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Evaluation of semen quality parameters in Tellicherry chicken breed of Kerala

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Abstract
The evaluation of fresh semen quality is an important prerequisite for artificial insemination and cryopreservation in poultry. This study was conducted to characterize the macroscopic and microscopic semen parameters of Tellicherry roosters, an indigenous dual-purpose chicken breed of Kerala. Twenty roosters aged 20 weeks were maintained under individual housing and fed a standard breeder diet. Semen was collected by abdominal massage at three-day intervals and six ejaculates per bird were analysed. The mean ejaculate volume was 0.36±0.02 mL, with semen colour predominantly white (86.67±0.98 percent). The mean semen pH was slightly alkaline (7.27±0.03). Mass activity averaged 4.67±0.27, progressive motility was 89.69±0.39 percent and sperm concentration was 4106.25±149.00 million/mL. Sperm viability was 89.55±0.28 percent, abnormalities averaged 6.82±0.26 percentand hypo-osmotic swelling test reactivity was 90.01±0.36 percent. These values confirmed that Tellicherry semen is of high quality and suitable for artificial insemination and cryopreservation.

Keywords: Tellicherry chicken, semen evaluation, motility, viability, sperm concentration, HOST

Introduction
Indigenous chickens form an integral part of India's poultry biodiversity, providing livelihood support, genetic resources and adaptability to harsh environments (Haunshi *et al*., 2011) [9]. Among the 19 recognised breeds, Tellicherry is the only one from Kerala. However, the reproductive biology of Tellicherry remains poorly characterised despite its regional importance in Kerala.

Semen quality is one of the most important parameters influencing fertility, hatchability and breeding success. Characteristics such as semen volume, colour, pH, sperm motility, viability, morphology and plasma membrane integrity directly determine fertilising capacity (Getachew, 2016) [7]. These parameters also guide dilution rates during semen preservation and the number of doses for artificial insemination.

Characterizing semen parameters in Tellicherry roosters will establish baseline values, provide reference for breeding and conservation programs and aid in cryopreservation studies. The objective of this study was to evaluate macroscopic and microscopic semen parameters of Tellicherry roosters under intensive management.

Materials and Methods
Experimental Site and Birds
The study was carried out at the Department of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, with facilities provided under the All India Coordinated Research Project (AICRP) on Poultry Breeding. Twenty Tellicherry roosters aged 20 weeks were selected for the experiment. Birds were healthy, active and free from any reproductive disorders. Each rooster weighed approximately 1.2 kg at the start of the trial. The roosters were housed individually in California cages, which allowed easy monitoring and collection.

Feeding and Management
All birds were fed a male breeder ration containing 16 percent crude protein and 2600 kcal metabolizable energy/kg formulated as per BIS (2007). Feed was offered ad libitum in linear trough feeders and fresh drinking water was available at all times through automatic nipple drinkers. Routine health care and prophylactic measures were followed as per the institutional schedule. Birds were accustomed to handling for one month prior to the commencement of semen collection to minimize stress.

Semen Collection
Semen was collected by the abdominal massage method as described by Lake (1985) [10]. Collections were carried out at three-day intervals during morning hours to avoid heat stress. Each bird was gently restrained and the abdomen and back were massaged until the cloacal protuberance was everted. The semen was collected into a pre-warmed graduated collection tube.
A total of six ejaculates were collected from each rooster. Immediately after collection, samples were examined macroscopically and any ejaculates contaminated with faeces, uric acid crystals or blood were discarded.

Macroscopic Evaluation
The following macroscopic traits were assessed:

Ejaculate volume (mL)
Measured directly using a graduated tuberculin syringe with accuracy of 0.01 mL.

Semen colour
Semen samples were visually categorized as yellowish white, white or chalky white (Peters *et al*., 2008) [15].

Semen pH
The pH was determined using narrow range pH paper strips (Merck, 6.5-9) and recorded by matching against the manufacturer's colour chart.

Microscopic Evaluation
Microscopic assessments were carried out immediately after collection.

Mass activity
A drop of undiluted semen was placed on a pre-warmed glass slide and observed under low power (10× objective). The swirling motion of spermatozoa was graded on a 0-5 scale, where 0 indicated no movement and 5 indicated vigorous, generalized oscillations (Wheeler and Andrews, 1943) [18].

