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Gonadosomatic and Histomorphological Bases of Sterility in Intergeneric Hybrid Male Mule and Hinny Ducks

Research Article

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ABSTRACT

Reproductive failure due to hybrid sterility is common among vertebrates, including birds. This study examined the causes of sterility in intergeneric hybrid male Mule and Hinny ducks by comparing their gonadosomatic index (GSI) and testicular histo-architecture with those of their parental species: Muscovy (*Cairina moschata*) and Mallard (*Anas platyrhynchos*) ducks. Testes from ten males of each genotype were collected, weighed, and processed histologically using Hematoxylin and Eosin staining. Analysis of variance showed that genotype significantly ($P<0.05$) influenced testis size (TS) and GSI. Mallard ducks had the highest TS (24.30 g) and GSI (1.74%), followed by Mule (TS: 9.73 g; GSI: 0.30%) and Hinny ducks (TS: 8.06 g; GSI: 0.27%), while Muscovy ducks recorded the lowest (TS: 2.49 g; GSI: 0.09%). Left testes were generally larger than right ones across all genotypes, though not significantly ($P>0.05$). Histological analysis revealed normal testicular organization in Muscovy and Mallard ducks, with all spermatogenic stages (spermatogonia, spermatocytes, spermatids, and spermatozoa) present, as well as mature sperm cells in the seminiferous tubules and epididymides. Conversely, Mule and Hinny males exhibited impaired spermatogenesis characterized by production of only primary spermatocytes and epididymal vacuoles devoid of mature sperm cells. It is concluded that the sterility of Mule and Hinny hybrid males is not related to testis size or GSI but results from their inability to complete spermatogenesis and produce mature sperm cells capable of fertilization of ova.

KEY WORDS epididymal semen, Muscovy duck, spermatogonia, testicular section, testis size.

INTRODUCTION

The occurrence of sterile male hybrids resulting from intergeneric and interspecific crossings has been widely documented across both vertebrates and invertebrates (Liang and Sharakhov, 2019; Niayale *et al.* 2021; Molnar *et al.* 2024). Such hybrids often display phenotypic, behavioural, and physiological traits that may resemble those of their parents or differ from them either subtly or conspicuously. Hybridisation between closely related taxa has long intrigued biologists because of its implications for the biological species concept, the unique nature of the resulting offspring, and its potential role as a rapid mechanism for genetic evolution through introgression (Deviche *et al.* 2011). The incidence of hybridisation varies considerably among avian orders, with Anseriformes (waterfowl: ducks, geese, and swans) showing the highest propensity to hybridise (Ottenburghs *et al.* 2016). Indeed, Ottenburghs *et al.* (2015)

reported that over 60% of waterfowl species have hybridised with at least one other species, a figure that rises to nearly 77% when captive hybrids are included.

The Mule duck is a well-known intergeneric hybrid, produced from crossing a male Muscovy duck (*Cairina moschata*) with a female Common duck (*Anas platyrhynchos* var. *domestica*) (Li *et al*. 2020). This hybrid exhibits marked heterosis, expressed as enhanced disease resistance, superior feed efficiency, and improved meat quality relative to its parental species. In addition, Mule ducks show no pronounced sexual dimorphism, lack typical sexual behaviours, and are sterile. Owing to their high meat yield and fatty liver production, Mule ducks have become one of the most commercially important avian hybrids worldwide.

Contrary to Mule ducks, Hinny ducks are produced through the intergeneric cross between a male Common duck and a female Muscovy duck. This hybrid is less common and less popular than the Mule duck and has not been widely exploited for commercial duck meat production, possibly due to its relatively smaller body size. Unlike Mules, Hinnies exhibit sexual dimorphism in traits such as growth rate, plumage colour, adult body weight, and expression of secondary sexual behaviours. Nevertheless, both Mule and Hinny ducks demonstrate hybrid vigour in terms of growth performance and lean meat production when compared with their parental species. A shared characteristic of both hybrids is their sterility, which remains a major limitation to their large-scale multiplication, commercial production, and utilisation.

