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1 # Developmental changes in the histological structure of the testes, and
2 testosterone profiles in male guinea fowls (Numida meleagris)

3

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14

15 **Abstract**
16 Owing to the paucity of information on the reproductive biology of guinea fowls, a
17 study involving a total of 66 males was conducted, and documented the
18 developmental changes in histological structure of the testes of guinea cocks from
19 hatching until adulthood. Changes in testosterone synthesis during sexual
20 development were also determined. Age-related changes were analysed using
21 univariate analysis for completely randomised design and means separated using

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Tukey's test/Kruskal-Wallis test and medians separated by Mann-Whitney U-test. Total germ cell population per testis and testicular histological morphometric parameters increased significantly ($p<0.0001$) from 12 weeks of age **(WOA)**, and stabilised between 20 and 24 WOA. Peripheral testosterone concentrations increased gradually from 4 WOA, and peaked at 20 WOA. Correlations among all the testicular morphometric parameters were positive and highly significant ($p<0.01$). Similarly, significant ($p<0.05$) positive correlations existed between testicular weight and testicular sperm production, tubular diameter, Sertoli cell population, tubular length and peripheral testosterone concentration. Testicular sperm production was positively correlated with meiotic index ($p<0.01$) and round spermatids population ($p<0.05$). The correlations between peripheral testosterone concentrations, tubular diameter and Sertoli efficiency were also significant ($p<0.05$) and positive. Testicular morphometric parameters stabilized between 20 and 24 WOA, while peripheral testosterone concentrations showed two patterns of secretion, initial and final phases of increasing and decreasing testosterone secretions, respectively, and may be implicated in the development of histological structures of the testes and spermatogenesis.

Keywords: Guinea cock; histology; sexual development; testosterone; testis

1. Introduction

Avian testes are surrounded by a fibrous capsule that includes connective tissue and contractile fibers [1]. They contain interstitial tissue and seminiferous tubules, which are the site of spermatogenesis and, in developed testes, make up most of the testicular mass. Interstitial tissue includes Leydig or interstitial cells, the main source

47 of testicular androgens [2, 3, 4, 5, 6]. The testes in some bird species are of identical
48 sizes (e.g., tree swallow, *Tachycineta bicolor*, [7]; chicken, *Gallus domesticus,* [8]),
49 but many species show testicular size asymmetry, with one testis normally being
50 larger in adulthood than the other [9, 10].

51 The testis of the mature bird is organized into discrete, easily discernible
52 cellular associations and functional compartments. However, during embryonic and
53 early post-hatch development this organization is less apparent [11]. The post-hatch
54 development of the fowl's testis can be divided into three distinct phases: (1)
55 proliferation of spermatogonia and the somatic cells that support spermatogenesis
56 (Sertoli, peritubular myoid, and interstitial cells); (2) differentiation and the
57 acquisition of functional competence by somatic support cells; and (3) spermatogonial
58 differentiation resulting in the initiation of meiosis. While the boundaries of these
59 phases are not clearly defined, this three-step process results in functional
60 seminiferous tubules that can maintain spermatogenesis when the appropriate
61 hormonal cues are present [11].

62 The growth and histological development of the testes of White Plymouth
63 Rocks has been described by Kumaran and Turner [12, 13]. Their account serves as a
64 general description of the sequence of changes that occur in the seminiferous tubule
65 during the sexual maturation of the male bird. However, they reviewed observations
66 made on other breeds and emphasized that interbreed differences are to be found in
67 the relation between age of a male and a particular histological structure displayed in
68 the seminiferous tubule. For instance, spermatids appeared at about 12 WOA in the
69 exotic breed of guinea cock [14], compared to 20 WOA [15] in the local breeds.
70 Kumaran and Turner [12, 13], however, noted that in general, light breeds mature
71 earlier than heavy breeds.

72 Several androgens and other steroids have been found in the fowl's testis [16].
73 Testosterone is considered the most important mammalian testicular androgen and has
74 been identified in the extracts of testis of fowls and other birds [17]. Driot and
75 associates [18] described changes occurring in plasma testosterone concentrations in
76 the domestic fowl during sexual development. The authors noted three stages
77 including i. a stationary phase, observed in cockerels less than 12 weeks old, ii. an
78 augmentation phase lasting approximately 12 to 22 WOA, and ii. an adult phase,
79 consisting of marked fluctuations in testosterone concentrations. Testosterone in the
80 male is essential for spermatogenesis, maintenance of the excurrent duct and
81 secondary sexual attributes, the expression of specific behaviours, and, altering the
82 pattern of GnRH secretion [11].
83 Even though a preliminary study documented some histological descriptions of
84 the age-related changes in the reproductive organs of male guinea fowls, these were
85 not detailed, and only involved qualitative descriptions, and small sample size [15].
86 Also, the endocrine profiles associated with these changes are unknown. For example,
87 testosterone concentrations have only been documented in breeding and non-breeding
88 males [19]. Besides, there is a general paucity of information on the reproductive
89 system of guinea fowls. The objective of the present study, therefore, was to
90 determine the developmental changes in histology of the reproductive organs of
91 guinea cocks from hatching until adulthood (32 weeks), and associated testosterone
92 profiles.
93
94 **2. Materials and methods**
95 *2.1 Experimental Site*
96 The study was conducted at the Poultry Unit of the Department of Animal Science,

97 University for Development Studies, Nyanpkala, Tamale (Ghana). Nyanpkala lies on
98 latitude 9° 69'N and longitude 0° 83'W. Temperatures are generally high with
99 minimum and maximum values of 22 °C and 35 °C recorded in March and December,
100 respectively (Savannah Agricultural Research Institute (SARI, 2008) cited by Abdul-
101 Rahman *et al.* [19]). Rainfall is monomial with mean annual rainfall varying from
102 1,000-1,500 mm and peaks from August to September, with a relatively long dry
103 season extending from November to April. The area lies in the Guinea Savannah
104 zone, and has nearly equal amounts of light and darkness (12L: 12D) throughout the
105 year. The guinea fowls used in the present study are indigenous to this area, hence the
106 name guinea fowl [20].
107
108 *2.2 Animals and Management*
109 A total of 66 local guinea cocks (*Numida meleagris*), of the pearl variety, were used
110 for the study. Birds were brooded for 6 weeks [21], and then transferred to a deep
111 litter house (floor spacing: 1.8 sq ft/bird; Lohmann LSL, Germany) until the end of
112 the experiment. They were individually identified using tags placed through their
113 inner wings to prevent detection by other birds and thus avoid pecking. Keets were
114 brooded at 35°C from hatching until three WOA, and then at 32°C until six WOA
115 [21]. Birds were then maintained at ambient temperatures of between 22°C and 35°C
116 until the end of the experiment. Feed and water were supplied *ad libitum.* Day old
117 keets were fed ground maize in flat feeders followed by a starter ration from day 2
118 until 6 WOA. This was followed by a grower ration from 6 WOA until 21 WOA and
119 then a layer feed until the end of the experiment. The starter (22% crude protein and
120 3,000 Kcal ME/kg diet), grower (14% crude protein and 2,800 Kcal ME/kg diet), and

121 breeder (17.5% crude protein and 2,800 Kcal ME/kg diet) rations were obtained from
122 a commercial feed supplier (Agricare Ghana Limited, Kumasi, Ghana).
123 Information on lighting requirements of the local guinea fowls from hatching are
124 unavailable, and those used for chicken, are usually employed. In this case, however,
125 the "golden rule" to follow in designing lighting programmes for pullets [22] was
126 followed. All birds received 24 h light from day old until one-WOA, and this was
127 reduced to 16 h until birds were 3 weeks old. These longer light periods during the
128 first 3 weeks of life were to ensure maximum feed consumption, enough to ensure
129 maximum growth, initially. This was gradually reduced to a minimum of 12 h by the
130 7th WOA, marking the phase of constant light [22]. Thereafter, birds were maintained
131 under natural photoperiods (12L: 12D) until the end of the study.
132

133 *2.3 Experimental procedure*

134 All procedures used followed approved guidelines for ethical treatment of
135 experimental animals.
136 A total of 56 male guinea fowls (7 per age group) were bled at 4, 8, 12, 16, 20, 24,
137 28, and 32 WOA. Two ml of blood was collected into EDTA vacutainer tubes from
138 the wing vein, and spun at 7100 x g for 3 min at room temperature (18-25 ^{o}C). Plasma
139 was then pipetted into a 1.5 ml microcentrifuge tube and stored at –20 °C until
140 subsequently analysed for testosterone.
141 Prior to bleeding, however, 5 birds at each age were weighed, and then following
142 bleeding, were sacrificed by cervical dislocation. Their testes and reproductive tracts
143 were completely freed from the adjoining ligaments and fascia, weighed and fixed in
144 Bouin's solution overnight for histology.

