aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2016.411.416

Research Article
Effect of Semen Diluents, Dilution Rates and Storage Periods on Live and Abnormal Spermatozoa of Pearl Guinea Fowls

G.H. Hudson, A.V. Omprakash and K. Premavalli

Department of Poultry Science, Madras Veterinary College, Chennai-600 007, India

Abstract

Background: Guinea fowls are neglected poultry with lower fertility rates compared to other avian species. Semen quality is one of the major determinants of fertility which deteriorates soon after collection. Therefore, the ability of different semen extenders in maintaining the livability and morphology of Guinea fowl spermatozoa under short term storage was studied. **Materials and Methods:** A total of 16 male Guinea fowls were housed in individual cages and was trained for semen collection by abdominal massage technique. Pooled semen samples were then diluted with two different semen extenders namely the beltsville poultry semen extender and instruments for veterinary medicine poultry semen preservation media each in the ratio 1:2 and 1:3. The diluted samples were then stored at 5°C and the percent spermatozoa livability and abnormality were assessed at 0, 3 and 6 h of storage. **Results:** Based on the study, the livability and morphologically normal spermatozoa were maintained above 80% with both the extenders until 6 h of storage. With respect to dilution rates, the semen in lower dilution had shown a higher percentage of live and morphologically normal spermatozoa irrespective of extenders and storage periods. The percent dead spermatozoa and abnormal spermatozoa significantly increased with increasing storage periods. **Conclusion:** Based on the results, it was concluded that the Guinea fowl semen may be extended with either of the diluents at lower dilution rates and the diluted semen must be inseminated within 3 h after collection which may have positive impact on fertility by artificial insemination.

Key words: Guinea fowl spermatozoa, livability, abnormality, BPSE, IMV

Received: March 27, 2016 Accepted: May 21, 2016 Published: June 15, 2016

Citation: G.H. Hudson, A.V. Omprakash and K. Premavalli, 2016. Effect of semen diluents, dilution rates and storage periods on live and abnormal spermatozoa of pearl Guinea fowls. Asian J. Anim. Vet. Adv., 11: 411-416.

Corresponding Author: G.H. Hudson, No. 7/134, Hyma Sadan, Vayalankarai, Methukummal P.O, Kanyakumari District, 629172, India Tel: 9944372582

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

From a biological point of view, only viable and morphologically normal spermatozoa carrying intact genetic information may contribute to egg fertility. Hence, the assessment of spermatozoa livability and abnormality are the most often used parameters for semen evaluation. Guinea fowls are semi wild birds that are yet to be genetically improved for commercial meat and egg production. However, the fertility and hatchability in Guinea fowls are less compared to other species of poultry leading to slower genetic gain and narrow genetic improvement. This may be due to narrow sex ratio, sexing problems[1], seasonality of breeding[2-4] and probable inbreeding. Hence artificial insemination, a novel tool of assisted reproductive technology may be a solution for this problem. To achieve better fertility by artificial insemination, fresh semen should be inseminated within 1 h after collection or the semen quality deteriorates and fertility will be low[5]. This is nearly impossible while inseminating large number of birds. Further poultry spermatozoa are fragile and cryopreservation techniques are not in par successful as in with other domestic animals. It was reported that cryopreservation of rooster semen retain only <2% of fertilizing ability of fresh semen[6]. The semen diluents are reported to be used to extend semen, maintain the livability and fertilizing capacity *in vitro* and maximize the number of hens that can be inseminated[7]. Therefore, the efficiency of different semen diluents in maintaining the livability and morphology of Guinea fowl spermatozoa under short term storage was studied.