Sperm progressive motility (percent)
A drop of semen was diluted with 0.9 percent saline and placed under a coverslip. The proportion of spermatozoa exhibiting forward linear motion was recorded under 40× objective and results were expressed as percentage. At least 200 spermatozoa were counted under oil immersion (100× objective)

Sperm concentration (million/mL)
Estimated using Neubauer's hemocytometer (Sutiyono *et al*., 2021) [17]. Fresh semen was diluted with eosin-formalin-saline solution in a red blood cell pipette at a ratio of 1:200. After thorough mixing, the sample was charged into the counting chamber and spermatozoa were enumerated in 80 small squares. The concentration was calculated as millions per mL.

Sperm viability (percent)
Assessed using eosin-nigrosin staining (Lake and Stewart, 1978) [11]. A smear was prepared by mixing one drop of semen with a mixture of 2 percent eosin and 10 percent nigrosin. At least 300 spermatozoa were counted under oil immersion (100× objective). Unstained spermatozoa were considered viable, while pink-stained or partially stained spermatozoa were recorded as dead.

Sperm morphological abnormalities (percent)
The same stained smears were used to assess abnormalities in head, midpiece and tail. A total of 200 spermatozoa were evaluated per sample and results expressed as percentage abnormal forms (Raghavendra *et al*., 2022) [16].

Hypo-osmotic swelling test (HOST, percent)
Functional integrity of sperm plasma membrane was assessed using a hypo-osmotic solution (100 mOsm/L trisodium citrate). One drop of semen was incubated with the solution at 37°C for one hour. A minimum of 200 spermatozoa were examined under phase contrast microscopy and sperm showing coiled or swollen tails were recorded as positive (Chauhan *et al*., 2017) [4].

Statistics
Descriptive statistics were done using Microsoft Excel 2007

Results and Discussion
Macroscopic characteristics
Semen volume
The mean semen volume of Tellicherry roosters was 0.36±0.02 mL, with values ranging from 0.20 to 0.43 mL (Table 1). Akshay *et al*. (2023) [1] observed lower volumes in Aseel (0.27±0.01 mL) and Kadaknath (0.36±0.02 mL), while native chicken of Kerala recorded slightly higher values (0.38±0.01 mL). Similarly, Haunshi *et al*. (2011) [9] reported semen volumes of 0.25-0.40 mL in indigenous roosters. The present results confirm that Tellicherry roosters produce semen volumes adequate for artificial insemination, as 0.2-0.4 mL per ejaculate is considered optimal in poultry.

Semen colour
Semen colour was predominantly white (86.67±0.98%), followed by chalky white (7.91±0.88%) and yellowish white (5.69±0.77%). White semen is generally associated with higher sperm density (Peters *et al*., 2008) [15]. Akshay *et al*. (2023) [1] reported similar findings in native Kerala chicken (91.67 percent white) and Kadaknath (88.89% white), whereas Aseel exhibited slightly lower proportion of white semen (83.33%). The predominance of white semen in Tellicherry roosters thus indicates good semen quality comparable with native Kerala and Kadaknath breeds.

Semen pH
The pH of Tellicherry semen ranged between 7.17 and 7.35, with a mean of 7.27±0.03. This is consistent with the slightly alkaline range considered favourable for sperm motility and survival (Latif *et al*., 2005) [12]. Comparable values have been reported in Aseel (7.24), Kadaknath (7.25) and native Kerala chickens (7.22) (Akshay *et al*., 2023) [1]. Earlier studies in exotic breeds such as Rhode Island Red and White Leghorn also reported semen pH values between 7.1 and 7.3 (Mavi *et al*., 2019) [14]. These results indicate that Tellicherry semen pH

falls within the physiological value, optimum for poultry.

Microscopic characteristics

Mass activity

The average mass activity score in Tellicherry roosters was 4.67±0.27. This denotes vigorous oscillatory motion and is similar to scores reported for native Kerala (4.75) and Kadaknath roosters (4.65) (Akshay *et al*., 2023) [1]. Aseel roosters showed slightly lower mass activity scores (4.45), suggesting relatively lower sperm vigor compared to Tellicherry. Higher mass activity is positively correlated with fertility outcomes (Donoghue *et al*., 1998) [6].

Progressive motility

Tellicherry semen exhibited high progressive motility (89.69±0.39 percent), ranging from 86.74 to 92.54 percent. This is markedly higher than Aseel (77.19±0.72 percent) and Kadaknath (82.05±0.68%) and slightly superior to native Kerala roosters (89.71±0.66%) (Akshay *et al*., 2023) [1]; Chauhan *et al*. (2012) [5] reported lower values (84%) in Gramapriya, while exotic breeds such as Rhode Island Red and Punjab Red showed 85-87 percent motility (Mavi *et al*., 2019) [14]. Thus, Tellicherry semen is at par with native Kerala chickens and superior to Aseel and Kadaknath in terms of sperm motility.