145

146 *2.3.1 Histological preparation, cell identification, stereological analyses and cell*

147 *counts*

148 The histological techniques used in the present study have been described previously

149 [23-24], therefore, only a brief description is given here. The testes (with capsule

150 intact) were each divided into 2 halves. One half of each testis was fixed in Bouin's

151 solution, dehydrated in absolute ethanol and embedded in paraffin wax. They were

152 sectioned (5 µm) using microtome (Leica RM2125RT), floated onto Poly-1-lysine

153 subbed slides (Polysine; VWR International Leuven, Germany), and stained in eosin

154 and Mayer's haematoxylin. Germinal cell counts were restricted to preleptotene

155 primary spermatocyte, type I spermatocyte in prophase I and step I spermatids [25-

156 26]. Sertoli and Leydig cell nuclei were also counted. The Sertoli cells were identified

157 on the basis of their nuclei following the descriptions given by Zlotnik [27] and de

158 Reviers [24], while Leydig cells were identified by their characteristic location as

159 clusters in the interstitial region and by nuclear diameter. In all cases, the location,

160 relative size, shape and nuclear morphology of germ and somatic cells helped in cell

161 identification. Nuclear diameters of testicular germ and somatic cells were obtained

162 with previously calibrated calipers (this was calibrated using graticule under

163 immersion oil) under immersion oil, using sections from 5 males and counting 20

164 nuclei/cell type/male. Cell counts/transverse section were determined from 10

165 sections of individual seminiferous tubules/slide and 10 interstitial areas (surface area

166 determined)/slide for Leydig cells. Germ cell counts were determined for all testes

167 involved. The numbers of fragmented nuclei were relatively high, and partially

168 sectioned nuclei were counted as seen, if their cell type were clearly recognizable. To

169 compensate for possible overestimation of cell numbers under such conditions, initial

170 cell counts were corrected using Abercrombie's [28] correction factor as follows: Nc
171 = N × e/(e + d), where:
172 Nc = The corrected number of cells in the preparation
173 N = The number of nuclei counted/tubular section
174 e = The thickness of the histological preparation
175 d = the diameter of the nucleus of a given cell type.
176 This correction determines the number of cells with nuclei effectively present in the
177 preparation.
178 Total number of cell (Nt): Total cell numbers for germ and Sertoli cells per
179 testis were determined using the formula Nt = Lt × Nc / e
180 Where Lt = Length of seminiferous tubules (estimated below), and e and Nc defined
181 as in the above. Total Leydig cell numbers were determined in relation to the
182 interstitial area occupied by the cells, and expressed as number of cells per 1000 µm²
183 of interstitial area.
184
185 *2.3.2 Dimensions of Seminiferous tubule (ST)*
186 Total length of seminiferous tubule (Lt) was estimated based on the formula Lt = Vr ×
187 (100-C) •10^{-1}/S [23, 29], where: Vr = percentage of testicular tissue occupied by the
188 ST as measured by a modification of the Chalkley's [30] technique. This was
189 determined by taking a picture of an entire cross section of each testis under the light
190 microscope at ×4 magnification. Each cross section therefore yielded several pictures
191 depending on the size of the cross section. Each picture was subsequently opened
192 with previously calibrated ImageJ software (National Institutes of Health, USA), and
193 grids 50 µm apart were superimposed on the entire image. With a pencil tool plug-in,
194 the grids on each image were grouped into 25 points grids (as obtained with 25-point

195 grid graticule) and each field labeled, in ascending order, until the entire cross section
196 was covered. Forty fields were then randomly chosen per cross section and counted as
197 in the Chalkley's [30] technique. Points that fell on the tubes (including the basement
198 membrane) were considered as tubular while those that fell outside the tube were
199 considered as non-tubular. This also represents the ratio of tubular to non-tubular
200 tissue [14]. Vr is expressed as a percentage of testicular tissue occupied by
201 seminiferous tubules. From this therefore, Vr could be determined according to the
202 formula: Vr =TW × %tubes/p where TW =testis weight (g), p = specific gravity of the
203 testis (in guinea fowls p = 1.05g/cm^3, as in the male chicken, [23]). % tubes =
204 (number of ST points within the eye piece/total number of points of the eye piece)
205 100. C = the histological contraction of the testes, is given by (Volume of fresh tissue-
206 Volume of embedded tissue/Volume of fresh tissue) x100 [29]. For guinea fowls, C in
207 both immature and mature birds was estimated as 33.4±13.1 [14]. S = mean area of a
208 transverse section of ST. The ImageJ software (National Institutes of Health, USA)
209 was used to measure the surface area of the tubules directly instead of deriving it from
210 the diameter. Tubules tended to elongate with age, and diameters may therefore not be
211 accurate when measured directly. Nonetheless, in tubules with minimum and
212 maximum diameter differences not exceeding 20% [14], diameters and surface areas
213 were measured in order to compare apparent diameters (diameters measured directly)
214 to actual diameters (diameters derived from the surface area using the formula D =
215 √surface area ×4/ π). LT was expressed in meters (m).
216

217 *2.3.3 Sertoli efficiency and quantitation of spermatogenesis*

218 Other parameters estimated were ratio of round spermatids to Sertoli cells, Sertoli
219 efficiency (total number of germ cells beyond the spermatogonia stage, supported by

220 each Sertoli cell) and meiotic index. Meiotic index, which measures the rate of
221 spermatogenesis, was expressed as a theoretical ratio based on the mean ratio for 5
222 males, and was calculated as follows: given that each type I spermatocyte should
223 provide 4 round spermatids during meiosis (MI = 4), and that ultimately, the actual
224 ratio of type I spermatocytes to round spermatids is dependent on the life span of each
225 cell type, %MI is therefore given as 100 (Number of round spermatids/life span of
226 round spermatids)/4(number of type I spermatocyte/life span of type I spermatocyte)
227 [31]. The life spans of primary spermatocyte and round spermatid in the guinea fowl
228 (*Numida meleagris*) are 4.5 and 2.5 days, respectively, as obtained from BrdU
229 observations and reported by Hein *et al*. [32].
230 Total reading for a parameter per testis was presented as average for the 2 testes
231 (i. e left testis reading + right testis reading/2).
232
233 *2.3.4 Testicular sperm production*
234 A total of ten 32-week old guinea cocks were involved. A fragment of testis (of
235 volumes ranging between 28.3 mm^3-265 mm^3) from each testis was weighed (fwt),
236 homogenised in 0.25M sucrose (1:200; testes: sucrose), and elongated spermatids (el)
237 and testicular spermatozoa (tspz) were counted using haemocytometer (10 replicates
238 per testes). Results for each male were estimated as follows:
239 TSP/male = right TSP + left TSP = (el + tspz)/fwt × testicular weight [31]
240
241 *2.3.5 Testosterone assay*
242 The testosterone assay had been previously validated for guinea fowl [19]. The assay
243 was a RIA using tritiated tracer (Amersham Int., Amersham, Bucks, UK) and a
244 procedure as originally described by Sheffield and O'Shaughnessy [33]. The

245 testosterone antibody was obtained from Guildhay Antisera, Surrey, UK. The
246 detection limit was 0.06 ng/ml, and intra-assay coefficient of variation was 9.5%.
247 Cross reactivity with androstenedione and androstanediol were 0.3% and 3.9%,
248 respectively. The assays were performed after sample extraction using diethyl ether in
249 duplicate of 50 µl aliquots. Peripheral testosterone concentrations in all the samples
250 assayed were determined using the standard curve generated by the Assayzap
251 software (Biosoft®, USA). All samples were evaluated for testosterone in one assay.
252

