RESEARCH ARTICLE

Comparative Assessment of Post-Extension Sperm Motility in Aseel, Kadaknath and Native Chicken of Kerala using Various Semen Diluents

Akshay Dharaniyedath[1], Harikrishnan Sreekumar[2]*, Hiron M Harshan[3], Anitha Pallikkattil[1], Vineetha Puthanmadathil Gopi[4]

Abstract

The present study aimed to compare the preservability of poultry semen using four extenders, *viz.,* Normal Saline (NS), Phosphate Buffered Saline (PBS), Lake Poultry Semen Extender (LPSE), and Beltsville Poultry Semen Extender (BPSE) across three indigenous chicken breeds (Aseel, Kadaknath, and native chicken of Kerala) at both room and refrigeration temperatures. After 8 h at room temperature, Aseel and Kadaknath semen diluted with LPSE demonstrated significantly better progressive sperm motility. For the native chicken of Kerala, both LPSE and BPSE showed promising results. In contrast, the preservability of PBS and NS was notably lower ($p<0.01$) compared to LPSE and BPSE across all breeds at room temperature. At refrigeration, LPSE outperformed other extenders in retaining progressive sperm motility with BPSE following closely. Notably, after 48 h all samples diluted with NS exhibited zero motility, while the spermatozoa of native chicken of Kerala extended in PBS showed feeble motility. Overall, LPSE emerged as the superior extender for maintaining sperm motility at both temperatures, with BPSE also performing well. Additionally, refrigeration significantly enhanced sperm viability, increasing it up to six fold compared to room temperature storage.

Keywords: BPSE, Cock semen, LPSE, PBS, Refrigeration temperature, Room temperature, Sperm motility

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Introduction

The poultry industry has increasingly adopted artificial insemination (AI) as a key reproductive technology to enhance breeding efficiency and genetic improvement. This trend highlights the critical importance of ensuring the distribution of high-quality spermatozoa, which is vital for successful fertilization outcomes. Due to the inherent high concentration and limited volume of chicken semen, effective extension with appropriate diluents is essential. This process not only facilitates uniform distribution of sperm but also enhances their viability during storage and subsequent use (Getachew, 2016). Semen extenders are formulated through empirical trials, incorporating various components that provide energy and maintain physiological parameters such as pH and osmolarity, which are crucial for sperm survival. The process of semen dilution significantly increases the number of inseminations per ejaculate, allowing for more effective management of breeding programs (Siudzinska and Lukaszewics, 2008). Despite the established importance of suitable semen extenders in AI, research focusing on the preservability of sperm from various indigenous chicken breeds remains sparse. This gap is particularly notable for the native chicken of Kerala and other indigenous breeds such as Aseel and Kadaknath, which are valued for their unique genetic traits and adaptability. This study was planned to investigate and compare the preservability of semen from valued indigenous chicken breeds such as Aseel, Kadaknath and native chicken of Kerala reared under intensive management conditions in Kerala. By evaluating different semen extenders, this research seeks to contribute valuable insights into optimizing AI practices for these local breeds, thereby supporting sustainable poultry production and genetic conservation efforts.

[1]Department of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680 651, Kerala Veterinary and Animal Sciences University (KVASU), Kerala, India

[2]University Poultry and Duck Farm, Mannuthy-680 651, KVASU, Kerala, India

[3]Department of Animal Reproduction Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy-680 651, KVASU, Kerala, India

[4]College of Avian Sciences and Management, Thiruvazhamkunnu-678 601, KVASU, Kerala, India

Corresponding Author: Harikrishnan, University Poultry and Duck Farm, Mannuthy-680 651, KVASU, Kerala, India. e-mail: harikrishnans@kvasu.ac.in