Two semen diluents namely the BPSE and IMV were used for the current study. Undiluted semen may not be suitable for short term storage as higher percentage of dead spermatozoa in undiluted semen was reported compared to diluted broiler breeder semen[8]. In Guinea fowls the semen diluted with beltsville poultry semen extender was reported to shown better seminal parameters in terms of spermatozoa motility, livability, abnormality and Methylene Blue Reduction Test (MBRT) in all varieties of Guinea fowl compared to Lakes Semen Extenders (LSE) and Modified Beltsville Poultry Semen Extenders (MBPSE)[9]. Seminology and fertility studies with beltsville poultry semen extender and IMV extender in Turkey and white leghorn semen were reported. Fertility percentage of above 88% were reported in white leghorn hens inseminated weekly with diluted with BPSE (1:4) semen containing as few as 20 million sperm which reflects on the quality of diluted semen inseminated[10]. In addition, fertility percentage of 77 and 38% were reported with the Turkey semen diluted with BPSE and IMV in the ratio 1:1 stored for 24 h, which shows the superiority of BPSE over IMV[11]. Further, fertility of semen held 6 h in BPSE was reported to be lower than their respective un stored controls[12]. Hence, the spermatozoa livability and abnormality were studied till 6 h of storage in the current study. Storage temperature of 5°C was reported to be optimum as, no significant differences were observed in candling fertility (85 vs. 82%) of Turkey hens AI with the semen diluted with BPSE (1:1) with unstored semen or semen held at 5°C for 18 h whereas higher storage temperatures have shown loss in fertility[13]. Dilution studies with IMV diluents in Guinea fowl semen cannot be traced. The current study was carried out during October-December months where the average daily high temperature in the study area (13.1623° N, 80.2433° E) was below 31°C. The birds used for the study were housed in individual cages and maintained under standard feeding and manage mental conditions.

MATERIALS AND METHODS

Sixteen male Guinea fowls of pearl variety, aged 1 year were housed in individual cages providing a floor space allowance of 1.5 sqft per bird. They were given a period of 2 weeks to accustom in the cages. Feed and water were provided *ad libitum*. Feed contained about 18.11% crude protein and 2657 kcal metabolizable energy. A standard 16 h day length was provided throughout the study period. The feathers around the soft part of the abdomen were clipped off and the birds were trained for semen collection for a period of about 1 month. Semen was collected during early hours of the day between 07.30-08.30 h, twice a week following abdominal massage technique[14]. Pooled semen sample collected was then diluted with two different diluents namely the Beltsville Poultry Semen Extender (BPSE) and Instruments for Veterinary Medicine (IMV) poultry semen preservation media. The BPSE extender was prepared with the following chemical composition[15].

Components	Company	Catalogue No.	g/100 mL
Sodium glutamate (monohydrate)	Sigma Aldrich	G1626	0.867
Fructose	Sigma Aldrich	F0127	0.500
TES buffer	Sigma Aldrich	T1375	0.195
Potassium citrate (monohydrate)	Sigma Aldrich	P1722	0.064
Potassium diphosphate (trihydrate)	Sigma Aldrich	P5504	1.270
Potassium monophosphate	Sigma Aldrich	P0662	0.065
Sodium acetate (anhydrous)	Sigma Aldrich	S2889	0.430
Magnesium chloride	Sigma Aldrich	M2670	0.034

The chemicals were weighed and dissolved in the above order in 100 mL of deionized triple distilled water in a conical flask under sterile conditions. The pH and osmolarity of the

extender were maintained at 6.5 and 353 mOsm kg^{-1} H_2O, respectively[15]. The BPSE prepared was then filtered in 0.2 µm syringe filter and stored at 5°C for further use. The IMV poultry semen preservation media is a commercial poultry semen extender purchased from IMV Technologies, Gurgaon, Haryana, India. Exactly 100 µL of raw semen was diluted with 100 and 200 µL of each diluent in sterile eppondorf tubes to get the final dilution of 1:2 and 1:3, respectively. The tubes were then stored at 5°C and the spermatozoa livability and abnormality were analyzed at 0, 3 and 6 h of storage. A total of 20 samples were analyzed over a period of 3 months.