253 *2.4 Statistical analysis*

254 Data were analysed using the SPSS software, version 20.0 [34]. Age-related changes
255 in histology of the reproductive organs and testosterone profiles in male guinea fowls
256 were analysed using univariate analysis for completely randomised design, and means
257 separated using tukey's test. Where variances were not homogenous, Kruskal-Wallis
258 test was used instead and medians separated using Mann-Whitney U test. Data were
259 presented either as mean±standard error of mean or median (Interquartile range). All
260 comparisons were done at 5% level of significance.
261

262 **3. Results**

263 *3.1 Testicular histology*

264 The testes of the guinea fowl were contained in a covering, the tunica albuginea. The
265 capsule did not give off septa, and therefore no separation of testes into lobules was
266 seen in any of the birds. The seminiferous tubules were not separated by true septa,
267 but rather only fine strands of connective tissues passed inwards from the tunica to
268 separate the tubules. Occasionally, larger amounts of connective tissue were found
269 surrounding a blood vessel passing towards the tunica. In the testes of a mature

270 breeding male guinea fowl, there were 4 germ and 2 somatic cell types. The germ cell
271 types were spermatogonia, primary spermatocytes, secondary spermatocytes and
272 round spermatids, which lined the basement membrane in a stratified manner. Three
273 different types of spermatogonia were seen in mature testes which could be
274 distinguished based on heterochromatin appearance and distribution, and nuclei
275 diameter. The somatic cells were Leydig and Sertoli cells.

276 At 8 WOA, only spermatogonia and Sertoli cells were present in the
277 seminiferous tubule of the birds, and the tubular lumen was absent or poorly
278 developed. These cells lined the basement membrane. There were no changes in the
279 tubular epithelium until at 12 WOA when both round and elongated spermatids (in
280 some samples) were visible. At this age, the lumen was generally well formed, but
281 tubules were widely separated by abundant interstitial tissue. By 16 WOA fully
282 formed spermatozoa could be found in both the tubular lumen and ductuli efferentes
283 of the epididymis, marking the onset of sexual activity. At this age, the interstitium
284 had decreased considerably in size and Leydig cells had become organized into
285 compact groups lying in the angular areas between adjacent seminiferous tubules
286 (Figure 1).

287 Age-related changes in testicular histological morphometric traits are shown in
288 Table 1. Round spermatid population size in the seminiferous tubules increased
289 significantly (Kruskal-Wallis $X^2 = 183.003$, df = 5, $p < 0.0001$) between 12 and 20
290 WOA. Cumulatively, the increase in round spermatid population size between week
291 20 and 28, and 24 and 32 were significant ($p<0.05$). Type I spermatocyte population
292 size on the other hand remained constant between 12 and 16 WOA, and saw
293 significant (Kruskal-Wallis test $X^2 = 169.975$, df = 5, $p<0.0001$) increases thereafter
294 until 20 WOA, dipped at 24 weeks, and increased ($p<0.05$) until 32 WOA. Total germ

295 cell numbers in the seminiferous tubule increased significantly (Kruskal-Wallis test
296 $X^2 = 186.147$, df = 5, $p<0.0001$) between 12 and 20 WOA. It remained constant
297 thereafter until 24 weeks of age and then increased significantly ($p<0.05$) between 24
298 and 32 WOA. Sertoli cell population size in the tubule also increased significantly
299 (Kruskal-Wallis test $X^2 = 214.116$, df = 6, $p <0.0001$) between 8 and 20 WOA. This
300 was followed by a significant decrease ($p<0.05$) at 24 weeks and thereafter, a
301 significant rise at 28 and 32 WOA.

302 Number of round spermatids per Sertoli cell increased significantly (Kruskal-
303 Wallis test $X^2 = 142.834$, df = 5, $p <0.0001$) between 12 and 24 WOA. The value then
304 dropped ($p<0.05$) between this age and 28 WOA, and rose ($p<0.05$) again to the level
305 similar to that observed at 24 weeks, between 28 and 32 WOA. Similarly, total
306 number of germ cells supported by each Sertoli cell differed ($p< 0.0001$) among age
307 groups. It decreased significantly ($p<0.05$) between 12 and 16 WOA, then increased
308 ($p<0.05$) cumulatively between 16 and 24 WOA. This was followed by a dip ($p<0.05$)
309 at 28 weeks and finally, a significant rise ($p<0.05$) at 32 WOA.

310 Meiotic index, which is an indication of the rate of cellular death during the first
311 and second meiotic divisions increased significantly (Kruskal-Wallis test X^2 =
312 141.059, df = 5, $p <0.0001$) between 12 and 24 WOA. This was followed by a highly
313 significant drop at 28 WOA, and finally, a significant rise ($p<0.05$) between 28 and 32
314 WOA. The highest value was at 24 WOA{83.5 (71.3-95.8)%} and the lowest {6 (0-
315 17.5)%} at 12 WOA.

316 Both apparent and actual seminiferous tubular diameters exhibited the same
317 pattern of growth between 8 and 32 WOA. Significant increases were recorded in
318 apparent (Kruskal wallis test $X^2 = 189.885$, df = 6, $p <0.0001$) and actual (Kruskal-
319 Wallis test $X^2 = 206.497$, df = 6, $p <0.0001$) seminiferous tubular diameters between 8

320 and 24 WOA. From this point onward, there were no significant increases in both
321 cases, however, there were cumulative increases ($p<0.05$) in both parameters between
322 24 and 32 WOA. Actual tubular diameter was significantly bigger ($p<0.05$) than
323 apparent tubular diameter {526.6 (481.0-576.0) μm vs 383 (348.6-419.9) μm}.

324 Relative volume of seminiferous tubule in the testes increased significantly
325 (Kruskal-Wallis test X^2 = 348.574, df = 6, p <0.0001) between 8 and 20 WOA. It then
326 stabilised for the next 8 weeks before increasing at 32 WOA. Seminiferous tubular
327 length, on the other hand, significantly (Kruskal-Wallis test X^2 = 623.228, df = 6,
328 p<0.0001) increased between 8 {2.5 (1.8-5.0) m} and 20 {9.8 (9.1-10.5) m} WOA,
329 followed by a dip ($p<0.05$) at 24 WOA. It then increased ($p<0.05$) between 24 and 32
330 WOA. Testicular sperm production in the adult breeding guinea cock averaged 9.9
331 $x10^7$ (8.5 $x10^7$-18.0 $x10^7$)

332 The Sertoli cells were located on the basement membrane. The Leydig cells had
333 spherical nuclei and occurred as clusters in the interstitial region. They possessed
334 prominent nucleoli. In the guinea fowls, the Sertoli cells were quasi-circular in most
335 cases, and were significantly bigger ($p<0.05$) than the Leydig cell nuclei (4.3±.07 μm
336 vs 3.0±.07 μm).

337 Correlations among all the testicular morphometric parameters were positive
338 and highly significant ($p<0.01$). Similarly, significant correlations existed between
339 testicular weight and testicular sperm production, actual tubular diameter, Sertoli cell
340 population, tubular length ($p<0.01$) and Sertoli efficiency (number of round
341 spermatids per Sertoli cell and total number of germ cells per Sertoli cell) ($p<0.05$).
342 The correlations between testicular weight and all the parameters except Sertoli
343 efficiency were positive. Testicular sperm production was not correlated with any of

344 the testicular morphometric parameters except meiotic index ($p<0.01$) and round
345 spermatids population ($p<0.05$). These were positively related to testicular sperm
346 production (Table 2).
347

348 *3.2 Changes in peripheral testosterone concentration*

349 Generally, no significant increases were recorded in peripheral testosterone
350 concentrations measured monthly. Testosterone concentrations, however, tended to
351 increase from 4 to 20 WOA when it peaked. Testosterone levels at sexual maturity
352 (16 WOA) were significantly higher ($p<0.05$) than the levels in 4-week old birds.
353 Similarly, the peak testosterone concentrations at 20 weeks were higher ($p<0.05$) than
354 the concentrations at 4 and 8 WOA. Testosterone concentration decreased after 20
355 WOA to a level similar to that seen at 12 WOA and remained at that level until the
356 end of the study (Figure 2).

