COMPARATIVE EFFICIENCY OF FOUR POULTRY SEMEN EXTENDERS FOR DILUTION AND STORAGE OF DRAKE SEMEN

Stella Cyriac[1], Leo Joseph[2] and T. Gibin George[3]
Department of Poultry Science
College of Veterinary and Animal Sciences,
Mannuthy 680 651, Thrissur, Kerala.

Received:20.06.2014
Accepted:18.09.2014

Abstract

An experiment was conducted in White Pekin, Kuttanad and Muscovy drakes with the objective of comparing the efficiency of four different poultry semen extenders for dilution and storage of semen. Semen samples were diluted in four different extenders viz. Normal Saline, Phosphate Buffer Saline, Lake Poultry Semen Extender and Beltsville Poultry Semen Extender and were kept at room temperature and also at 4^{0}C. The post dilution motility was assessed at 0, 2, 4, 8, 16 and 24 h after dilution. The results of post dilution motility revealed that storage at refrigeration temperature after dilution with diluents containing buffer and energy prolonged the livability of spermatozoa.

Key words: *Drake, diluents, motility, spermatozoa*

The semen of birds is highly concentrated and the volume is too low to handle conveniently. Many poultry semen extenders have been developed and their efficiency has been evaluated in chicken and other avian species. However, research work on storage and preservation of drake semen is limited and the viability of drake sperms after dilution in different extenders is least studied. Hence, a study was conducted with the objective of comparing the efficacy of common poultry semen diluents namely, Normal Saline (NS), Phosphate Buffer Saline (PBS), Lake Poultry Semen Extender (LPSE) and Beltsville Poultry Semen Extender (BPSE) in maintaining the motility of drake spermatozoa after dilution.

Materials and Methods

Twenty four adult drakes of 36 weeks of age, eight each from Kuttanad, White Pekin (*Anasplatyrhynchosdomesticus)*and Muscovy (*Cairinamoschatamomelanotus*)breeds were utilized for the study. All drakes were reared under uniform housing and management with adlibitum feed and water. Collections were done after clipping the feathers around the vent region in order to avoid contamination. The method of semen collection followed, was the massage procedure outlined by Lake and Stewart (1978).

Pooled semen samples from the three breeds were collected at weekly intervals for six weeks and were diluted in1:1 ratio with four poultry semen diluents *ie* Normal Saline (NS), Phosphate Buffered Saline (PBS), Lake Poultry Semen Extender (LPSE) and Beltsville Poultry Semen Extender (BPSE). The diluted semen samples were stored at room temperature and at 4ºC and the motility was examined at 0, 2, 4, 8, 16 and 24 h. Motility of spermatozoa was scored as per the scoring system suggested by

1.Assistant Professor, Dept. of Poultry Science, CVAS, Mannuthy
Corresponding Author: stellacyriac03@gmail.com
2.Director,Centre for Advanced Studies in Poultry Science, CVAS, Mannuthy
3.Assistant Manager, Kerala Livestock Development Board, Kattappana, Idukki

Wheeler and Andrews (1943). Fresh diluents prepared every week were used for the study and the pH of the diluents was between 6.8 and 7.4.The data were analysed statistically as per the method described by Snedecor and Cochran (1994). All the tests of difference between means were conducted at five per cent probability level.

Normal Saline was prepared bydissolving0.9 g Sodium Chloride in 100 ml distilled water and PBS by dissolving1.0078 g PBS powder in 100 ml double distilled water.The composition (g/100ml double distilled water) of Lake Poultry Semen Extender and Beltsville Poultry Semen Extender prepared are given in Table 1.

Results and Discussion

The results of post dilution motility after storage at room temperature and refrigeration temperature of White Pekin, Kuttanad and Muscovy arepresented in Table 2 and 3. The samples diluted with NS showed significantly ($p<0.05$) lower initial motility than that diluted with PBS, LPSE and BPSE. At two hours of storage at room temperature, the motility of samples diluted with NS dropped down to zero but under refrigeration temperature, samples diluted with NS showed a motility of 3.33 per cent after 4 hours. Samples diluted with BPSE lost their motility completely after four hours of storage at room temperature. But, under refrigeration conditions the samples diluted with BPSE showed motility similar to that diluted with PBS and LPSE even after eight hours. Samples diluted with LPSE and PBS showed numerically higher motility under room temperature and refrigeration conditions at four and eight hours of storage, respectively.

The motility of samples diluted with PBS and LPSE was significantly ($p<0.05$) greater than the motility of samples diluted with NS and BPSE. After two and four hours of storage at room temperature and refrigeration temperature, the samples diluted with NS showed significantly ($p<0.05$) lower motility percentage and samples diluted with LPSE showed significantly ($p<0.05$) higher motility percentage. The spermatozoa retained their motility after 8 hours of storage under refrigeration temperature in samples diluted with PBS (13.33 per cent) and LPSE (35.00 per cent) whereas after 4 hours of storage at room temperature, 16.67 per cent motility was observed in samples diluted with LPSE.

