Seasonal Morphometric and Stereological Changes in the Testes of Cattle Egret (Bubulcus Ibis)

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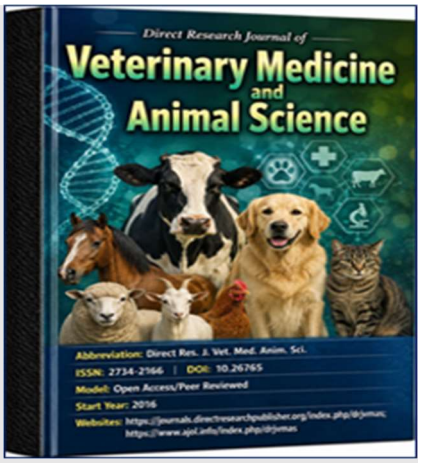
ABSTRACT

Cattle egrets are important stocky heron seasonal breeder involved in bio-control of ticks and lice in cattle. Despite research documented on morphometric parameters of avian testes including cattle egret there is need for documentation of data on the seasonal changes in the male organ of this species to further buttress the existing literature. The study was undertaken to document the seasonal changes in morphometry, gonadosomatic index and stereology of male sex organ in cattle egret. The mean ± standard error of mean of the weight, length and width of the reproductive organs were compared within and between seasons with student t test using GraphPad prism version 8. The Mean ± SEM weight of the whole testes (1.47 ± 0.10g breeding and 0.68 ± 0.11g non-breeding) was statistically significant (p ≤ 0.05), whole testis gonado-somatic index (0.42 ± 0.03 breeding and 0.25 ± 0.13 non-breeding season) was statistically significant (p ≤ 0.05), left and right testis (0.72 ± 0.06g, 0.76 ± 0.04g breeding and 0.37 ± 0.06g, 0.31 ± 0.05g non-breeding season) not statistically significant within each season (p ≥ 0.05). Testis left and right length (4.33 ± 0.14mm, 4.64 ± 0.14 breeding and 2.80 ± 0.13mm, 3.49 ± 0.16mm non-breeding season) not statistically significant within each season (p ≥ 0.05) but statistically significant (p ≤ 0.05) between breeding and non-breeding season, testis left and right width (4.78 ± 0.30mm, 5.12 ± 0.32mm breeding and 2.46 ± 0.29mm, 2.20 ± 0.15mm non-breeding season) were statistically significant (p ≤ 0.05) between breeding and non-breeding season and left and right vas deferens length (45.44 ± 0.90mm, 46.00 ± 0.96mm breeding and 35.13 ± 0.98mm, 36.28 ± 1.36mm non-breeding season) obtained were significantly increased during the breeding season compared to the non-breeding season (p ≤ 0.05). In conclusion, the result indicated heavier, wider and longer male sex organ, larger germ cell volume and germ cell number in the breeding season than non=breeding season. The study also revealed that the right testis has higher germ cell volume than the left testis in both seasons. Hence, these findings provide baseline quantitative morphometric data on seasonal reproductive dynamics in the cattle egret, with potential applications in comparative anatomy, reproductive biology and species management.

Keywords: *Bubulcus ibis, morphometry, stereology, germ cell, reproductive biology, gonado-somatic index,*



