Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

e-ISSN: 1806-9061 2025 / v.27 / n.1 / 001-010

http://dx.doi.org/10.1590/1806-9061-2024-1942

Original Article

Effects of Various Semen Extenders on the Hatching Characteristics of Japanese Quail

■Author(s)

Raziq F[I] https://orcid.org/0000-0003-2021-4201
Khan MT[II] https://orcid.org/0000-0002-4734-3428
Ullah A[III] https://orcid.org/0000-0001-5443-382X
Farooq U[IV] https://orcid.org/0000-0003-1575-9979
Akhtar R[V] https://orcid.org/0000-0003-0501-3989
Siddique B[VI] https://orcid.org/0009-0000-3226-5415
Abbas G[VII] https://orcid.org/0000-0002-2741-994X
Ali F[VIII] https://orcid.org/0000-0002-2001-2928
Aslam S[IX] https://orcid.org/0000-0001-7465-5358
Khaliq H[X] https://orcid.org/0000-0002-4806-7349
Ali H[XI] https://orcid.org/0009-0007-8864-9160
Khalid MF[IV] https://orcid.org/0000-0003-1601-3642
Ullah MI[XII] https://orcid.org/0000-0002-8769-3086
Aljawdah HM[XIII] https://orcid.org/0009-0002-7484-0398
Farah MA[XIII] https://orcid.org/0000-0001-7616-7542

*Affiliations

■Mail Address

Corresponding author e-mail address
Muhammad Tahir Khan
Cholistan University of Veterinary and Animal Sciences, Faculty of Animal Production and Technology, Department of Poultry Science, Bahawalpur - 63100, Pakistan.
Phone: +92 300 836 0008
Email: mtahirkhan@cuvas.edu.pk

■Keywords

Fertility, hatchability, quail, semen extenders, semen evaluation.

Section Editor: Tatiana Carlesso dos Santos

Submitted: 21/April/2024
Approved: 17/February/2025

ABSTRACT

Reproductive efficiency is a cornerstone of success in poultry production, particularly in species such as the Japanese quail (*Coturnix coturnix japonica*), which are valued for their high productivity and adaptability. Semen extenders have been evaluated for their efficacy in preserving sperm quality and improving reproductive outcomes. This study assessed the effects of different semen extenders on the reproductive performance of Japanese quail, focusing on key parameters such as fertility, hatchability, and semen quality. Over a period of twenty-eight days, a total of 300 adult female breeder quails (5 to 6 weeks of age) with an average weight of 200 ± 8 g were divided into four experimental groups, under a completely randomized design. The treatment groups included T_1 (proctodeal gland foam), T_2 (quail egg albumen), T_3 (Shit's extenders), and T_4 (control group with undiluted semen). Each group comprised five replicates with 15 birds per replicate. Additionally, 75 male breeder quails with an average weight of 170 ± 7 g were housed separately for semen collection and analysis. The results showed that birds in the T_1 group, treated with proctodeal gland foam as a semen extender, exhibited the highest fertility and hatchability rates. This group also displayed improved semen quality, with increased sperm motility and a higher percentage of live sperm count. These findings suggest that proctodeal gland foam is an effective semen extender for enhancing reproductive outcomes in Japanese quails.

INTRODUCTION

Japanese quails, locally known as "Batair", have recently gained prominence in Pakistan's poultry industry (Ghayas *et al*., 2017; Hussain *et al*., 2019). The high profitability of quail farming has made it an attractive option in this sector (Arshad *et al*., 2021). Approximately 40 years ago, hybrid Japanese quails, recognized for their exceptional egg production and quality, were introduced to Pakistan (Jatoi *et al*., 2013). Despite their potential, the commercial expansion of quail farming has faced challenges, particularly due to issues related to low fertility and hatchability rates (Reddish *et al*., 1996; Khalifa *et al*., 2023). Enhancing reproductive performance in these birds is therefore a key focus for both researchers and producers, as it directly impacts the profitability and sustainability of poultry operations. Artificial insemination (AI) is a transformative tool in poultry breeding programs, optimizing reproductive performance and enhancing genetic diversity (Wang *et al*., 2024). By enabling the controlled use of superior genetic material, AI reduces reliance on natural mating, which can be inconsistent and less efficient. Semen extenders play a crucial role in this process, preserving sperm viability, motility, and functionality during storage

and transportation (Bustani & Baiee, 2021). Typically composed of nutrients, buffers, and antimicrobial agents, these extenders create an optimal environment for sperm cells, thereby increasing the success rates of artificial insemination (Bustani & Baiee, 2021; Tvrdá *et al*., 2023).

Among the various options available, natural and synthetic extenders have shown varying levels of effectiveness in preserving sperm quality. For instance, egg yolk-based extenders have traditionally been utilized for their ability to protect sperm membranes from damage (Alkali *et al*., 2022). More recently, novel options such as proctodeal gland foam and protein-rich substrates have gained attention for their potential to enhance reproductive outcomes in quails. The proctodeal gland foam, a natural secretion from male quails, contains a unique composition of proteins, enzymes, and antimicrobial agents that may enhance sperm preservation and functionality (Farooq *et al*., 2015). This foam plays a critical role during copulation, facilitating sperm transfer and increasing fertilization success. As an artificial semen extender, it has shown promising results in early studies, particularly in maintaining sperm motility and viability (Farooq *et al*., 2015; Abuoghaba *et al*., 2024). Similarly, quail egg albumen has been investigated for its protective and nutritional properties, which sustain sperm cells during storage and artificial insemination (Mohan *et al*., 2023).

In contrast, synthetic extenders such as Shit's extenders have been developed to provide precise control over the composition of the medium, ensuring consistent protection and optimized conditions for sperm preservation. Although synthetic extenders are widely used in livestock breeding, their efficacy in quail reproduction remains underexplored. Comparing synthetic extenders with natural alternatives such as proctodeal gland foam and egg albumen can provide valuable insights into the most effective strategies for enhancing fertility and hatchability in Japanese quails. Therefore, this study was designed to evaluate the impact of different semen extenders on the reproductive performance of Japanese quail (*Coturnix coturnix japonica*), with a focus on fertility, hatchability, and semen quality.