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Materials and Methods

Nine adult roosters, 30 weeks of age (three each from Aseel, Kadaknath and native chicken of Kerala), procured from University Poultry and Duck farm of Kerala Veterinary and Animal Sciences University, Mannuthy (India) were utilised for the study. The roosters were maintained in individual cages with dimension of 39 cm × 46 cm × 44 cm. All birds were provided with male breeder diet containing 16 % crude protein and 2600 kcal/kg metabolisable energy (BIS, 2007) with *ad-libitum* water. The semen was periodically collected from the roosters at four days interval by abdominal massage method outlined by Lake *et al.* (1985). Pooled semen samples from the 3 roosters of a breed were diluted with poultry semen extenders such as Normal Saline (NS), Phosphate Buffered Saline (PBS), Lake Poultry Semen Extender (LPSE) and Beltsville Poultry Semen Extender (BPSE) in a ratio of 1:10. The composition of LPSE and BPSE used are given in Table 1 and 2, respectively. The extended semen samples were stored at room temperature and at 4°C and the progressive motility was examined at 0, 2, 4, 8, 16, 24 and 48 h interval (Stella, 2011). Fresh extenders prepared every week were used for the study and the pH of the diluents was between 5.6 and 7.4.

Composition of the Diluents Used

Normal Saline: 0.9 g Sodium chloride dissolved in 100 mL distilled water (pH 5.6).

Phosphate Buffered Saline: Sodium chloride 8.0 g, Potassium chloride 0.2 g, Sodium phosphate dibasic 1.44 g and Potassium phosphate monobasic 0.245 g were dissolved in double distilled water, and the volume was made up to 1000 mL with double distilled water with a final pH of 7.4 **(**Ambily, 2021).

Lake Poultry Semen Extender (pH 6.8-7.1) and **Beltsville Poultry Semen Extender (**pH 6.8-7.1) were formulated using components presented in Tale 1 and 2, respectively.

Table 1: Composition of Lake poultry semen extender (Stella, 2011)

Component	Level (g/100 mL double-distilled water)
Monosodium glutamate	1.350
Tripotassium citrate	0.128
Sodium acetate	0.510
Magnesium acetate tetra hydrate	0.080
Glucose	0.800

Table 2: Composition of Beltsville poultry semen extender (Stella, 2011)

Component	Level (g/100 mL double-DW)	Primary function
Dipotassium phosphate	1.270	Buffer
Monosodium glutamate	0.867	Chelator
Fructose	0.500	Metabolic substrate
Sodium acetate	0.430	Osmotic balance
TES*	0.195	Buffer
Tripotassium citrate	0.064	Osmotic balance
Monopotassium phosphate	0.065	Buffer
Magnesium chloride	0.034	Osmotic balance

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

The data obtained from three indigenous breeds of poultry were assessed statistically by one-way ANOVA using SPSS version 24.0.

Results and Discussion

The data on post-extension progressive motility of sperms from three indigenous breeds (Aseel, Kadaknath and native chicken of Kerala) in four different extenders at different time intervals of dilution and storage temperatures are presented in the Tables 3 and 4.

In Aseel, no significant difference was observed in initial progressive sperm motility when extended with NS, PBS, LPSE and BPSE. At 8 h of storage in room temperature, significantly ($p<0.01$) higher progressive motility was measured in LPSE followed by BPSE and PBS and the trend was the same in case of refrigeration temperature. When the semen samples were extended with NS, the motility dropped down to zero at ambient temperature, but on refrigeration, the progressive sperm motility was retained up to 38 %. Similarly, under refrigeration temperature, the motility was zero in case of NS and PBS at 48 h of storage, while significantly ($p<0.01$) higher progressive motility was retained in LPSE followed by BPSE. The samples extended with LPSE had significantly ($p<0.01$) higher progressive motility at various time intervals both in room and refrigeration temperature and the progressive motility in LPSE was retained up to 48 h in refrigeration compared to 8 h at room temperature (Tables 3, 4).

There was no difference in the initial progressive motility of spermatozoa of Kadaknath roosters between the four extenders. However on preservation, the samples extended with LPSE had significantly ($p<0.01$) higher progressive motility than BPSE at the different time intervals both in refrigeration and room temperature. However, a comparable progressive motility was observed between NS and PBS at different time intervals. At 8 h of storage under room temperature, the progressive motility of semen extended with NS and PBS dropped to zero, but a comparable progressive motility of 45.00 and 44.83 %, respectively, was retained at refrigeration temperature. After 48 h of storage at refrigeration temperature, the progressive motility in NS and PBS was zero. However, LPSE retained a significantly ($p<0.01$) higher progressive sperm motility than BPSE at 48 h (Tables 3, 4).

