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Assessment of spermatogenesis in camels *via* seminiferous tubule staging and testosterone profilingSaif Ullah¹, Abdul Quddus², Quanwei Wei¹, Wael Ennab¹, Sheeraz Mustafa³, Jiahao Sun¹, Rahmani Mohammad Malyar¹, Abdul Baseer², Dagan Mao¹, Fangxiong Shi^{1✉}¹College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China²Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture Water and Marine Sciences, Balochistan, Pakistan³Faculty of Veterinary and Animal Sciences Ziaddin University Karachi, Pakistan

ABSTRACT

Objective: To investigate the successive morphological stages of spermatogenesis, hormonal regulation, and testosterone profile in dromedary camel reproduction.

Methods: Testicular tissue samples were obtained from 12 dromedary bull camels aged 7 to 8 at a local abattoir. The histological assessment involved tissue processing, hematoxylin and eosin (H & E) staining, and examination under a microscope. Stereological analysis, germ cell identification, and assessment of seminiferous tubules and maturation were conducted. Testosterone assay was performed by radioimmunoassay using blood samples collected at regular intervals.

Results: The study revealed 12 phases of the dromedary camel's seminiferous epithelium cycle, highlighting distinct morphological characteristics and cellular processes. Acrosomal migration, maturation, cap formation, and the Golgi-mediated synthesis of proacrosomal vesicles were also explained in dimension, as were the steps of acrosome biogenesis. Spermatids and mature sperm cells were present when spermatogenesis phases were examined. An analysis of the dimensions of seminiferous tubules revealed specific measures for diameter, area, and epithelial height about luminal characteristics. Moreover, there were noticeable variations in the serum testosterone concentrations during the study period, indicating temporal dynamics.

Conclusions: This study outlines the spermatogenesis process in dromedary camels across 12 stages, emphasizing cellular dynamics and acrosomal biogenesis. It also provides seminiferous tubule measurements and observes seasonal testosterone fluctuations, offering insights into reproductive regulation and potential strategies for camel breeding conservation.

KEYWORD: Spermatogenesis; Camel; Testosterone profile; Morphological staging

1. Introduction

Studying spermatogenesis and hormonal dynamics in dromedary camels is crucial for gaining insights into their unique reproductive processes and has impacts on veterinary medicine, conservation, and agriculture[1–3]. Dromedary camels are an important part of many people's livelihoods, especially in arid areas. They are well-known for their economic value and adaptability.

The production of spermatozoa, or spermatogenesis, is an essential component of the male reproductive system[4,5]. To evaluate the health and potential of dromedary camels' reproductive systems,

Significance

Before this study, a detailed understanding of spermatogenesis was primarily focused on common laboratory and livestock animals, with limited specific insights into the process in dromedary camels. This research pioneers a detailed description of the spermatogenesis process in dromedary camels, identifying twelve stages of the seminiferous epithelium cycle and four distinct phases of acrosome biogenesis. It offers a comprehensive histological and stereological analysis of camel testicular tissue, enhancing our understanding of germ cell maturation and testosterone dynamics critical for camel fertility and reproductive behavior.

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it is essential to comprehend the exact morphological staging of spermatogenesis and the hormonal regulation in these animals[6]. The management and conservation of this species depend on having a thorough understanding of this complex process[1,2,6].

Spermatogenesis is a carefully controlled, long-term process that occurs in the seminiferous tubules of the testes. It is divided into three main stages, meiosis, spermiogenesis, and spermatocytogenesis, which involves mitosis and differentiation without cell division[6–8]. The initial phase is called spermatocytogenesis, which is defined by mitotic cell divisions that increase the number of spermatogenic cells and give rise to primary spermatocytes and stem cells, both of which go through meiosis to produce haploid cells[6,9]. The last stage, spermiogenesis, is a prime example of cell differentiation, in which germ cells differentiate into self-propelled, enzyme-packed spermatozoa that carry the male genome for fertilization[6]. It is crucial to note that there is some degree of germ cell degeneration during the whole spermatogenesis process. But, this degradation is most pronounced during meiosis and spermatocytogenesis[10]. The complexity of the spermatogenic process is shown by the fact that the degree of germ cell degradation varies depending on age, species, and pubertal development.

During distinct stages of growth and activity, the testes have unique morphological characteristics: sexually matured testes, sexually matured and active testes, and sexually matured but resting testes[11]. The histomorphological changes seen in the seminiferous tubules indicate these morphological variances. When these tubules are examined cross-sectionally in the majority of mammals, they show that several generations of germ cells are present, arranged distinctly and sequentially from the basement membrane to the lumen of the tubule[7,8]. The developmental stages of each generation of germ cells are precisely synchronized, and they usually cohabit with previous generations in a predictable and species-specific way[12,13]. Since any errors at any of these developmental stages may have a major effect on the entire process, this synchronization is important to spermatogenesis.

Male camels engage in unique rutting behaviors that last 50 to 100 days, driven by high testosterone levels. Camels breed seasonally, usually in the colder winter months[12]. These unique behaviors, which are linked to high testosterone levels, are more like those of some wild species, such as deer than they are of other domesticated animals. After 11 to 13 weeks during the rut, camels return to their normal calm temperament[12]. This phenomenon emphasizes the link between increased testosterone levels and camel rut symptoms.

Research on the systematic understanding of the successive morphological stages of spermatogenesis in dromedary camels is very limited. Furthermore, although hormonal regulation, particularly testosterone profiles, has been studied in a variety of animal species, little is known about its function in dromedary camel reproduction.