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INTRODUCTION

Cattle egrets are important stocky heron involve in bio-control of cattle parasites and also feed on insects like grasshopper, maggot and grains (John, et al., 2016, Abou shaky, 2012). Cattle egret (*Bubulcus ibis*) is a cosmopolitan avian species (Ayodeji, *et al.,* 2022, Ahmad, *et al.,* 2024). They are found in Africa, Europe, America, Asia, and Australia (Metallaoui, *et al.,* 2020) They are versatile species that can survive and adapt to different environment and food sources (Talbi et al., 2023). Egret are good subject for breeding study as they are abundant, consume easily identify prey and are consumed by rural dwellers as meat (Lie, et al., 2016). Studies has shown that environmental changes have effects in motivating them in their distribution, habitat use, reproductive parameters and foraging behavior (Robert, *et al.,* 2000, Arendt,1988, Sodhi,1989). The non-breeding adult has mainly white plumage, yellow bill and greyish-yellow legs (Katzir, *et al.*, 1999) whie during breeding season, adult species develop orange buff plumes on the back, breast and crown. The bill, legs and iris become bright red for a slight period before pairing (Rojas, *et al*., 1999). Research has also shown that cattle egret laid few numbers of eggs ranging between 6-7 per pair with 80% (3-5) hatchability (Samraoui, et al., 2009). Other research documented on reproductive organs of cattle egret include, ultrastructural features of the excurrent ducts of the testis of the cattle egret (*Bubulcus ibis*) (Roopnarine, et al., 2026), Spermiogenesis in the cattle egret (Roopnarine, et al., 2021), Sperm structure of the cattle (Roopnarine, et al., 2020), Therefore, not much information has been documented on the morphometric seasonal changes of the male reproductive organs of this species hence the intention of this study Stereology is a three-dimensional interpretation of two-dimensional cross sections of tissues that utilizes random systematic sampling to provide unbiased and quantitative data; it is based on fundamental principles of geometry like Cavalieri principle and statistics (Howard and Reed, 2005). Many studies conducted using stereological approach on testis are testis stereology, seminiferous epithelium cycle length, and daily sperm production in the ocelot (Silva, *et al*., 2010), the human testis studied using stereological methods (Petersen, *et al*., 1996), quantification of germ cells and seminiferous tubules by stereological examination of testicles from 50 boys who suffered from sudden death (Muller and Niels, 1983), but no similar study has been conducted on the stereological evaluation of cattle egret testis. The aim of this study was to quantitatively estimate the testicular volume and germ cell number in the testes of cattle egret using physical dissector method.

METHODOLOGY

Animal Used for the Study

A total number of twenty (10 for breeding and 10 for non-breeding season) cattle egrets were used for this study. The cattle egrets were sourced from Zuru town, Kebbi State in North-western Nigeria between May to September (for breeding season) and November to February (non-breeding season). The birds were captured by trap with the use of fishing nets around the top branches of tree were the birds nest at night as described by Danmaigoro, *et al,* 2014. The birds were held in plastic cages and was transported to the Gross Anatomy Laboratory of Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Kaduna state. Ethical approval was sought from the committee on animal welfare and handling of Ahmadu Bello University Zaria with approval no: (ABUCAUC/2025/004).

Necropsy and Organ Harvesting

The organs were examined in-situ, photograph and thereafter exteriorized and trimmed. Gross features of the male reproductive system were carefully examined and recorded. The length, width, weight and gonadomatic index of the male reproductive organs was measured using a digital Vernier caliper (MG6001DC, General Tools and Instruments Company, NewYork; sensitivity: 0.01mm) and weighing balance (Shimadzu AW320, Germany), respectively. Gonadosomatic index was evaluated by dividing the gonad mass or weight by the body weight and multiply by 100 (%) as described by Geoff, *et al*., (2018).

Stereological Methodology

Isotropic uniform random (IUR) samples were obtained from the fixed sample by the orientator method as described by (Ali, *et al*., 2012) (Figure 1).

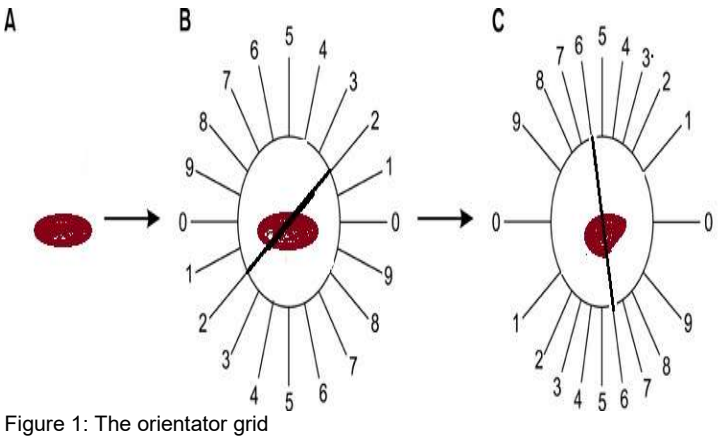

Figure 1: The orientator grid

First, the left and right testes each was placed at the center of the circle with equal divisions and a random number (2) was calculated and selected from the random number table and the sample was cut. Secondly, each part of the cut sample was again placed on a second circle with unequal divisions and another random number (6) was selected and the samples was cut and then a trochar was

used for getting the isotropic sections; then these sections were measured before and after processing with a digital vernier caliper (MG6001DC, General Tools and Instruments Company, NewYork; sensitivity: 0.01mm) as described by Braendgaard, *et al.,* (1990) and modified by Ajeigbe, *et al.,* (2021). Degree of shrinkage will be estimated by subtracting the final volume after processing from the initial volume before processing and then divided by the initial volume before processing (S_1-S_2/S_1) Braendgaard, *et al.,* (1990).