MATERIALS AND METHODS

Animal care

The experiment followed protocols approved by the Ethical Review Committee for Biomedical Research at the University of Agriculture Peshawar (UAP), Pakistan, with authorization granted by Letter No. 687-A/UAP, dated 06/01/2023.

Location and duration of the experiment

The trial took place from March to April 2023 at the Poultry Unit of the Department of Poultry Science at the University of Agriculture, Peshawar, Pakistan. The experimental site is situated at coordinates 34°N, 71°E, at an altitude of 450 m above sea level. Peshawar, the city where the trial was conducted, has a typically hot and humid tropical climate, with temperatures ranging from 5°C in the winter to +45°C in the summer. The study lasted for four weeks.

Experimental birds and husbandry

A total of 300 adult female breeder quails (5 to 6 weeks of age) with an average weight of 200 ± 8 g were divided into four experimental groups under a completely randomized design. The treatment groups included T_1 (proctodeal gland foam), T_2 (quail egg albumen), T_3 (Shit's extenders), and T_4 (control group with undiluted semen). Each group consisted of five replicates with 15 birds per replicate. Additionally, 75 male breeder quails with an average weight of 170 ± 7 g were housed separately for semen collection and analysis. The female birds were housed in colony laying cages (60 × 60 × 24 cm) (Chelmońska *et al*., 2007; Arif *et al*., 2022), with a stocking density of 240 cm^2 per bird. They were 7 weeks old at the time of egg production, and were exposed to 16 hours of illumination during the laying periods, aided by artificial light (Arcadia 20 W Fluorescent Bird Lamp, 2-4% UVA; Arcadia Products plc, Redhill, UK). The Arcadia 20 W Fluorescent Bird Lamp typically produces a cool light designed to simulate natural daylight. These lamps usually have a color temperature of around 5500K to 6000K, closely resembling daylight. During the growing period, all treatment groups were given a quail grower diet (1–5 weeks) containing 22% crude protein and 2900 kcal/kg metabolizable energy (Table 1). From the sixth week onwards, a layer diet containing 18% crude protein and 2800 kcal/kg metabolizable energy was provided to the birds (Farooq *et al*., 2015; Ghayas *et al*., 2017). The breeder ration was provided to the flock at a rate of 30 g per day per bird (Table 1). The male birds were kept in cages (Ventury Welders cages measuring 22 × 20 × 15 cm) equipped with feeders and drinkers.

Table 1 – Composition of the diet.

Ingredients (%)	Phases	
	Grower	Production
Yellow corn	47.60	44.80
Wheat	15.32	20.50
Soybean meal (48%)	29.58	20.50
Protein concentrate	5.00	5.00
Fat	0.00	0.70
Calcium carbonate	1.00	7.00
Di-calcium phosphate	1.20	1.20
Salt	0.30	0.30
Total	**100.00**	**100.00**
Nutrient		
Metabolizable energy (kcal/kg)	2900	2800
Crude protein (%)	22.00	18.00
Crude fiber (%)	3.72	3.18
Lysine (%)	1.15	0.92
Methionine (%)	0.47	0.47
Methionine + Cysteine (%)	0.81	0.70
Calcium (%)	0.90	3.03
Phosphorus (%)	0.51	0.51

To record daily variations in temperature (°C) and relative humidity (%), a wet and dry bulb hygrometer (Mason's type, Zeal, England) was placed in the middle of the house. The house had dimensions of 6.10 × 6.10m (37.21 m^2). Readings were taken at 6:00 AM and 6:00 PM. The minimum and maximum mean temperature and humidity ranged from 15.4 to 31.2°C, and 50 to 75%, respectively.

Extender's composition

The Shit extender used in the study consisted of the following components per 100 mL of distilled water: 0.90 g of sodium chloride (NaCl), 0.10 g of sodium bicarbonate ($NaHCO_3$), 0.02 g of potassium chloride (KCl), 0.02 g of calcium chloride ($CaCl_2$), 0.01 g of magnesium chloride ($MgCl_2$), and 0.87 g of fructose. The pH of the extender was 7.3 (Shit *et al*., 2010).

Semen collection

Males were provided 14 hours of light. The timing of the collection was carefully planned to ensure that no more than 20 minutes passed between semen collection and insemination. A bird holder was used to collect the semen, allowing for easy manipulation of the males with free hands. To extract the frothy secretion from the cloacal gland, the left hand was used to apply pressure and force the secretion out, while the right hand cleared the secretion deposited at the vent using a clean towel. The right hand's second finger was placed below the pubic bones and gentle upward pressure was applied. The thumb and index finger of the left hand applied lateral pressure to the cloacal region (Singh *et al*., 2011b; Wong *et al*., 2024). The semen, extruded from the vasa differentia, was drawn into a 30 mL sterile (10 × 75 mm) glass test tube (Fisher Scientific, Pittsburgh, Pennsylvania 15105, USA). To prevent sperm dehydration due to the small ejaculate volume, the semen from each male was immediately diluted two-fold with different semen extenders, and then the diluted samples were pooled (Blanco *et al*., 2009).

Semen extenders

One gram of freshly collected semen was cleansed of foam and then mixed with 1 mL of normal saline. The mixture was homogenized for 10 minutes by centrifugation at 3000 revolutions per minute. The resulting supernatant was used as 100% foam extract. To create a 5% foam extract, the extract was diluted in a ratio of 1:20 with normal saline. For insemination of the birds, a two-fold dilution of this extender was used, with 30 µL of diluted semen (Biswas *et al*., 2010).

Quail eggs albumen

The albumen from quail eggs was collected and homogenized. This was done by using a two-fold dilution of an extender, or by insemination using a 30 µL artificial insemination gun.

New semen diluent

A new semen diluent for quail semen was prepared using ingredients available in the local market. The diluent contained NaCl at a concentration of 0.90 g per 100 mL of distilled water, $NaHCO_3$ at a concentration of 0.10 g, KCl at a concentration of 0.20 g, $CaCl_2$ at a concentration of 0.02 g, $MgCl_2$ at a concentration of 0.01 g, and fructose at a concentration of 0.08 g. The pH of the diluent was 7.3. For the insemination of the birds, a two-fold dilution was prepared and 10 µL of semen was used (Shit *et al*., 2010).