This study aims to delineate the morphological stages of

spermatogenesis and analyze the hormonal regulation in dromedary camels, focusing particularly on testosterone profiles. Through histological assessments and testosterone assays of testicular tissue samples from dromedary bull camels, we seek to enhance our understanding of reproductive physiology, which could inform effective breeding and management strategies for the conservation and sustainability of this species.

2. Materials and methods

2.1. Experimental location and animals

Testicular samples were collected from 12 dromedary bull camels of the Lassi breed aged 7 to 8 years. These animals were gently slaughtered at a local abattoir located in Bela City, Balochistan, Pakistan, in the late autumn and early winter of the year 2021. The abattoir was located at 26°13'37.56" N latitude and 66°18'39.96" E longitude in Bela city, Balochistan, Pakistan. November through March is considered the winter season. January is the coldest month with mean maximum and lowest temperatures of 17 °C and 3 °C, respectively. The summer season lasts from April to October, with June being the hottest month. June's average high temperature is 38 °C, while its average low temperature is 24 °C. All experimental animals were healthy and fit before slaughter, and no diseased animals were used in our study. After the camels were slaughtered in an abattoir that followed exact standard operating procedures, Testis samples were collected immediately post-mortem from slaughtered animals to prevent tissue degradation. Following collection, each sample was submerged in 10% neutral buffered formalin for fixation. The fixed samples were then stored at room temperature to ensure proper preservation for hematoxylin and eosin (H & E) analysis.

2.2. Histologic assessment

The histological analysis was conducted in compliance with previously described protocols[14–16]. The tissue samples were subjected to a variety of steps, starting with a graded sequence of ethanol and xylene for dehydration, and ending with paraffin wax cleaning and penetration. The tissue slices were then sectioned to a thickness of 5 µm after being fixed in 4% paraformaldehyde for 24–48 h. The tissue slices were stained with H & E. Examining the histopathological alterations was done at 100× magnification using an oil immersion microscope (Nikon ECLIPS 80i and OLYMPUS BX51 Tokyo, Japan).

2.3. Germ cell identification and stereological analysis

Identification of germ cells and stereological analysis followed

rigorous instructions. According to known techniques, nuclear features such as the presence and distribution of heterochromatin were used to predominantly characterize spermatogonia[17,18]. The relative size, shape, and nuclear morphology of each analyzed slide were carefully analyzed to identify the germ cells. Furthermore, careful microscopic analysis of morphological changes in germ cell developmental stages and their cellular relationships made it easier to distinguish between the various phases of the seminiferous epithelium.

2.4. Diameters of the seminiferous tubule and tubular lumen, epithelium height, and number of seminiferous tubules per field

Tissue sections were cut perpendicular to the left testis's longest axis to determine histological parameters. Following their attachment to glass slides, these sections were stained with H & E. The Olympus BX63 light microscope (OLYMPUSBX63, Olympus Corporation, Tokyo, Japan) was used to carefully examine each stained section at 10 and 40 magnifications. This allowed for the observation of minute changes in the diameter of the seminiferous tubule, lumen, epithelium height, and the number of seminiferous tubules per field.

By averaging two opposed measurements, the height of the seminiferous epithelium was determined. Similarly, the luminal tubular diameter was determined by calculating the difference between the epithelium height and the total diameter. Using the accessible Image J analysis, ten histological fields were assessed for every testicular region in every group to ensure thorough data collection.

2.5. Assessment of seminiferous tubules and maturation using histology

The Johnsen scoring system was conducted to assess the sperm production in the seminiferous tubules[19]. A total of thirty tubules from each cross-section were analyzed and given a score ranging from 0 to 5 (Table 1). The score was then recorded and described to provide a comprehensive understanding of the results obtained from the evaluation.

2.6. Hormonal assay

Using jugular venepuncture, blood samples were taken from 12 bull camels in a cohort into EDTA tubes at regular intervals of fifteen days, from November to May. After centrifuging the obtained samples at 4000×g for 10 min, the supernatant was separated to produce sera. A commercial RIA kit (TKTT5; Diagnostic Product Corporation, California, USA) was used to measure the amount of testosterone in the serum. The assay sensitivity was 5 pg/mL, and the intra-CV and inter-CV were less than 10% and 15%, respectively.

Table 1. Criteria of scores for the evaluation of camel spermatogenesis.

Score	Description
5	Complete spermatogenesis with mature sperm cells
4	Some sperm cells, with a disorganized epithelium
3	Presence of few sperm (5 to 10)
2	Absence of sperm cells, presence of spermatids
1	Absence of sperm cells, presence of a few spermatids
0	Absence of sperm cells or spermatids, presence of spermatocytes

2.7. Statistical analysis

To perform statistical analysis, GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA) was used. The data were evaluated for normality distribution using the Shapiro-Wilk test. We used a one-way analysis of variance (ANOVA) and *t*-test to evaluate different parameters, and then we performed a *post hoc* Tukey multiple-comparison analysis. Data were presented as mean±standard deviation (mean±SD). For all statistical studies, a significance criterion of $P<0.05$ was established.

2.8. Ethics statement

The experimental protocols were carried out by the guidelines specified in the Guide for the Care and Use of Laboratory Animals, which was authorized by the Lasbela University of Agriculture, Water, and Marine Sciences (LUAWMS), Pakistan, and Animal Care and Use Committee of Nanjing Agricultural University, Nanjing, China, with permission Numbers: (Permit Number: SYXK (Su) 2019–0036).