Histological Procedures for Light Microscopy

After fixation in 10% neutral buffered formalin, tissue samples were processed manually using copling (staining) jars. The tissues were processed using graded concentrations of alcohol for dehydration (50%, 60%, 80%, 95% and absolute), then it was cleared in xylene (two changes), infiltration as well as embedding in molten paraffin wax. Serial Sections (5µm thick) were cut with a rotatory microtome (LEICA) at 5µ. A random number 3 was selected from the random number table and sections were randomly picked and floated out in a hot water bath, mounted on glass slides, left to air dry, stained with haematoxylin and eosin and covered with cover-slips (Baker, *et al.,* 2001). Photomicrographs were taken with a light microscope (Amscope, T120B) and a digital microscope camera (DCM 510 megapixel, ScopePhoto® China) at ×25 and x400

Volume Estimation

A test point counting grid (Cavalieri estimator) was superimposed on the testis tissue sections (Figure 2) and single test points hitting the testis were counted and summed.

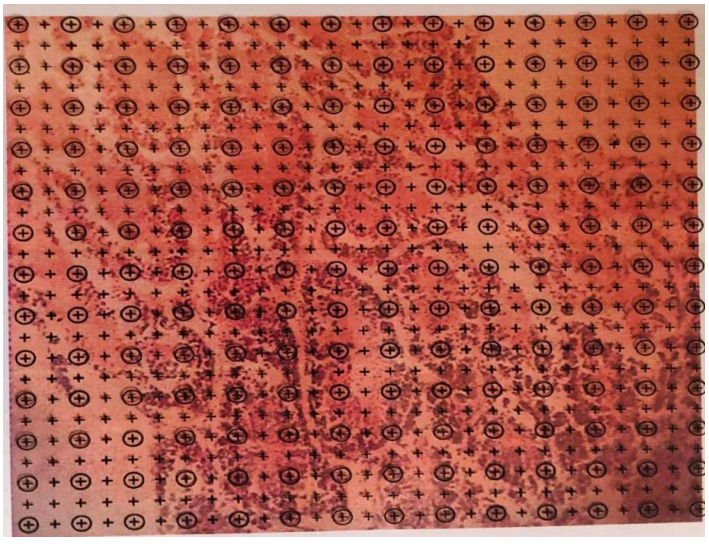

Figure 2: The Cavalieri estimator grid.

The volume changes of the testis were calculated as previously described by Gundersen *et al*., 1988 using the following computations:

$V\ (mm^3) = \overline{T} \times a/p \times \sum P_i$ ($\overline{T}$= distance from the 1st section to the 13th section; a/p = area per point; $\sum P_i$ = sum of test points).

The procedure used for estimating testicular volume by the Cavalieri point-counting method is summarized in Table 1.

Table 1: Volume estimation (Gundersen, *et al.,* 1988), Cavalieri estimator point counting method.

Sections	P_1	$P_1 \times P_1$	$P_1 \times P_{1+1}$	$P_1 \times P_{1+2}$
1	171	29,241	26,676	48,051
2	156	24,336	43,836	27,768
3	281	78,961	50,018	50,861
4	178	31,684	22,218	31,328
5	181	32,761	31,856	32,942
6	176	30,976	32,032	26,224
7	182	33,124	27,118	30,576
8	149	22,201	25,032	20,797
9	168	28,224	25,704	-----
10	153	23,409	------	-----

$\sum P_1$ = 1,745 $\sum P_1 x P_1$=334,917(A)$\sum P_1$ x P_{1+1}=294,490(B)$\sum P_1 x P_{1+2}$= 270,547(C)

Noise due to errors in the sampling:

Noise = 0.0724 x B/√A x √ n x $\sum P_1$ ($\sum P_1$= sum of test points).