Over the past decade, multiperspective approaches including molecular genetics, proteomics, transcriptomics, physiology, and endocrinology have been employed in efforts to elucidate the mechanisms underlying male hybrid sterility (Niayale *et al*. 2021). Despite these efforts, the nature and complexity of hybrid incompatibilities remain poorly understood, largely because of the difficulty in identifying interacting loci that influence complex phenotypes. Hybrid sterility, a postzygotic reproductive isolation mechanism, occurs across all sexually reproducing organisms and results from the accumulation of genetic incompatibilities. It plays a critical role in maintaining species boundaries (Forejt and Jansa, 2023) by preventing successful interbreeding between distinct species (Islam *et al*. 2013).

In male birds, the reproductive system consists of paired testes, epididymides, and ductus deferens (Bachmid *et al*. 2019). The testes are the primary reproductive organs, responsible for testosterone synthesis and sperm production (Mfoundou *et al*. 2022). They comprise interstitial tissue and seminiferous tubules, the latter serving as the sites of spermatogenesis and accounting for the majority of testicular mass in fully developed testes (Deviche *et al*. 2011). Empirical studies investigating sterility phenotypes in animals often assess testis shape, size, and weight, alongside sperm morphology, density, and motility, as key indicators of male sterility (Liang and Sharakhov, 2019).

The gonadosomatic index (GSI) refers to the relative weight of the gonads compared to total body weight and is commonly used to assess sexual maturity in relation to testicular development (Tamilselvan *et al*. 2018). This reproductive index reflects the proportional increase in gonad size relative to body weight during development (Chakraborty *et al*. 2024). It has been widely employed to evaluate sperm production efficiency and is regarded as a reliable indicator of reproductive performance in livestock (Mfoundou *et al*. 2022), including chickens (Bachmid *et al*. 2019), guinea fowl (Tamilselvan *et al*. 2018), and turkeys (Masyitha *et al*. 2021), among others.

The GSI is a critical reproductive performance index because reproduction is a fundamental biological process that determines the survival and continuity of a species (Al-Deghayem *et al*. 2017). Similarly, Yang *et al*. (2021) reported that GSI serves as an indicator of sexual maturity, with testis weight, body weight, and GSI values in poultry correlating strongly with sexual maturity and fecundity. Consequently, any testicular defect is expected to adversely affect the physiological functions of the testes and, in turn, impair the reproductive performance of the affected animal. Although numerous scientific approaches have been applied to investigate sterility across different animal classes particularly mammals and avian species, including waterfowl; comparative studies focusing on GSI and testicular histomorphology in ducks and their intergeneric hybrids remain limited. In light of this, the present study was designed to investigate the GSI and testicular histomorphological bases of sterility in intergeneric hybrid male Mule and Hinny ducks.

MATERIALS AND METHODS

Experimental ducks and management

A total of forty (40) healthy, sexually mature adult male ducks were used for this study, comprising ten (10) males each of Muscovy, Mallard, Hinny, and Mule ducks. The source of the birds, acclimatization period, and management prior to data collection followed the procedures described by Oguntunji *et al*. (2019).

Body weight measurement and gonadosomatic index

The body weight of each bird was recorded using a sensitive weighing scale, accurate to two decimal places (g). Following ethical sacrifice via jugular vein incision, both the left and right testes were carefully harvested from the abdominal cavity and weighed using an electronic balance,

also to the nearest two decimal places (g).

The gonadosomatic index (GSI) was calculated as the relative weight of paired testes to body weight, according to Muzaffar *et al*. (2024), using the formula:

Gonadosomatic index= (weight of paired testes (g)×weight of bird (g)) × 100

Histological analysis of the testes and epididymis

Testicular tissues collected from all forty (40) ducks were fixed in Bouin's fluid and processed using the routine paraffin embedding technique. Sections of 5 µm thickness were obtained with a Reichert Jung 2030© microtome, mounted on slides, and stained with Harris' Hematoxylin and Eosin (H&E). The stained sections were examined under an Acuscope® (China) light microscope. Similarly, epididymides were harvested, fixed in Bouin's fluid, and processed for histological examination following standard protocols. Paraffin sections of 5 µm thickness were prepared, stained with Hematoxylin and Eosin, and photomicrographs were captured at ×400 magnification.