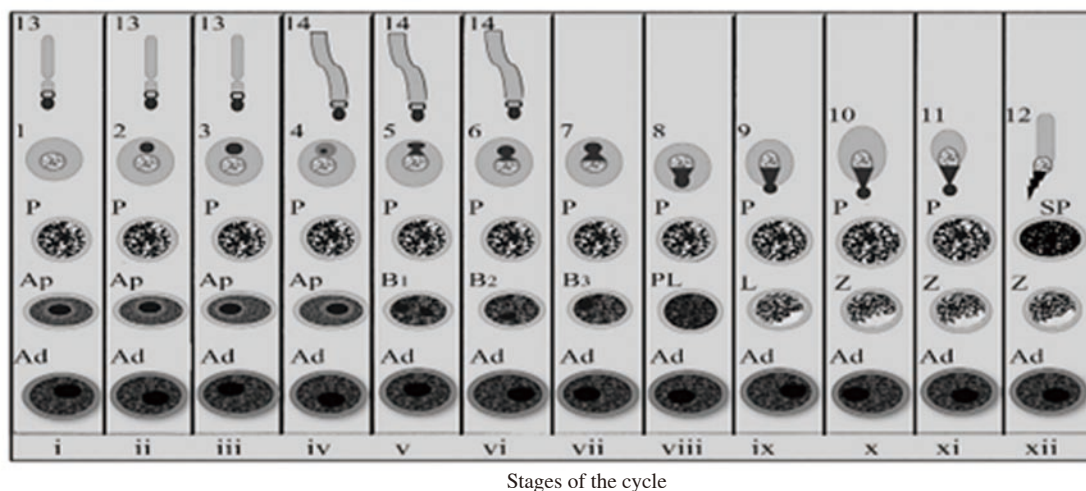
3. Results

3.1. Stages of the cycle

Our study clarified the particular context of twelve seminiferous epithelium stages and fourteen spermatid steps in dromedary camels. Starting with the complex process of spermatogenesis in dromedary camels, our research revealed a thorough examination of the seminiferous epithelium cycle throughout 12 stages. We have observed in Bull Lassi Camels that there are twelve stages of seminiferous epithelium and fourteen distinct processes in spermiogenesis. Every stage, exhibiting distinct morphological characteristics and cellular dynamics, was distinct, ranging from the first division of secondary spermatocytes to the elongation and maturation of spermatids (Figure 1, 2).

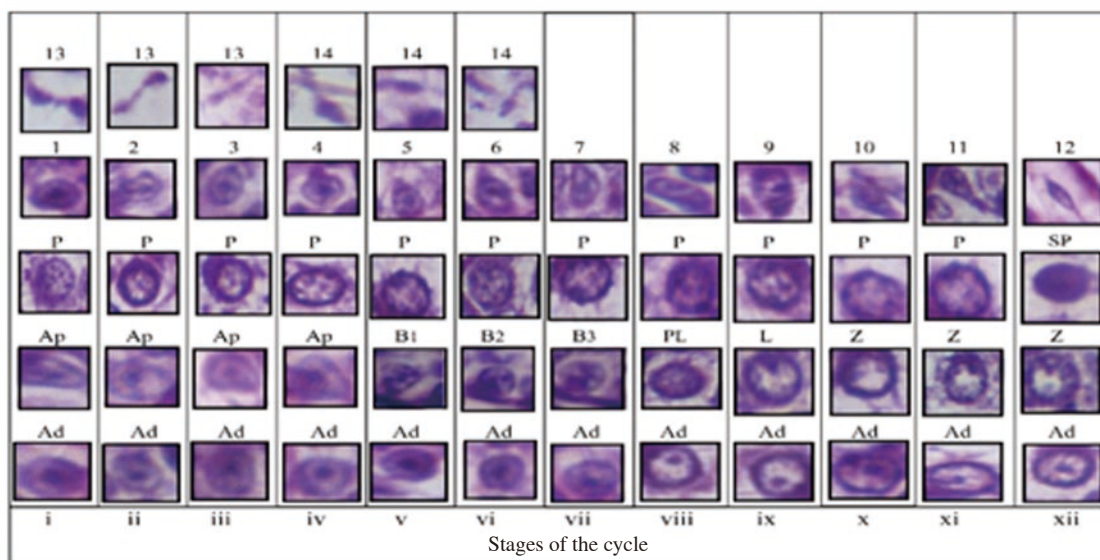
In stage 1, secondary spermatocytes were divided to produce a new batch of spermatids during the first stage of the seminiferous epithelium cycle. The Golgi zone, which was spherical and slightly stained, was located opposite the nuclear surface of these developing spermatids. Interestingly, two or more strongly stained chromatin spheres were seen inside the nucleus, which was

A



Stages of the cycle

B



Stages of the cycle

Figure 1. Determination of cellular relationships at each of the 12-dromedary camel seminiferous epithelium cycle phases (Roman numbers i through xii), through schematic representation (A) and H & E staining (B) as explained by the developmental pathway of the acrosome during spermiogenesis. Ad (type A spermatogonia), Ap (pale type spermatogonia), B1 (type B1 spermatogonia), B2 (type B2 spermatogonia), B3 (type B3 spermatogonia), PL (preleptotene primary spermatocytes), L (leptotene primary spermatocytes), Z (zygotene primary spermatocytes), P (pachytene primary spermatocytes), SP (secondary spermatocytes), and 1–14 (spermatids) are among the identified germ cell types.

positioned in the center. This stage included two different kinds of spermatogonia: dark type (Ad) and pale type (Ap) spermatogonia, and pachytene spermatocytes. Furthermore, spermatogonia and spermatids were scattered with primary spermatocytes in the early pachytene phase, which were distinguished by thick chromatin strands and a noticeable nucleolus. In step 13 of spermiogenesis, stage 1 simultaneously hosts spermatids, which had flat, paddle-shaped nuclei partially covered by the acrosomic system (fused head cap and acrosome). These step 13 elongated spermatids had a black look and were completely condensed.