Sperm concentration

The mean sperm concentration of Tellicherry semen was 4106.25±149.00 million/mL (range: 3246-4921 million/mL). This value is within the physiological range of 3-7 billion/mL reported in domestic fowl (Gordon, 2005) [8]. Akshay *et al*. (2023) [1] recorded similar concentrations in Aseel (4.41 billion/mL) and Kadaknath (4.15 billion/mL), while native Kerala chicken exhibited higher concentrations (4.71 billion/mL). Slight inter-breed variation may be attributed to body size, testicular weight and spermatogenic efficiency (Bah *et al*., 2001) [2]. The present results demonstrate that Tellicherry semen contains sufficient sperm concentration for effective insemination.

Sperm viability

Sperm viability in Tellicherry semen averaged 89.55±0.28 percent, with individual values ranging from 88.11 to 91.93 percent. This was comparable with native Kerala chickens (90.32%) and superior to Aseel (84.40%) and Kadaknath (87.26%) (Akshay *et al*., 2023) [1]. The high viability indicates that a large proportion of spermatozoa remain metabolically active, which is essential for fertilization success. Similar values have been reported in Gramapriya (91%) (Chauhan *et al*., 2012) [5].

Sperm abnormalities

Morphological abnormalities in Tellicherry semen averaged 6.82±0.26 percent, ranging from 4.45 to 9.13 percent. This was lower than Aseel (7.78%) and Kadaknath (7.42%) (Akshay *et al*., 2023) [1] and comparable to native Kerala chickens (6.25%). Abnormalities below 10 percent are generally acceptable for fertile semen (Getachew, 2016) [7]. Higher levels of abnormalities, such as those reported in Bantam (38%) and Red Junglefowl (31.42%) (Malik *et al*., 2013) [13], are associated with reduced fertility. Thus, the relatively low abnormality rates in Tellicherry semen indicate normal spermatogenesis and healthy reproductive status.

Hypo-osmotic swelling test (HOST)

The mean HOST reactivity was 90.01±0.36 percent, with a range of 87.24-92.45 percent. This indicates a high proportion of spermatozoa with intact plasma membranes. Comparable values were reported in native Kerala chickens (92.10%), while Aseel and Kadaknath showed lower values (88.22% and 89.41%, respectively) (Akshay *et al*., 2023) [1]; Chauhan *et al*. (2017) [4] reported 92.98 percent HOST reactivity in Gramapriya roosters, further supporting the suitability of indigenous breeds for semen cryopreservation. High HOST values are predictive of better freezability and fertility (Chauhan *et al*., 2017) [4].

Table 1: Macroscopic fresh semen evaluation of Tellicherry chicken

Bird No.	Volume (mL)	Colour			pH
		Yellowish White (%)	Chalky White (%)	White (%)	
1	0.38±0.02	5.55± 0.62	8.33±3.73	91.67±3.73	7.18±0.03
2	0.38±0.01	2.78 ±0.53	11.11±5.55	88.89±5.56	7.32±0.06
3	0.35±0.03	2.78± 0.64	2.78±2.78	86.11±5.12	7.25±0.04
4	0.36±0.02	5.55±0.47	2.78±2.78	86.11±5.12	7.37±0.06
5	0.36±0.01	5.55±0.68	2.78±2.78	83.33±6.09	7.23±0.02
6	0.34± 0.01	2.78±0.69	13.88±5.12	77.77±7.03	7.27±0.02
7	0.33± 0.02	2.78±0.63	8.33±5.69	88.89±3.51	7.22±0.03
8	0.37± 0.02	2.78±0.71	13.88±5.12	88.89±5.56	7.35±0.03
9	0.35± 0.02	11.11±0.34	5.55±3.51	91.67±5.69	7.22±0.03
10	0.38±0.01	11.11±0.48	5.55±3.51	80.55±2.78	7.38±0.07
11	0.39± 0.01	5.55± 0.73	2.78±2.78	86.11±6.69	7.25±0.03
12	0.37±0.01	2.78 ±0.68	11.11±5.55	91.67±3.73	7.35±0.03
13	0.37± 0.02	11.11 ±0.83	2.78±2.78	88.89±5.56	7.20±0.03
14	0.34±0.01	11.11 ±0.42	13.88±5.12	80.55±5.12	7.30±0.04
15	0.37±0.02	11.11±0.56	11.11±3.51	83.33±6.09	7.20±0.03
16	0.34±0.02	2.78±0.34	11.11±5.55	83.33±6.09	7.42±0.04
17	0.37±0.02	2.78±0.46	8.33±5.69	94.44±3.51	7.17±0.02
18	0.35±0.02	5.55 ±0.24	8.33±3.73	88.89±5.56	7.17±0.03
19	0.36±0.02	2.78±0.35	5.55±3.51	88.89±5.56	7.22±0.03
20	0.37±0.00	5.55±0.14	8.33±3.73	83.33±4.30	7.32±0.04
Mean ± SE	0.36±0.02	5.69±0.77	7.91±0.88	86.67±0.98	7.27±0.03