Statistical analysis

Data on body weight, testicular weight, and gonadosomatic index were subjected to one-way analysis of variance (ANOVA), with genotype as the fixed effect. The general linear model applied was:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where:

Y_{ij}: individual observation.

μ: general mean.

G_i: fixed effect of genotype ($_i$=Muscovy, Mallard, Hinny and Mule ducks).

e_{ij}: experimental error assumed to be independently, identically and normally distributed, with zero mean and constant variance, i.e. nd (0, σ^2).

A 5% probability level was adopted, and analyses were performed using the Statistical Package for the Social Sciences (SPSS, 2024). Differences between means were separated using Duncan's New Multiple Range Test (DNMRT).

RESULTS AND DISCUSSION

The present study is the first cohort investigation to compare the gonadosomatic and histomorphological bases of sterility in parental and intergeneric hybrid (Mule and Hinny) ducks. Consequently, there is a paucity of literature for direct and critical comparison of the findings reported herein.

Genotype had a significant (P<0.05) effect on testis size (TS) and gonadosomatic index (GSI) across the four duck genotypes (Table 1). Mallard ducks exhibited the highest testicular mass (24.37 g) and GSI (1.74%), intermediate values were observed in the hybrids: Mule ducks (TS: 9.73 g; GSI: 0.30%) and Hinny ducks (TS: 8.06 g; GSI: 0.27%). Muscovy ducks had the lowest testicular mass (2.49 g) and GSI (0.09%). Across all four genotypes, the left testes were consistently heavier than the right; however, this asymmetry was not statistically significant (P>0.05) within genotypes.

The trend observed in this study, whereby the testis size of hybrid male ducks fell between those of the parental genotypes, aligns with the findings of Snapir *et al*. (1998), who reported average testis weights of 34.60 g, 19.40 g, and 7.00 g for Khaki Campbell (a Common duck breed), hybrid Mule, and Muscovy ducks, respectively. The intermediate TS and GSI recorded for Mule and Hinny ducks relative to their parental genotypes suggest a heterotic effect on testis size. In contrast, Castillo *et al*. (2012) observed no heterosis in testis size in interspecific hybrids of chicken and pheasant.

The observed testicular asymmetry across the four duck genotypes corroborates earlier reports in ducks (Gerzilov *et al*. 2016; Khatun *et al*. 2019; Kareem *et al*. 2020; Yang *et al*. 2021). Similar patterns have also been documented in other avian species, with Tamilselvan *et al*. (2018) reporting that the left testis was slightly larger and heavier than the right in guinea fowl, and Calhim and Birkhead (2009) reporting comparable asymmetry in Zebra finches. More recently, Molnar *et al*. (2024) noted that in an interspecific hybrid between chicken and guinea fowl, the left testis was longer and broader at the anterior end compared to the right one.

Contrastingly, some avian studies have reported results that differ from the left-biased testicular asymmetry observed in the present study. For instance, in Khaki Campbell drakes, the length and width of the right testis were greater than those of the left (Khatun *et al*. 2019). Yang *et al*. (2021) similarly reported that the right testes of Mule ducks were slightly heavier than the left. Castillo *et al*. (2012) also documented a marginally higher mass of the right testis compared with the left in chickens, pheasants, and their interspecific hybrids. Likewise, Calhim and Birkhead (2009) found no significant difference in red-billed quelea but noted that the right testis tended to be larger than the left.

Directional asymmetry in gonadal size is common among vertebrates and is especially pronounced in birds, where the left testis is most often larger than the right (i.e. left-biased directional testis size asymmetry) (Calhim and Montgomerie, 2015).