Measurements

Post dilution semen parameters

After diluting the semen with the aforementioned semen extenders at a ratio of 1:2, the semen was evaluated for various parameters, including volume, motility, sperm concentration, live/dead sperm count, and morphological defects. The volume of the semen

was measured by collecting it in a sterile glass test tube (10 × 75 mm, Fisher Scientific, Pittsburgh, Pennsylvania 15105, USA) with a capacity of 30 µL. To determine motility, fresh semen was diluted at a ratio of 1:200 (Tabatabaei *et al*., 2009), and the different semen extenders were used for different time intervals of 0 min, 30 min, 60 min, and 90 min (Shit *et al*., 2010). One drop of diluted semen was placed on a slide at a temperature of 27°C, and a cover slip was placed over it to ensure even distribution. The observation was conducted using a microscope with a magnification of 400x (Model N-400ME, CEL-TECH Diagnostics, Hamburg, Germany). Sperm concentration was determined using a hemocytometer (Levy chamber with double Neubauer ruling, Clay-Adams, A-2900). The entire semen sample was diluted in a spermicidal solution (Qureshi, 2011). A drop of diluted semen was placed on both ends of the hemocytometer, and the sperm count was calculated using the following formula:

$$C = 50000 \times N \times D$$

Where;

C = Concentration of spermatozoa per volume (mL)

N = number of spermatozoa

D = dilution rate

This observation was also performed using a microscope with a magnification of 400x (Model N-400ME, CEL-TECH Diagnostics, Hamburg, Germany).The live/dead sperm count was determined using smear staining with eosin-nigrosin, based on 300 spermatozoa (Tabatabaei *et al*., 2009; Qureshi, 2011). To perform the staining, the slide was pre-warmed to body temperature and a drop of eosin-nigrosin stain was placed on the slide. A small drop of semen was then added near the stain. The edge of another slide was placed on top of drop of stain and semen, and the two were mixed by sliding them back and forth a few times at 27°C. A second smear of stain was then prepared over the surface of the first one. The live/dead sperm count was determined by observing the stained slide under a microscope at 400x magnification (Model N-400ME, CEL-TECH Diagnostics, Hamburg, Germany). Live sperm appeared white, while dead sperm appeared red (Qureshi, 2011). Morphological defects were also assessed by observing 300 cells for each preparation, including defects in the head, mid-piece, tail, and other deformities (Tabatabaei *et al*., 2009).

Artificial insemination

The female quails were inseminated once a week between 9:00 and 10:00 AM, following egg-laying (Malecki & Martin, 2004). To perform artificial insemination, gentle pressure was applied to the left side of the abdomen to cause vaginal eversion. Once the vaginal eversion occurred, the AI gun (adjusted to 30 µL) loaded with diluted semen was inserted into the vagina. The pressure was then released, allowing the cloaca to return to its original position. Finally, the diluted semen was carefully delivered through the pipette, reaching a depth of 1 centimeter (cm) into the oviduct, following the established protocol (Shit *et al*., 2010).

Egg collection and incubation

Eggs from each of the four groups were collected separately, 3-4 times a day. They were then stored for three days at room temperature (18°C) with a relative humidity of 70-80%. Once all the eggs were gathered, they were set in the incubator (Circulated Air Hova-Bator Incubator, Model No. 1590, Savannah, GA, USA) for incubation. The eggs were incubated at the university poultry farm using an automatic incubator. The incubator maintained a temperature of 99.6°F (37.6°C) and a relative humidity of 50 to 60%. To ensure proper development, the eggs were manually turned 3-4 times a day at a 45° angle. After 15 days, all the eggs were transferred to a hatcher (Humidaire Incubator Company, New Madison, OH, Belgium) for hatching. In the hatcher, the eggs were kept at a temperature of 98°F (36.67°C) and a relative humidity of 75 to 80% (Seker *et al*., 2004).

The following egg's parameters were studied:

Hatching traits

The percentage of egg fertility was calculated based on 20 eggs per replicate using the following formula;

$$\% \text{ fertility} = (\text{The number of fertile eggs}) / (\text{Total number of eggs laid}) \times 100$$

Similarly, the percentage of egg hatchability was determined using the formula below:

$$\% \text{ hatchability} = (\text{The number of hatched chicks}) / (\text{Number of fertile eggs}) \times 100$$

Data analysis

The data collected were analyzed using the one-way analysis of variance technique. This was done through the general linear model procedure in the statistical package for social sciences (SPSS) software. To determine significant differences among treatments, Duncan's multiple range test was used with a significance level of $p \leq 0.05$ (Duncan, 1955). The following mathematical model was applied:

$$Yij = \mu + \tau i + \epsilon ij$$

Where;

Yij = observation of dependent variable recorded on the ith treatment

μ = Population mean; τi = Effect of ith treatment (i = 1, 2, 3, 4)

ϵij = Residual effect of jth observation in ith treatment NID ~ 0, σ 2.

RESULTS

Semen volume and sperm concentration

This study assessed various parameters of quail semen (Table 2). The average semen volume observed was 18.73 µL, with individual ejaculates ranging from 13 µL to 22 µL. Similarly, the concentration of spermatozoa varied among individuals, averaging 686 × 10^6 sperm/mL, with a range from 580 × 10^6 to 800 × 10^6 sperm/mL.

Table 2 – The impact of male quail breeders on semen volume, sperm concentration, and the presence of morphological defects.

Nº of males	SV (µmL)	SC (n×10^6/mL)	LNS (%)	Deformities in spermatozoa (%)			
				MC	BN	MPC	OC
1	21.11	800.00	81.44	2.50	1.60	1.20	5.20
2	19.65	650.00	72.68	3.00	2.10	3.60	4.40
3	16.64	730.00	79.59	3.22	1.40	1.30	4.20
4	22.31	580.00	80.89	3.30	2.10	1.10	5.10
5	13.98	670.00	77.03	2.60	1.30	1.00	4.30
Mean	18.73	686.00	78.32	2.92	1.70	1.64	4.64

SV*: semen volume,* SC*: sperm concentration,* LNS*: live normal spermatozoa,* MC*: macrocephalic,* BN*: bent neck,* MPC*: mid-piece changes,* OC*: other changes.*

Morphological defects

In the present study, the average percentage of morphologically normal spermatozoa per ejaculate was 78.32%, with a range from 72.68% to 81.44%. The observed deformities included macrocephalic, bent-neck, mid-piece, and other deformities. Macrocephalic deformities ranged from 2.50% to 3.30%, while bent-neck deformities varied from 1.30% to 2.10%. The average percentages for mid-piece and other deformities were 1.64% and 4.64%, respectively. The range for mid-piece deformities was 1.10 to 3.60%, and for other deformities it was 4.20% to 5.20%. Overall, the total deformities observed in all birds amounted to 10.90% (Table 2).