In Muscovy drakes, the motility of the semen samples diluted with NS and BPSE was significantly ($p<0.05$) lower than that of samples diluted with PBS and LPSE initially and also after two hours of storage at room temperature and refrigeration temperature. Similar results were observed after four hours of storage under refrigeration conditions also. After four hours of storage at room temperature, samples diluted with LPSE showed significantly ($p<0.05$) higher motility and the motility in samples diluted with

Table 1. Composition of Lake and Beltsville Poultry Semen Extender

Sl. No.	Component	LPSE	BPSE	Sl. No.	Component	LPSE	BPSE
1.	Monosodium glutamate	1.350	0.867	6.	Dipotassium phosphate	–	1.270
2.	Tripotassium citrate	0.128	0.064	7.	Fructose	–	0.500
3.	Sodium acetate	0.510	0.430	8.	TES*	–	0.195
4.	Magnesium acetate tetra hydrate	0.080	-	9.	Monopotassium phosphate	–	0.065
5.	Glucose	0.800	-	10.	Magnesium chloride	–	0.034

*N-tris [Hydroxymethyl] methyl-2-Aminoethane Sulfonic Acid.

PBS was similar to that in samples diluted with LPSE, BPSE and NS. Samples diluted with PBS showed significantly ($p<0.05$) higher motility after eight hours of storage under refrigeration temperature and the motility of samples diluted with LPSE was similar to that diluted with PBS, NS and BPSE.

The spermatozoa became non motile after eight hours of dilution and storage at room temperature but, when stored at refrigeration temperature very small percentage (< 1 per cent) of spermatozoa with weak oscillatory movement were observed at 16 and 24 hours in the samples diluted with PBS and LPSE.

Reports on dilution and storage of drake semen are scanty and similar studies have not been conducted in Kuttanad ducks so far. Results similar to the present study were reported by Stunden (1996) who compared Beltsville Poultry Semen Extender (BPSE) and Lake Poultry Semen Extender (LPSE) in Leghorns and found that semen stored in either BPSE or LPSE performed better than undiluted control (3 per cent). The findings of Kasai and Izumo (1997) also agree with the present findings. Similar results were reported by Ax and Lodge (1975) and Austin and Natarajan (1991) in rooster spermatozoa and they found that the motility of diluted semen reduced with increase in storage time. Literatures with similar diluents, storage time and temperature in ducks are scanty. From the available literatures, it could be concluded that in addition to ambient temperature several other factors like the composition of diluents, dilution rate and pH can also affect the motility of diluted semen. Hypotonic solutions cause sperm swelling and bursting and hence osmotic pressure also appears to be a crucial factor in maintaining motility. Maintenance of fertilizing capacity of poultry sperm for up to 48 hours requires the provision of oxygen, a diluent that

Table 2. Post dilution motility (per cent) of drake semen after dilution with Normal Saline (NS), Phosphate Buffer Saline (PBS), Lake Poultry Semen Extender (LPSE), Beltsville Poultry Semen Extender (BPSE) and storage at room temperature for different time duration

Storage time duration	White Pekin				Kuttanad				Muscovy			
	NS	PBS	LPSE	BPSE	NS	PBS	LPSE	BPSE	NS	PBS	LPSE	BPSE
0 hour	40.00^{b} ± 0.00	66.67^{a} ± 6.67	81.67^{a} ± 4.77	68.33^{a} ± 5.43	50.00^{b} ± 6.83	80.00^{a} ± 4.47	88.33^{a} ± 1.67	53.33^{b} ± 4.22	46.67^{b} ± 4.22	78.33^{a} ± 4.01	71.67^{a} ± 5.43	46.67^{b} ± 4.22
2 hours	0.001^{b} ± 0.00	20.00^{ab} ± 9.66	35.00^{a} ± 9.92	25.00^{a} ± 7.19	3.33^{c} ±3.33	23.33^{b} ± 7.60	50.00^{a} ± 7.30	21.67^{bc} ± 6.54	15.00^{b} ± 3.42	36.67^{a} ± 3.33	33.33^{a} ± 8.43	10.00^{b} ± 4.47
4 hours	0.001^{a} ± 0.00	1.67^{a} ± 1.67	5.00^{a} ± 3.42	0.001^{a} ± 0.00	0.001^{b} ±0.00	5.00^{ab} ± 3.42	16.67^{a} ± 8.03	1.67^{b} ±1.67	1.67^{b} ± 1.67	3.33^{ab} ± 2.11	8.33^{a} ± 3.07	0.001^{b} ± 0.00

Means bearing different superscripts in a row within a breed differ significantly ($p< 0.05$)

