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Photoperiodic-dependent histomorphological changes in the excurrent duct system of helmeted guinea fowl subjected to short day (8L:16D), long-day (16L:8D) light/dark cycles and exogenous melatonin

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ABSTRACT

In the present study, the influence of varying photoperiods [short day light 8L:16D, long day light 16L;8D] and exogeneous melatonin on the excurrent duct system of male helmeted guinea fowl was investigated using histo-morphological and histometric approaches. A total of twenty-eight (28) guinea fowl birds were randomly divided into Group I: Short daylight (SD; 8 HL), Group II: (SD +1mg/kg melatonin; 8 HL+ Mel), Group III: Long daylight (LD; 16 HL) and Group IV: (LD +1mg/kg; 16 HL + Mel)] and comprises of seven birds (n=7) per group. At the end of the 8 weeks of experimentation, the excurrent ducts were excised and processed for routine histological examination and the variations in histo-morphometrical parameters were determined using the GIMP2 software. Histologically, apart from the moderate cellular degeneration observed in efferent duct epithelia of the SD subgroups: (8 HL and 8 HL + Mel), there was remarkable spermatozoa presence in the lumens of the epididymal duct and ductus deferens of both 16 HL and 16 HL + Mel groups. The histo-morphometric data (luminal, ductal diameters and epithelial heights) were significantly increased ($p < 0.05$) in the excurrent ducts of guinea fowl exposed to 16 HL and 16 HL + Mel, as compared to other groups. There was significant decrease ($p < 0.05$) in stereocilia height (SH) in 16 HL compared to 8 HL sub-groups of lower segments. Although, a non-significant ($p > 0.05$) increase in SH was observed in melatonin-treated groups, regardless of photoperiod. Taken together, these sets of data from this study indicate the importance of artificial light and exogenous melatonin in the control of seasonality of reproduction and which could be used to influence the reproductive cycle of the guinea fowl.

1. Introduction

The helmeted Guinea fowl (*Numida meleagris*) is a poultry bird with seasonal reproductive cycles. This opportunistic breeder, breeds only in spring and rainy seasons in Europe and Africa, respectively. The species has not attracted much attention principally because it is considered uneconomic due to its seasonal egg production, low fertility and slow growth rate (Ayorinde, 1991; Houndonougbo et al., 2017). In seasonal breeders, the reproductive activity is inhibited by reducing organs weight and volume and by lowering sperm and spermatocyte production during the non-reproductive season (Onuora, 1982; Abdul-Rahman et al., 2016; Mohan et al., 2016; Alli et al., 2016). Unlike temperature or rainfall, photoperiod is one of the most important factors affecting seasonal reproductive behaviour and is invariant from year to year, day length therefore provides a highly reliable anticipatory cue for future or distant seasonal conditions (Dawson et al., 2001; Bradshaw and Holzapfel, 2007; Dixit and Singh, 2012).

Photoperiodic signals are translated into effects on the reproduction system by alteration in the pattern of secretion of melatonin from the pineal gland, which in-turn affects the pulsatile release of gonadotropin releasing hormone (GnRH) from the hypothalamus. This results in alterations in the release of gonadotropic hormones regulating spermatozoa production and maturation in epididymis (Sancho et al., 2004, Rani and Kumar, 2014) and also, the seasonal variations associated with

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semen production (Azubuike et al., 2017).

Recent mammalian studies on seasonal reproductive rhythms have shown that the epididymis of Viscacha (*Lagostomus maximus maximus*) exposed to long daylight exhibited a low epithelium and a wide lumen totally occupied by dense sperm mass. On the contrary, lower presence of stored sperm cells and abundant detached epithelial cells were examined under the short daylight (Cruceño et al., 2013). In addition, it was reported that short daylight stimulation induced morphological changes in the epididymis of Syrian hamster, and changes included a decrease in luminal diameter and the disappearance of spermatozoa (Calvo et al., 1997).

The photo-dependent pineal hormone, melatonin, plays an important role in the neuroendocrine control of reproductive cycle of seasonal breeders in a pro- or anti-gonadotropic, depending on the species (Koziol et al., 2020). However, membrane receptors (MT1 and MT2) of the hormone, melatonin have been demonstrated in the testis and epididymis of some species of non-seasonal (Izzo et al., 2010; Yang et al., 2014) breeder mammals. This suggests, melatonin could have a direct regulatory effect on reproductive processes independent of the hypothalamic-pituitary-gonadal/epididymal axis. In Leydig cells, it regulates the synthesis of testosterone (Frungieri et al., 2005; Deng et al., 2018), whereas in Sertoli cells it plays a role in cellular growth, proliferation, energy metabolism and oxidation state (Yang et al., 2014; Rocha et al., 2014). Similarly, in the epididymis, it induces the proliferation of epithelial cells (Shiu et al., 2000) and regulates their function (Mukherjee and Haldar, 2014).

Artificial lighting regimes and exogenous melatonin administration may be used to influence the reproductive functions of birds with respect to annual changes. Recently, biostimulation using photoperiod (Ogawa et al.,1993; Calvo et al., 1997; Cruceño et al., 2013; Mou et al., 2020; Baso et al., 2022) and melatonin (Juss et al., 1993; Shiu et al., 2000; Mousa-Balabel and Mohamed, 2011; Assia and Boulakoud, 2014) to improve reproductive efficiency in male reproductive organs of mammals and birds have become common practice.

Unlike mammals, most of the avian species do not have accessory sex glands which add secretory products to the semen, but does have secretory cells in the epithelium of the excurrent ducts (Tingari and Lake, 1972). The excurrent duct system of birds comprises the epididymal region and ductus deferens (Aire et al., 1979; Maruch et al., 1998; Kirby and Froman, 2021), and these ducts are no longer regarded as mere conduit pipes for the transportation of spermatozoa to the exterior (Ilio and Hess, 1994), but have profound influences on the subtle process of sperm maturation and viability (Clulow and Jones, 1988; Cooper et al., 2002). It is well established that the combined secretory and absorptive functions performed by the ductal epithelial cells allow the maintenance of a microenvironment in which gametes interact with the secretion produced by the ducts cells and acquire a potential capacity to fertilize (Robaire and Hermo, 1988; Amann et al., 1993; Elbashir et al., 2021).

The functional maturation of spermatozoa results from their exposure to the luminal environment of the epididymis and ductus deferens where they undergo a series of morpho-biochemical modifications and is a fundamental step in the acquisition of their fertilizing ability (Zhou et al., 2018; James et al., 2020). In spite of numerous reports and increasing body of evidence on the morpho-physiological adaptations of spermatozoa along epididymal transit in mammalian and avian species, there are few or no reports on the effects of photoperiodism and exogenous melatonin on the avian excurrent duct system. Therefore, the present study, was designed to investigate the histo-morphological changes in the excurrent duct system of guinea fowl subjected under controlled artificial photoperiod conditions and melatonin administration using both histological and histo-morphometric approaches.

2. Materials and methods

2.1. Study area

The experiment was carried out at the Avian & Aqua Culture Research Center of the Department of Veterinary Anatomy, Ahmadu Bello University, Zaria (11°4′N, 7°42′E), located in the Northern Guinea Savannah Zone of Nigeria, with a mean (± standard error) monthly photoperiod of 12.13 ± 0.13 h (Kowal and Knabe, 1972; Dzenda et al., 2011). The zone has nearly equal amounts of light and darkness (12L:12D) within a 24 h period throughout the year and is characterized by three major seasons; Harmattan (November- February), Hot-Dry (March- May) and Rainy (June – October) seasons (Ayo et al., 1999, Dzenda et al., 2011).