Live/dead sperm count and sperm motility

This study assessed the effects of various semen extenders on live sperm count and motility in quail semen (Table 3). The extenders tested included proctodeal gland foam (T_1), quail egg albumen (T_2), Shit's extenders (T_3), and a control group with undiluted semen (T_4). Results indicated that the T_1 group (proctodeal gland foam) had the highest live sperm count, followed by T_2, T_3, and T_4. Additionally, Table 4 presents the sperm motility results for all four groups. The type of semen extender was found to significantly affect sperm motility, with the T_1 group demonstrating the highest motility, followed by T_2, T_3, and the control group (T_4).

Table 3 – Effect of various semen extenders on live and dead sperm count at varying time intervals.

Effect[1]	0 (min)		30 (min)		60 (min)		90 (min)	
	Live	Dead	Live	Dead	Live	Dead	Live	Dead
T_1	92.88^a± 0.39	7.10^d± 0.39	84.55^a± 0.86	15.44^d± 0.86	79.66^a± 0.69	20.33^d± 0.68	67.77^a± 0.48	32.22^d± 0.47
T_2	87.88^b± 0.58	12.11^c± 0.58	72.00^b± 0.57	28.00^c± 0.57	38.77^b± 0.58	61.22^c± 0.58	26.40^b± 0.43	73.66^c± 0.42
T_3	81.33^c± 0.57	18.66^b± 0.57	60.00^c± 0.57	40.00^b± 0.57	26.40^c± 0.43	74.90^b± 0.42	10.33^c± 0.53	89.33^b± 0.53
T_4	74.88^d± 0.77	24.99^a± 0.77	32.10^d± 0.77	67.33^a± 0.80	10.83^d± 0.50	88.66^a± 0.50	00.00^d± 0.00	100.00^a± 0.00

$^{a\text{-}d}$ *Means followed by different superscripts are statistically significant at alpha = 0.05 levels.*

[1]T_1*: Proctodeal gland foam extender,* T_2*: Shit's extender,* T_3*: Quail eggs albumen extender,* T_4*: control group.*

Table 4 – Effect of various semen extenders on sperm motility (%) at varying time intervals.

Effect[1]	0 (min)	30 (min)	60 (min)	90 (min)
T_1	90.28a±1.02	85.16a±1.42	72.98a±0.95	66.29a±1.70
T_2	85.42ab±.98	65.16b±1.92	15.35b±1.02	00.00b±0.00
T_3	72.23b±1.14	55.58c±1.41	10.55b±1.02	00.00b±0.00
T_4	74.46b±1.01	40.12d±0.61	00.00c±0.00	00.00b±0.00

$^{a\text{-}d}$ *Means followed by different superscripts are statistically significant.*

[1]T_1*: Proctodeal gland foam extender,* T_2*: Shit's extender,* T_3*: Quail eggs albumen extender,* T_4*: control group.*

Hatching traits

Table 5 summarizes the fertility and hatchability outcomes for the four experimental groups: T_1 (proctodeal gland foam), T_2 (quail egg albumen), T_3 (Shit's extenders), and T_4 (control group with undiluted semen). The findings revealed that the T_1 group exhibited the highest fertility rates, followed by T_2, T_4, and T_3. Similarly, hatchability was significantly higher in the T_1 group compared to the others.

Table 5 – Effect of various semen extenders on quail egg fertility and hatchability.

Effect[2]	Parameters[1]	
	F (%)	H (%)
T_1	87.18^{a}±2.21	62.28^{a}±2.08
T_2	74.03^{b}±2.28	49.50^{b}±3.27
T_3	33.21^{d}±1.36	48.07^{b}±1.63
T_4	51.01^{c}±3.40	46.44^{b}±1.68

[a-d] *Means followed by different superscripts are statistically significant.*

[1]F*: fertility,* H*: hatchability.*

[2]T_1*: Proctodeal gland foam extender,* T_2*: Shit's extender,* T_3*: Quail eggs albumen extender,* T_4*: control group.*

DISCUSSION

Semen volume and sperm concentration

Semen production and volume in poultry are influenced by factors such as diet, age, and environmental conditions (Zaghari *et al*., 2011). In the present study, the average semen volume observed was 18.73 µL, with individual ejaculates ranging from 13 µL to 22 µL. The variations in semen volume among individual quails may be attributed to genetic differences, since diet, management, and temperature were kept consistent. These results are consistent with those of Chelmońska *et al*. (2008), who collected semen from different male quails and observed variations in volume based on factors such as cloacal gland size, age of the males, collection procedure, and frequency of collection. Similar findings were also reported by another study (Shit *et al*., 2010). In their experiment, Qasimi *et al*. (2017) collected semen from quails with different sizes of cloacal glands and discovered that cloacal gland size significantly influenced the volume and concentration of quail spermatozoa. In contrast to our findings, Wentworth & Millen (1963) reported an average volume of 10 µL per ejaculate, which was lower than what we observed. This difference in volume may be attributed to the use of a different method for semen collection.