Table 1 Testicular size and gonadosomatic indices of testis of four duck genotypes

Duck genotype	Body weight (g)	Right testis (g)	Left testis (g)	Mean (g)	Gonadosomatic index (%)
Muscovy	2700	2.01±0.43^{ax}	2.97±0.10^{ax}	2.49±0.30^{x}	0.09^{x}
Mallard	1400	23.10±0.80^{bz}	25.63±0.90^{bz}	24.37±0.84^{z}	1.74^{y}
Mule	3230	9.34±1.40^{cy}	10.12±0.70^{cy}	9.73±1.12^{y}	0.30^{y}
Hinny	3020	7.00±1.40^{cy}	9.11±1.60^{ay}	8.06±1.40^{y}	0.27^{z}

$^{a, b, c.}$: the means within the same row with different letter, are significantly different (P<0.05).
$^{x, y, z:}$ the means within the same column with different letter, are significantly different (P<0.05).

According to Calhim and Montgomerie (2015) and Calhim *et al*. (2019), approximately 75% of bird species exhibit this left-biased pattern. Nevertheless, the underlying basis of testicular asymmetry remains unresolved, and its functional significance for reproduction is unclear (Arian *et al*. 2024). Several hypotheses have been proposed by avian biologists to explain this phenomenon (Delehanty and O'Hearn, 2005; Calhim and Birkhead, 2009; Arian *et al*. 2024).

In recent years, numerous empirical studies have explored the relationship between testicular size, gonadosomatic index (GSI), and sperm production. A high GSI has been suggested as an indicator of greater sperm production efficiency (Tamilselvan *et al*. 2018). This observation builds on earlier reports that, in domestic animals, testicular size is highly correlated with sperm output; thus implying that larger testes typically produce more sperm (Omari *et al*. 2018). Similarly, Dharani *et al*. (2018) described GSI as a measure of the efficiency with which males produce spermatozoa, while Masyitha *et al*. (2021) reported a direct relationship between testis weight and the volume of seminiferous tubules (ST).

Contrary to the direct relationship between testis size (TS), gonadosomatic index (GSI), and sperm production reported by previous researchers, the present findings revealed that intergeneric hybrid males had significantly higher TS and GSI; approximately four- and three-fold greater, respectively, than the corresponding values recorded for non-sterile parental Muscovy ducks. Based on this, it can be inferred that testis size, testicular asymmetry, and GSI are not directly associated with the sterility observed in hybrid male ducks. This suggests that the sterility of Mule and Hinny drakes is not a function of testicular size, asymmetry, or GSI, but may instead be attributed to other, as yet undetermined, genetic or non-genetic factors.

Normal testicular histoarchitecture, characterised by well-organised germinal stages and intact seminiferous tubule (ST) structures, was observed in Muscovy (Figure 1A) and Mallard (Figure 1B) ducks, but not in their hybrid counterparts. The STs of Muscovy and Mallard ducks displayed lumina filled with abundant germ cells at various stages of spermatogenesis including spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa anchored to Sertoli cells.

Thin intertubular connective tissues separated adjacent tubules, and mature spermatozoa were evident in the epididymal sections (Figures 2A and 2B).

In contrast, the testicular architecture of Mule (Figure 1C) and Hinny (Figure 1D) ducks was similar to each other but markedly different from that of the parental species. In both hybrids, germ cells were arrested at the primary developmental stage (spermatogonia), with no evidence of spermatocytes or spermatozoa. Their STs were fewer in number, irregular in shape, more constricted, and frequently vacuolated. Many lumina contained few germ cells or were devoid of spermatocytes altogether. The interstitial connective tissues were noticeably thicker compared to those in parental ducks. Furthermore, no spermatozoa were detected in the epididymal sections of either Mule (Figure 2C) or Hinny (Figure 2D) males.

Comparative studies on the testicular histoarchitecture of parental and hybrid male ducks are limited. However, the presence of all stages of spermatogenesis in the testicular tissues of Mallard and Muscovy ducks observed in this study is consistent with earlier reports. Gerzilov *et al*. (2016) documented the presence of spermatids at four months of age and mature sperm cells at five months in Muscovy ducks. Similarly, Islam *et al*. (2013) reported that all stages of germ cells were present in Muscovy and Mallard ducks by six months of age. In another related study, Snapir *et al*. (1998) observed complete spermatogenesis, including mature spermatozoa, in the testicular sections of 27-week-old Khaki Campbell and Muscovy ducks.