The spermatozoa livability was determined by eosin-nigrosin staining procedure[16], with staining solution containing 1% eosin and 5% nigrosin. The same slides stained for the assessment of livability were used for the assessment of percentage of abnormal spermatozoa. In every slide, more than 200 cells were counted each for livability and abnormality. With respect to livability the spermatozoa were classified as live (unstained cells) and dead (every cell stained by eosin). Spermatozoa within the fraction of live cells were classified as morphologically normal (normal head with well-marked acrosome and visible tail) or deformed (swollen head, bent neck, defective mid-piece, coiled tail, lack of tail etc.). The results of livability and morphological evaluation were expressed as the percentage of live spermatozoa and abnormal spermatozoa, respectively. The statistical analysis of the data was carried out by one way ANOVA[17] using statistical package for the social sciences 20.0 software.

RESULTS

The effect of different semen extenders, dilution ratio and storage periods on percent live spermatozoa and abnormal spermatozoa of pooled Guinea fowl semen at 5°C (Mean±SE) was presented in Table 1-3.

Table 1: Effect of semen diluents (1:2 dilution) and storage periods on percent live and abnormal spermatozoa of pooled Guinea fowl semen (Mean±SE)

	Percent livability			Percent abnormality		
Storage periods (h)	BPSE	IMV	Significance	BPSE	IMV	Significance
0	90.65 ± 0.65^{Aa}	88.44 ± 0.41^{Ba}	**	12.74 ± 0.75^{a}	13.53 ± 0.64^{a}	NS
3	87.39 ± 0.69^{b}	86.02 ± 0.39^{b}	NS	14.58 ± 0.76^{ab}	14.63 ± 0.82^{a}	NS
6	84.29 ± 0.62^{c}	83.64 ± 0.55^{c}	NS	16.72 ± 0.89^{b}	17.41 ± 0.68^{b}	NS
Significance	**	**		**	**	

n = 20, means bearing different superscripts in uppercase letter in a row and lowercase letter in a column differ significantly, **Highly significant ($p\leq0.01$), NS: Non Significant ($p>0.05$)

Table 2: Effect of semen diluents (1:3 dilution) and storage periods on percent live and abnormal spermatozoa of pooled Guinea fowl semen (Mean±SE)

	Percent livability			Percent abnormality		
Storage periods (h)	BPSE	IMV	Significance	BPSE	IMV	Significance
0	88.28 ± 0.46^{a}	88.24 ± 0.51^{a}	NS	13.47 ± 0.52^{a}	13.66 ± 0.57^{a}	NS
3	86.23 ± 0.52^{b}	85.18 ± 0.34^{b}	NS	14.68 ± 0.78^{a}	15.23 ± 0.55^{a}	NS
6	83.53 ± 0.61^{c}	82.68 ± 0.61^{c}	NS	16.55 ± 0.39^{Ab}	18.46 ± 0.74^{Bb}	*
Significance	**	**		**	**	

n = 20, means bearing different superscripts in uppercase letter in a row and lowercase letter in a column differ significantly, **Highly significant ($p\leq0.01$), *Significant ($p\leq0.05$), NS: Not significant ($p>0.05$)

Table 3: Effect of dilution ratios on percent live and abnormal spermatozoa of pooled Guinea fowl semen diluted with BPSE and IMV diluents under short term storage (Mean±SE)

		Percent livability		Percent abnormality	
Storage period (h)	Dilution ratio	BPSE	IMV	BPSE	IMV
0	1:2	90.65±0.65	88.44±0.41	12.74±0.75	13.53±0.64
	1:3	88.28±0.46	88.24±0.51	13.47±0.52	13.66±0.57
Significance		**	NS	NS	NS
3	1:2	87.39±0.69	86.02±0.39	14.58±0.76	14.63±0.82
	1:3	86.23±0.52	85.18±0.34	14.68±0.78	15.23±0.55
Significance		NS	NS	NS	NS
6	1:2	84.29±0.62	83.64±0.55	16.72±0.89	17.41±0.68
	1:3	83.53±0.61	82.68±0.61	16.55±0.39	18.46±0.74
Significance		NS	NS	NS	NS

n = 20, **Highly significant ($p\leq0.01$), NS: Not significant ($p>0.05$)