Sperm concentration is closely linked to testicular health and the efficiency of spermatogenesis (Aire, 2007; Santiago-Moreno *et al*., 2016). These processes are regulated by testosterone and other gonadotropins, such as luteinizing hormone and follicle-stimulating hormone, which help maintain a high density of spermatozoa (Sengupta & Elbardisi, 2019). Quails with well-developed testes and an optimal hormonal balance typically exhibit higher sperm concentration levels (Parkhurst *et al*., 1998).The sperm concentration of the quail semen collected in this study ranged from 580 to 800 × 10^6 per mL, which is similar to the results reported by Chelmońska *et al*. (2008). In their experiment, they collected semen from different individual quails and found a spermatozoa concentration ranging from 592 to 800 × 10^6 per mL. However, the sperm concentration in our trial was higher than that reported by Buxtton & Orcut (1975) (469 × 10^6 per mL), Fujihara & Koga (1991) (42.92 × 10^6 per mL), and Bunaciu *et al*. (1994) (220-230 × 10^6 per mL). This difference could be attributed to variations in age or the method of semen collection. In the experiment by Chelmońska *et al*. (2006), the highest spermatozoa concentration (2240-2640 × 10^6 per mL) was reported, which could be due to breed differences in their study.

Morphological defects

Sperm morphology is affected by spermiogenesis (Biagi *et al*., 2016) and by processes that occur after spermiation (Khalil *et al*., 2019). Inexperienced handling of semen samples can lead to issues during cooling and freezing, which may result in acrosomal damage and tail abnormalities (El-Bahrawy *et al*., 2017). Abnormal sperm shapes may include head defects like microcephaly and macrocephaly, as well as mid-piece defects such as proximal cytoplasmic droplets, distal mid-piece reflexes, and segmental aplasia of the mitochondrial sheath (Gruhot *et al*., 2019).

The findings of this study indicated that the average percentage of morphologically normal spermatozoa per ejaculate was 78.32%, with deformities ranging from 72.68 to 81.44% across individual birds. Observed abnormalities included macrocephalic spermatozoa, bent-neck deformities, mid-piece abnormalities, and other defects. The reported range (72.68–81.44%) reflects individual variation within the population. Overall, the total deformities for all birds were 10.90%, which is consistent with the findings of Chelmońska *et al*. (2008), who observed an average of 11% defects in

different birds. Our results also align with the findings of Bunaciu *et al*. (1994), who specifically studied sperm head deformities and reported a percentage ranging from 4.05% to 6.63%. In a study by Fujihara *et al*. (1989), the morphological defects of quail spermatozoa were examined at different time intervals, and it was found that after 24 hours of storage, 70% of the defects observed were broken necks, similarly to what is observed in poultry semen.

Live/dead sperm count and sperm motility

Sperm viability is crucial for motility and the ability to fertilize. As sperm viability declines, their potential to achieve fertilization is reduced (Kumaresan *et al*., 2017; Baiee *et al*., 2018). In the present study, different semen extenders had a significant impact on the live/dead sperm count, as shown in Table 3. T_1, which utilized proctodeal gland foam, had the highest live sperm count in both fresh and storage conditions. This can be attributed to the foam's biochemical properties, which closely mimic the natural reproductive environment of birds. As a secretion from specialized male bird glands, proctodeal foam provides an ideal medium for sperm preservation (Abuoghaba *et al*., 2024). Its antioxidant content neutralizes reactive oxygen species, protecting sperm membranes from oxidative damage (Kaltsas, 2023). Additionally, the foam's lipid content serves as a readily available energy source, supporting ATP production that is essential for sperm survival and motility (Islam *et al*., 2021).

These findings are consistent with Chelmońska *et al*. (2008), who observed 94.60% live spermatozoa in quail semen. Similarly, Biswas *et al*. (2010) conducted research on different concentrations of foam as quail semen extenders and found a higher live sperm count at 5% concentration. Our results also align with those of Shit *et al*. (2010), who reported similar findings with Shit's extender. However, our findings for the control group contradict the results of Chelmońska *et al*. (2008), who reported a higher live sperm count in their research. This difference may be attributed to variations in environmental temperature or storage conditions.

Sperm motility is a key indicator of male fertility potential (Bergeron & Manjunath, 2006). To achieve optimal fertility, sperm cells must maintain high motility levels (Martínez-Pastor *et al*., 2010; Amann & Waberski, 2014). Poor motility or the presence of immotile sperm are significant indicators of male infertility (Longobardi *et al*., 2017). In the present study, semen extenders had a significant impact on sperm motility. Group T_1, which utilized proctodeal gland foam, had the highest number of motile spermatozoa. This high motility in group T_1 may be attributed to the lipids in the foam, which provide a readily available energy source, supporting motility through ATP production (Farooq *et al*., 2015). Additionally, the antioxidants present in the foam help preserve the axoneme, the structural core of sperm motility, from oxidative damage (Kowalczyk, 2022). Biswas *et al*. (2010) conducted a study on different foam concentrations and their effect on quail sperm motility. They reported that a 5% foam concentration significantly affected sperm motility under both fresh and storage conditions, which aligns with our own research findings. Similarly, Shit *et al*. (2010) examined different semen extenders at various time intervals in quails and obtained comparable results. The findings of Chelmońska *et al*. (2008) were also consistent with our own research.

Hatching traits

The primary objective of poultry breeding operations is to produce healthy and viable chicks. Since only fertile eggs can hatch into chicks, egg fertility is a crucial factor. Low fertility or infertility can significantly impact the productivity of breeder flocks, leading to economic challenges. Since both male and female birds contribute equally to egg fertility, a decline in fertility may arise from factors related to either or both sexes (Farooq, 2014).

Group T_1, which utilized proctodeal gland foam, exhibited the highest fertility rates, likely due to its unique biological composition and compatibility with the avian reproductive system. This foam closely mimics the natural secretions of male birds, providing an ideal medium for sperm storage, transport, and survival. It contains bioactive components such as lipids, proteins, and antioxidants that enhance sperm viability (Agarwal *et al*., 2016). *In vitro* studies have shown that quail sperm tend to cluster without foam (Kobayashi *et al*., 1972; Ogawa *et al*., 1974). In contrast, when foam is added, they disperse effectively in the medium, sustain motility for longer durations, and exhibit high metabolic activity (Fujihara *et al*., 1989; Chelmońska *et al*., 2006; Biswas *et al*., 2010; Singh *et al*., 2011a), which may positively impact fertility. Our findings support this, as fertility rates in the T_1 group were the highest among the experimental groups. Cheng *et al*. (1989) found that, when stored with foam at room temperature, sperm maintain motility for 90 minutes, whereas motility is lost within 10 minutes without foam. Similarly, our results align with those of Fujihara

& Koga (1991), who found that quails inseminated with foam-mixed semen had higher fertility rates than those inseminated with semen alone. Furthermore, Chelmońska *et al*. (2008) achieved comparable fertility using undiluted fresh semen and diluted semen with a proctodeal gland foam extender in quails. Shit *et al*. (2010) also reported similar fertility rates when using Shit's extender. Similarly, Farooq *et al*. (2015) found that proctodeal gland foam enhances fertilization by improving the motility of the sperm. However, our results differ from the findings of Chelmońska *et al*. (2006), who reported higher fertility with fresh undiluted semen and lower fertility with diluted semen, possibly due to the type of extender or the high dilution rate used in their study.