357 Correlation between testicular weight and peripheral testosterone
358 concentration was positive and highly significant ($p< 0.0001$). Similarly, there were
359 significant ($p<0.05$) positive correlations between testosterone concentrations and
360 actual tubular diameter, total number of germ cells per Sertoli cell and number of
361 round spermatids per Sertoli cell (Sertoli efficiency) and tubular length (Table 2).
362

363 **4. Discussions**

364 *4.1 Changes in the histology of the testes*

365 In agreement with the observations made by Awotwi [15] and Brillard [14] in the
366 local and exotic breeds of guinea fowls, respectively, the testes of a growing male
367 guinea keet could only be detached for decent histological sections from 8 WOA. At
368 this age, the seminiferous tubules had poorly-formed lumen or none at all; only

369 Sertoli cells and spermatogonia lined the basement membrane, and abundant
370 interstitial tissue separated the tubules. Puberty, characterized by the presence of
371 primary and secondary spermatocytes and round spermatids in the tubular lumen, was
372 attained at 12 WOA in the birds studied by Brillard [14]. The author noted that
373 elongated spermatids were seen in the tubular lumen of a few birds. The results of the
374 present study confirm this earlier report by Brillard [14]. Awotwi [15], however,
375 found only primary spermatocytes at 12 weeks and secondary spermatocyte at 16
376 WOA, an indication of late attainment of puberty in those birds. Guinea fowls used in
377 this study attained sexual maturity at 16 WOA when fully formed spermatozoa were
378 present both in the tubular lumen and the lumen of excurrent duct system. This was
379 earlier than the 20 weeks reported by Awotwi [15] in the same breed. This result is
380 not surprising considering the fact that the processes of spermatogenesis started
381 earlier in the birds used in this study than those in the study by Awotwi [15]. The
382 differences in the time of sexual maturity between the 2 flocks of birds may be
383 attributed to possible differences in management, as management factors including
384 feeding [35] and photoperiod [36] have been cited to alter dramatically the onset of
385 meiosis and sustained spermatogenesis.

386 Seminiferous tubular diameter was measured in two ways during the present
387 study. The actual seminiferous tubular diameter (estimation method developed during
388 this investigation) was much larger than the apparent diameter (conventional method
389 of tubular diameter estimation). This indicates that tubular diameters are usually
390 underestimated using the conventional method of measurement. Another disadvantage
391 of the conventional method is that not all tubules are given equal chances of being
392 selected for measurement since the tubule has to be quasi-circular in order to be
393 considered. Where a software package is employed for area measurement from which

394 the diameter is determined, all these problems are avoided. Even though the use of the
395 apparent tubular diameter underestimates the diameter of the tubule, it is still
396 reflective of the true situation when comparing across groups or conducting trend
397 analysis, as evidenced by the relationship between the trends of age-related variations
398 in the two tubular diameters in the present study. The use of the actual tubular
399 diameter approach is particularly useful when estimating tubular diameters in a
400 situation where transverse sections of seminiferous tubules tend to elongate in
401 growing animals, making it difficult to obtain the number of tubules required for the
402 estimation of tubular diameters and other tubular parameters.

403 Several quantitative histological changes occurred in the testes of male guinea
404 fowl during the period before sexual maturity. Both the apparent and actual
405 seminiferous tubular diameters increased from 74.4 µm and 87.2 µm, respectively, at
406 8 weeks to 326.8 µm and 387.7 µm, respectively, at 20 WOA. Tubular length also
407 increased from 2.5 m at 8 weeks to 9.8 m at 20 weeks. These reflected in massive
408 increase in the relative volume of the seminiferous tubules. These figures tended to
409 plateau after 20 WOA. Brillard [14], therefore, defined 20 weeks as the beginning of
410 adulthood in the guinea fowl. The fluctuations seen after 20 weeks was attributable to
411 the fact that these birds attained sexual maturity during the minor breeding season,
412 and this may have influenced subsequent readings. The modifications seen in the
413 seminiferous tubules led to early onset of spermatogenesis and rapid development of
414 the spermatocytes population between 8 to 12 WOA (0 to 0.503 x 10^8). Round
415 spermatids were also present in all samples analysed at 12 WOA. It increased from
416 this age and tended to stabilise from 20 WOA. Puberty in these birds therefore
417 commenced from 12 WOA. A similar observation was made by Brillard [14]. This
418 study, found some type I spermatocyte at 8 WOA, however, this was not noticed in

419 the present study.

420 The Sertoli cells were quasi-circular in the guinea fowl. This is in agreement
421 with the earlier observation by Brillard [14]. Sertoli cell population increased even
422 during adulthood and was linearly correlated with total germ cell numbers. This is
423 consistent with the report of Brillard [14] in the exotic breeds of guinea fowls. The
424 author evoked 2 hypotheses to explain the increase in Sertoli cell population during
425 adulthood in the guinea fowl. First, even at sexual maturity, a low level of mitotic
426 activity may persist among the Sertoli population. Secondly, some undifferentiated
427 Sertoli cells might remain in the testes after sexual maturity. These cells could play
428 the role of reserves proliferating and differentiating slowly during adulthood.

429 The fluctuations in the total number of germ cells per Sertoli cell may be
430 attributed to the attainment of sexual maturity in the non-breeding season and cellular
431 deaths. The reduced meiotic rate occurring during this period may account for the
432 fluctuating numbers of germ cells supported by each Sertoli cell. It is currently
433 accepted that the number of Sertoli cells established during testicular development
434 determines the rate of spermatogenesis in sexually mature animals [37-38]. This
435 assumption is based on the fact that each Sertoli cell supports a limited number of
436 germ cells in a species-specific manner [39-40]. Studies have shown that
437 spermatogenic efficiency, expressed as the number of sperm produced daily per gram
438 of testis, is usually positively correlated with the number of germ cells supported by
439 each Sertoli cell [39-41]. This was evidenced by the positive correlation between
440 testicular sperm production and Sertoli efficiency in the present study. Other
441 important factors that were reported to have correlated with spermatogenic efficiency
442 were the volume density of the seminiferous tubule, the length of spermatogenic
443 cycle, the number of spermatogonial generations, the rate of germ cell loss during

444 spermatogenesis (supported by the strong positive correlation between testicular
445 sperm production and meiotic index in this study), the number of Sertoli cells per
446 gram of testis and the size of Sertoli cells [40, 42]. Contrary to the reports of Franca
447 and Godinho [43], Sertoli cell population positively correlated with actual and
448 apparent tubular diameter, and total germ cells per testis. The average number of
449 round spermatids per Sertoli cell and total germ cell per Sertoli cell (Sertoli
450 efficiency) in the adult guinea fowl were 12.5 and 7.2, respectively.

451 Germ cell apoptosis constitutes a normal process during spermatogenesis [44]
452 and can occur in different developmental phases. It is considered mainly to function
453 in density regulation of spermatogonia and to eliminate cells with chromosomal
454 damage (meiotic phase), whereas cell loss during spermiogenesis is less prominent
455 [40]. The quantitative significance of germ cell loss becomes clear when considering
456 that only two to three spermatozoa of 10 theoretically possible cells are produced
457 from type A1 spermatogonia [40, 45]. In the present study, the highest percentage of
458 cell deaths was 94% at 12 WOA, while the least was 16.5% at 24 WOA. The high
459 initial cell deaths at 12 WOA was not surprising considering the fact that these birds
460 attained puberty at this age, and maximal efficiency of spermatogenesis, as indicated
461 by quality of spermatozoa produced, is not achieved until several weeks after puberty
462 has been attained [46]. The lower percentage of cell deaths (16.5%) observed in the
463 present study at 24 WOA indicates a more efficient spermatogenesis in these birds at
464 this age. The significant and positive correlations between testicular sperm production
465 and number of round spermatids per testis, meiotic index and testicular weight, was
466 an indication that these parameters could be good predictors of spermatogenic
467 efficiency in guinea fowls. The lack of a significant correlation between Sertoli
468 efficiency and Sertoli cell populations with testicular sperm production was possibly