In contrast, the absence of spermatozoa in the testicular tissues of Mule and Hinny ducks in the present study agrees with previous findings on intergeneric hybrid sterility. A pioneering investigation by Rigdon and Mott (1965) reported the absence of spermatozoa in the testes of Mule ducks produced from male Muscovy and female Common (Pekin and Mallard) ducks. They further noted the presence of many primary spermatocytes with abnormally condensed chromosomes but no spermatids or spermatozoa in the F1 hybrid testis. Later, Snapir *et al*. (1998) confirmed these observations, showing that Mule ducks possessed only spermatogonia and primary and secondary spermatocytes in their testicular tissues. Mature spermatozoa were absent, spermatids were rare, and some contained multiple nuclei within a single cell.

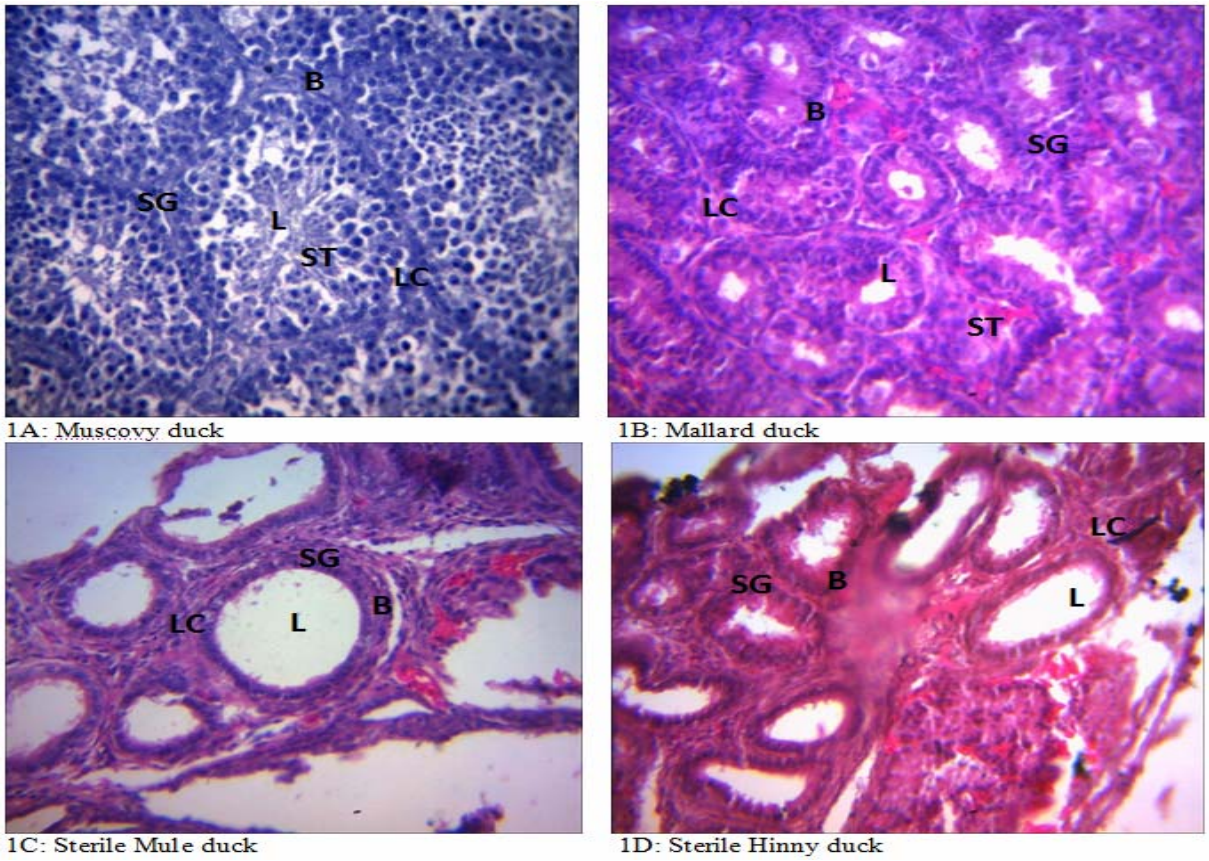

Figure 1 Representative micrographs of the testes of four adult male duck genotypes
L: lumen; B: basement membrane; SG: spermatogonia; LC: leydig cells and ST: spermatids