Stage 2 was characterized by pachytene primary spermatocytes, pale and dark type spermatogonia, and spherical spermatids in step 2 that showed proacrosomal granules within proacrosomal vesicles that were first created during this stage. A single, big vesicle with a fused granule was formed when small proacrosomal vesicles fuse. In

this stage, step 13 elongated spermatids were notable for persisting.

In stage 3, the appearance of a single, centrally positioned acrosomic granule in the step 3 spermatids' Golgi zone characterizes stage III. The proacrosomic granules seen in the stage before fused to form this granule. Furthermore, a decrease in the spermatids' cytoplasmic content was noted, which results in the development of residual bodies inside the tubular lumen. The morphological characteristics of pachytene spermatocytes and spermatogonia were essentially unchanged.

In stage 4, round spermatids had an acrosomal vesicle that flattens on the nuclear surface to form a structure resembling a head cap in step 4. Step 14 elongated spermatids are present concurrently. The morphological characteristics of pachytene spermatocytes and spermatogonia were essentially unchanged.

In stage 5, the acrosomal granule started to expand over the

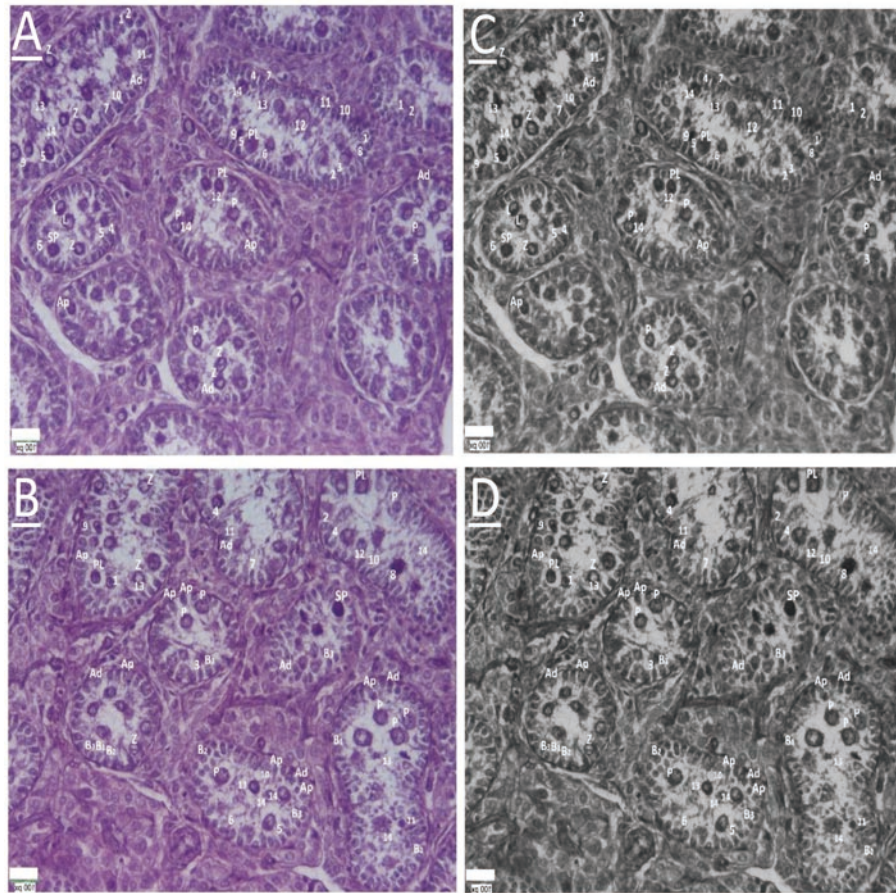


Figure 2. Figure A-D depicts the 12 stages of the seminiferous epithelium cycle and tubular morphology, stained with H & E, in dromedary camels. Each stage is characterized by specific morphological changes and cellular dynamics, detailing the progression from secondary spermatocyte division to spermatid maturation. The following are the abbreviations for germ cells: L (leptotene primary spermatocytes), PL (preleptotene primary spermatocytes), P (pachytene primary spermatocytes), Z (zygotene primary spermatocytes), Ad (dark type spermatogonia), Ap (pale type spermatogonia), and B (type B spermatogonia), SP (secondary spermatocytes). The 20 µm scale bar is in the set.

nuclear membrane in step 5 round spermatids, generating a little head cap. In step 14, spermiation was indicated by the discharge of elongated spermatids into the lumen. There was just one generation of spermatids detected after this point. B1 spermatogonia were the predominant morphological feature of this stage, but the morphology of Ad spermatogonia and pachytene spermatocytes do not change.

In stage 6, round spermatids in step 6 had an acrosomal head cap that covered 25% of the nuclear surface. Parallel to this, fully grown step 14 elongated spermatids align consistently, a crucial feature before sperm discharge from the testis. B2 spermatogonia was indicative of this stage.