2.2. Pzocurement, preparation and administration of melatonin

The melatonin (Mel) Melatonin (Mel) [CAS number 73-31-4, analytical grade, 99.73%] used in this study, was obtained from Med-Chem Express (Princeton, NJ, USA). It was dissolved in 100% ethanol at 0.1 M for stock solution and further diluted to 25 Mm with PBS (Hu et al., 2017). Thereafter, 1mg/kg bwt of melatonin was administered via intramuscular (IM) route, according to the modified method of Soni et al. (2017).

2.3. Animals and management

A total of twenty-eight (28) sexually-mature helmeted guinea fowls (*Numida meleagris*) were purchased from the local market in Zaria, Kaduna State, Nigeria. Prior to purchase, the sexes were distinguished through visualization of the vent and the use of helmet shape as well as wattle size and shape; according to the selection criteria described by Umosen et al. (2008) and Yakubu et al. (2022). The selected male birds were transferred using standard transport basket stackable of 600 × 400 × 300 mm dimensions and transported to the Avian Research and Aqua Culture Centre in the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, where the experiment was conducted. The birds were acclimatized for two weeks prior to the commencement of the study. The birds were intensively managed on deep-litter system in a fenced poultry house, in order to reduce the stress of confinement or restricted movement, and commercial feed (Vital feed® Jos, Nigeria) and water provided *ad libitum*.

2.4. Experimental design

Twenty-eight (28) apparently normal sexually-mature helmeted guinea fowls (weighing between 1.0-1.48kg) were randomly divided into photoperiodic regimes, as described by Thiele (2009) (based on hours of light exposure (light/dark cycle), into experimental groups namely: [Group I: Short daylight (SD; 8 HL (Hour of light); Group II: SD +1mg/kg melatonin; 8 HL+ Mel) lights on 7:00 h, lights off 15:00 h; Group III: Long daylight (LD; 16 HL) and Group IV: (LD +1mg/kg melatonin; 16 HL + Mel) lights on 7:00 h, lights off 23:00 h] of seven birds per group (n=7), conditioned for 8 weeks of experimentation. Each group was demarcated by placement in a black light-tight chamber, with each partition of dimension of 200 × 110 × 100 cm; illuminated by two cool-white fluorescent lights (12W) that provided light intensity of approximately 350 lux, for short-day (8L:16D) and long-day (16L:8D) photoperiods. The room temperature was kept constant at 27±2°C. The exogeneous melatonin was injected daily intramuscularly at least 2 h before the end of light phase of each group (Juss et al.,1993). The birds were treated humanely in strict compliance with protocols approved by the Ahmadu Bello University Institutional Animal Care and Use Committee vide approval number of (ABUAUC/2022/021). In addition, the animal experimental data reported in this study are in compliance with the ARRIVE (Animal Research: Reporting In Vivo Experiments)

guidelines. (Kilkenny et al., 2010). Fig. 1

2.5. Sample collection

The guinea fowls were euthanized using intramuscular injection of ketamine and xylazine combination at a dose of 35mg/kg and 5mg/kg respectively (Flecknell, 2015). Thereafter, the thoraco-abdominal cavity of each bird was dissected gently to exteriorize the reproductive organs. The testes together with their attached epididymis were removed (along their longitudinal axis). The epididymides (i.e., left and right) were then excised off the body of the testes, freed of all adhering fat and connective tissues, and the ductus deferens also separated from adjoining structures for histo-morphological studies.

2.6. Tissue preparation

The specimens from different regional segments of the excurrent duct system (i.e., epididymis and ductus deferens) were used for histo-morphological studies. Samples were fixed in Bouin's solution for 24 h. The fixed tissues were dehydrated through a series of graded concentrations of ethanol (70%, 80%, 90%, 95% and 100%) for 1.5 hrs with gentle shaking and soaked in absolute ethanol overnight. The tissues were immersed in xylene three times for 30 min and infiltrated in molten paraffin wax (Bancroft and Gamble, 2007). Sections of 5 μm thick were cut from the embedded tissues and mounted on clean grease-free glass slides and stained at room temperature using Haematoxylin (Sigma-Aldrich) and Eosin (Sigma-Aldrich) stains for 5 min for routine histological studies (Bancroft and Gamble, 2007). The stained slides were viewed for histo-architectural changes in the excurrent duct system using a bright-field optical microscope (Olympus^{TM}, Japan) attached to digital camera equipped with imaging software, MII ImageView version 3.7.9229 (YSC Technologies, Fremont, CA, USA).

2.7. Histomorphometry

The histo-morphometric measurements of the components of excurrent duct system (efferent duct, connecting duct, ductus epididymis and ductus deferens) were performed by modified methods of Omirinde et al. (2021). From the photographed (X10 objective lens) sections of each segment of the duct in different groups of guinea fowl (n=7), about twenty (20) round or nearly round ductal profiles were randomly selected for the evaluation of following parameters: the epithelial height (EH), stereocilia height (SH), luminal diameter (LD) and ductal diameter (DD) using GIMP2 software. The epithelial height was estimated using linear measurement between the ductal basement membrane and its apex. Also, the ductal and luminal diameters were determined from four orthogonal lines with a common intersection. However, stereocilia height was determined by a linear measurement on epithelia surface modification (stereocilia) from photomicrographs captured at x40 objective lens. Data were finally expressed in micrometers (μm).

2.8. Data analyses

The histometric data generated were expressed as mean (± standard error of the mean) (SEM). One-way analysis of variance (ANOVA) (SPSS version 16.0) was performed followed by the Tukey's post hoc for multiple comparisons. Value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Histology

The excurrent duct system in guinea fowl used for this study was generally observed to be constituted by the rete testis, efferent (proximal and distal) duct, connecting duct, epididymal duct and ductus deferens (Fig. 2). The effects of varying photoperiod and exogenous melatonin administration on the proximal efferent duct histology is shown in Fig. 3 A-D. Both guinea fowl exposed to 8 h only (8 HL) and those co-administered melatonin (8 HL + Mel) displayed moderate cellular degeneration in the proximal efferent duct parenchyma cells. However, efferent duct in guinea fowl exposed to 16 HL only and those exposed to 16 HL and exogenously treated with melatonin (16 HL + Mel) displayed normal histoarchitecture typified by distinctly folded pseudostratified columnar epithelium with patent lumen.

Also, the histological profile of the distal efferent duct in guinea fowl exposed to varying photoperiod and exogenous melatonin is presented