More recently, Li *et al.* (2020) compared the testicular morphology of Mule and Pekin ducks, reporting that spermatogonia and spermatocytes in Mule ducks were disorderly arranged, with no mature sperm cells present in the testis.

Comparative investigative research on seminiferous tubules (STs) in relation to the fertility of parental (Muscovy and Mallard) and intergeneric hybrid (Mule and Hinny) ducks remains sparse or virtually non-existent. Consequently, there is a scarcity of literature available for critical comparison of findings. In line with the present study, which reported the presence of mature sperm cells in the STs of parental (Muscovy and Mallard) ducks, previous histological examinations of Muscovy ducks similarly revealed numerous mature spermatozoa within significant portions of the seminiferous tubules. These spermatozoa were observed as clusters attached to Sertoli cells, while the lumen of the STs appeared large, irregular, and filled with spermatozoa as well as desquamated younger germ cell generations (Gerzilov *et al.* 2016). Likewise, different stages of spermatozoon development were identified in the STs of the Pekin duck (Li *et al.* 2020), a breed of the Common duck belonging to the same genus (*Anas*) as the Mallard.

In parallel with reports on the histomorphology of seminiferous tubules (STs) in male Mule and Hinny ducks, investigations into the widely documented sterility of hybrid male Mule ducks revealed that their STs were poorly developed, indistinct, and characterized by thick connective tissue (Li *et al.* 2020). Supporting this finding, Islam *et al.* (2013) observed a marked accumulation of primary spermatocytes within the seminiferous epithelium of F_1 hybrid ducks, many of which were extensively exfoliated into the lumina of the STs. Notably, neither spermatids nor spermatozoa were detected in the seminiferous tubules. A later report by Yang *et al.* (2021) further emphasized these abnormalities, describing the STs of intergeneric hybrid male Mule ducks as structurally abnormal compared with those of sexually mature poultry. The study highlighted pronounced individual variations in ST structure, with a complete absence of mature sperm cells capable of fertilizing ova.