Spermatozoa livability: There observed a highly significant difference ($p \leq 0.01$) in percent live spermatozoa between the semen diluted with BPSE (90.65±0.65%) and IMV (88.44±0.41%) diluents in the ratio 1:2, immediately after dilution. Whereas, numerically higher percentage of live spermatozoa was observed in all other combinations with the semen diluted with BPSE than with IMV diluent. Further, the semen in lower dilution had shown a higher percentage of live spermatozoa irrespective of diluents and storage periods. In addition, highly significant ($p \leq 0.01$) difference was found between the semen diluted with BPSE in the ratio 1:2 (90.65±0.65%) and 1:3 (88.28±0.46%) immediately after dilution, whereas in all other combinations of dilution ratios and storage periods no significant differences were noticed. Storage period significantly ($p \leq 0.01$) reduced the percentage of live spermatozoa irrespective of diluents and dilution ratios. Higher percent live spermatozoa at 6 h of storage was found with the semen diluted with BPSE (84.29±0.62%). All the treatment combinations have shown above 80% of live spermatozoa which is required for artificial insemination. This shows the efficiency of the BPSE and IMV diluents in maintaining the livability of Guinea fowl spermatozoa under short term storage.

Spermatozoa abnormality: The semen diluted with IMV diluent in the ratio of 1:3 stored for 6 h have shown significantly ($p \leq 0.05$) higher percentage (18.46±0.74%) of morphologically abnormal spermatozoa than with the semen diluted with BPSE (16.55±0.39%). Although non-significant, similar trend was observed in all other treatment combinations, where the BPSE had shown its superiority over IMV in maintaining morphology of Guinea fowl spermatozoa under short term storage. Dilution ratio do not have any significant ($p > 0.05$) effect on spermatozoa abnormality. In general, as the dilution ratio increased mean percent abnormal spermatozoa also increased numerically. Irrespective of the diluents and dilution ratios, percent abnormal spermatozoa was increasing over storage periods especially at 6 h of storage.

DISCUSSION

The results of the study carried out to assess the livability and morphology of Guinea fowl spermatozoa were discussed below in the light of earlier reports available so as to draw the inferences even though there were scanty references available for Guinea fowl.

Immediately after dilution, the semen diluted with IMV in the ratio 1:2 had shown significant ($p \leq 0.01$) reduction in percentage of live spermatozoa compared to the semen diluted with BPSE in the same dilution ratio. This may be due to differing chemical compositions between the extenders which may have impacted on spermatozoa livability during dilution. With the increase in storage periods, irrespective of dilution ratio there observed no significant difference between the extenders in maintaining the livability of Guinea fowl spermatozoa. However, the Guinea fowl semen diluted with BPSE showed numerically higher livability in all treatment combinations compared to the semen diluted with IMV extender. Maximum livability at 6 h of storage was found with the semen diluted with BPSE in the ratio 1:2 (84.29±0.62%). The superiority of BPSE over other extenders namely LSE and MBPSE were also reported which have been found to be comparable with the present study[9]. The values obtained in the current study may also be correlated with the previous studies where lower fertility was reported in Turkey semen diluted with IMV extender[11].

The percent live spermatozoa observed in this study in all treatment combinations was higher than that observed in Turkey semen diluted with BPSE in the ratio 1:3 (81.36±0.87%) at oh of storage[18]. Lower livability (79.01%) than observed in this study was also reported in Rhode Island Cockerels with the semen diluted with BPSE[19] in the ratio 1:3. A higher spermatozoa livability (89.1%) than obtained in this study was also reported in broiler breeder semen[8]. The variations observed in the study may be due to species difference, age of birds used in the study, climatic conditions etc. Further, in the current study the livability of the spermatozoa in BPSE (1:3) was significantly lower ($p \leq 0.01$), immediately after dilution, compared to BPSE (1:2) which was in agreement with previous studies where the lower livability was observed with the semen diluted with BPSE[18,19] in the ratio 1:3. As the storage period increased, with respect to the dilution rates, there observed no significant difference in livability of Guinea fowl spermatozoa with both the diluents. This indicates the suitability of both the diluents in maintaining the livability of Guinea fowl spermatozoa under short term storage.