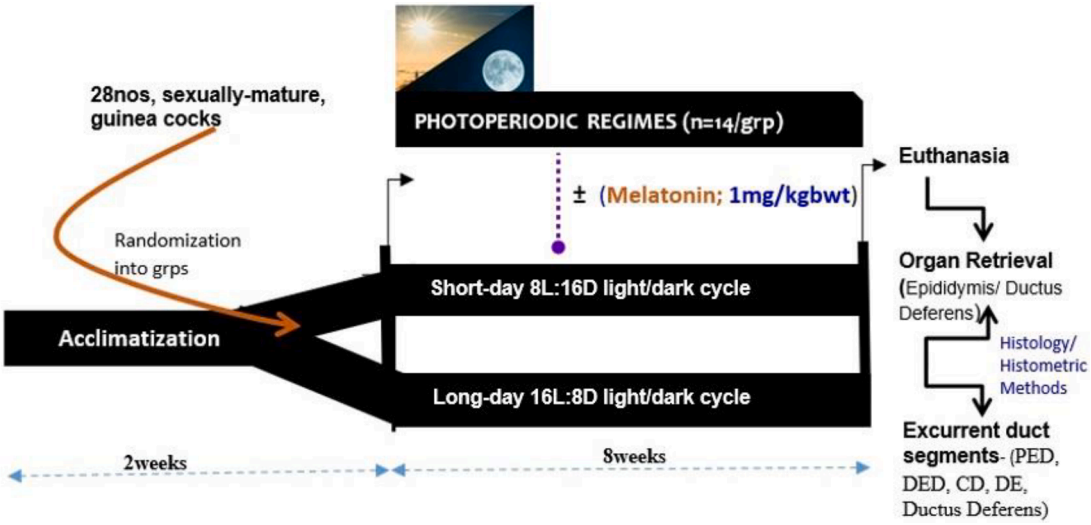

Fig. 1. Schematic representation of the Experimental design used in this study. Twenty-eight (28) sexually-mature male guinea fowl cocks (*Numida meleagris*) were acclimatized for 2 weeks, and randomly assigned into two main groups (n =14/group); and thereafter, subjected to varying photoperiodic regimes: viz; short-day (SD; 8L:16D) and long-day (LD; 16L:8D), with or without exogenous melatonin (Mel) [i.e. 1 mg/kgwt-^{d}] administered daily, throughout 8 wks period of experimentation. The harvested testes were dissected and freed from the adherent connective tissues and subsequently, the retrieved organs (i.e epididymal/ductus deferens tissues) were analyzed using histological and histometric approaches. Excurrent duct segments studied include: Proximal Efferent Duct (PED), Distal Efferent Duct (DED), Collecting Duct (CD), DE-Ductus Epididymis and Ductus Deferens.

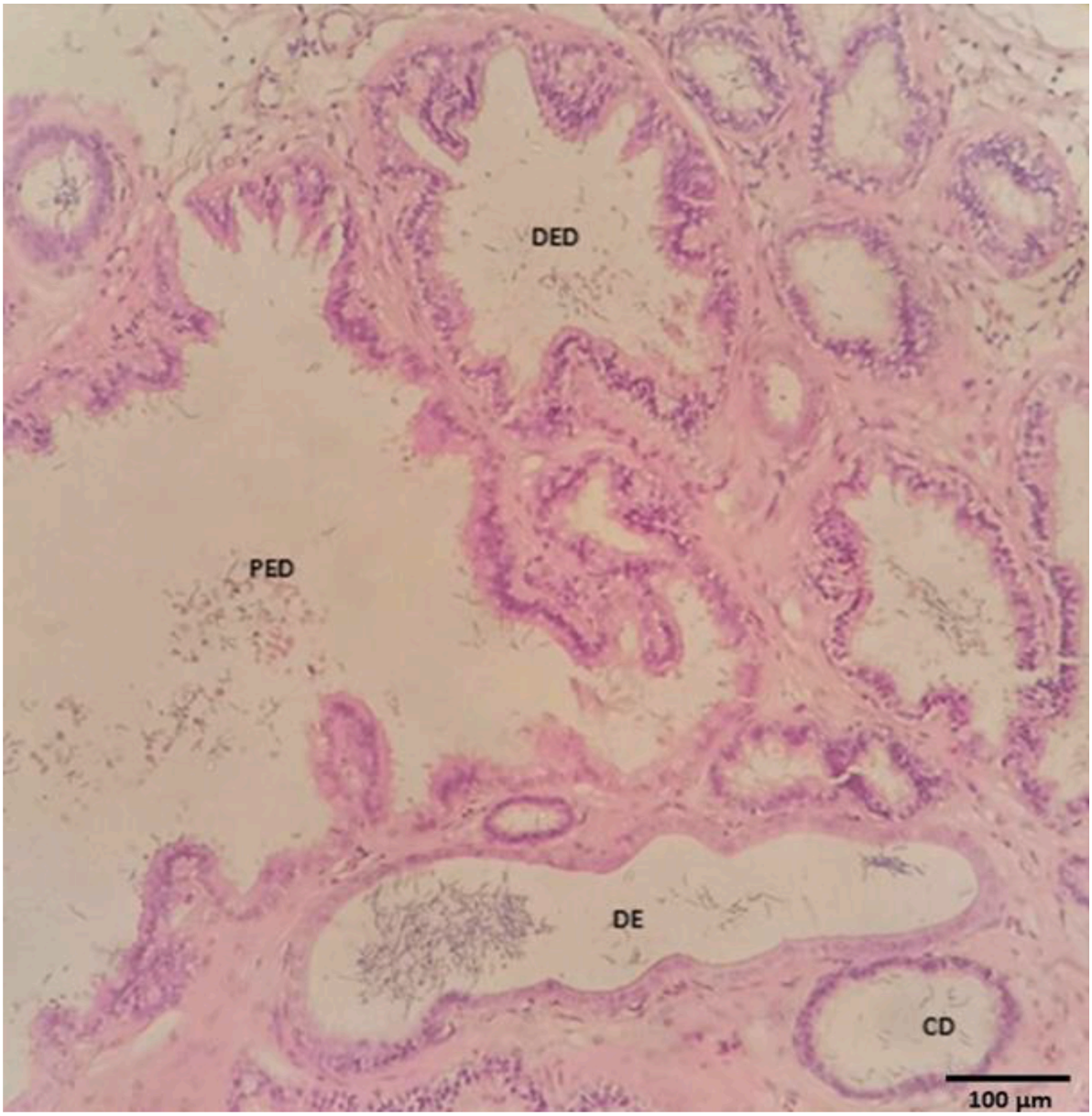

Fig. 2. Survey photomicrograph of the excurrent duct of guinea fowl (*Numida meleagris*) showing the different regional segments: (proximal efferent duct (PED), distal efferent duct (DED), connecting duct (CD) and ductus epididymis (DE) of the duct systems. Stain: Haematoxylin and Eosin; **Scale bar: 100 µm**.

in Fig. 4 A-D. The distal efferent duct in 8 HL only group guinea fowl bear no visible lesion except for scanty luminal content. On the contrary, the guinea fowl exposed to 8 HL and exogenously administered melatonin (8 HL+ Mel) had moderate epithelial cell degeneration with some distinct cytoplasmic vacuolation and scanty luminal content. For the two categories of 16 HL exposed guinea fowl (16 HL+ Mel and 16 HL), the distal efferent duct showed normal histoarchitecture.

In addition, the histological effect of varying photoperiod and exogenous melatonin on the connecting duct of guinea fowl is presented in Fig. 5 A-D. Interestingly, the connecting duct in the two subdivisions of 8 HL (8 HL+ Mel. and 8 HL only) groups displayed scanty spermatozoa in their lumen with exclusive presence of mild cellular degeneration (black arrow) in the connecting duct epithelium of the melatonin-treated groups. However, both the two subgroups of 16 HL (16 HL + Mel and 16 HL only) retain their normal architecture; though, there was a distinct presence of numerous luminal spermatozoa in 16 HL + Mel compared to 16 HL.

For the epididymal duct histological profile in guinea fowl exposed to varying photoperiod and exogenous melatonin (Fig. 6 A-D). The ductus epididymis across the different groups of guinea fowl bears normal histoarchitecture characterized by tortuous epithelium made up of pseudostratified columnar epithelium with cilia. Of note is the substantial presence of spermatozoa in the lumen of 16 HL+ Mel compared to its counterpart 16 HL only.

The histological profile of the ductus deferens in the different groups of guinea fowl exposed to varying photoperiods and exogenous melatonin is shown in Fig. 7 A-D. The ductus deferens across the different groups of guinea fowl showed normal histoarchitecture. However, appreciable spermatozoa contents (asterisk) were exclusively found in the ductus deferens lumens in 16 HL+ Mel. and 16 HL sub groups.