In native chicken of Kerala, the initial progressive motility of the semen samples extended with LPSE was comparable with PBS and was significantly ($p<0.05$) higher than the other two extenders. At 8 h of storage at room temperature, the

progressive motility of sperm extended with NS became zero and there was no difference in the progressive motility between the other three extenders. At 48 h storage in refrigeration temperature, the semen extended with LPSE showed significantly ($p<0.01$) higher progressive motility and a comparable motility in BPSE and PBS extenders, while the motility was zero in case of NS (Tables 3, 4).

Table 3: Post-extension progressive motility of sperm (mean ±SE %) from roosters of Aseel, Kadaknath and native Chicken of Kerala diluted with different extenders and stored at room temperature (n=6 samples /breed)

Breed of poultry	Time (h)	Semen extenders				F-value	p-value
		NS	PBS	LPSE	BPSE		
Aseel	0	93.83±0.98	94.33±0.92	96.67±0.49	95.17±0.70	2.42^{ns}	0.10
	2	51.83^{c}±3.07	53.17^{c}±2.81	73.17^{a}±2.40	64.17^{b}±1.90	15.11**	0.001
	4	23.50^{b}±3.20	28.50^{b}±1.52	50.50^{a}±4.33	43.83^{a}±5.90	9.72**	0.001
	6	6.17^{b}±1.28	12.50^{b}±2.16	29.83^{a}±3.35	25.17^{a}±3.16	17.61**	0.001
	8	0	1.00^{c}±3.35	13.67^{a}±2.29	8.83^{b}±2.04	16.37**	0.001
Kadaknath	0	95.50±0.22	94.33±0.84	96.33±0.67	95.33±0.42	1.95^{ns}	0.15
	2	45.00^{c}±2.92	54.33^{b}±3.49	68.50^{a}±2.87	63.50^{a}±2.25	12.66**	0.00
	4	22.50^{c}±4.09	28.00^{c}±3.91	54.67^{a}±3.26	40.33^{b}±3.70	14.51**	0.00
	6	10.33^{c}±2.06	11.33^{c}±3.01	34.33^{a}±2.91	24.00^{b}±1.81	20.80**	0.00
	8	0	0	18.67^{a}±2.30	7.83^{b}±1.85	35.74**	0.00
Native chicken of Kerala	0	94.00^{b}±0.45	95.50^{ab}±0.67	96.00^{a}±0.56	93.83^{b}±0.70	3.16*	0.04
	2	48.50^{b}±4.897	55.17^{b}±3.06	69.00^{a}±2.28	65.00^{a}±1.03	8.76**	0.001
	4	26.00^{c}±2.71	32.83^{bc}±4.25	44.00^{a}±3.97	38.17^{ab}±2.43	5.00*	0.01
	6	11.17^{c}±2.26	18.17^{bc}±3.31	26.67^{a}±2.36	22.67^{ab}±2.50	6.34**	0.003
	8	0	7.17^{a}±3.00	11.33^{a}±1.36	11.83^{a}±2.74	6.52**	0.003
	16	0	0	1.67±1.67	1.67±1.67	0.67^{ns}	0.58

NS-Normal saline, PBS-Phosphate buffered saline, LPSE-Lake poultry semen extender and BPSE-Beltsville poultry semen extender. Mean values bearing different superscripts in a row within the breed differ significantly, ns-non significant, **highly significant ($p<0.01$), * significant ($p<0.05$)

Table 4: Post-extension progressive motility of sperm (Mean ±SE, %) from rosters of Aseel, Kadaknath and native Chicken of Kerala diluted with different extenders and stored at refrigeration temperature (n=6 samples /breed)