In stage 7, spherical spermatids displayed a maximum-extending acrosomal head cap that covered one-third of the nuclear surface. The presence of B3 spermatogonia distinguishes this stage from others.

In stage 8, spermatids exhibited round to oval-shaped nuclei that were positioned eccentrically within the cytoplasm during the seventh stage of spermiogenesis. Interestingly, in step 8 spermatids,

the head cap was facing the basal membrane. Preleptotene primary spermatocytes (PL) were present at this stage, which is characteristic.

In stage 9, spermatids extended their nuclear structures and took on a funnel-like conical form in the head cap-covered area. The presence of leptotene primary spermatocytes identified this stage.

In stage 10, step 10 involved the spermatids' nuclei lengthening and the head cap-covered portion developing a tapered, peg-top-like form. The existence of zygotene primary spermatocytes characterized this stage.

In stage 11, the nucleus of spermatids continued to elongate and flatten, taking on a form like a paintbrush or paddle. The existence of zygotene primary spermatocytes indicated this stage.

In stage 12, spermatids continued to lengthen their nucleus, and the tip became noticeably pointed. The secondary spermatocytes present during this stage were characteristic (Figure 1, 2). Our study clarifies the particular context of twelve seminiferous epithelium stages and fourteen spermatid steps in dromedary camels.

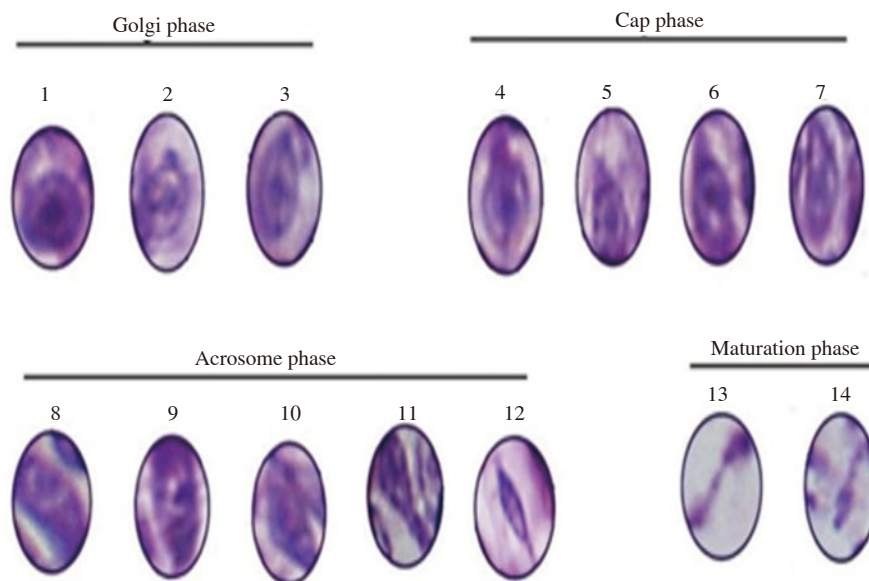


Figure 3. Illustration of spermatid development across four key phases: Golgi phase (1-3), where small proacrosomal vesicles form from the trans-Golgi network; cap phase (4-7), featuring acrosomal granules forming a thin layer over one-third of the nuclear surface; acrosome phase (8-12), marked by cytoplasm thinning and manchette microtubule production near the nuclear ring; and maturation phase (13-14), where the acrosome covers nearly the entire nuclear surface, with the acrosome apex developing in the anterior segment. The comprehensive process of spermiogenesis in camels encompasses 14 sequential steps.

3.2. Phases of acrosome biogenesis in camel

3.2.1. Phase 1: Proacrosomal vesicle formation mediated by the Golgi

The Golgi apparatus actively participated in the early phases of spermiogenesis during the first stage of acrosome biogenesis in camels, which is known as the Golgi phase. This essential organelle is responsible for coordinating the production of various glycoproteins, which result in the formation of several small proacrosomal vesicles that emerge from the trans-Golgi network. These vesicles are essential for the mature acrosome's subsequent assembly (Figure 3).

3.2.2. Phase 2: Golgi relocation and cap formation

As the acrosomal granule entered the second phase, known as the cap phase, it significantly enlarged and began to accumulate a high concentration of glycoproteins. At the same time, it compressed against the nuclear membrane and spread out to cover the nucleus like a cap. At the same time, the Golgi complex moved to the developing neck area at the distal end. At the end of this stage, the acrosomal granules become a thin, fragile layer that covers about one-third of the nuclear surface (Figure 3).

3.2.3. Phase 3: Spermatid elongation and acrosomal migration

The acrosomal system migrated along the ventral surface of the elongating spermatid nucleus during the next acrosomal phase,

and it completed its migration by the 14th spermatid step. During this phase, the cytoplasm is thinned concurrently with the start of manchette microtubule production close to the nuclear ring area. Furthermore, a slow reorientation of the acrosome in the direction of the adjacent plasma membrane was observed (Figure 3).

3.2.4. Phase 4: Acrosome maturation and changes in nuclear morphology

The last stage of development included unique changes in nuclear shape and acrosomal migration. The acrosomal granule covered the whole acrosomal membrane, and nucleus condensation continued. The anterior and posterior parts of the acrosome were defined by further differentiation. Except for the area connected to the sperm tail, the remaining acrosome covered almost the whole nuclear surface when the anterior segment developed into the acrosome apex (Figure 3).