3.2. Histomorphometric results

3.2.1. Proximal efferent duct

There was a significant increase ($p < 0.05$) in luminal diameter (LD) in guinea fowl exposed to 16 HL+ Mel and 16 HL groups compared to

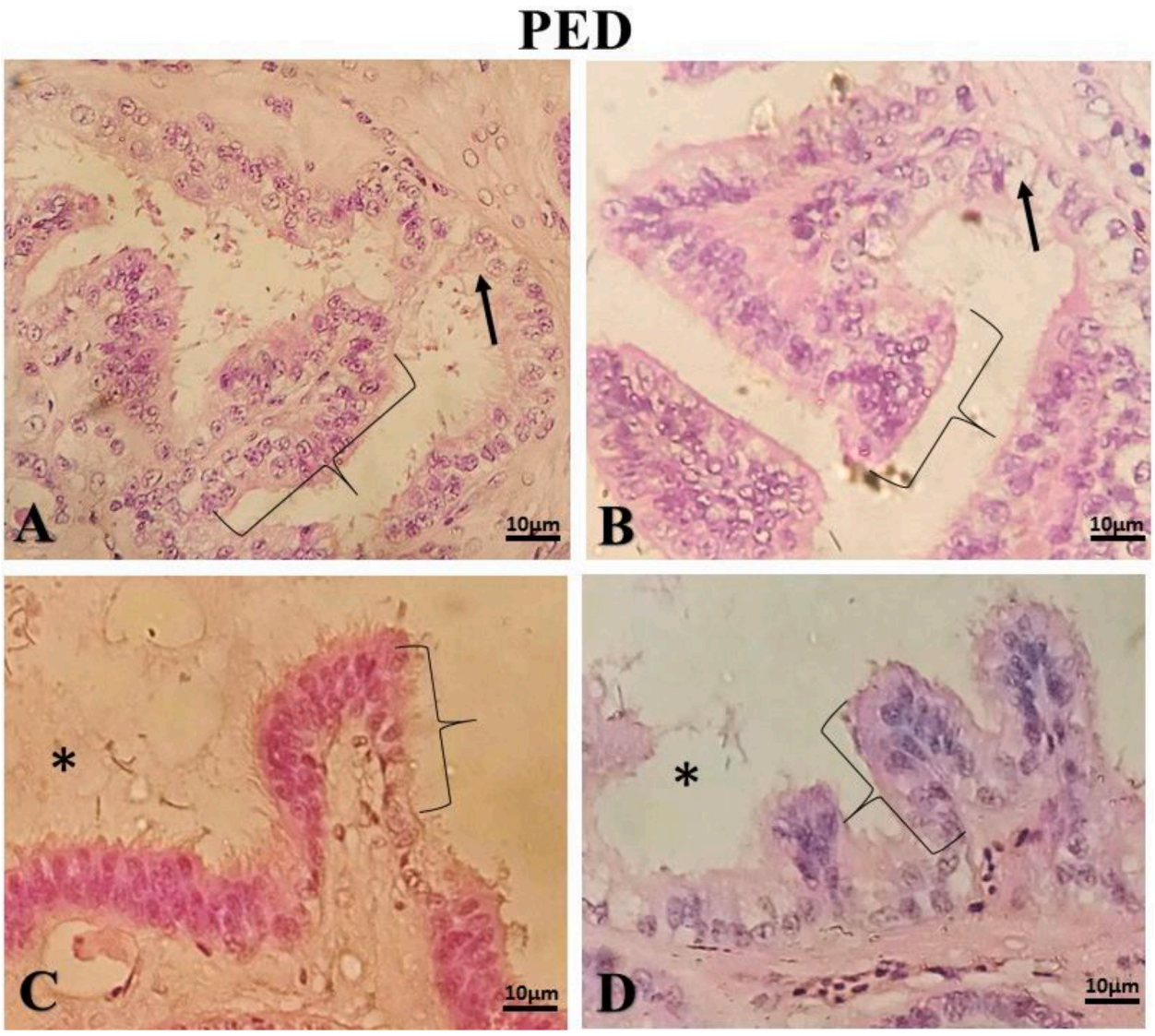

Fig. 3. Photomicrograph of the proximal efferent duct (PED) in the different groups of guinea fowl (*Numida meleagris*) exposed to photoperiod and exogenous melatonin. **A**). 8 HL+ Mel and **B**). 8 HL: Apart from the typical epithelial folding (brace), there is presence of moderate cellular degeneration in the efferent duct parenchyma cells, **C**). 16 HL+ Mel and **D**). 16 HL: No visible lesion as evidenced by distinctly folded pseudo-stratified columnar epithelium with patent lumen. Stain: Haematoxylin and Eosin; **Scale bar: 10 µm**.

others. The mean ductular diameter (DD) of guinea fowl exposed to 16 HL+ Mel and 16 HL groups increased significantly ($p < 0.05$) compared to other groups. Similarly, the epithelial height (EH) was significantly increased ($p < 0.05$) in guinea fowl exposed to 16 HL+ Mel relative to other treatments. However, there was no significant difference ($p > 0.05$) in stereocilia height (SH) across the groups, though an insignificant increased value was observed in the 8 HL+Mel group compared to others (Table 1).

3.2.2. *Distal efferent duct*

The distal efferent LD values in guinea fowl exposed to 8 HL+Mel and 8 HL were significantly decreased ($p < 0.05$) compared to other treatment groups. Also, the DD of guinea fowl in 8 HL+ Mel and 8 HL groups decreased significantly ($p < 0.05$) when compared to other treatments. However, the distal efferent duct EH was significantly increased ($p < 0.05$) in guinea fowl exposed to 16 HL+ Mel relative to other groups. Regarding SH, there was no significant difference ($p > 0.05$) in the SH values across all the groups; though an insignificantly increased value was displayed by 8 HL+ Mel groups relative to others. (Table 1).

3.2.3. *Connecting duct*

There was a significant increase ($p < 0.05$) in connecting duct LD in guinea fowl exposed to 16 HL+ Mel and 16 HL groups compared to others. Similarly, the connecting duct DD values were increased significantly ($p < 0.05$) in 16 HL+Mel and 16 HL groups compared to other treatments. On the contrary, the connecting duct EH values were not significantly different ($p > 0.05$) across the various treatment groups. On the profile of stereocilia height (SH), significantly higher SH value was recorded in 8 HL+ Mel group compared to others (Table 1).

3.2.4. *Ductus epididymidis*

The ductus epididymis LD values were significantly increased ($p < 0.05$) in 16 HL+ Mel and 16 HL groups of guinea fowl compared to others. Also, the epididymal DD values were increased in groups exposed to 16 HL+Mel and 16 HL, compared to others. The ductus epididymidis EH was significantly decreased ($p < 0.05$) in guinea fowl exposed to 16 HL, compared to others. Similarly, the SH values were