Hatchability is a key economic trait in the poultry industry due to its significant impact on chick production (Wolc *et al*., 2010). In the current trial, group T_1 demonstrated significantly higher hatchability compared to the other groups. This increased hatchability in group T_1 may be attributed to the high fertility observed in the group, as only fertile eggs can successfully hatch into chicks. In a study by Chelmońska *et al*. (2006), a hatchability of 69% was reported with the use of a foam extender, which is higher than what we observed in our experiment. The lower hatchability in our study may be due to a lower frequency of insemination, as they conducted it twice and thrice per week. Similarly, Shit *et al*. (2010) reported findings that are comparable to our research.

CONCLUSION

These findings suggest that using proctodeal gland foam as a semen extender in Japanese quails may enhance hatching traits. The positive results with this extender also support further studies on quail semen storage and cryopreservation. However, the research on the effect of various semen extenders on the reproductive performance of Japanese quail has certain limitations. Firstly, due to species-specific reproductive biology, the findings may not be widely applicable to other poultry species. Secondly, the effectiveness of semen extenders may vary, since factors such as extender composition, storage conditions, and quail genetics can influence the outcomes.

ACKNOWLEDGEMENTS

The authors would like to extend their sincere appreciation to the Researchers Supporting Project number (RSPD2025R694), King Saud University,Riyadh, Saudi Arabia, and the faculty staff of the Poultry Science department at the University of Agriculture Peshawar, Pakistan, for their assistance in conducting the trial.

AUTHOR CONTRIBUTIONS

Conceptualization, FR and AU; methodology, AU, FR and MTK; software, BS, GA and FA; validation, UF, RA and SA; formal analysis, AU and MTK; investigation, AU and FR; resources, BS, HA and HK; data curation, AU; writing—original draft preparation, AU, FR, HK and MTK; writing—review and editing, FA, HMA, MAF, and MFK; visualization, MIU; supervision, FR and MTK; project administration, AU and FR; funding acquisition, HMA and MAF. All authors have read and agreed to the published version of the manuscript.

FUNDING

NA.

DATA AVAILABILITY STATEMENT

Data will be available upon request.

CONFLICT OF INTEREST

All authors of the present study declare that there is no conflict of interest, financial or otherwise.

REFERENCES

Abuoghaba AA, Abdelfattah MG, Sayed MA, *et al*. The relationship of the male's proctodeal gland size to sperm-egg interaction and the duration of fertility in Japanese quail. Poultry Science 2024;103(8):103809. https://doi.org/10.1016/j.psj.2024.103809

Aire TA. Spermatogenesis and testicular cycles. Reproductive biology and phylogeny of birds 2007;1:279-348.

Alkali IM, Asuku SO, Colombo M, *et al*. Spermatozoa survival in egg yolk-based and soybean-based extenders at ambient and chilling temperature in domestic turkeys (Meleagris gallopavo). Animals 2022;12(5):648. https://doi.org/10.3390/ani12050648

Amann RP, Waberski D. Computer-assisted sperm analysis (CASA): capabilities and potential developments. Theriogenology 2014;81(1):5-17. https://doi.org/10.1016/j.theriogenology.2013.09.004

Arif M, ur Rehman A, Naseer K, *et al*. Effect of Aloe vera and clove powder supplementation on growth performance, carcass and blood chemistry of Japanese quails. Poultry Science 2022;101(4):101702. https://doi.org/10.1016/j.psj.2022.101702

Arshad L, Naqvi SZ, Hussain J, *et al*. Effect of Neem (Azadirachta indica) and Tulsi (Ocimum sanctum) leaves powder on growth performance, hemato-biochemical profile and carcass traits of Japanese quail (Coturnix japonica). Turkish Journal of Veterinary & Animal Sciences 2021;45(3):388-95. https://doi.org/10.3906/vet-2010-113

Baiee F, Haron W, Yusoff R, *et al*. Modification of electro-ejaculation technique to minimise discomfort during semen collection in bulls. Pakistan Journal of Zoology 2018;50(1):83-9. https://doi.org/10.17582/journal.pjz/2018.50.1.83.89

Bergeron A, Manjunath P. New insights towards understanding the mechanisms of sperm protection by egg yolk and milk. Molecular Reproduction and Development 2006;73(10):1338-44. https://doi.org/10.1002/mrd.20565

Biagi F, Piras F, Farina V, *et al*. Testis structure, spermatogenesis and sperm morphology in pipefishes of the genus Syngnathus. Acta Zoologica 2016;97(1):90-101 https://doi.org/10.1111/azo.12108

Biswas A, Ranganatha OS, Mohan J. The effect of different foam concentrations on sperm motility in Japanese quail. Veterinary Medicine International 2010:564921. https://doi.org/10.4061/2010.564921

Blanco JM, Wildt DE, Höfle U, *et al*. Implementing artificial insemination as an effective tool for ex situ conservation of endangered avian species. Theriogenology 2009;71(1):200-13. https://doi.org/10.1016/j.theriogenology.2008.09.019

Bunaciu M, Bunaciu P, Cimpeanu L. The influence of mating design on the reproductive performance in Japanese quail (*Coturnix coturnix japonica*). Proceedings of the 9th European Poultry Conference; 1994. Glaskow, UK: World's Poultry Science Association; 1994. p.314-6.