Breed of poultry	Time (h)	Semen Extenders				F-value	p-value
		NS	PBS	LPSE	BPSE		
Aseel	0	93.83±0.98	94.33±0.92	96.67±0.49	95.17±0.70	2.416^{ns}	0.10
	2	74.17^{d}±1.47	78.00^{c}±1.34	87.17^{a}±0.54	82.67^{b}±0.88	25.29**	0.001
	4	59.67^{c}±2.23	63.50^{c}±1.15	76.83^{a}±1.33	72.00^{b}±1.15	26.09**	0.001
	6	48.33^{c}±1.33	54.00^{b}±1.93	65.50^{a}±1.78	60.83^{a}±2.41	15.70**	0.001
	8	38.33^{d}±0.76	46.33^{c}±1.12	58.50^{a}±2.23	52.83^{b}±1.90	41.30**	0.001
	16	15.00^{d}±1.24	28.67^{c}±2.37	46.50^{a}±1.41	36.00^{b}±1.06	68.51**	0.001
	24	3.17^{d}±0.79	15.83^{c}±0.60	33.00^{a}±1.26	24.17^{b}±1.87	105.95**	0.001
	48	0	0	9.50^{a}± 0.96	2.67^{b}± 1.31	30.62**	0.001
Kadaknath	0	95.50±0.22	94.33±0.84	96.33±0.67	95.33±0.42	1.95^{ns}	0.15
	2	77.83^{c}±1.33	80.50^{bc}±1.48	86.50^{a}±0.67	83.00^{ab}±1.37	8.70**	0.001
	4	63.00^{c}±2.58	65.00^{bc}±3.14	78.50^{a}±0.89	70.67^{b}±2.12	8.84**	0.001
	6	54.33^{b}±1.69	54.33^{b}±2.55	68.67^{a}±2.04	60.33^{b}±2.39	9.57**	0.001
	8	45.00^{c}±1.53	44.83^{c}±2.07	61.70^{a}±1.60	52.17^{b}±1.72	19.60**	0.001
	16	26.67^{c}±3.09	27.33^{c}±3.68	46.17^{a}±1.22	35.67^{b}±0.67	13.34**	0.001
	24	15.17^{c}±2.70	11.00^{c}±2.18	33.33^{a}±1.33	23.50^{b}±1.20	25.53**	0.001
	48	0	0	11.17^{a}±1.56	1.33^{b}±0.99	34.22**	0.001

Native chicken of Kerala	0	94.00^{b}±0.47	95.50^{ab}±0.67	96.00^{a}±0.58	93.83^{b}±0.70	3.16*	0.04
	2	82.00^{c}±1.65	84.33^{bc}±0.67	89.00^{a}±0.86	86.67^{ab}±0.84	7.85**	0.001
	4	68.67^{bc}±1.93	66.33^{c}±3.36	79.33^{a}±0.84	73.67^{ab}±1.41	7.51**	0.001
	6	59.67^{b}±1.87	56.67^{b}±3.02	69.67^{a}±2.15	62.17^{b}±2.76	4.96*	0.01
	8	47.17^{b}±1.22	48.00^{b}±3.49	60.17^{a}±1.797	52.67^{ab}±3.37	5.018**	0.009
	16	29.67^{b}±1.43	29.33^{b}±4.66	45.83^{a}±1.83	37.67^{b}±1.80	8.06**	0.001
	24	13.83^{c}±0.87	15.67^{c}±3.33	33.17^{a}±1.38	22.50^{b}±2.45	15.51**	0.001
	48	0	0.83^{b}±0.83	8.67^{a}±1.65	3.33^{b}±1.54	10.55**	0.001

NS-Normal saline, PBS-Phosphate buffered saline, LPSE-Lake poultry semen extender and BPSE-Beltsville poultry semen extender. Mean values bearing different superscripts in a row within a breed differ significantly, ns-non significant, **highly significant ($p<0.01$), * significant ($p<0.05$)