3.3. Seminiferous tubule scores

A thorough examination of the several phases of sperm development was revealed by counting spermatogonia, primary and secondary spermatocytes, round sperms, elongated sperms, and spermatozoa in the 12 camels. The study showed all phases of spermatogenesis, including the existence of spermatids in the samples under investigation and mature sperm cells (Table 2). Values are expressed as seminiferous tubule scores and percentages in each column.

Table 2. Frequency and percentage of seminiferous tubule score 5, 4, 3, 2, 1, and 0 in multiple cross-sections in all 12 animals [*n* (%)].

Animal No.	Scores					
	5	4	3	2	1	0
1	8 (40)	4 (20)	5 (25)	2 (10)	1 (5)	0 (0)
2	10 (50)	3 (15)	2 (10)	3 (15)	1 (5)	1 (5)
3	13 (65)	2 (10)	3 (15)	2 (10)	0 (0)	0 (0)
4	14 (70)	3 (15)	1 (5)	2 (70)	0 (0)	0 (0)
5	11 (55)	2 (10)	2 (10)	3 (10)	1 (5)	1 (5)
6	13 (65)	4 (20)	1 (5)	1 (5)	1 (5)	0 (0)
7	9 (45)	4 (20)	3 (15)	2 (10)	2 (10)	0 (0)
8	15 (75)	4 (20)	1 (5)	0 (0)	0 (0)	0 (0)
9	13 (65)	5 (25)	1 (5)	1 (5)	0 (0)	0 (0)
10	12 (60)	3 (15)	2 (10)	2 (10)	1 (5)	0 (0)
11	8 (40)	5 (25)	3 (15)	2 (10)	1 (5)	1 (5)
12	6 (30)	3 (15)	5 (25)	1 (5)	4 (20)	1 (5)

Values are expressed as seminiferous tubule scores and percentages in each column.

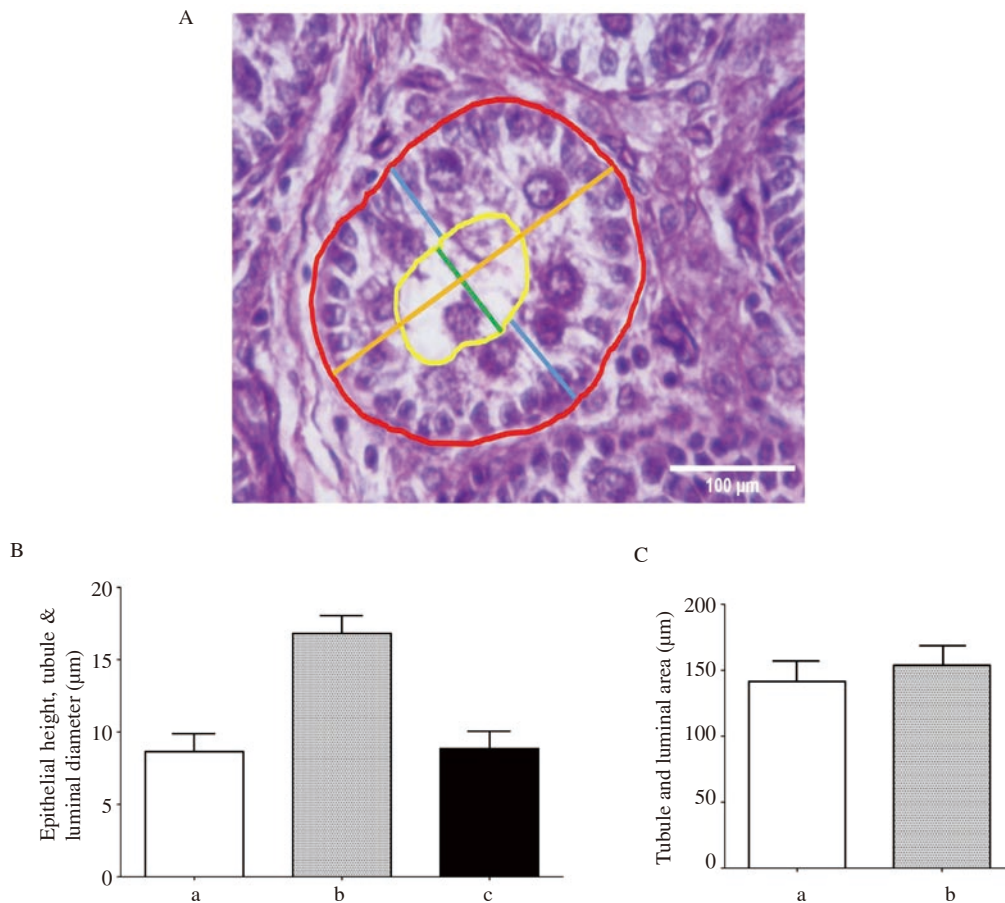


Figure 4. A: Histometric evaluation of the seminiferous tubules in the testicles of dromedary camels. Height of the seminiferous epithelium, measured by the mean of two opposed measures; Photomicrograph of camel testis showing histologic measurements. Area of seminiferous tubules (red circle), diameter of seminiferous tubules (orange line), area of the lumen (yellow circle), diameter of the lumen (green line), epithelial height (blue lines). We measured all areas and diameters based on the geometric constant “Pi” square root ($A = \pi \sqrt{2}$) ($A = 3.14 \sqrt{2}$) represents 100 µm. **B:** (a) Epithelial height of seminiferous tubule (µm); (b) Diameter of seminiferous tubule (µm); (c) Luminal diameter of seminiferous tubule (µm). **C:** (a) Area of seminiferous tubule (µm²); (b) Luminal area of seminiferous tubule (µm²).