469 because of the relatively small sample size of 10 birds (for testicular sperm
470 production).
471
472 *4.2 Changes in peripheral testosterone concentration*
473 The rise in peripheral testosterone concentrations between 12 and 20 WOA in the
474 present study may be related to the early onset of puberty in these birds. Spermatozoa
475 were first seen at 16 WOA. It is probable therefore that the phase of rising plasma
476 testosterone levels occurred several weeks before the onset of sexual activity in the
477 local guinea cocks.
478 In the present study, the peak testosterone concentrations in sexually mature
479 guinea cocks were low (0.284 ng/ml). Abdul-Rahman *et al.* [19] also reported a low
480 peak testosterone concentration (0.471 ng/ml) in breeding males. These results were
481 not surprising considering earlier reports that male tropical birds have low plasma
482 testosterone concentrations, involving low amplitude cycles with possible slight
483 variations during times of breeding [47-49]. It is thought that these low concentrations
484 are a way of avoiding the potential detrimental effects of elevated concentrations of
485 testosterone, since there is a trade-off between testosterone concentration and
486 immunity [50]. Consequently, selection in the tropics may have favoured birds with
487 low concentrations of testosterone, in line with a slow pace of life, with more
488 resources being allocated to immune function [51]. The guinea fowl is a tropical bird
489 [20].
490 The peak testosterone concentrations recorded in the present study is several
491 fold lower than those reported in exotic breeding guinea cocks [52-53]. A possible
492 reason for this massive difference is that the guinea fowls used in the present study
493 are indigenous breeds, small in stature, and have not undergone any intensive

494 selection and breeding compared to their exotic counterparts. The exotic breeds are
495 much higher in weight at all ages than the local breeds [54]. Several workers [55-57]
496 have reported positive relationship between body and testicular weight. Positive
497 relationship has also been reported between testes size and testosterone titer [58-60],
498 with some authors inferring that the link is a consequence of the phenotypic
499 integration of spermatogenic and endocrine functions of the testes [58-59]. The
500 testicular weight reported for the exotic guinea fowl is two fold higher [14, 52-53]
501 than that found in the indigenous guinea fowls in the present study. The lower
502 testicular weight and corresponding lower testosterone concentrations in the
503 indigenous guinea fowls are, therefore, not surprising.

504 Rising plasma testosterone levels in the guinea fowls corresponded to
505 increasing seminiferous tubular diameters and volume. Sertoli and germ cell
506 populations also increased from 12 WOA. All these parameters did not see any
507 significant rise after the peak testosterone concentration was attained at 20 WOA,
508 implicating this hormone in spermatogenesis and the development of the seminiferous
509 tubules. A role for testosterone in adult testicular function is suggested by the finding
510 in mature hypophysectomized quail that administration of large doses of testosterone,
511 while insufficient to maintain spermatogenesis, retards testicular regression resulting
512 from the surgery [61]. Germ cell development started between 8 and 12 WOA when
513 the concentrations of testosterone were low, while spermatids and spermatozoa were
514 observed between 12 and 16 WOA when testosterone had nearly peaked. Low doses
515 of testosterone have also been implicated in the maturation of the germinal epithelium
516 in intact immature cockerels [62-63].

517 The significant positive correlations between plasma testosterone
518 concentrations and Sertoli efficiency, actual seminiferous tubular diameter and

519 seminiferous tubular length is an indication that plasma testosterone concentrations in
520 the local guinea fowls could be highly related to these histological morphometric
521 parameters.

522 In conclusion, puberty and sexual maturity were attained at 12 and 16 weeks
523 of age, respectively, in male guinea cocks. The pattern of testosterone secretion in the
524 guinea cock may be divided into two, initial phase of increasing testosterone
525 concentrations prior to 20 WOA, and a final one of decreasing peripheral testosterone
526 concentrations after 20 WOA, and may be implicated in the development of
527 histological structures of the testes and spermatogenesis in the guinea cock.

528

529 **Declaration of interest**

530 The authors declare that there is no conflict of interest that could be perceived as
531 prejudicing the impartiality of the article.

532

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539

540 **References**

541 [1] Aire TA, Ozegbe, PC. The testicular capsule and peritubular tissue of birds:
542 morphometry, histology, ultrastructure and immunohistochemistry. J Anat. 2007;
543 210: 731-40.

544 [2] Halse SA. Gonadal cycles and levels of luteinizing hormone in wild spur-winged
545 geese, *Plectropterus gambensis.* J. Zool. 1985; 205: 335-55.
546 [3] Dufty AM, Wingfield JC. Temporal patterns of circulating LH and steroid
547 hormones in a brood parasite, the brown-headed cowbird, Molothrus ater. 1.
548 Males. J. Zool. 1986; 208: 191-203.
549 [4] Mauget R, Jouventin P, Lacroix A, Ishii S. Plasma LH and steroid hormones in
550 king penguin (Aptenodytes patagonicus) during the onset of the breeding cycle.
551 Gen. Comp. Endocrinol. 1994; 93: 36-43.
552 [5] Rosenstrauch A, Weil S, Degen AA, Friedlander M. Leydig cell functional
553 structure and plasma androgen level during the decline in fertility in aging roosters.
554 Gen. Comp. Endocrinol. 1998; 109: 251-58.
555 [6] Madekurozwa MC, Chabvepi TS, Matema S, Teerds KJ. Relationship between
556 seasonal changes in spermatogenesis in the juvenile ostrich (Stuthio camelus) and
557 the presence of the LH receptor and 3β-hydroxysteroid dehydrogenase.
558 Reproduction 2002; 123: 735-42.
559 [7] Kempenaers B, Peer K, Vermeirssen ELM, Robertson RJ. Testis size and
560 asymmetry in the tree swallow Tachycineta bicolor: a test of the compensation
561 hypothesis. Avian Sci. 2002; 2: 115-22.
562 [8] Hocking PM. Bilateral testicular asymmetry and supernumerary testes in the
563 domestic fowl (Gallus domesticus). Br. Poult. Sci. 1992; 33: 455-60.
564 [9] Yu ZH. Asymmetrical testicular weights in mammals, birds, reptiles and
565 amphibian. Int. J. Androl 1998; 21: 53-5.
566 [10] Gunn MR, Champion Z, Casey ME, Teal P, Casey PJ. Testicular and
567 spermatozoan parameters in the pukeko (Porphyrio porphyrio melanotus). Anim.
568 Reprod. Sci. 2008; 109: 330-42.

569 [11] Kirby JD, Froman DP. Reproduction in male birds. In: Whittow CG, editor.
570 Sturkie's Avian physiology, 5th ed. New York: Academic Press; 2000.
571 p. 597-615.
572 [12] Kumaran JDS, Turner CW. The normal development of testes in the White
573 Plymouth Rock. Poult Sci 1949a; 28: 511-20.
574 [13] Kumaran JDS, Turner CW. Endocrine activity of the testis of the
575 White Plymouth Rock. Poult Sci 1949b; 28: 636-40.
576 [14] Brillard JP. Age-related variations in seminiferous tubule dimensions and
577 germinal and Sertoli cell numbers in guinea fowl raised under a 14L:10D
578 photoperiod. Poult Sci 1986; 65: 369-74.
579 [15] Awotwi EK. Some aspects of the reproductive physiology of male and
580 female guinea fowls. MSc. Thesis, University of Ghana, Legon, Ghana.1975.
581 [16] Lake PE, Furr, JA. The endocrine testis in reproduction. In: Bell DJ, Freeman
582 BM, editors. Physiology and biochemistry of the domestic fowl, vol. 3. London
583 and New York: Academic Press; 1971. p. 1469-88.
584 [17] Cardinali DP, Tramezzani JH, Cuello AE, Rosner, JM. In: James VHT, Martini
585 L, editors. Proceedings of the 3rd International Congress on Hormonal Steroids,
586 Hamburg. Amsterdam: Excerpta Medica Foundation; 1970. p. 231
587 [18] Driot FJM, de Reviers M, Williams J. Plasma testosterone levels in intact and
588 hemicastrated growing cockerels. J. Endocr. 1979; 81: 169-174.
589 [19] Abdul-Rahman II, Robinson JE, Obese FY, Jeffcoate IA, Awumbila B. Effects of
590 season on reproductive organ and plasma testosterone concentrations in guinea
591 cocks. Poult Sci 2016; 95(3): 636-644.
592 [20] Awotwi EK. A review of studies on guinea fowls in Ghana. Leg. Agric. Res.
593 Bull. 1987; 2: 1-4.