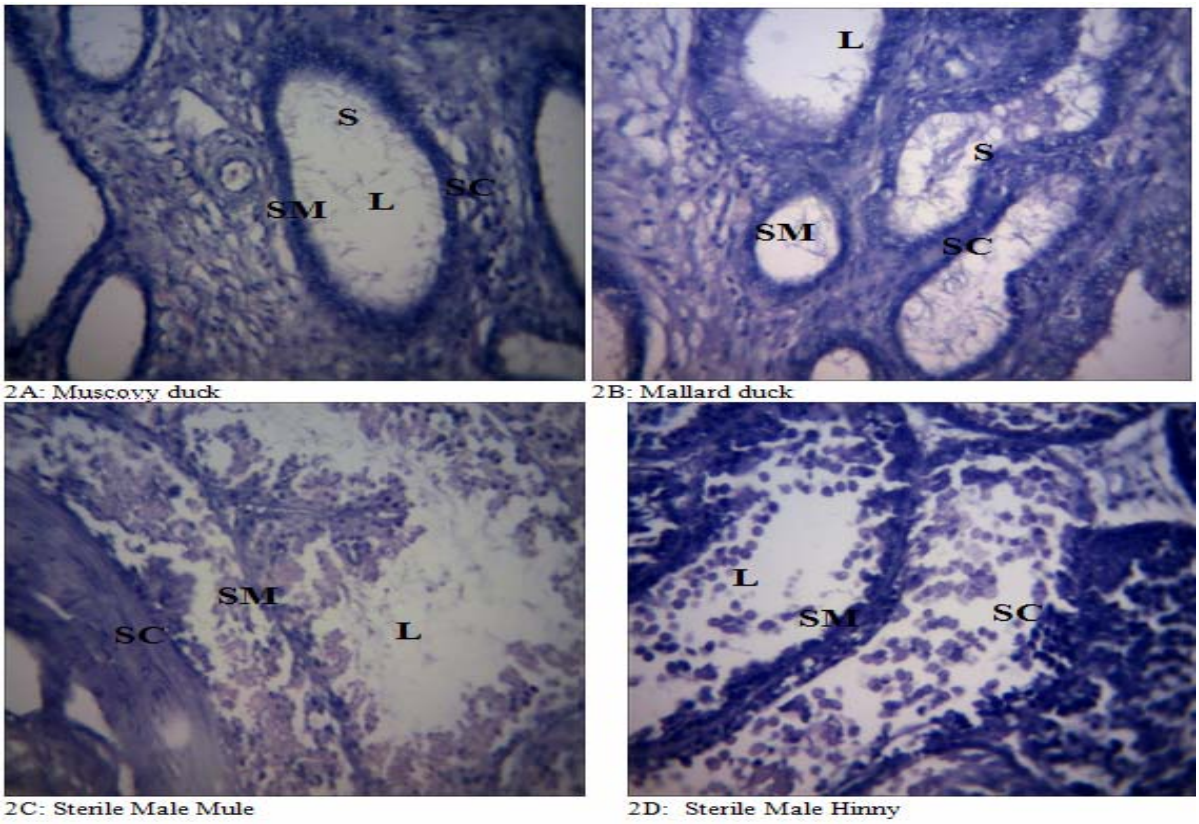

Figure 2 Representative micrographs of the epididymides of four adult male duck genotypes
L: lumen; S: spermatozoa; SC: stereocilia epithelium and SM; smooth muscle

Similar histological findings have been reported in the seminiferous tubules (STs) of sterile interspecific hybrids of guinea fowl and chickens between 16 and 20 weeks of age. These studies revealed tubular structures of irregular diameter with only two to three cell layers, minimal proliferative activity, and predominantly inactive or immature testicular cells.

Evidence of spermiocytomorphogenesis was absent, and the available germ cells failed to develop into spermatozoa during sexual maturation (Molnar *et al*. 2019; Molnar *et al*. 2024). However, in one interspecific hybrid, spermatid cells at different maturation stages and even mature sperm cells were observed (Molnar *et al*. 2024).

Seminiferous tubules play multifaceted roles in spermatogenesis, including germ cell proliferation, differentiation, and maturation, as well as the transport of spermatozoa within the testes. Their physiological integrity is therefore essential for fertility and reproductive efficiency in male animals. Structurally, STs consist of somatic cells (Sertoli and myoid cells) and germ cells (spermatogonia, spermatocytes, and spermatids) (Denk and Kempenaers, 2006; Mescher, 2016). During spermatogenesis, germ cells divide along the seminiferous epithelium and undergo differentiation while migrating from the basal lamina toward the tubular lumen (Deviche *et al*. 2011).

Spermatogenesis is the process of cell division and differentiation through which spermatozoa are produced within the seminiferous tubules (Obeid *et al*. 2021). This highly coordinated process involves intricate stages of germ cell transformation: spermatogonia in the germinal epithelium undergo self-renewal and differentiate into spermatocytes, which then undergo meiosis to generate haploid spermatids (Otuechere *et al*. 2020). Broadly, spermatogenesis can be divided into three principal stages: mitotic proliferation of spermatogonia, meiotic genome reduction of spermatocytes, and morphological transformation of haploid spermatids into mature spermatozoa (Yang *et al*. 2022).