The results of Kadaknath semen extended with NS and LPSE are in agreement with the findings of Shinde *et al.* (2013) at 48 h of storage. Jabbar *et al.* (2015) reported a similar progressive motility when extended with LPSE in Aseel chicken at 6, 12 and 24 h of storage at 4°C. Due to scarcity of literature, the results of the post-dilution progressive sperm motility of indigenous chicken breeds are compared with other chicken breeds. At room temperature, a similar progressive motility with LPSE was reported by Blank *et al.* (2021) and Ghaniei *et al.* (2019) at 2, 4 and 8 h of storage. The results documented in the present study with LPSE and BPSE at 48 h of incubation under refrigeration temperature was in accordance with the findings of Latif *et al.* (2005). The progressive sperm motility measured with Lake extender after 24 h at 4°C in the current study was in accordance with the findings of Al-Daraji (2013) and Siudzinska and Lukaszewics (2008). Ahangari *et al.* (2013) reported a similar progressive motility in BPSE (1:1 dilution) at 24 h of storage. Rakha *et al.* (2016) reported similar motility at 24 and 48 h of storage in BPSE extender at refrigeration temperature, which is closely in agreement with the present findings.

Contrary to the present findings, Sexton *et al.* (1980) and Blesbois *et al.* (1999) reported higher progressive sperm motility at 48 h of storage with BPSE at refrigeration temperature. Similarly, Eslami *et al.* (2016), Ola *et al.* (2020) and Balogun (2021) measured a higher progressive motility after 48 h of storage at refrigeration temperature.

The normal ejaculated volume of chicken semen is 0.2-0.5 mL with a high concentration of spermatozoa. Proper processing and using suitable semen extenders are helpful to increase the semen volume (Getachew, 2016). The semen extenders are buffered salt solutions designed to "stretch" the semen volume, ensure homogeneous distribution of spermatozoa and provide an ideal environment. The composition of the semen extenders should be in agreement with the biochemical characteristics of poultry semen and for long-term storage, semen should be exposed to storage temperature as low as 4-10°C up to 24 h. This helps the sperm metabolism to slow down without affecting the viability and ability to fertilize spermatozoa (Siudzinska and Lukaszewicz, 2008; Brillard, 2009). This must be the reason for the higher progressive sperm motility at refrigeration temperature compared to room temperature.

The results obtained in the present study indicated that good quality semen could be collected by massage method from Aseel, Kadaknath and native chicken of Kerala. Upon studying the efficacy of four extenders in Aseel, it was found that LPSE is superior compared to BPSE followed by PBS and NS. A similar trend was evident in case of Kadaknath too with the exception that a comparable motility was observed between NS and PBS at different time intervals of extension and storage. In case of native chicken of Kerala, up to 6 h storage at room temperature, comparable results were observed between LPSE and BPSE and between NS and PBS. However, on prolonged storage at refrigeration temperature, LPSE was found better than BPSE followed by PBS.

The higher sperm motility in LPSE and BPSE might be due to the presence of chemical components that can provide energy, better pH and osmolarity for the sperms to survive. Blesbois and Reviers (1992) stated that the LPSE and BPSE are equally effective in preserving the sperm motility of roosters at 4°C. Comparatively lower motility was observed in NS and PBS extenders and this may be due to the lack of energy sources and buffering agents (in NS). Further studies are warranted to determine the efficacy of preserved spermatozoa in the said 4 extenders by ascertaining the fertility of eggs by artificial insemination technique.

Conclusion

Upon comparing the efficacy of various poultry semen extenders, LPSE was found superior in retaining the progressive sperm motility of indigenous chicken breeds at room and refrigeration temperature followed by BPSE. The NS and PBS diluents can be used where short-term semen storage options are only available. PBS was found obviously a better option than NS due to its additional buffering capacity which helps to maintain motility of spermatozoa for slightly longer-term storage. The study also concluded that the preservation of semen in refrigeration enhanced the viability of sperm up to six times possibly due to the slowing down of metabolic processes, reducing the rate of cell death and preventing damage from thermal stress.

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References

Ahangari, Y.J., Parizadian, B., & Zamani, M. (2013). The impact of organic selenium supplementation on rooster semen quality in liquid condition. *The Journal of Poultry Science, 1,* 23-31.

Al-Daraji, H.J. (2013). Effect of diluent supplementation with garlic extract on semen quality of cocks during liquid storage. *Theriogenology Insight, 3*(1), 21-29.

Ambily, K.G. (2021). Freezability of specific fractions of large White Yorkshire boar semen supplemented with sodium dodecyl sulphate. *M.V.Sc Thesis*, Kerala Veterinary and Animal Sciences University, Pookode, India, pp. 38.