3.4. Diameter, area, and epithelial height investigated about luminal area and luminal diameter in seminiferous tubule dimensions

Histological diagrams in Figure 4A–C showed the methodology used to measure luminal diameter, epithelial height, and

seminiferous tubule diameter. The findings indicated that the diameter of the seminiferous tubules is (16.44 ± 0.07) µm and the average area is (141.86 ± 3.22) µm². Furthermore, the seminiferous tubules had a luminal width of (8.90 ± 0.19) µm and a luminal area of (156.15 ± 4.32) µm². It was found that the epithelium height is (8.39 ± 0.40) µm (Figure 4).

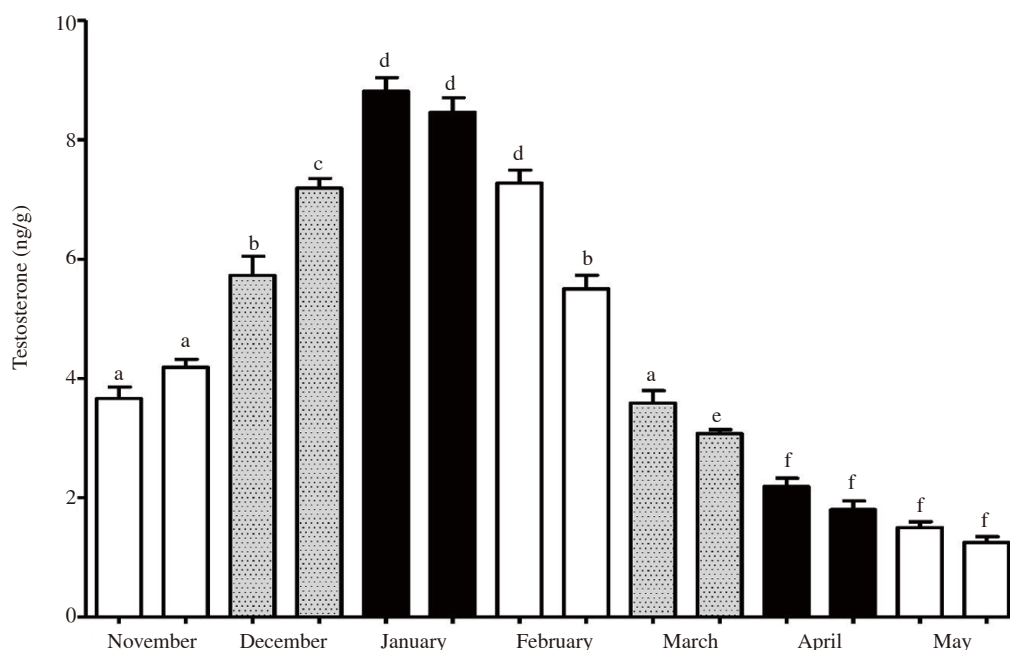


Figure 5. Testosterone profile of dromedarian camels ($n=12$) at 15-day intervals from November to May. Values are expressed as mean $\pm$ SD, using one-way ANOVA. Bars with different letters (a, b, c, d, e, f) differ significantly ($P<0.05$).

3.5. Serum concentrations of testosterone

Throughout the research, 12 camels were continuously monitored for changes in their serum testosterone levels from November to May. Over this period, samples of testosterone were taken every 15 days. A notable increase in testosterone concentration was noted in November, and this elevated level persisted into December. The hormone then peaked at the beginning of January till the end of January, then began to gradually decrease from February until it finally reached its lowest point in May. These results offer a thorough comprehension of the temporal dynamics of testosterone levels in the camel population under research, providing insightful knowledge about the reproductive physiology of dromedary camels in this particular period (Figure 5).

4. Discussion

This study describe the complex process of spermatogenesis and categorize the seminiferous epithelium cycle in the testes of active adult dromedary camels. By methodically evaluating the histological factors controlling the growth of seminiferous tubules, we have been able to clarify important stages that are involved in the morphological development of germ cells, specifically spermatogonia, spermatocytes, and spermatids. These germ cells must mature properly to increase camel fertility. We have observed in Bull Lassi Camels that there are twelve stages of seminiferous epithelium and fourteen distinct processes in spermiogenesis. Five varieties of spermatogonia were distinguished, three B spermatogonia (B1, B2, and B3), pale types Ap, and dark type A (Ad). Furthermore, we identified the Golgi phase, cap phase, acrosome phase, and maturation phase as the four stages of acrosome

formation. Interestingly, when we looked into the testosterone profile, we found that the concentration peaked in January. All of these results highlight the critical roles that germ cells—more especially, spermatogonia, spermatocytes, and spermatids play in fertility and, consequently, in the production of high-quality and abundant semen in dromedary camels.

Spermatogenesis is a complex procedure that occurs continuously in the seminiferous tubules and involves the conversion of highly differentiated haploid spermatozoa from undifferentiated diploid stem cell spermatogonia[20]. This complex process consists of three main stages: spermiogenesis, which is the morphological change of immature spermatids into spermatozoa, with nuclear DNA condensation, the formation of a glycoprotein-covered acrosome, and the development of a whip-like tail enclosing a flagellum with tightly packed mitochondria, marks the proliferative phase[21]. The large number of distinctive animal spermatozoa that result from these phases work together to highlight the importance of this process in reproductive biology.