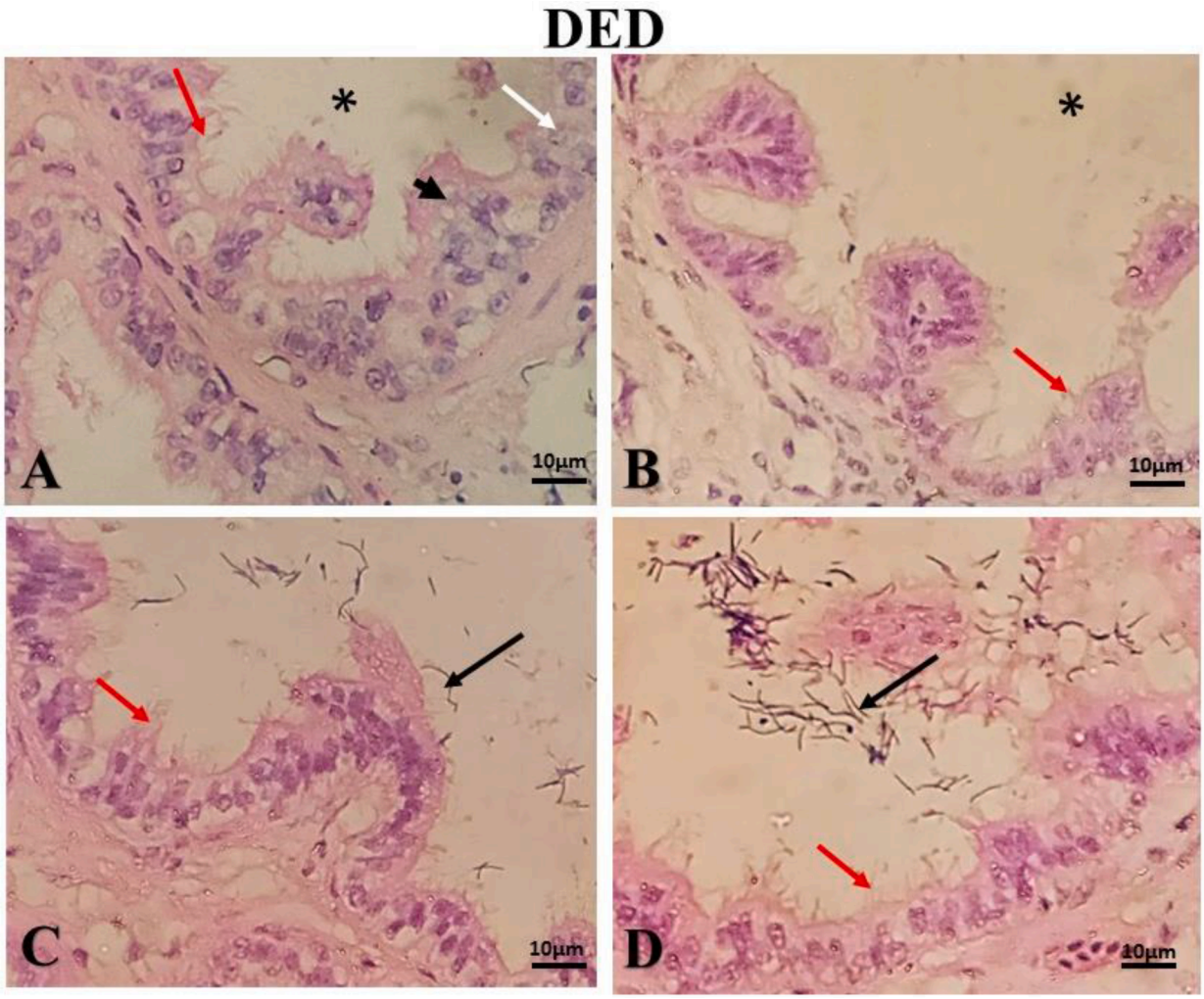

Fig. 4. Photomicrograph of the distal efferent duct (DED) in the different groups of guinea fowl (*Numida meleagris*) exposed to photoperiod and exogenous melatonin showing **A**). 8 HL+ Mel.: There is moderate epithelial cell degeneration (white arrow) with some distinct cytoplasmic vacuolation (black arrowhead) and very scanty luminal content (black asterisk). **B**). 8 HL: with the exception of scanty lumen (black asterisk), ductal histoarchitecture bear no visible lesion as evidenced by remarkable epithelial folding (brace), pseudostratified epithelial nature (black line) and presence of distinct stereocilia (red arrow) on the apical part of the epithelium. **C**). 16 HL+ Mel and **D**). 16 HL: with the exception of the presence of scanty lumina spermatozoa (black arrow), there is no visible lesion in the distal efferent duct parenchyma as typified by prominent epithelium with intact stereocilia (red arrow). Stain: Haematoxylin and Eosin; **Scale bar: 10 µm**.

higher in groups exposed to 8 HL+Mel and 8 HL when compared to other treatment groups (Table 1).

3.2.5. *Ductus deferens*

3.2.5.1. Cranial segment. The LD, DD and EH values of the cranial ductus deferens were significantly increased ($p < 0.05$) in the 16 HL+Mel group when compared to others. However, SH values were significantly reduced in 16 HL+Mel and 16 HL groups compared to others (Table 1).

3.2.5.2. Middle and caudal segment. The similar trend of significant increase ($p < 0.05$) in LD, DD and EH values noted in cranial segment was recorded in guinea fowl exposed to 16 HL+Mel relative to others. It is important to mention that there was no significant difference ($p > 0.05$) between the LD, DD and SH values of 16 HL and 16 HL+Mel groups across all the segments of ductus deferens (Table 1).

4. Discussion

The excurrent duct system in guinea fowl used for this study was generally observed to be constituted by the rete testis, efferent (proximal and distal) duct, connecting duct, epididymal duct and ductus deferens. This finding corroborated plethora of documentations on components of avian excurrent duct system in roosters (Razi et al., 2010), domestic fowl (Tingari, 1971, 1972; Budras and Sauer, 1975; Aire et al., 1979), Japanese quail (Aire, 1979), pigeon (Stefanini et al., 1999), turkey (Hess et al., 1976; Noori et al., 2018), other domestic duck (Aire, 1982; Simões et al., 2004) and ostrich (Aire and Soley, 2000; Freneau et al., 2021).

The histopathological lesions precipitated in the proximal efferent duct of the short daylight subgroups (8 HL and 8 HL + Mel) are suggestive of the potential of this photoperiodic duration in the impairment of histoarchitecture of the efferent duct. Again, the exclusive observation of histoarchitectural distortion in the epithelium of distal efferent duct of guinea fowl exogenously administered melatonin (8 HL+ Mel) appeared to be surprising. As available reports on exogenous melatonin studies (Seymen et al., 2021) have shown remarkable histo-architectural enhancement of the excurrent duct system. However, in the present study, this morphological distortion could be attributed to shorter duration of light exposure and exogeneous melatonin administration.

The presence of appreciable luminal spermatozoa in the connecting duct of 16 HL + Mel compared to 16 HL could be associated with intense