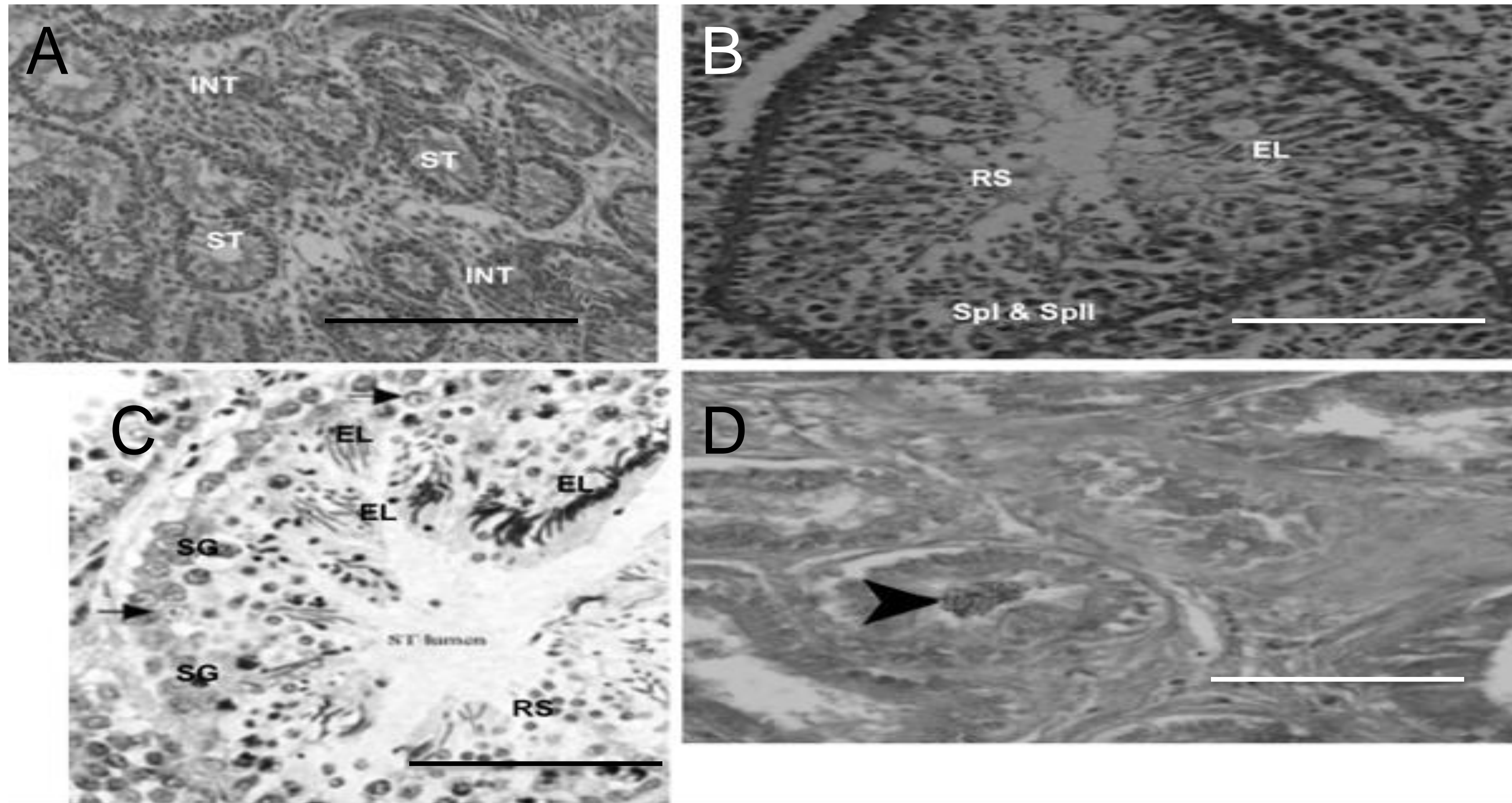
594 [21] Teye GA, Gyawu P. A guide to guinea fowl production in Ghana. Tamale:
595 Muetpress; 2002.
596 [22] Thiele HH. Light stimulation of commercial layers, Vol 44 (2). In: Thiele HH,
597 editor. Lohmann information. pp. 39–48. (Lohmann-
598 information.com/content/l i 44artikel13.pdf.; 2009 [accessed 15.06.14]
599 [23] de Reviers M. Le développement testiculaire chez le coq. l, Criissance
600 Ponderale des testicules et développement des tubes seminiferes. Ann. Biol.
601 Anim. Biochim. Biophys. 1971a; 11: 519-30.
602 [24] de Reviers, M. Le développement testiculaire chez le coq. ll. Morphologie de
603 l'epithelium séminifere et établissement de la spermatogenese. Ann. Biol.
604 Anim. Biochim. Biophys. 1971b; 11: 531-46.
605 [25] Aire TA, Olowo-okorun MO, Ayeni JS. The seminiferous epithelium in the
606 guinea fowl (Numida meleagris). Cell Tissue Res. 1980; 205: 319-25.
607 [26] Abdul-Rahman II, Obese FY, Robinson JE. Spermatogenesis and cellular
608 associations in the seminiferous epithelium of Guinea cock (Numida meleagris)
609 Can J Anim Sci 2017; 97: 241–249.
610 [27] Zlotnik I. The cytoplasmic components of germ cells during spermatogenesis in
611 the domestic fowl. Quat. J. Morphol. Sci. 1947; 88: 353-65.
612 [28] Abercrombie M. Estimation of nuclear population from microtome sections.
613 Anat Rec. 1946; 94: 238-48.
614 [29] Attal J, Courot, M. Developpement testiculaire et etablissement de la
615 spermatogenese chez le taureaux. Ann. Biol. Anim. Biochim. Biophys.
616 1963; 3: 219-41.
617 [30] Chalkley HW. Method for the quantitative morphologic analysis of tissues. J.
618 Natl. Cancer Inst. (1943; 4: 47-73.

619 [31] Noirault J, Brillard J-P, Bakst MR. Spermatogenesis in the turkey (Meleagris
620 gallopavo): Quantitative approach in immature and adult males subjected to
621 various photoperiods. Theriogenology 2006; 65: 845–859.
622 [32] Hein OC, Diarra B, Brillard J-P, Boly H, Sawadogo L. Effects of improving
623 health status on testicular development of guinea fowl (Numida meleagris) reared
624 under natural photoperiod in the Sudanian zone of Burkina Faso. Intl. J. Poult
625 Sci. 2011; 10 (2): 113-119.
626 [33] Sheffield JW, O'Shaughnessy PJ. Effect of injection of gonadotrophin releasing
627 hormone on testicular steroidogenesis in the hypogonadal (hpg) mouse. J.
628 Reprod. Fertil. 1989; 86: 609-617.
629 [34] IBM Corp. IBM SPSS Statistics for Macintosh, Version 20.0. 2011; Armonk,
630 NY.
631 [35] Cheah YS, Nxi W, Ng Y. Functions of essential nutrition for high quality
632 spermatogenesis. Adv Biosci Biotechnol 2011; 2: 182-97.
633 [36] Ingkasuwan P, Ogasawara FX. The effect of light and temperature and their
634 interaction on the semen production of White Leghorn males. Poult Sci 1966;
635 45: 1199-204.
636 [37] Orth JM, Gunsalus GL, Lampert AA. Evidence from Sertoli cell-depleted rats
637 indicates that spermatid number in adults depends on numbers of Sertoli cells
638 produced during perinatal development. J. Endocrinol. 1988; 122: 787-794.
639 [38] Hess RA, Cooke PS, Bunick D, Kirby JD. Adult testicular enlargement induced
640 by neonatal hypothyroidism is accompanied by increased Sertoli cell and germ
641 cell number. J. Endocrinol. 1993; 132: 2607-2613.
642 [39] Russell LD, Peterson RN. Determination of the elongate spermatid-Sertoli cell
643 ratio in various mammals. J Reprod Fertil. 1984; 70: 635-64.