Balogun, A.S. (2021). Optimizing liquid storage duration of two poultry species semen with plant-based extender: semen preservation in poultry. *Letter to Animal Biology, 1,* 1-6.

BIS (2007). *Poultry Feed Specification*. 5th edn., Bureau of Indian Standards, New Delhi, 4p.

Blank, M.H., Ruivo, L.P., Novaes, G.A., Lemos, E.C., Losano, J.D., Siqueira, A.F., & Pereira, R.J. (2021). Assessing different liquid-storage temperatures for rooster spermatozoa. *Journal of Animal Reproduction Science, 233,* 68.

Blesbois, E., & De-Reviers, M. (1992). Effect of different fractions of seminal plasma on the fertilizing ability of fowl spermatozoa stored *in-vitro*. *Journal of Reproduction, 95,* 263-268.

Blesbois, E., Grasseau, I., & Hermier, D. (1999). Changes in lipid content of fowl spermatozoa after liquid storage at 2 to 5°C. *Theriogenology, 52,* 325-334.

Brillard, J.P. (2009). Practical aspects of fertility in poultry. *Avian Biological Research, 2,* 41-45.

Eslami, M., Ghaniei, A., & Rad, H.M. (2016). Effect of the rooster semen enrichment with oleic acid on the quality of semen during chilled storage. *The Journal of Poultry Science, 95,* 1418-1424.

Getachew, T. (2016). A review article of artificial insemination in poultry. *World Poultry Science, 6*(1), 26-35.

Ghaniei, A., Eslami, M., Zadeh-Hashem, E., Rezapour, R., & Talebi, A. (2019). Quercetin attenuates H_2O_2 induced toxicity of rooster semen during liquid storage at 4°C. *Journal of Animal Physiology and Animal Nutrition, 103,* 713-722.

Jabbar, A., Abbass, W., Riaz, A., Sattar, A., & Akram, M. (2015). Effect of different concentrations of ascorbic acid on semen quality and hatchability of indigenous Aseel chicken. *Journal of Animal and Plant Science, 25,* 1222-1226.

Lake, P.E. (1985). Fowl semen as collected by the massage method. *Journal of Agricultural Sciences, 49,* 120-126.

Latif, A., Ijaz, A., Aleem, M., & Mahmud, A. (2005). Effect of osmotic pressure and pH on the short-term storage and fertility of broiler breeder sperm. *Pakistan Veterinary Journal, 25,* 179.

Ola, S.I., Faleye, O., Adeyemi, A., & Adeyosoye, O. (2020). Evaluation of egg yolk plasma as replacement for whole egg yolk in chicken semen extender. *Journal of Poultry Research, 17,* 96-101.

Rakha, B.A., Ansari, M.S., Hussain, I., Anwar, M., Akhter, S., & Blesbois, E. (2016). Comparison of extenders for liquid storage of Indian Red Jungle Fowl (*Gallus gallus murghi*) spermatozoa. *Avian Biological Research, 9,* 207-212.

Sexton, T.J., Jacobs, L.A., & Mc-Daniel, G.R. (1980). A new poultry semen extender and effect of antibacterials in control of bacterial contamination in chicken semen. *Poultry Science, 59,* 274-281.

Shinde, A.S., Mohan, J., Sastry, K.V.H., Singh, R.P., Chouhan, L., & Tyagi, J.S. (2013). CARI diluent for short-term preservation of Kadaknath chicken semen. *Indian Journal of Poultry Science, 48,* 240-243.

Siudzinska, A., & Lukaszewicz, E. (2008). Effect of semen extenders and storage time on sperm morphology of four chicken breeds. *Journal of Applied Poultry Research,* 17, 101-108.

Stella, C. (2011). Semen quality characteristics of Kuttanad, White Pekin (*Anasplatyrhynchos domesticus*) and Muscovy (*Cairinamoschatamo melanotus*) ducks. *M.V.Sc.Thesis.* Kerala Veterinary and Animal Sciences University, Pookode, India, pp. 122.