Values are mean ± SE of six observations per male

Table 2: Microscopic fresh semen evaluation of Tellicherry chicken

Birds No.	Mass motility (%)	Progressive motility (%)	Sperm Concentration (millions/mL)	Sperm Viability (%)	Sperm Abnormality (%)	HOST (%)
1	4.67±0.27	88.25±0.47	3946.00±164.42	89.15±0.18	7.54±1.16	90.01±0.36
2	4.67±0.27	90.06±0.58	3755.67±276.13	89.59±0.21	8.90±0.92	90.40±0.39
3	4.83±0.17	89.56±0.79	4141.67±101.14	89.11±0.18	5.49±0.76	90.21±0.55
4	4.65±0.26	90.82±0.34	4210.33±156.24	89.10±0.27	7.46±1.08	89.04±0.21
5	4.83±0.17	89.52±0.20	4233.33±237.85	90.21±0.20	6.98±0.48	89.26±0.36
6	4.83±0.17	89.75±0.45	3373.33±158.48	89.27±0.26	7.34±0.64	90.21±0.55
7	4.83±0.17	90.13±0.31	4652.83±214.47	90.39±0.39	7.50±0.84	88.33±0.35
8	4.67±0.27	89.44±0.46	4287.33±173.48	89.26±0.36	4.45±1.52	90.33±0.46
9	4.83±0.17	89.27±0.26	4245.83±191.83	90.21±0.55	6.86±0.52	90.17±0.39
10	4.83±0.17	90.40±0.39	4076.33±50.04	89.04±0.21	9.10±0.68	89.86±0.22
11	4.67±0.27	90.82±0.34	3954.50±79.83	88.64±0.12	7.66±0.84	89.78±0.45
12	4.67±0.27	88.64±0.12	3898.50±125.99	89.52±0.20	5.65±0.84	90.21±0.20
13	4.83±0.17	89.52±0.20	4124.33±76.77	89.75±0.31	6.06±0.56	89.44±0.46
14	4.83±0.37	89.57±0.25	4313.00±115.37	89.75±0.43	5.57±0.80	89.27±0.26
15	4.83±0.17	89.37±0.28	4030.33±197.42	89.84±0.16	6.78±0.64	90.40±0.39
16	4.67±0.27	89.54±0.37	4082.50±86.69	89.53±0.40	7.62±0.84	89.26±0.36
17	4.83±0.17	89.69±0.44	4358.00±191.31	89.79±0.35	5.78±0.40	90.21±0.55
18	4.67±0.27	89.76±0.47	4257.50±83.62	89.81±0.30	5.69±0.84	89.04±0.21
19	4.67±0.27	89.85±0.76	4276.17±92.32	89.57±0.25	7.42±1.20	90.21±0.55
20	4.83±0.17	89.76±0.47	3907.50±206.72	89.44±0.37	6.14±0.76	89.04±0.21
Mean ± SE	4.67±0.27	89.69+ 0.39	4106.25±149.00	89.55±0.28	6.82±0.26	90.01±0.36

Values are mean ± SE of six observations per male

Conclusion

The study established baseline semen parameters of Tellicherry roosters. Ejaculates were characterized by good volume, predominantly white colour, slightly alkaline pH, high progressive motility and viability, optimal sperm concentration, low morphological abnormalities and excellent membrane integrity. These findings confirm that Tellicherry semen is of good quality and fit for artificial insemination and cryopreservation, comparable to other indigenous breeds.

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Conflict of Interest

Not available.

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