Variations due to the systematic random sampling of the serial sections were calculated:

VAR_{SURS} = 3 (A-Noise) – 4(B + C) + C
Total variance (TVAR) = Noise + VAR_{SURS}

Coefficient of error due to the entire sampling process (CE) was calculated:
CE= √TVAR/$\sum P_1$.

Total germ cell number estimation

These was done on the testis of cattle egret (Bubulcus ibis) using an unbiased counting frame called the physical dissector

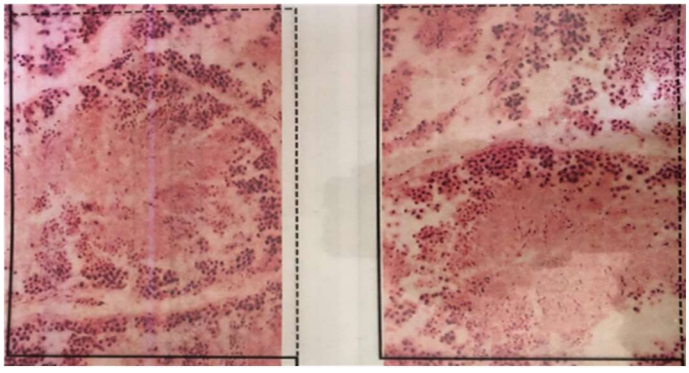

Figure 3**:** The physical dissector counting frames

Two unbiased counting frames were superimposed on systematically random tissue sections (10 sections from each half of the testis), as shown in Figure 3. According to

the dissector rules; the counting frame defines germ cells to be completely outside the frame if the cells touch the exclusion line as being outside the frame and not counted (black arrow); whereas germ cells that are completely within the frame or that touch the inclusion line were counted as being within the frame (white arrow). In addition, only distinct germ cells which are seen in the sampling section frame but are not seen in the reference section frame were counted and summed as Q^-, Each half, left or right is sample and reference section to each other i.e when counting the left as sample section, the right is reference section and vice versa. The total germ cell number was therefore estimated from the computations below as proposed by Moller, *et al.,* 1991.

The procedure used for estimating the total germ cell number using the physical dissector method is summarized in Table 2.

Table 2: Estimating total germ cell number with the physical dissector

Dissector	Q- Left	Q- Right
1	287	213
2	212	282
3	123	204
4	239	212
5	245	202
6	145	182
7	154	226
8	273	260
9	249	275
10	248	271
Total (∑Q)	2,175	2,327

$N = N_v \times V_{ref}$ $Vref = \sum Q^- / n \times vdis$ $Vdis = t \times a(frame)/magnification^2 = 21 \times 110 \times 175 / 600 \times 600$ $n = 10 + 10$ $\sum Q^- = 2,175 + 2,327$

Where Nv = numerical density, V(ref) = volume of the structure estimated with the cavalieri estimator, V(dis) = volume of all dissector probes placed in the structures, a(fra) = area of counting frame which is standardize from the grid as 110 x 175.

Noise due to errors in the sampling:

$Noise = 0.0724 \times B/\sqrt{A} \times \sqrt{n} \times \sum Q_1$.

Variations due to the systematic random sampling of the serial sections was calculated:

$VAR_{SURS} = 3 (A\text{-}Noise) – 4(B + C) + C$

Total variance (TVAR) = Noise + VAR_{SURS}

Coefficient of error due to the entire sampling process (CE) was calculated:

$CE = \sqrt{TVAR}/\sum Q_1$.