644 [40] França LR, Russell LD. The testis of domestic animals. In: Martínez F, Regadera
645 J, editors. Male reproduction: a multidisciplinary overview. Madrid, Spain:
646 Churchill Livingstone; 1998. p.197-219.
647 [41] Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neill JD, editors. The
648 Physiology of Reproduction, 2nd ed, vol. 1. New York: Raven Press; 1994.
649 p. 1363-434.
650 [42] Johnson L. Spermatogenesis. In: Cupps PT, editor. Reproduction in domestic
651 animals, 3rd ed. New York: Academic Press; 1991. p.173-219.
652 [43] França LR, Godinho CL. Testis morphometry, seminiferous epithelium cycle
653 length, and daily sperm production in domestic cats (Felis catus). Biol Reprod**.**
654 2003; 68: 1554-561.
655 [44] Baum JS, St George JP, McCall K. Programmed cell death in the germ line.
656 Semin Cell Dev. Biol. 2005; 16: 245–59.
657 [45] De Rooij DG, Russell LD. All you wanted to know about spermatogonia but
658 were afraid to ask. J Androl. 2000; 21: 776–98.
659 [46] Dyrmundsson OR. Puberty and early reproductive performance in sheep. I. Ewe
660 lambs. Anim. Breed. Abstr. 1973; 416: 273-89.
661 [47] Hau M, Wikelski M, Soma KK, Wingfield JC. Testosterone and year-round
662 territoriality in a tropical bird. Gen. Comp. Endocrinol. 2000; 117: 20–33.
663 [48] Moore IT, Perfito N, Wada H, Sperry TS, Wingfield, JC. Latitudinal variation in
664 plasma testosterone levels in birds of the genus Zonotrichia. Gen. Comp.
665 Endocrinol. 2002; 129: 13–19.
666 [49] Moore IT, Wada H, Perfito N, Busch DS, Hahn TP, Wingfield JC. Territoriality
667 and testosterone in an equatorial population of rufous-collared sparrows,
668 Zonotrichia capensis. Anim. Behav. 2004; 67: 411–20.

669 [50] Wingfield JC, Lynn SE, Soma KK. Avoiding the "costs" of testosterone:
670 ecological bases of hormone-behavior interactions. Brain Behav and Evol.
671 2001; 57: 239–51.
672 [51] Wikelski M, Ricklefs RE. The physiology of life histories. Trends Ecol. Evol.
673 2001; 16: 479– 81.
674 [52] Ali MZ, Qureshi AS, Rehan S, Akbar SZ, Manzoor A. Seasonal variations in
675 histomorphology of testes and bursa, immune parameters and serum testosterone
676 concentration in male guinea fowl (Numida meleagris). Pak Vet J 2015; 35(1):
677 88-92.
678 [53] Qureshi AS, Saif-Ur-Rahman HM, Ali MZ, Kausar R. Changes in testicular
679 histomorphology and serum testosterone concentration of helmeted guinea fowl
680 (Numida meleagris) during different reproductive phases in Pakistan. J. Anim
681 Plant Sci 2016; 26(2): 564-568.
682 [54] Teye GA, Gyawu P, Agbolosu AA. Growth potential and carcass yields of
683 exotic and indigenous guinea fowls in Ghana. Dev Spectrum 2001; 1 (1): 34-40.
684 [55] Aviagen (Ross). Testis weight, fertility and bodyweight,
685 http://www.thepoultrysite.com; 2004 [accessed 11.06.17].
686 [56] Osama MA, Abd-El-Hamid EA, Wagdey ZA. Effect of crossing on the
687 performance of local strains. 4. Blood hematology and biochemical traits
688 and some organs relative weights of chicken cocks. J. Agric. & Env. Sci. Alex.
689 Univ. 2006; 5 (1): 57-71.
690 [57] Sarabia FJ, Pizarro DM, Abad MJ, Casanovas IP, Rodriguez-Bertos A, Barger
691 K. Relationships between fertility and some parameters in male broiler
692 breeders (body and testicular weight, histology and immunohistochemistry of

693 testes, spermatogenesis and hormonal leve**ls).** Reprod Domest Anim. 2013; 48
694 (2): 345–352.
695 [58] Garamszegi LZ, Eens M, Hurtrez-Bousses S, Moller AP. Testosterone, testes
696 size, and mating success in birds: a comparative study. Horm Behav 2005; 47:
697 389-409.
698 [59] Malo AF, Roldan ERS, Garde JJ, Soler AJ, Vicente J, Gortazar C, Gomendio
699 M. What does testosterone do for red deer males? Proc R Soc Biol Sci Ser B
700 2009; 276: 971-980.
701 [60] Preston BT, Stevenson IR, Lincoln GA, Monfort SL, Pilkington JG, Wilson K.
702 Testes size, testosterone production and reproductive behaviour in a natural
703 mammalian mating system. J Anim Ecol 2012; 81: 296-305.
704 [61] Brown NL, Follett BK. Effects of androgens on the testes of intact and
705 hypophysectomized Japanese quail. Gen. Comp. Endocrinol. 1977; **33**: 267-277.
706 [62] Kumaran JDS, Turner CW. The endocrinology of spermatogenesis in birds II.
707 Effect of androgens. Poult Sci 1949c; 28: 739-746.
708 [63] Siegel HS. Nitrogen metabolism in cockerels treated with 17-$\propto$-methyl-17-β-
709 hydroxyandrosta-Δ1, 4-3-one (methandrostenolone). Gen Comp Endocrinol.
710 1964; 4: 132-143.
711
712
713
714



727 Figure 1: Cross section of guinea fowl testes at various developmental stages: 8 (A), 12 (B), and 20 (C) weeks old. Note Interstitial tissue (INT),
728 Seminiferous tubule (ST), Spermatogonia (SG), Sertoli cells (arrow), primary and secondary spermatocytes (SpI and II), round (RS) and
729 elongated (EL) spermatids, Seminiferous tubular lumen (ST. Lumen), HE x20 (Scale bar = 100 µm). Plate D shows the distal ductule efferentes
730 of guinea cock at 16 weeks indicating the first appearance of spermatozoa (arrow head) in the lumen, HE x20 (Scale bar = 100 µm).

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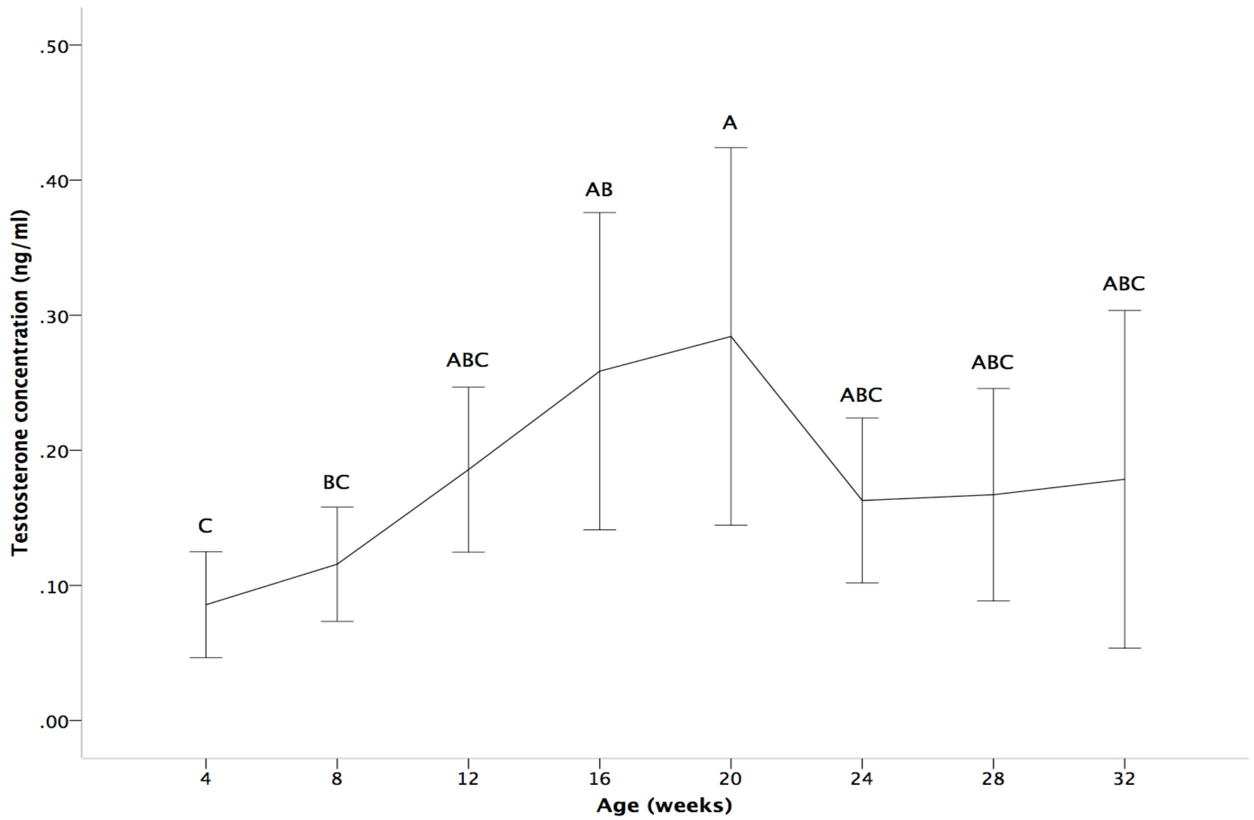