In the present study, it was evident that spermatogenesis in intergeneric hybrid male ducks was initiated but arrested at an early stage. The process commenced with limited mitotic proliferation of immature primary spermatocytes in the seminiferous tubules. However, these spermatogonia were unable to progress to the subsequent phases of spermatocytogenesis (meiotic division) and spermiogenesis (morphological maturation into spermatids and spermatozoa). This incomplete developmental transition represents a critical physiological impairment underlying the sterility of hybrid male ducks, as mature sperm cells capable of fertilization were entirely absent from their testes and ejaculates.

It is noteworthy that the three stages of spermatogenesis are sequentially dependent, with each stage serving as a prerequisite for the next. Consequently, disruption at any stage adversely affects the completion of the process and, ultimately, male fertility. In this context, the histomorphological anomalies observed in the seminiferous tubules of Mule and Hinny drakes characterized by developmental arrest at the spermatogonial and spermatocyte stages play a central role in their reproductive sterility.

In view of the foregoing, the reported histomorphological anomalies in the STs of hybrid males where immature spermatogonia and spermatocytes fail to progress into mature spermatozoa and are unable to complete spermatogenesis play a central role in the sterility observed in intergeneric hybrid male Mule and Hinny ducks.

The observation of only spermatogonia and the absence of mature sperm cells in both sectional and structural epididymides of hybrid male Mule and Hinny ducks in this study aligns with earlier reports. Rigdon and Mott (1965) described the epididymis of Mule ducks as empty, containing only granules. Similarly, Oguntunji *et al.* (2019) reported that neither viable nor motile spermatozoa were present in the epididymal semen of hybrid Mule ducks.

The epididymis functions as a critical site for sperm maturation and as a storage reservoir for fully developed spermatozoa prior to ejaculation. Thus, the emptiness of the epididymis observed in Mule and Hinny drakes indicates that early-stage germ cells (spermatogonia) failed to progress into mature sperm cells. In the absence of mature spermatozoa, the semen released during copulation consists solely of seminal fluids and immature germ cells lacking fertilizing capacity.

Therefore, the inability of hybrid male Mule and Hinny ducks to accumulate and store functional spermatozoa in the epididymis, with only immature spermatogonia present, represents a key physiological basis for their sterility. This failure explains why these hybrids neither ejaculate viable sperm nor fertilize ova, thereby confirming their reproductive incapacity. This inability of hybrid male Mule and Hinny ducks to store functional spermatozoa in the epididymis constitutes a crucial physiological basis for their sterility, explaining their failure to ejaculate viable sperm and fertilize ova.

Explaining the genetic basis of sterility in avian male hybrids, Castillo *et al.* (2012) reported that gametogenic cells in hybrids failed to progress beyond the pachytene stage of meiosis, subsequently undergoing cellular degeneration, with meiotic division absent and gametogenesis arrested. Similarly, Li *et al.* (2020) attributed the non-reproductive nature of hybrid Mule ducks to disrupted regulation of gene expression and cell differentiation in their reproductive organs. They emphasized that hybrid sterility, particularly in distantly related crosses, represents a complex biological phenomenon likely governed by multiple genetic pathways. Olsen *et al.* (2023) further suggested that hybrid male sterility in many animal taxa is linked to genetic incompatibilities associated with sex chromosomes.

CONCLUSION

Applying lactation curve functions provides valuable information for genetic selection and management practices such as DHI programs. Wilmink produces better results compared to Wood function in our study. Overall, our results confirm that the Pollott's mechanistic function outperforms the other two functions for fitting individual lactation curves. It is more robust in terms of: (1) maximum number of standard curves, (2) lowest AICc, (3) independent curve parameters, and (4) biological interpretation of typical curves. Moreover, the Pollott function allows for independent selection of different aspects of the lactation curve, improving genetic evaluations and production performance. Therefore, we recommend employing this function for fitting test day records and standardizing (national) milk production for Simmental and Jersey breeds.

ACKNOWLEDGEMENT

This study demonstrated that sterility in intergeneric hybrid male Mule and Hinny ducks is not related to testis size, testicular asymmetry, or gonadosomatic index. Rather, it is primarily attributable to their inability to complete spermatogenesis and produce mature spermatozoa capable of fertilizing ova.

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