Result

Morphometric Result of Male Reproductive Organ of Cattle Egret

The mean body weight and body length of cattle egret (Bubulcus Ibis) during breeding were 354.1 ±12.80 g and 59.48 ±0.60 mm respectively. At the same time the mean body weight and body length for non-breeding season were 274.6 ±12.80 g and 57.13 ±1.39 mm (Table 3 and 4). The mean whole testis weight for breeding and non-breeding season were 1.47 ± 0.10g and 0.68 ± 0.11g (table 3 and 4), the mean left and right testis weight for breeding and non-breeding season were 0.72 ± 0.06 g, 0.76 ± 0.04 g and 0.31 ± 0.05 g, 0.37 ± 0.06 g (Table 3 and 4) respectively. The mean left and right testis length during breeding season was 4.64 ±0.14 mm, 4.33 ± 0.14 mm and 3.49 ± 0.16 mm, 2.80 ±0.13 mm during non-breeding season (Table 3 and 4). The mean left and right testis width during breeding season was 4.78 ±0.30 mm, 5.12 ± 0.32mm and 2.46 ± 0.29 mm, 2.20 ± 0.15 mm for non-breeding season (Table 3 and 4). The mean left and right vas deferens length during breeding season was 45.44 ±0.90 mm, 46.00 ± 0.96 mm and 35.13mm ± 0.98, 36.28mm ± 1.36 for non-breeding season (table 3 and 4). The gonadosomatic index (GSI) value estimated for whole testis weight of cattle egret was about 0.4% while the left and right testis weight constituted about 0.20% and 0.21% of the body weight, the left and right testis length constituted about 7.28% and 7.80% of the body length and the left and right vas deferens length constituted about 76.4% and 77.34% during breeding season (Table 3) while the gonadosomatic index value for the non-breeding season was whole testis weight 0.25%, left and right testis weight 0.11% and 0.13% of the body weight, the left and right testis length constitute 6.11% and 4.90% of the body length and the vas deferens constituted 61.49% and 63.50% of the body length (Table 4)

The result showed that the volume estimated for the left and right testis during breeding season were 32540 mm^3 and 35900 mm^3, and 13960 mm^3 and 15300 mm^3, for non-breeding season respectively (Table 5). The total germ cell estimates of the testis during breeding and non-breeding season respectively were $426.7x10^4$ and $100.4x10^4$ (Table 6). However, data obtained for the volume and germ cell number estimated for the testis showed that the breeding season had higher volume and germ cell number than the non-breeding season (Table 5 and 6) Also data obtained from the volume estimation also showed that the right testis has a larger volume than the left testis in breeding and non-breeding season (Table 5).

The mean body weight of cattle egrets was significantly ($P < 0.05$) higher during the breeding season (354.1 ± 12.80 g) compared to the non-breeding season (274.6 ± 17.51 g); and the body length did not show a significant ($P > 0.05$) difference between the seasons, with values of 57.13 ± 1.39 mm in the non-breeding season and 59.48 ± 0.60 mm in the breeding season (Table 6).

There was a significance ($P < 0.05$) higher in whole testicular weight during the breeding season (1.47 ± 0.10

Table 3: Morphometric data of the reproductive organs of cattle egret during breeding season

S/N	Body Weight (g)	Body length (mm)	Whole testis weight(g)	Testis weight(g)		Testis Length (mm)		Testis Width (mm)		Vas deferens Length (mm)	
				Left	Right	Right	left	Left	Right	Left	Right
1	399.	62.61	1.76	0.96	0.78	4.70	4.82	5.66	5.90	44.67	43.55
2	369	55.85	1.903	1.00	0.89	4.28	4.33	5.84	6.42	45.30	42.42
3	374	60.24	1.754	0.91	0.85	4.40	4.53	3.31	3.59	48.20	48.80
4	322	58.27	1.579	0.70	0.88	4.00	4.20	3.60	4.02	45.00	48.00
5	310	58.21	1.204	0.58	0.57	4.60	4.80	5.10	6.00	47.09	48.01
6	376	61.18	0.851	0.50	0.48	3.84	4.78	5.75	5.78	44.10	43.12
7	299	60.37	1.405	0.60	0.81	3.60	4.73	3.60	3.80	40.50	48.60
8	305	58.45	1.291	0.60	0.79	4.90	4.62	4.88	4.90	49.83	46.31
9	401	60.18	1.445	0.68	0.76	4.91	5.59	5.00	5.10	42.10	41.60
10	386	59.45	1.545	0.67	0.83	4.03	4.01	5.05	5.65	47.58	49.60
Mean	354.1	59.48	1.47	0.72	0.76	4.33	4.64	4.78	5.12	45.44	46.00
±SEM	±12.80	±0.60	±0.10	±0.06	±0.04	±0.14	±0.14	±0.30	±0.32	±0.90	±0.96
GSI (%) of Organ parameter			0.42%	0.20%	0.21%	7.28%	7.80%			76.4%	77.34 %