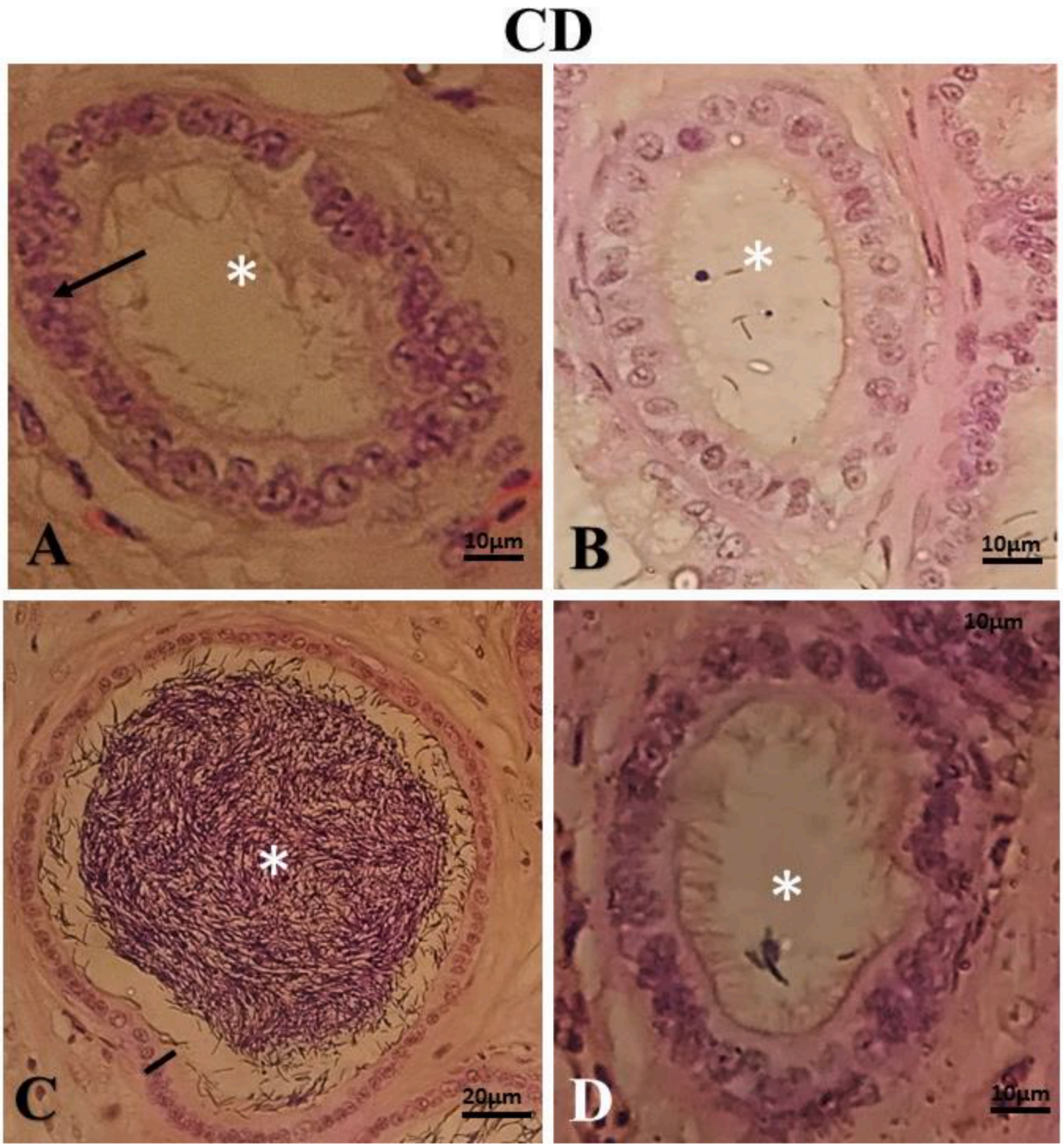

Fig. 5. Photomicrograph of the connecting duct (CD) in the different groups of guinea fowl (*Numida meleagris*) exposed to photoperiod and exogenous melatonin showing **A**). 8 HL+ Mel and **B**). 8 HL: bear scanty spermatozoa in their lumens (white asterisk) and exclusive presence of mild cellular degeneration (black arrow) in the connecting duct epithelium of the Mel-treated groups; **C**). 16 HL+ Mel: No visible lesion as shown by the typical pseudostratified columnar epithelium (black line) and presence of numerous luminal spermatozoa (white asterisk); **D**). 16 HL: Except for scanty luminal spermatozoa (white asterisk), there is no visible lesion in the connecting duct parenchymal histoarchitecture. Stain: Haematoxylin and Eosin; **Scale bars: 10 µm and 20 µm**.

morpho-physiological activeness of this group, occasioned most probably by longer duration of light and melatonin administration. While the scanty spermatozoa distribution in the connecting ducts of the short-day light counterparts implied a condition of reduced reproductive vigour or vitality.

The normal epididymal histological appearance observed across all the groups concurs with the typical epididymal histo-morphology earlier reported in avian species (Abdul-Rahman and Jeffcoate, 2018; Freneau et al., 2021). Also, the functional relevance of this normal epididymal histoarchitecture in all the groups studied is that both photoperiodism and melatonin administration seemed not to induce histoarchitectural distortion. The presence of numerous spermatozoa in the epididymal duct lumen of 16 HL+ Mel, compared to others most especially, the 16 HL only group, could be ascribed to the potential of melatonin in boosting reproductive vigour of the guinea fowl in this group. This finding corroborates the reports of Mousa-Balabel and Mohamed (2011), Assia and Boulakoud (2014), Egerszegi et al. (2014). The histomorphological features of lack of luminal spermatozoa within epididymal duct of guinea fowl exposed to short photoperiod could probably be a consequence of lack of photoinduction. These findings are similar to the report of Cruceño et al. (2013) on photoperiodic treatments in Viscacha, a south American wild rodent.