Bustani GS, Baiee FH. Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. Veterinary World 2021;14(5):1220-33. https://doi.org/10.14202/vetworld.2021.1220-1233

Buxtton JR, Orcutt FSJ. Enymes and electrolytes in the semen of Japanese quail. Poultry Science 1975;54(5):1556-66. https://doi.org/10.3382/ps.0541556

Chelmońska B, Jerysz A, Lukaszewics E, *et al*. Semen collection from Japanese quail (*Coturnix coturnix japonica*) using a teaser female. Turkish Journal of Veterinary & Animal Sciences 2008;32(1):19-24. Available from: https://journals.tubitak.gov.tr/veterinary/vol32/iss1/4

Chelmońska B, Jerysz A, Lukaszewics E, *et al*. The effect of proctodeal gland foam, diluent and dimethylacetamide addition on morphology and fertilising ability of Japanese quail (*Coturnix coturnix japonica*) spermatozoa. Journal of Poultry Science 2006;43(1):54-9. https://doi.org/10.2141/jpsa.43.54

Chelmonska B, Jerysz A, Lukaszewicz E, *et al*. The effect of proctodeal gland foam, and depth and frequency of artificial insemination on fertility and hatchability of Japanese quail (Coturnix japonica). Turkish Journal of Veterinary & Animal Sciences 2007;31(3):171-8. Available from: https://journals.tubitak.gov.tr/veterinary/vol31/iss3/5

Cheng KM, McIntyre RF, Hickman AR. Proctodeal gland foam enhances competitive fertilization in domestic Japanese quail. The Auk 1989;106(2):286-91 https://doi.org/10.1093/auk/106.2.286

Duncan DB. Multiple range and multiple F tests. Biometery 1955;11(1):1-42. https://doi.org//10.2307/3001478

El-Bahrawy K, Rateb S, Khalifa M, *et al*. Physical and kinematic properties of cryopreserved camel sperm after elimination of semen viscosity by different techniques. Animal Reproduction Science 2017;187:100-8. https://doi.org/10.1016/j.anireprosci.2017.10.011

Farooq U, Cho S, Rybnik-Trzaskowska PK, *et al*. Effect of proctodeal gland foam on sperm kinetics in Japanese quail (Coturnix japonica). Theriogenology 2015;83(2):162-7. https://doi.org/10.1016/j.theriogenology.2014.09.006

Farooq U. Investigation of factors controlling fertility in Japanese quail (Coturnix Japonica) [thesis]. Crawley (WA): University of Western Australia; 2014.

Fujihara N, Koga O. Physiological function of the dorsal proctodeal gland foam of the male quail. Proceedings of the World Quail Conference; 1991. Taru-Tallin (EE): World's Poultry Science Association; 1991. p.7-83.

Fujihara N, Nishiyama H, Koga O. Physiological characteristics of quail (*Coturnix coturnix japonica*) spermatozoa. Proceedings of the 4th International Congress of Andrology. Florence. Italy; 1989. New York: Raven Press; 1989, p.225-9.

Ghayas A, Hussain J, Mahmud A, *et al*. Productive performance, egg quality, and hatching traits of Japanese quail reared under different levels of glycerin. Poultry Science 2017;96(7):2226-32. https://doi.org/10.3382/ps/pex007

Gruhot T, Gray K, Brown V, *et al*. Genetic relationships among sperm quality traits of Duroc boars collected during the summer season. Animal Reproduction Science 2019;206:85-92. https://doi.org/10.1016/j.anireprosci.2019.05.012

Hussain J, Mehmood S, Ullah I, *et al*. Post hatch performance of meat type Japanese quail influenced by time of offering feed and ambient environment. Journal of Animal and Plant Sciences 2019;29(2):365-9.

Islam MM, Umehara T, Tsujita N, *et al*. Saturated fatty acids accelerate linear motility through mitochondrial ATP production in bull sperm. Reproductive Medicine and Biology 2021;20(3):289-98. https://doi.org/10.1002/rmb2.12381

Jatoi AS, Sahota AW, Akram M, *et al*. Effect of different body weight categories on the productive performance of four close-bred flocks of Japanese quails (*Coturnix coturnix japonica*). The Journal of Animal and Plant Sciences 2013;23(1):7-13.

Kaltsas A. Oxidative stress and male infertility: the protective role of antioxidants. Medicina 2023;59(10):1769. https://doi.org/10.3390/medicina59101769

Khalifa WH, Sallam MG, Kamel NN, *et al*. Using in-ovo injection of olive pulp extract and vitamin C to improve hatchability, post hatch growth performance, carcass traits and some biochemical blood analysis in broiler chickens. International Journal of Veterinary Science 2023;12(3):353-359. https://doi.org/10.47278/journal.ijvs/2022.200

Khalil WA, El-Harairy MA, Zeidan AE, *et al*. Impact of selenium nano-particles in semen extender on bull sperm quality after cryopreservation. Theriogenology 2019;126:121-7. https://doi.org/10.1016/j.theriogenology.2018.12.017

Kobayashi S. The influence of the foamy materials on the fertilizing capacity of semen in the Japanese quail, *Coturnix coturnix japonica*. Agricultural Bulletin of Sage University 1972;32:89-95.

Kowalczyk A. The role of the natural antioxidant mechanism in sperm cells. Reproductive Sciences 2022;29(5):1387-94. https://doi.org/10.1007/s43032-021-00795-w

Kumaresan A, Johannisson A, Al-Essawe EM, *et al*. Sperm viability, reactive oxygen species, and DNA fragmentation index combined can discriminate between above-and below-average fertility bulls. Journal of Dairy Science 2017;100(7):5824-36. https://doi.org/10.3168/jds.2016-12484

Longobardi V, Salzano A, Campanile G, *et al*. Carnitine supplementation decreases capacitation-like changes of frozen-thawed buffalo spermatozoa. Theriogenology 2017;88:236-43. https://doi.org/10.1016/j.theriogenology.2016.09.031

Malecki IA, Martin GB. Artificial insemination in the emu (Dromaius novaehollandiae): effects of numbers of spermatozoa and time of insemination on the duration of the fertile period. Animal Science Papers and Reports 2004;22(3):315-23.