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735 Figure 2: Peripheral testosterone concentrations in guinea cocks during sexual

736 development.

Age-related changes in testicular histology

Table 1: Developmental changes in testicular histological morphometric traits in local guinea cocks

Testicular morphometric trait {Median(Interquartile range)}		Age (weeks)						
		8	12	16	20	24	28	32
nSpdR	($x10^8$)		0.1 (0.1 - 0.6)^{e}	0.5 (0.3 - 0.8)^{d}	1.6 (0.9 - 2.3)^{c}	1.9 (1.1 - 3.0)^{bc}	2.2 (1.7 - 2.8)^{b}	3.8 (2.4 - 4.8)^{a}
nSpcI	($x10^8$)		0.5 (0.2 - 1.3)^{e}	0.8 (0.5 - 0.9)^{e}	1.3 (1.1 - 1.7)^{c}	1.1 (0.6 - 1.6)^{d}	2.0 (1.6 - 2.6)^{b}	3.1 (2.1 - 3.9)^{a}
spdR/Sert			0.4 (0-3.2)^{d}	2.0 (1.6-2.7)^{c}	4.0 (1.8-4.6)^{b}	6.0 (4.8-7.4)^{a}	3.9 (3.1-5.4)^{b}	7.2 (5.3-8.6)^{a}
***tGm/Sert**			7.3±0.5^{cd}	5.4±0.5e	6.5±0.5^{ed}	9.9±0.6b	8.4±0.6^{c}	12.7±0.5^{a}
Mind	(%)		6.0 (0-17.5)^{e}	35.5 (20.8-57.5)^{d}	58.5 (35.7-72.2)^{bc}	83.5 (71.3-95.8)^{a}	46.9 (46.3-47.5)^{c}	59.2 (48.2-75.6)^{b}
tGcPlp	($x10^8$)		0.6 (0.2 - 1.9)^{e}	1.3 (1.0 - 1.6)^{d}	2.8 (2.2 - 3.8)^{c}	3.2 (1.9 - 4.7)^{c}	4.1 (3.2 - 5.3)^{b}	7.2 (5.3 - 9.3)^{a}
appØ	(μm)	74.4 (65.0-77.2)^{f}	134.1 (108.2-247.6)^{e}	266.0 (247.5-300.3)^{d}	326.8 (257.6-364.0)^{c}	312.5 (288.4-418.1)^{b}	384.0 (340.0-423.6)^{ab}	397.8 (362.1-426.7)^{a}
actØ	(μm)	87.2 (59.2-102.7)^{f}	199.2 (165.8-331.3)^{e}	335.2 (318.0-372.8)^{d}	387.7 (341.4-413.5)^{c}	451.5 (392.6-493.0)^{b}	492.7 (403.6-556.1)^{ab}	501.1 (465.3-534.2)^{a}
nSert	($x10^7$)	0.7 (0.4-1.6)^{f}	1.5 (0.6 - 2.0)	2.3 (1.9 - 2.8)^{d}	4.7 (3.7 - 6.4)^{b}	3.3 (1.9 - 4.1)^{c}	4.9 (4.2 - 5.8)^{b}	5.7 (4.3 - 7.1)^{a}
Vr	(%)	60.0 (55.5-68.5)^{e}	86.0 (72.0-94.0)^{d}	90.0 (86.0-94.0)^{c}	96.0 (94.0-98.0)^{b}	96.0 (92.0-99.0)^{b}	96.0 (92.0-99.0)^{b}	98.0 (92.0-99.0)^{a}
Lt	(m)	2.5(1.8-5.0)^{g}	4.9 (3.9-6.8)^{f}	6.1 (5.4-6.8)^{e}	9.8 (9.1-10.5)^{c}	8.5 (4.8-10.2)^{d}	10.7 (8.3-11.7)b	11.3 (8.7-13.2)^{a}
TW	(mg)	5.0 (2.8-7.8)^{e}	38.5 (23.0-91.5)^{d}	94.5 (82.5-133.5)^{c}	192.5 (131.5-241.3)^{b}	170.5 (86.5-304.5)^{bc}	365.5 (226.1-428.6) ^{a}	351.0 (246.0-408.5)^{a}

*Mean±SEM. Abbreviations: nSpdR: Round spermatids population, nSpcI : TypeI spermatocyte population, spdR/ Sert: Round spermatids/Sertoli cell, tGM/Sert: Total number of germ cells per Sertoli cell, Mind: Meiotic index, tGcPlpn: Total germ cell population, nSert: Sertoli cells population, act□: actual tubular diameter, app□: Apparent tubular diameter, Vr: Relative volume of seminiferous tubules, Lt: Seminiferous tubular length, TW: Testicular weight

Table 2: Correlations among testicular morphometric characteristics, testicular sperm production and peripheral testosterone concentrations in guinea cocks

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	nSpdR	nSpcI	spdR/Sert	tGM/Sert	Mind	tGcPlp	appØ	actØ	nSert	Vr	Lt	TSP	Testo Conc
nSpcI	.678***												
spdR/Sert	.610***	.535***											
tGm/Sert	.541***	.586***	.926***										
Mind	.455***	.204**	.697***	.448***									
tGcPlp	.750***	.937***	.712***	.675***	.438***								
appØ	.582***	.548***	.607***	.560***	.488***	.587***							
actØ	.610***	.509***	.602***	.560***	.481***	.543***	.853***						
nSert	.583***	.792***	.289***	.198**	.310***	.771***	.473***	.433***					
Vr	.272***	.249***	.319***	.236***	.398***	.281***	.453***	.529***	.341**				
Lt	.426***	.360***	.324***	.182**	.395***	.435***	.361***	.386***	.537***	.429***			
TSP	.297*	-.195	.291*	.258*	.472**	.171	-.129	-.105	.184	-.006	.195		
Testo Conc	.156	-.010	.260*	.238*	.160	.107	.039	0298*	.004	-.020	.239*	-.157	
TW	.035	-.033	-.247*	-.403**	.061	.012	.212	.354**	.327**	.098	.500***	.459**	.563**

Abbreviations: nSpdR: Round spermatids population, nSpcI : TypeI spermatocyte population, spdR/ Sert: Round spermatids/Sertoli cell, tGM/Sert: Total number of germ cells per Sertoli cell, Mind: Meiotic index, tGcPlpn: Total germ cell population, nSert: Sertoli cells population, act□: actual tubular diameter, app□: Apparent tubular diameter, Vr: Relative volume of seminiferous tubules, Lt: Seminiferous tubular length, Testo Conc: Peripheral testosterone concentration, TW: Testicular weight