The decrease in spermatozoa livability with increase in storage periods recorded in this study is in accordance with the earlier studies in different breeds of Chickens and Guinea fowl varieties[8,9,20,21]. Complementary results were also obtained in pearl Guinea fowl semen in CARI diluent (1:1) where the spermatozoa livability decreased significantly from 87.22±3.17% to 74.36±4.31% with 24 h of storage[22]. The results obtained may be due to lactic acid buildup during storage which further reduces the oxygen uptake in spermatozoa thereby affecting the livability[20].

A total spermatozoa abnormality of less than 15% was reported to be desirable for carrying out artificial insemination[9]. The semen diluted with BPSE in the ratio 1:2 and 1:3 and IMV in the ratio 1:2, stored for 3 h had maintained the morphology of spermatozoa within desirable levels. A lower percent abnormal spermatozoa (9.43±0.67%) than obtained in the study was reported with the semen diluted with BPSE in the ratio 1:3 in Turkey semen[18].

Time dependent increase in percent abnormal spermatozoa irrespective of the extenders was observed in this study. This is in agreement with the earlier reports in Italian Patridge and Guinea fowls[9,21,22]. A initial non significant increase in spermatozoa abnormality between 0 and 3 h of storage period was observed. But significant differences were observed between 3 and 6 h storage period except for the semen diluted with BPSE in the ratio 1:2. It is known that fertilizing ability of undiluted neat fowl semen stored *in vitro* usually decreases within 1 h of collection[5]. The effect of semen extenders was well evident in this study as the morphology was maintained in desirable levels up to 3 h of storage. The reduction in normal spermatozoa over time may be due to differing osmolarity[23] and nutrient environment in maintaining the integrity of spermatozoa in the diluent. Further, an increase in bend spermatozoa was reported with the fowl semen diluted with Ringer's solution at 5°C in which, the researcher attributed it to the increase in chloride ion over storage[24]. The effect of ionic and osmotic changes on morphology of spermatozoa reported by previous authors needs to be further investigated with Guinea fowl semen in specific diluents under consideration.

CONCLUSION

Based on this study it is concluded that artificial insemination of semen diluted with either of the extenders at low dilution rates may have positive impact on seminal parameters and subsequent fertility by artificial insemination in Guinea fowls. Storage time had negative implication spermatozoa livability and abnormality and it is recommended that diluted semen should be used for artificial insemination within 3 h of collection.

ACKNOWLEDGMENTS

The authors are highly thankful to Dean, Madras Veterinary College, Chennai-07 and Hon'ble Vice chancellor, Tamil Nadu Veterinary and Animal Sciences University for providing all the necessary support and facilities for conducting this study.