Note: GSI-Gonadosomatic index, SEM-Standard error of mean

Table 4: Morphometric data of the reproductive organ of cattle egret during non-breeding season

SN	Body weight (g)	Body length (mm)	Whole testis weight (g)	Testis (g)	weight	Testis (mm)	length	Testis width (mm)		Vas length (mm)	deferens
				Right	Left	Right	Left	Right	Left	Right	Left
1	237	61.50	0.527	0.280	0.250	2.40	4.53	3.40	2.53	35.0	38.0
2	210	57.76	0.347	0.160	0.140	2.10	4.12	4.15	2.62	30.0	30.0
3	234	62.30	0.661	0.400	0.260	2.50	3.60	2.58	2.21	38.0	38.5
4	260	55.30	0.968	0.520	0.450	2.59	3.31	2.59	2.31	32.0	39.0
5	291	59.16	1.234	0.630	0.600	3.10	3.30	1.12	1.12	38.0	45.0
6	214	60.25	0.756	0.500	0.300	2.96	3.40	1.50	1.60	39.0	34.0
7	290	59.60	1.160	0.610	0.550	2.81	2.92	2.67	2.45	35.6	36.0
8	302	48.92	0.350	0.188	0.158	3.48	3.53	2.23	2.41	35.1	32.5
9	318	55.20	0.320	0.160	0.161	2.88	2.96	1.60	2.13	31.4	32.3
10	390	51.32	0.452	0.211	0.243	3.17	3.20	2.71	2.66	37.2	37.4
Mean	274.6	57.13	0.68	0.37	0.31	2.80	3.49	2.46	2.20	35.13	36.28
±SEM	±17.51	±1.39	±0.11	±0.06	±0.05	±0.13	±0.16	±0.29	±0.15	±0.98	±1.36
GSI (%) of organ parameter			0.25%	0.13%	0.11%	4.90%	6.11%			61.49%	63.50%

Note: GSI-Gonadosomatic index, SEM-Standard error of mean

Table 5: Volume estimation of Testis of Bubulcus Ibis during breeding and non-breeding season

Side	Season	Vol. (mm^3)	Noise	VAR_{SURS}	TVAR	CE
Left	Breeding	32540	$45803.3*10^6$	$-137407.7*10^6$	$-91604.3*10^6$	8.02
	Non-breeding	13960	$1562.8*10^6$	$-4688.2*10^6$	$-3125.3*10^6$	8.09
Right	Breeding	35900	$70872.3*10^6$	$212613.6*10^6$	$141741.2*10^6$	9.70
	Non-breeding	15300	$2257.7*10^6$	$-6773.3*10^6$	$-4515.6*10^6$	8.80

g) compared to the non-breeding season (0.68 ± 0.11 g); and the organo-somatic index (which relates the testes weight to body weight) also showed a significantly ($P < 0.05$) higher value during the breeding season (0.42 ± 0.03) compared to the non-breeding season (0.25 ± 0.13) (Table 6).

Table 6: Mean body weights and lengths, testicular weights, and organo-somatic indices of egret during breeding and non-breeding season

Parameter	Non-breeding season	Breeding season
Body weight (g)	274.6 ± 17.51^a	354.1 ± 12.80^b
Body length (mm)	57.13 ± 1.39^a	59.48 ± 0.60^a
Whole testes weight (g)	0.68 ± 0.11^a	1.47 ± 0.10^b
Organo-somatic index	0.25 ± 0.13^a	0.42 ± 0.03^b

Mean values with different superscript letters in the same row differ significantly at $P < 0.05$.

During the breeding season, both the left and right testes exhibited significantly ($P < 0.05$) higher weights, with the left testis weighing 0.72g ± 0.06 and the right 0.76g ± 0.04, compared to 0.31g ± 0.05 and 0.37g ± 0.06 respectively, during the non-breeding season. There was no significant ($P > 0.05$) difference between the left and right testes weight during each season (Table 7).