Table 3. Post dilution motility (per cent) of drake semen after dilution with Normal Saline (NS), Phosphate Buffer Saline (PBS), Lake Poultry Semen Extender (LPSE), Beltsville Poultry Semen Extender (BPSE) and storage at refrigeration temperature for different time duration

Storage time duration	White Pekin				Kuttanad				Muscovy			
	NS	PBS	LPSE	BPSE	NS	PBS	LPSE	BPSE	NS	PBS	LPSE	BPSE
0 hour	40.00^{b} ± 0.00	66.67^{a} ± 6.67	81.67^{a} ± 4.77	68.33^{a} ± 5.43	50.00^{b} ± 6.83	80.00^{a} ± 4.47	88.33^{a} ± 1.67	53.33^{b} ± 4.22	46.67^{b} ± 4.22	78.33^{a} ± 4.01	71.67^{a} ± 5.43	46.67^{b} ± 4.22
2 hours	11.67^{c} ± 4.01	25.00^{bc} ± 11.48	51.67^{a} ± 6.01	36.67^{ab} ± 8.03	8.33^{c} ± 4.01	43.33^{ab} ± 7.60	56.67^{a} ± 6.15	26.67^{b} ± 6.15	16.67^{b} ± 3.33	51.67^{a} ± 7.03	51.67^{a} ± 7.03	21.67^{b} ± 7.03
4 hours	3.33^{b} ± 3.33	11.67^{ab} ± 7.49	26.67^{a} ± 5.58	13.33^{ab} ± 6.15	4.167^{b} ± 3.27	23.33^{ab} ± 8.43	40.00^{a} ± 8.56	6.67^{b} ± 3.33	1.67^{b} ± 1.67	30.00^{a} ± 7.30	33.33^{a} ± 7.15	8.33^{b} ± 4.01
8 hours	0.001^{a} ±0.00	5.00^{a} ± 3.42	3.33^{a} ± 2.11	1.67^{a} ± 1.67	0.001^{b} ± 0.00	13.33^{b} ± 6.67	35.00^{a} ± 9.22	0.001^{b} ±0.00	0.001^{b} ±0.00	15.00^{a} ± 5.63	8.33^{ab} ± 4.01	0.001^{b} ±0.00

Means bearing different superscripts in a row within a breed differ significantly ($p< 0.05$)

supplies fructose or glucose as a substrate for production of ATP, sufficient buffering capacity to maintain pH and an ambient temperature of 5 to 7^0 C (Etches, 1996).

The results obtained in the present study indicate that good quality semen collected by massage method from White Pekin, Kuttanad and Muscovy breeds of ducks could be used for artificial insemination after dilution with 1:1 ratio. Among the four different diluents tested, LPSE was found to be better in maintaining the motility. The semen diluted with LPSE could be stored under refrigeration for upto eight hours retaining the motility. In poultry, artificial insemination is usually done within 30 minutes after semen collection. From the results, it is evident that samples diluted with PBS also showed motility similar to that diluted with LPSE at zero hour in all the three breeds. Hence, dilution with PBS could be recommended for dilution of drake semen that is to be used for insemination immediately after collection of semen. Readymade PBS powder is available in markets which make the preparation of PBS easier when compared with LPSE. But, if the semen is to be used later, dilution with LPSE in 1: 1 ratio under refrigeration conditions could be recommended. Further studies are to be conducted to determine the fertility of eggs by artificial insemination, the duration of fertility and time of insemination.

References

Austin, A.J.S. and Natarajan, N. 1991. Effect of buffers and dilution rates on motility percent of chicken spermatozoa. *Indian J. Poult. Sci.* **26**: 34-38.

Ax, R.L. and Lodge, J.R. 1975. Rooster spermatozoan motility, forward progression and fertility after storage at 25, 5 or -196^{0}C in various extenders. *Cryobiology* **12**: 93-97.

Etches, R.J. 1996. *Reproduction in Poultry.* 1st ed. CAB International, Wallingford, Oxon, U.K. 318p.

Kasai, K. and Izumo, A. 1997. *Basic studies on artificial insemination of ducks*. No.33. Bulletin of the Osaka Agricultural Research Center, AFFRC, Japan. 47p.

Lake, P. E. and Stewart, J. M. 1978. *Artificial Insemination in Poultry*. No. 213, Bulletin of Ministry of Agriculture, Fisheries and Food. Her Majesty's Stationary Office, London. 34p.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.* 8th ed. Affiliated East- West Press, Pvt. Ltd, New Delhi, India. 313 p.

Stunden, C. E. 1996. Studies on reproduction characteristics and artificial insemination in captive mallard ducks.*M.Sc.thesis*, The University of British Columbia. 149p.

Wheeler, N.C. and Andrews, F.N. 1943. The influence of season on semen production in the domestic fowl. *Poult. Sci*. **22**: 361-367.

❑