REFERENCES

1. Abdul-Rahman, I.I., B. Awumbila, I.A. Jeffcoate, J.E. Robinson and F.Y. Obese, 2015. Sexing in Guinea fowls (*Numida meleagris*). Poult. Sci., 94: 311-318.
2. Ayorinde, K.L., 1989. Effect of semen dosage and insemination frequency on the fertility of local pearly guinea fowl in Nigeria. Trop. Agric. (Trinidad), 66: 135-136.
3. Konlan, S.P., F.K. Avornyo, N. Karbo and A. Sulleyman, 2011. Increasing guinea fowl eggs availability and hatchability in the dry season. J. World Poult. Res., 1: 1-3.
4. Premavalli, K., 2013. Influence of strain, age and system of management on the productive and reproductive performance of guinea fowl. Ph.D. Thesis, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.
5. Carter, R.D, M.G. Mccartney, V.D. Chamberlin and J.W. Wyne, 1957. The effect of storage time and temperature on fertilizing capacity of turkey semen. Poult. Sci., 36: 618-621.
6. Wishart, G.J., 1985. Quantitation of the fertilising ability of fresh compared with frozen and thawed fowl spermatozoa. Br. Poult. Sci., 26: 375-380.
7. Lake, P.E., 1956. A retarding factor in the problem of fowl semen storage. Proceedings of the 3rd International Congress on Animal Reproduction, Volume 3, June 25-30, 1956, Cambridge, pp: 104-106.
8. Dumpala, P.R., H.M. Parker and C.D. McDaniel, 2006. The effect of semen storage temperature and diluent type on the sperm quality index of broiler breeder semen. Int. J. Poult. Sci., 5: 838-845.
9. Keerthy, A.J., 2014. Studies on semen characteristics and short term storage of diluted semen of guinea fowl. M.V.Sc. Thesis, Tamil Nadu Veterinary and Animal Sciences University., Chennai, India.
10. Sexton, T.J., 1977. A new poultry semen extender. 1. Effect of extension on the fertility of chicken semen. Poult. Sci., 56: 1443-1446.
11. Sexton, T.J., 1988. Comparison of commercial diluents for holding Turkey semen 24 hours at 5°C. Poult. Sci., 67: 131-134.
12. Giesen, A.F. and T.J. Sexton, 1983. Beltsville poultry semen extender. 7. Comparison of commercial diluents for holding turkey semen six hours at 15°C. Poult. Sci., 62: 379-381.
13. Giesen, A.F. and T.J. Sexton, 1983. Beltsville poultry semen extender. 9. Effect of storage temperature on Turkey semen held eighteen hours. Poult. Sci., 62: 1305-1311.
14. Burrows, W.H. and J.P. Quinn, 1937. The collection of spermatozoa from the domestic fowl and Turkey. Poult. Sci., 16: 19-24.

15. Sexton, T.J. and A.F. Giesen, 1982. Beltsville poultry semen extender. 6. Holding turkey semen for six hours at 15°C. Poult. Sci., 61: 1202-1208.
16. Bakst, M.R. and H. Cicil, 1997. Spermatozoa Viability, Eiosin/Nigrosin Stain for Determining Live, Dead and Abnormal Spermatozoa Counts. In: Techniques for Semen Evaluation, Semen Storage and Fertility Determination, Bakst, M.R. and H. Cicil (Eds.). Poultry Science Association, Illinois, USA., ISBN: 9780964981119.
17. Snedecor, G.W. and W.G. Cochran, 1994. Statistical Methods. 9th Edn., Iowa State University Press, Iowa.
18. Venkatesh, G., 2005. Effect of pre-insemination vaginal douching and different semen extenders on fertility and hatchability in Turkeys. M.V.Sc. Thesis, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.
19. Arshami, J. and K. Cheng, 2007. Effect of *rc* mutation on semen characteristics, spermatogenic tissues and testosterone profile in blind Rhode Island Red cockerels. Asian-Australasian J. Anim. Sci., 20: 701-705.
20. Donoghue, A.M. and G.J. Wishart, 2000. Storage of poultry semen. Anim. Reprod. Sci., 62: 213-232.
21. Siudzinska, A. and E. Lukaszewicz, 2008. Effect of semen extenders and storage time on sperm morphology of four chicken breeds. J. Applied Poult. Res., 17: 101-108.
22. Mohan, J., J.M. Khanday, R.P. Singh and J.S. Tyagi, 2013. Effect of storage on the physico-biochemical characteristics and fertility of Guinea Fowl semen. Adv. Anim. Vet. Sci., 1: 65-68.
23. Clarke, R.N., M.R. Bakst and M.A. Ottinger, 1984. Morphological changes in chicken and turkey spermatozoa incubated under various conditions. Poult. Sci., 63: 801-805.
24. Saeki, Y., 1960. Crooked-necked spermatozoa in relation to low fertility in the artificial insemination of fowl. Poult. Sci., 39: 1354-1361.