Table 7: Mean right and left testicular weight, lengths and width, and vas deferens lengths of egret during breeding and non-breeding season

Parameter	Side	Non-breeding season	Breeding season
Testis weight (g)	Left	0.37 ± 0.06^a	0.72 ± 0.06^b
	Right	0.31 ± 0.05^a	0.76 ± 0.04^b
Testis length (mm)	Left	2.80 ± 0.13^a	4.33 ± 0.14^b
	Right	3.49 ± 0.16^a	4.64 ± 0.14^b
Testis width (mm)	Left	2.46 ± 0.29^a	4.78 ± 0.30^b
	Right	2.20 ± 0.15^a	5.12 ± 0.32^b
Vas deferens length (mm)	Left	35.13 ± 0.98^a	45.44 ± 0.90^b
	Right	36.28 ± 1.36^a	46.00 ± 0.96^b

Mean values with different superscript letters in the same row differ significantly at $P < 0.05$, No significant difference existed between the left and right for each parameter in each season.

The right testis showed a significant ($P< 0.05$) increased length from 2.80 ± 0.13 mm (non-breeding season) to 4.33

± 0.14mm (breeding season), while the left testis length increased from 3.49 ± 0.16 mm to 4.64 ± 0.14mm. Testicular width followed the same trend, with the left testis widening from 2.46 ± 0.29mm to 4.78 ± 0.30mm and the right from 2.20 ± 0.15mm to 5.12 ± 0.32mm between seasons. No significant differences ($P > 0.05$) existed between the right and left testes length and width during each season (Table 7).

The vas deferens lengths also demonstrated a significant ($P < 0.05$) increase in the breeding season, with the left side measuring 45.44 ± 0.90 mm and the right 46.00 ± 0.96 mm, compared to 35.13 ± 0.98 mm and 36.28 ± 1.36 mm, respectively, during the non-breeding season. There was no significant difference ($P > 0.05$) between the right and left vas deferens lengths in each season (Table 7).

Stereology Result

The result showed that the volume estimated for the left and right testis during breeding season were 32540 mm^3 and 35900 mm^3, and 13960 mm^3 and 15300 mm^3, for non-breeding season respectively (Table 5). The total germ cell estimates of the testis during breeding and non-breeding season respectively were 426.7 x 10^4 and 100.4 x 10^4 (Table 8). However, data obtained for the volume and germ cell number estimated for the testis showed that the breeding season had higher volume and germ cell number than the non-breeding season (Table 5 and 8). Also data obtained from the volume estimation also showed that the right testis has a larger volume than the left testis in breeding and non-breeding season (Table 8).

Table 8: Estimated total germ cell number of Testis of Bubulcus Ibis during breeding and non-breeding season

Season	Total Germ Cell Estimate	NOISE	VARSURS	TVAR	CE
Breeding	426.7 × 10^4	6027.3 × 10^8	−18082.07 × 10^8	−12054.69 × 10^8	3.88
Non-breeding	100.4 × 10^4	255.1 × 10^8	−765.29 × 10^8	−510.19 × 10^8	1.12

Discussion

Our findings indicate that the weight of the testes significantly increased during the breeding season compared to the non-breeding season ($P \leq 0.05$). However, there was no significant difference between the left and right testes within each season. These results align with the study by Danmaigoro, *et al*., (2014), who reported no differences in weight, length, and width of male reproductive organs in bats. Additionally, our findings are consistent with Dharani, *et al.,* (2018) report, which noted a similar weight distribution in guinea fowl, where the right testis is smaller and wider than the left. This observation is not statistically significant, as it reflects the characteristics of seasonal breeders. A similar pattern was observed in the cattle egret, which is also a seasonal breeder.

Our result findings indicate that the left testis is significantly longer and comparatively narrower than the right testis. This finding corroborates the report of Jos Dorlan, *et al*., (2022), who reported a similar asymmetry in yellow-feathered broilers where the left testis was longer while the right testis exhibited greater width. In contrast, Romer, *et al*., (1977), reported that the left testis could be wider than the right, a finding that differs from the present study and mating system. Such discrepancies may reflect species specific differences, seasonal influences or variations associated with reproductive strategy and mating system. The morphometric variations observed between the testes during breeding and non-breeding periods further support the notion that reproductive organ dimensions are highly dynamic in seasonal breeders. Crane and Scott (2002), emphasized that testicular size is closely associated with mating behavior, with polygamous species generally exhibiting relatively larger testes than monogamous species because of increased reproductive demand. Similarly, Shackelford and Goetz (2007) reported that parameters such as testicular weight, length and width fluctuate considerably in seasonal breeders in response to reproductive activity and endocrine changes.