Martínez-Pastor F, Mata-Campuzano M, Álvarez-Rodríguez M, *et al*. Probes and techniques for sperm evaluation by flow cytometry. Reproduction in Domestic Animals 2010;45(s2):67-78. https://doi.org/10.1111/j.1439-0531.2010.01622.x

Mohan J, Kolluri G, Srivastava V, *et al*. Bacterial contamination of poultry semen, its dilution and storage. World's Poultry Science Journal 2023;79(3):593-617. https://doi.org/10.1080/00439339.2023.2225793

Ogawa K, Nakanishi Y, Tojo H, *et al*. Effect of frothy fluid from cloacal gland on fertility in the Japanese quail (*Coturnix coturnix japonica*). Bulletin of the Faculty of Agriculture-Kagoshima University 1974;24:35-40.

Parkhurst CR, Mountney GJ. Physiology and reproduction in poultry. In: Mountney GJ, Parkhurst CR. Poultry meat and egg production. Boston: Springer; 1998. p.31-48. https://doi.org/10.1007/978-1-4757-0683-3_3

Qasimi MI, Nagaoka K, Watanbae G. The effects of phytosterols on the sexual behavior and reproductive function in the Japanese quail (*Coturnix coturnix japonica*). Poultry Science 2017;96(9):3436-44. https://doi.org/10.3382/ps/pex161

Qureshi MS. Reproductive physiology of domestic animals. Islamabad (PK): Higher Education Commission; 2011. p.198-200.

Reddish JM, Kirby JD, Anthony NB. Analysis of poultry fertility data. 3. Analysis of the duration of fertility in naturally mating Japanese quail. Poultry Science 1996;75(1):135-9. https://doi.org/10.3382/ps.0750135

Santiago-Moreno J, Esteso MC, Villaverde-Morcillo S, *et al*. Recent advances in bird sperm morphometric analysis and its role in male gamete characterization and reproduction technologies. Asian Journal of Andrology 2016;18(6):882-8. https://doi.org/10.4103/1008-682X.188660

Seker I, Ekmen F, Bayraktar M, *et al*. The effects of parental age and mating ratio on egg weight, hatchability and chick weight in Japanese quail. Journal of Animal and Veterinary Advances 2004;3(7):424-30.

Sengupta P, Arafa M, Elbardisi H. Hormonal regulation of spermatogenesis. In: Singh R, editor. Molecular signaling in spermatogenesis and male infertility. London: CRC Press; 2019. p.41-9. .

Shit N, Singh RP, Sastry KVH, *et al*. Development of artificial insemination technology in Japanese quail. Indian Journal of Poultry Science 2010;45(1):50-4.

Singh RP, Sastry KV, Shit N, *et al*. Cloacal gland foam enhances motility and disaggregation of spermatozoa in Japanese quail (Coturnix japonica). Theriogenology 2011b;75(3):563-9. https://doi.org/10.1016/j.theriogenology.2010.09.028

Singh RP, Sastry KVH, Shit N, *et al*. Characterization of lactate dehydrogenase enzyme from semen of Japanese quail (Coturnix japonica). Theriogenolgy 2011a;75(3):555-62. https://doi.org/10.1016/j.theriogenology.2010.09.026

Tabatabaei S, Bataani RA, Talebi AR. Comparison of semen quality in indigenous and Ross breeder roosters. Journal of Veterinary and Animal Advances 2009;8(1):90-3.

Tvrdá E, Petrovičová M, Ďuračka M, *et al*. Short-term storage of rooster ejaculates: sperm quality and bacterial profile differences in selected commercial extenders. Antibiotics 2023;12(8):1284. https://doi.org/10.3390/antibiotics12081284

Wang Q, Wang Q, Wang C, *et al*. Genetic improvement of duration of fertility in chickens and its commercial application for extending insemination intervals. Poultry Science 2024;103(3):103438. https://doi.org/10.1016/j.psj.2024.103438

Wentworth BC, Mellen WJ. Egg production and fertility following various methods of insemination in Japanese quail (*Coturnix coturnix japonica*). Journal of Reproduction and Fertility 1963;6(2):215-20. https://doi.org/10.1530/jrf.0.0060215

Wolc A, White IM, Hill WG, *et al*. Inheritance of hatchability in broiler chickens and its relationship to egg quality traits. Poultry Science 2010;89(11):2334-40. https://doi.org/10.3382/ps.2009-00614

Wong I, Doneley R, Johnston S. Semen collection and baseline semen characteristics of the king quail (Synoicus chinesis). Theriogenology Wild 2024;5:100117. https://doi.org/10.1016/j.therwi.2024.100117

Zaghari M, Fazlali F, Gerami A, *et al*. Effects of environmental factors on the performance of broiler breeder hens. Journal of Applied Poultry Research 2011;20(3):383-9. https://doi.org/10.3382/japr.2009-00110

DISCLAIMER/PUBLISHER'S NOTE

*AFFILIATIONS

[I] Department of Livestock and Dairy Development (Extension), KPK, 25000, Pakistan.
[II] Cholistan University of Veterinary and Animal Sciences, Faculty of Animal Production and Technology, Department of Poultry Science, Bahawalpur, 63100, Pakistan.
[III] Civil Veterinary Hospital Gumbat, Kohat, 26120, Pakistan.
[IV] University of Agriculture, Toba Tek Sing, Sub Campus, Faisalabad, 36050, Pakistan.
[V] University of Veterinary and Animal Sciences, Department of Pathology, Lahore, 54000, Pakistan.
[VI] Poultry Research Institute, Livestock and Dairy Development Department, Rawalpindi, 46300, Pakistan.
[VII] Riphah College of Veterinary Sciences, Department of Animal Production, Lahore, 54000, Pakistan.
[VIII] The Islamia University of Bahawalpur, Faculty of Veterinary and Animal Sciences, Department of Theriogenology, Bahawalpur, 63100, Pakistan.
[IX] University of Veterinary and Animal Sciences, Department of Veterinary Surgery, Lahore, 54000, Pakistan.
[X] Cholistan University of Veterinary and Animal Sciences, Faculty of Veterinary Science, Department of Anatomy and Histology, Bahawalpur, 63100, Pakistan.
[XI] Food Safety Officer, Office of Human and Animal Food Operation, 3310, Chatham rd, Springfield, IL 62704, USA.
[XII] Bahauddin Zakariya University, Faculty of Veterinary sciences, Multan - 60800, Pakistan.
[XIII] King Saud University, College of Science, Department of Zoology, Riyadh, 11451, Saudi Arabia.