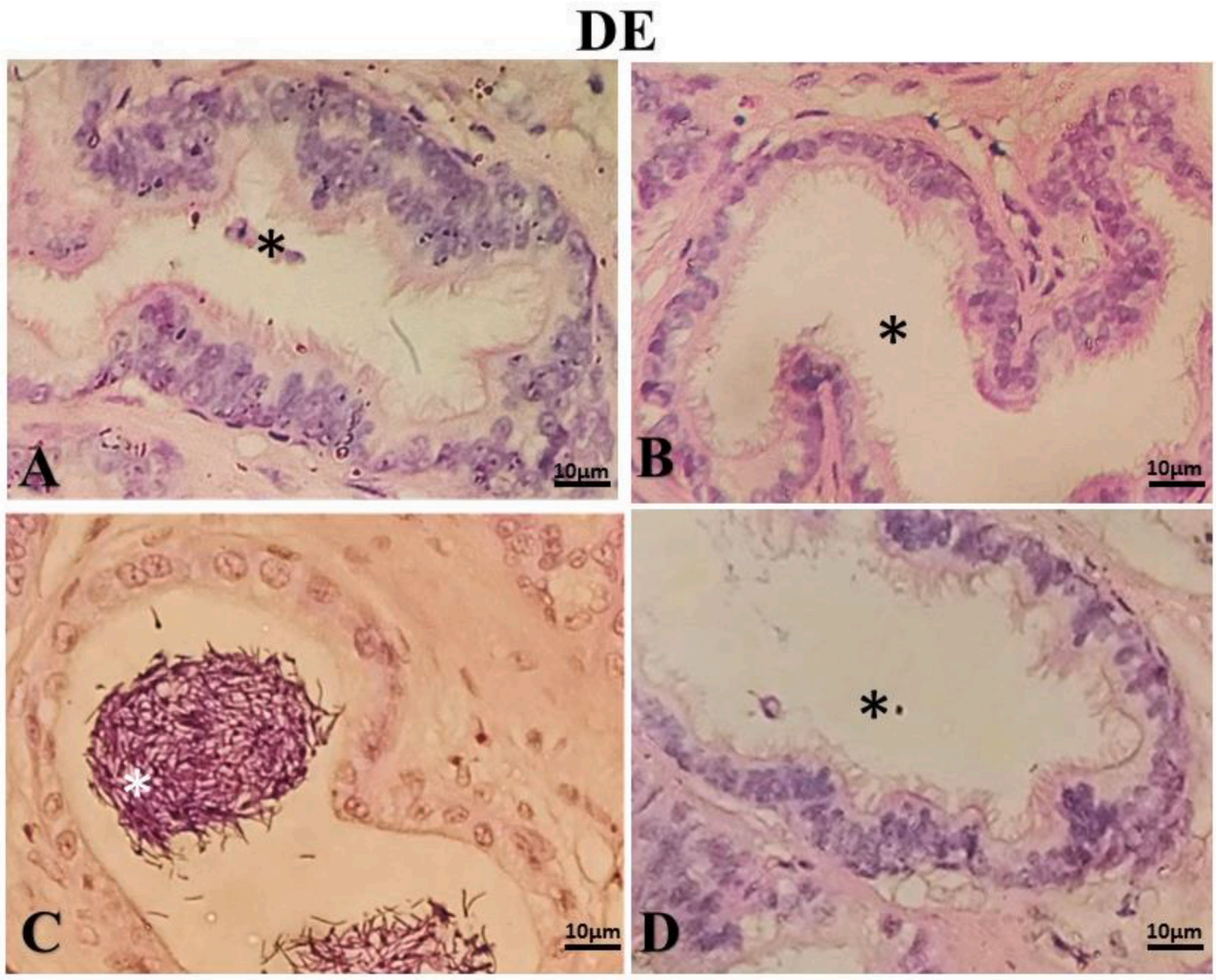

Fig. 6. Photomicrograph of the ductus epididymis (DE) in the different groups of guinea fowl (*Numida meleagris*) exposed to photoperiod and exogenous melatonin showing **A**). 8 HL+ Mel. and **B**). 8 HL: bear normal histoarchitecture characterized by tortuous epithelium made up of stereo ciliated pseudostratified epithelium. The luminal space was devoid of spermatozoa (asterisk). **C**). 16 HL+ Mel.: bears normal histological features with remarkable luminal spermatozoa content (white asterisk). **D**). 16 HL: Has normal epididymal duct histology but scanty luminal spermatozoa (asterisk). Stain: Haematoxylin and Eosin; **Scale bar: 10 µm**.

The finding on increased ductal spermatozoa in the ductus deferens of 16 HL+ Mel and 16 HL sub groups observed in this study, tallies wholly with the histological result in connecting duct and partially with epididymal duct in this group. This finding further substantiates the possible synergy between photoperiod and exogenous melatonin in the enhancement of reproductive organ vigour.

Regarding the histo-morphometric data, the observed increased luminal and ductal diameter of the various segments of the excurrent duct (proximal and distal efferent duct, connecting duct, epididymal ducts and ductus deferens) with increased photoperiod, regardless of melatonin treatment. This suggests that, longer photoperiod is an important phenomena that could exert morphological influence on the excurrent duct architecture, more particularly on the efferent duct and ductus deferens.

The notable findings on the efferent duct and ductus deferens histo-morphometric data from this study, concurs with the report of Calvo et al. (1997) in Syrian Hamster, Cruceno et al. (2013) in wild rodent Viscacha (*Lagostomus maximus maximus*) Grahmajo-Bühler et al. (2015) in *Chinchilla lanigera* Grey and Mou et al. (2020) in striped dwarf hamster. However, markedly lower luminal and ductal diameters were reported for proximal efferent duct by Aire et al. (1979) and Abdul-Rahman and Jeffcoate (2018) in the same guinea fowl cock. Of special note is the observed slight insignificant increase in nearly all of the luminal and ductal diameters of melatonin treated guinea fowl compared to their counterpart non-melatonin treated group. In addition, as revealed from this study, exogeneous melatonin resulted in an increased ductal epithelial height regardless of photoperiod regimes. Thus, the epithelial height increment which was more remarkable in the PED (of guinea fowl subjected to 16 HL) could be attributed to the epithelial cell stimulatory potential of melatonin. This is consistent with the observation of Li et al. (1999) and Shiu et al. (2000) in rats, where exposure of epididymal epithelial cells to melatonin beyond a critical length of time (12 to 24 h) under *ex vivo* conditions exerted stimulatory effect on epididymal epithelial cell proliferation.

Thus, the insignificant changes observed in SH of the efferent ductal segments, may be attributed to unique morpho-physiological adaptive characteristics of the highly-specialized epididymal segments. Although, a significant decrease ($p < 0.05$) in stereocilia height (SH) was observed in 16 HL compared to 8 HL sub-groups of lower segments. This observation concurs with the findings of Cruceño et al. (2013) in the epididymis of Viscacha (*Lagostomus maximus maximus*), while working on photoperiod treatments. The observed insignificant increase in SH in the melatonin-treated groups, in this study, could be attributed to the stimulatory role of melatonin on stereocilia on the luminal surface of epididymal micro-environment.

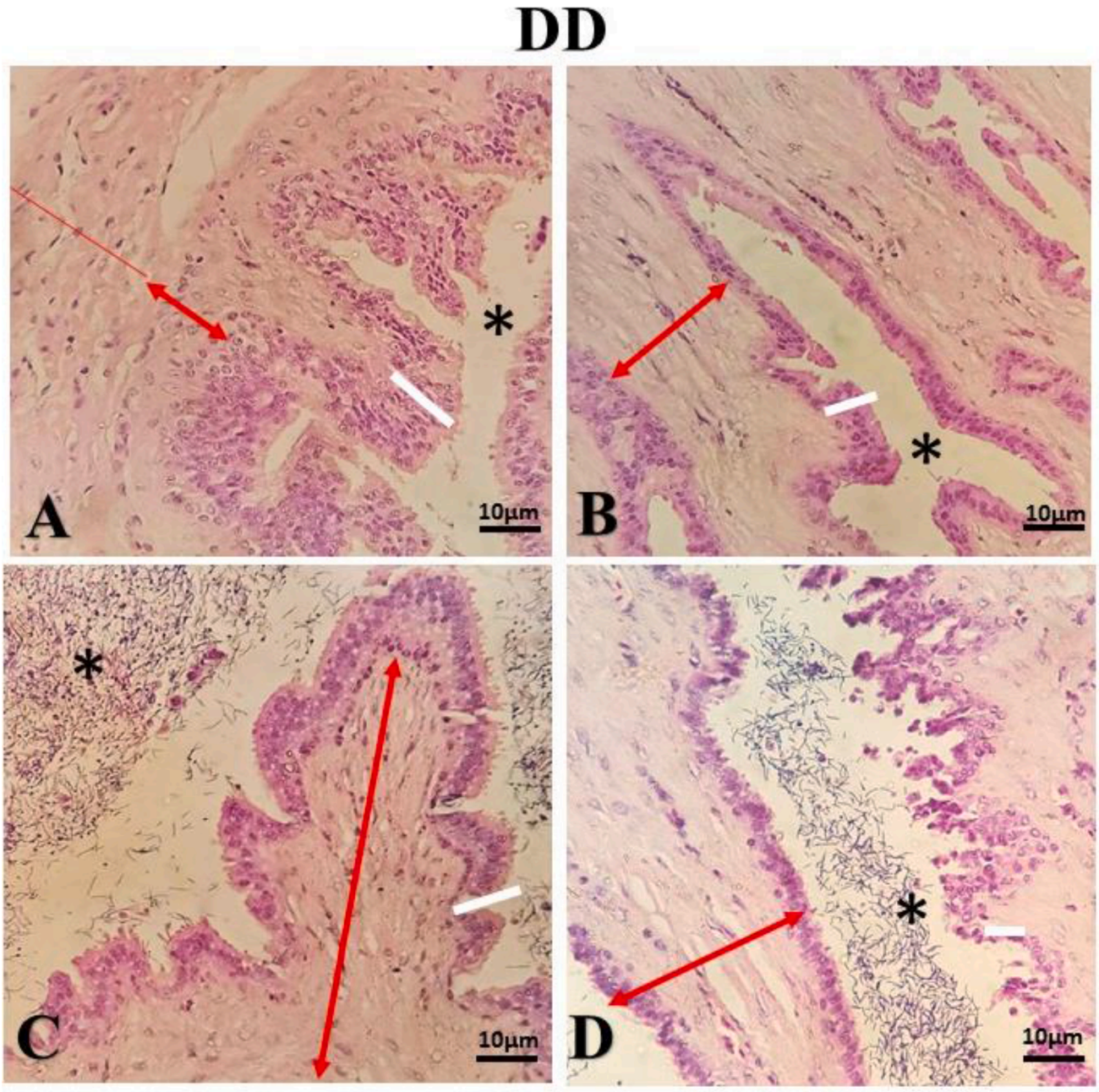

Fig. 7. Photomicrograph of the ductus deferens (DD) in the different groups of guinea fowl (*Numida meleagris*) exposed to photoperiod and exogenous melatonin showing **A**). 8 HL+ Mel and **B**). 8 HL: display normal histoarchitecture characterized by mucosa thrown into longitudinal folds (white line) with an irregular luminal outline and distinct lamina propria (red double arrow head) thick tunica muscularis (red line) with an overlapping tunica serosa. Also, the luminal space was devoid of spermatozoa (asterisk); **C**). 16 HL+ Mel and **D**). 16 HL: bears normal histological features with appreciable luminal spermatozoa content (asterisk). Stain: Haematoxylin and Eosin; **Scale bar: 10 µm**.