The findings on the gonadosomatic index (GSI) of this species based on total testicular weight revealed a significant difference between the breeding and non-breeding seasons. These results differ from those reported by Tamilselvan, *et al*., (2018), who recorded a gonadosomatic index of 2.33% for paired testes in adult turkeys, and from Papia, *et al*., (2019), who recorded a 3.79% gonadosomatic index for whole testes weight in adult khaki ducks. The authors attributed their findings to higher sperm production during the breeding cycle. In this study, the breeding season corresponds to the rainy period (May to September) while the non-breeding season extends from November to March in Northwestern Nigeria. In this study, the differences observed between the seasons may be attributed to increased spermatozoa formation during the reproductive season. Maddock and Baxter (1991) and (Kier and Isham, 2024) noted that the reproductive cycle of the cattle egret occurs once a year in arid zones and begins with the onset of the rainy season, which aligns with our findings. In this study, the breeding season coincides with the rainy period from May to September, while the non-breeding season extends from November to March in northwestern Nigeria.

Stereological studies have demonstrated that assessing testicular volume and cell count is one of the most effective indirect methods for predicting fertility in vertebrates (Varela, *et al.,* 2015). The volume and germ cell count estimates obtained through stereological methods, such as the Cavalieri estimator and physical dissector, have enhanced our understanding of reproductive fertility and clinical diagnoses related to reproductive disorders. The stereological estimates used in this study were unbiased, meaning they were not influenced by germ cell size, section thickness, or dimensional changes in testicular tissue caused by histological procedures. The application of the Cavalieri principle for volume estimation was found to be more accurate than the fluid displacement method (Yan, *et al*., 2003).

The physical dissector has been widely utilized in various studies to obtain unbiased estimates of testicular parameters, such as the number of Leydig and Sertoli cells in different animals. Notable examples include the mean total number of Sertoli and Leydig cells in normal men (Peter, *et al.,* 1996), a stereological study on the effect of vitamin E on testis structure in rats treated with paranonyl-phenol (Soleimani, *et al*., 2009), and a stereological and histometrical assessment of testicular parameters between Holstein and Simmental bulls (Murat, *et al*., 2013). Other studies have applied the technique to neural tissue including brown rat cortex (Korbo, *et al*., 1990), the rat hippocampus induced with penicillin epilepsy (Ilgaz, *et al*., 2002), neocortex of domestic pigs (Jelsing, *et al*., 2006), the mink whale (Eriksen and Pakkenberg, 2007), the Rhesus macaque brain (Christensen, *et al*., 2007), the harbor porpoise brain (Walloe, *et al*., 2010), and the right pons and medulla oblongata of African elephants (Suzana, *et al*., 2014).

The increased volume and germ cell estimates observed during the breeding season, as compared to the non-breeding season, may be linked to factors such as shrinkage and natural germ cell death (apoptosis). Additionally, the larger volume recorded in the right testis compared to the left in both seasons aligns with findings by Murat, *et al*., (2013), who also noted that the right testis had a greater volume than the left in Simmental and Holstein bulls. This suggests a potential advantage in reproductive performance and fertility associated with the right testis. Therefore, the volume and germ cell estimates obtained in this study for both the breeding and non-breeding seasons could serve as a comparative anatomical resource among avian species and provide baseline data for domestication efforts in this species

Conclusion

The study was done to estimate the reproductive cells of the testis of cattle egret and determine functional relationship between reproductive cells and performance using stereological approach. The result indicates higher volume and germ cell number in breeding season which range between May to September than in non-breeding season which range from November to February. These differences could be due to lack of frequent mating, feed availability (breeding season is more predispose to crop and insect availability than non-breeding season in this region) and climate adaptation (high temperate with low rainfall). In conclusion, the data obtained in this study provides the first stereological baseline data for seasonal reproductive changes in the testes of *Bubulcus ibis* and could also be used as comparative anatomy and clinical diagnosis among the order of the species.

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