide an effective and plausible method to harvest sperm cells from male local chicken and that could be successfully cryopreserved for subsequent use in artificial insemination programmes. This eliminates the need to keep live male chicken and their transportation burden in order to increase production. This study employed the latest technology to protect local chicken biodiversity through cryopreservation. Therefore, the objective of this study was to identify the most effective and efficient cryoprotectant to be used for local chicken spermatozoa.

Materials and Methods

Location and Samples: The experiment was conducted at the biotechnology and reproductive physiology laboratories of the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. Matured male chickens, *Gallus gallus domesticus*, of a minimum age of one year were selected and their semen was collected.

Preparation of extender-cryoprotectant: Two extenders of Phosphate Buffer Saline (PBS) and Ginzburg Fish Ringer (GFR) with pH of 7.4 and 7.6 respectively were utilized. Table 1 and 2 show how the extenders were prepared after which they

were sterilized for 20 minutes.

Sperm cryopreservation: Before cryopreservation of the spermatozoa, the motility of the fresh sperm was evaluated using high power objective (40x) and was diluted with 0.9% saline water using dilution ratio of 1:20 and the motility was evaluated. The semen was mixed with extender-cryoprotectant (E-CPA) at ratio 1:7 and stored in cryotubes. The cryotubes were gently shaken to allow semen to properly mix with E-CPA and placed in the refrigerator at 4°Cfor 10 minutes. This was later transferred into a Dewar container containing liquid nitrogen for preservation. 30 µl of cryoprotectant was added to 5 µl of semen for storage in the liquid nitrogen.

DP (Dimethyl sulfoxide and Phosphate buffered saline); DF (Dimethyl sulfoxide and Ginzburg fish ringer); GP (Glycerol and Phosphate buffered saline); GF (Glycerol and Ginzburg fish ringer); EY+P (Egg yolk and Phosphate buffered saline); EY+F (Egg yolk and Ginzburg fish ringer); QEY+P (Quail Egg yolk and Phosphate buffered saline); QEY+F (Quail Egg yolk and Ginzburg fish ringer).

Motility evaluation: After thawing the motility and sperm count was evaluated using an haemocytometer and viewed under light microscope. Sperm motility = (Number of motile sperm cells x 100)/total no of sperm cells. The sperm count was evaluated also using the haemocytometer after proper dilution with 0.9% saline solution. The sperm count was carried out in five replicates. The total sperm count was calculated as thus: Total sperm count = sperm count x dilution factor) / volume of sperm

Results and Discussion

The motility and concentration of freshly collected sperm respectively recorded 70% and 1.353×10^9 spermatozoa/ml. Table 3 shows the effect of cryopreservation on sperm motility and concentration stored in liquid nitrogen. The sperm motility of the semen deteriorates after the first week of preservation depending on the type of cryoprotective agent used for the preservation of the fresh semen and this agrees with the result obtained by Dumpala *et al.* (2006) that the motility of sperm reduces after one hour of semen collection if stored *in vitro*. For the first week the motility of the semen was seen to decrease slightly for some cryoprotective agents such as EY + F, and QEY + P. For some cryoprotective agents there was high decrease in the motility such as DF and GP. For the second week the reduction in the motility of the cryopreserved semen was higher than that of the second week, except E-CPA QEY+P and EY+P which have the same motility as the first week. It was also seen that the sperm concentration for the second week was lower than that of the first week. For the third week the motility of the sperm cell have reduced to a minimum level, the speed of the sperm cells that were moving is slow and the sperm concentration is also low, at this rate the viability of the sperm cell would also be at a minimal level. It was shown in this study that the QEY+P and EY+F, and EY+P preserved the motility of the semen better for the first week and also the sperm concentration. For the second week the QEY+P still maintained the motility of the sperm with no difference from that of the first week but the concentration was lower than that of the first week. EY+P also maintained the motility of the semen without decrease in the value obtained for the first week. The motility of the semen in the EY+F extender has reduced greatly by the second week. Therefore, by the third week the motility of the semen in all the extenders has all reduced to a minimal level and also the concentration. From the results obtained from this study it was shown that E-CPA QEY+P can preserve the motility of the semen best.

DP (Dimethyl sulfoxide and Phosphate buffered saline); DF (Dimethyl sulfoxide and Ginzburg fish ringer); GP (Glycerol and Phosphate buffered saline); GF (Glycerol and Ginzburg fish ringer); EY+P (Egg yolk and Phosphate buffered saline); EY+F (Egg yolk and Ginzburg fish ringer); QEY+P (Quail Egg yolk and Phosphate buffered saline); QEY+F (Quail Egg yolk and Ginzburg fish ringer)

Conclusion

Long-term cryopreservation of sperm has been proven to be of great importance in poultry farming; therefore, this study showed that that the combination QEY and PBS makes the best cryoprotecting agent and extender. Hence, farmers can make use of cryopreserved semen in order to reduce cost of operation and cost of acquiring male stock as well as conserving genetic resources of male animals.

References

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Table 1. Composition of extenders (g/l) tested for cryopreservation of the sperm of local chicken in liquid nitrogen

Composition (g/100ml)	Phosphate buffer saline (PBS)	Ginzburg Fish Ringer (GFR)
NaCl	0.8	0.7
KCl	0.002	0.028
CaCl	_	0.033
$NaHCO_3$	0.023	_
Na_2HPO_4	0.115	_
KH_2PO_4	0.02	_
$MgSO_47H_2O$	_	_
$C_6H_{12}O_6$	_	_

Table 2. Composition (%) of the cryoprotective agents used

Cryoprotective agent	DMSO	Glycerol	PBS	GFR	Glucose/sucrose
DP	10	_	90	_	_
DF	10	-	_	90	_
GP	_	10	90	_	_
GF	_	10	_	90	-
EY+P	_	_	90	_	-
EY+F	_	_	_	90	-
QEY+P	_	-	90	_	_
QEY+F	_	_	_	90	-

Table 3. Weekly evaluation of sperm motility and concentration stored in liquid nitrogen

Cryoprotective agent	*Motility per week(s) (%)*			*Sperm count/week(s) spermatozoa/ml*		
	1st	*2nd*	*3rd*	*1st*	*2nd*	*3rd*
DP	50	35	15	1.141×10^9	1.036×10^9	8.33×10^8
GP	30	20	20	1.134×10^9	1.085×10^9	9.1×10^8
DF	35	23	10	8.75×10^8	8.68×10^8	5.88×10^8
GF	40	15	10	1.121×10^9	4.9×10^8	3.57×10^8
EY + F	65	30	10	9.73×10^8	6.37×10^8	1.89×10^8
EY + P	50	41	20	7.91×10^8	5.95×10^8	3.64×10^8
QEY + F	40	20	20	1.183×10^9	6.79×10^8	6.65×10^8
QEY + P	60	53	25	1.351×10^9	8.4×10^8	4.55×10^8