5. Conclusion

Taken together, the study has established that the structural changes observed along the highly-specialized excurrent duct transit in the helmeted guinea fowl, were potentially due to the combined effects of photoperiodic regimes and exogeneous melatonin. The findings from this study would be useful, especially to guinea fowl breeders/producers in designing appropriate intervention programs necessary to influence the reproductive cycle, in this specie, regardless of the season.

Ethical approval and consent statement

The experimental procedure and animal protocols used this study were evaluated and approved by the Institutional Animal Use and Care Ethical Committee of Ahmadu Bello University, Zaria, and ethical clearance (vide approval #: ABU-IAUC/2022/021).

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Table 1
Histomorphometric changes in the excurrent duct system of helmeted guinea fowl exposed to photoperiods and exogenous melatonin.

Parameters	SD; 8 HL	Experimental groups SD; (8 HL+Mel)	LD; 16 HL	LD; (16 HL+Mel)	P-Value
		Proximal Efferent Duct			
DEH (μm)	103.52 ± 8.84[a]	86.62 ± 4.56[a]	105.69 ± 9.80[a]	145.54 ± 14.65[b]	0.00
DSH (μm)	8.85 ± 0.27[a]	9.30 ± 0.20[a]	8.45 ± 0.32[a]	8.99 ± 0.41[a]	0.19
DLD (μm)	223.75 ± 8.08[a]	215.97 ± 10.37[a]	451.00 ± 33.44[b]	509.38 ± 22.42[b]	0.00
DD (μm)	422.95 ±24.78[a]	406.47 ± 20.39[a]	596.36 ± 33.19[b]	631.38 ± 47.27[b]	0.00
		Distal Efferent Duct			
DEH (μm)	52.81 ± 3.44[ab]	39.60 ± 3.16[a]	64.72 ± 5.06[b]	76.08 ± 6.48[c]	0.00
DSH (μm)	8.57 ± 0.24[a]	9.28 ± 0.28[a]	8.02 ± 0.33[a]	9.01 ± 0.41[a]	0.00
DLD (μm)	156.96 ± 8.08[a]	138.41 ± 7.17[a]	255.79 ± 7.78[b]	251.46 ± 14.63[b]	0.00
DD (μm)	207.72 ± 9.63[a]	198.63 ± 9.77[a]	348.30 ± 9.93[b]	315.94 ± 16.20[b]	0.00
		Connecting Duct			
DEH (μm)	22.82 ± 0.91[a]	21.48 ± 1.24[a]	19.21 ± 0.72[a]	21.28 ± 0.88[a]	0.27
DSH (μm)	12.05 ± 0.39[b]	14.28 ± 0.40[a]	8.16 ± 0.34[c]	8.79 ± 0.37[c]	0.00
DLD (μm)	57.37 ± 2.15[a]	55.62 ± 2.02[a]	106.12 ± 6.58[b]	97.94 ± 7.58[b]	0.00
DD (μm)	95.26 ± 3.09[a]	93.16 ± 3.98[a]	137.29 ± 6.13[b]	124.59 ± 7.03[b]	0.00
		Ductus Epididymidis			
DEH (μm)	30.09 ± 1.77[a]	25.19 ± 1.99[a b]	20.71 ± 0.50[b]	30.58 ± 2.07[a]	0.00
DSH (μm)	12.06 ± 0.45[a]	12.86 ± 0.42[a]	8.87 ± 0.38[b]	8.78 ± 0.39[b]	0.00
DLD (μm)	112.35 ± 7.68[a]	104.97 ± 5.26[a]	176.38 ± 13.71[b]	165.06 ± 9.75[b]	0.00
DD (μm)	158.19 ± 8.17[a]	147.25 ± 4.90[a]	215.15 ± 13.78[b]	226.16 ± 10.55[b]	0.00
		Cranial Ductus Deferens			
DEH (μm)	35.27 ± 1.78[a]	43.15 ± 4.12[a b]	51.30 ± 4.62[b]	68.00 ± 3.46[c]	0.00
DSH (μm)	8.50 ± 0.46[a]	9.40 ± 0.38[a]	6.56 ± 0.52[b]	6.30 ± 0.39[b]	0.00
DLD (μm)	291.55 ± 40.12[a]	236.23 ± 46.08[a]	584.49 ± 55.34[b]	604.74 ± 35.53[b]	0 0.00
DD (μm)	551.39 ± 84.83[a]	531.27 ± 75.28[a]	623.12 ± 44.48[a]	630.43 ±39.30[a]	0.28
		Middle Ductus Deferens			
DEH (μm)	54.84 ± 7.92[a]	62.60 ± 4.83[a b]	61.67 ± 3.44[a b]	86.61 ± 9.71[b]	0.00
DSH (μm)	8.73 ± 0.54[a b]	9.38 ± 0.40[a]	6.05 ± 0.64[c]	6.69 ± 0.55[b c]	0.00
DLD (μm)	416.65 ± 54.25[a]	290.24 ± 58.17[a]	764.23 ± 65.16[b]	792.40 ± 61.24[b]	0.00
DD (μm)	727.71 ± 41.56[a,b]	576.59 ± 48.20[a]	961.82 ± 88.37[b]	886.11 ± 95.58[ab]	0.01
		Caudal Ductus Deferens			
DEH (μm)	57.86 ± 5.48[a]	80.49 ± 7.93[a]	81.78 ± 3.24[a b]	139.45 ± 31.19[b]	0.00
DSH (μm)	8.85 ± 0.37[a]	9.00 ± 0.50[a]	6.28 ± 0.76[b]	6.42 ± 0.40[b]	0.00
DLD (μm)	763.11 ±48.41[a,b]	428.61 ± 71.75[a]	815.33 ± 76.14[b]	1077.40 ± 99.36[b]	0.00
DD (μm)	1040.80 ±47.41[a,b]	726.29 ± 86.12[a]	1190.80 ± 81.10[b]	1110.09 ± 135.78[ab]	0.02

[a,b,c]Values with the different superscript alphabets in the same row differ significantly at $p < 0.05$. DEH- Ductal epithelial height, DSH- Ductal stereocilia height, DLD- Ductal luminal diameter, DD- Ductal diameter.

CRediT authorship contribution statement

Abdullahi Baso: Investigation, Writing – original draft, Visualization, Formal analysis, Resources. **Umar M. Bello:** Conceptualization, Supervision, Methodology, Writing – review & editing. **Mohammed H. Sulaiman:** Supervision, Project administration. **Innocent J. Gosomji:** Methodology, Formal analysis, Resources. **Oyewole J. Omirinde:** Formal analysis, Writing – review & editing. **Mansur Zubairu:** Methodology. **Muazu. T. Abubakar:** Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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