Secondary spermatocytes divide in stage 1 of the seminiferous epithelium cycle to produce new spermatids. Interestingly, the Golgi zones in these spermatids are located across from the nuclear surface and exhibit spherical staining. There are many kinds of spermatogonia and pachytene spermatocytes, as well as chromatin spheres inside the nucleus[8,11,13,22]. In step 13 of spermiogenesis, stage 1 also includes the presence of elongated spermatids, which are distinguished by flat, paddle-shaped nuclei that are partially buried in the acrosomic system. Proacrosomal granules start to form inside spermatids during stage 2, which causes the granules to merge and create a huge vesicle. A centrally located acrosomic granule in spermatids indicates the third stage, during which residual bodies arise as a result of a decrease in cytoplasmic content. Acrosomal vesicles flatten on the nuclear surface of spherical spermatids during

stage 4. In stage 5, acrosomal granules in round spermatids expand, and the discharge of elongated spermatids indicates spermiogenesis. The acrosomal cap morphology, nuclear elongation, and existence of certain spermatogonia and spermatocytes alter further in subsequent stages, culminating in stage 12, which has characteristic secondary spermatocytes and pointy tips on elongating spermatids.

Different spermatogenic cell generations end in distinct stages that create particular cell relationships in seminiferous tubules. Although there is significant variation in the appearance of germ cells and the timing of development between species, Russell *et al* have shown that there is a common organizational basis for these stages[23]. As a demonstration, referring to other animals, mice with twelve seminiferous epithelium stages and a sixteen-step spermatid formation process[6], bulls with twelve seminiferous epithelium stages and a fourteen-step spermatid formation process[13], and monkeys with twelve seminiferous epithelium stages and a fourteen-step spermatid formation process[24], our study clarifies the particular context of twelve seminiferous epithelium stages and fourteen spermatid steps in dromedary camels, defining a complex pattern that advances our knowledge of spermatogenesis.

It presents a detailed picture of the planned transition from Golgi to acrosomal phases, as seen in our work, and explores deeply the important stages of spermiogenesis. As per the groundbreaking studies of Hess *et al*[25] and Russell *et al*[20], our analysis validates the importance of the Golgi phase, emphasizing the active Golgi apparatus participation and the generation of proacrosomal vesicles as fundamental processes. By characterizing the cap phase and explaining acrosomal granule development and the emergence of the acroplaxome, our study validates the acrosomal phase's maturation milestone, which shows the acrosomal system's transit across the spermatid nucleus. Concurrently, our investigation reveals the subtle details of spermiogenesis, including chromatin condensation, inner acrosomal membrane attachment, and critical roles played by cytoskeletal proteins, which are consistent with the findings of Camatini *et al*[26]. The conversation continues into the maturation stage, where our findings provide insights into nuclear condensation, acrosomal differentiation, and the elimination of cytoplasmic constituents, so enhancing the works of Russell *et al*[20], De Franca *et al*[22] and Hess *et al*[25]. Although our study makes a substantial contribution to the understanding of acrosome morphogenesis, it also highlights the ongoing gaps in the complex molecular mechanisms governing sperm acrosome biogenesis, providing important information for future research in this area.

The reproductive physiology of twelve dromedary camels was illuminated by the unique temporal patterns seen in their plasma testosterone profiles, which were continuously monitored. The notable increase in testosterone concentration that was seen in November and persisted into December highlights increased and persistent androgenic activity in the pre-rut and rut stages. The peak that follows in January corresponds with the predicted peak of the rutting season, indicating that testosterone plays a crucial role in mediating reproductive processes and behaviors during this time. Beginning in February, there may have been a shift in testosterone

levels towards the post-rut phase, which is marked by a decrease in reproductive activity. This temporal pattern corresponds with related research on mammal reproductive cycles, where testosterone levels are correlated with particular stages of the mating season[19,27]. The lowest point recorded in May indicates a stop to increased reproductive activity, possibly signaling the end of the rutting season for dromedary camels. These results highlight the significance of testosterone in coordinating important reproductive events and advance our knowledge of the intricate reproductive physiology of dromedary camels[28,29]. The present study establishes the groundwork for subsequent inquiries into the structural aspects of testosterone control in dromedary camels, which may provide valuable perspectives on reproductive tactics and adaptations within dry settings. Additionally, research comparing camelids to other species may offer a more comprehensive understanding of the endocrine regulation of reproduction in these unusual mammals.

The limitation of this study is that this research focuses on a specific breed and age group which may not represent the diversity within the dromedary camel population. Furthermore, the study was conducted in a specific geographic site, which may restrict the applicability of the result to other regions. Further research should address these limitations to increase the comprehensiveness and robustness of the findings of this research in the reproduction field.

In conclusion, this study represents a major advancement in our knowledge of spermiogenesis and the seminiferous epithelium cycle in mature, fully developed dromedary camels. Our research clarifies important steps in the formation of germ cells, such as spermatogonia differentiation and acrosome biogenesis phases. Furthermore, the temporal dynamics of testosterone offer new perspectives on the biology of camel reproduction. Future studies should concentrate on filling in the identified knowledge gaps to enhance our comprehension of camel reproduction. The results present significant prospects for enhancing camel breeding methods and conservation endeavors.

Conflict of interest statement

The authors declare no conflicts of interest in this study.

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Authors' contributions

Saif Ullah was responsible for designing the study, collecting data, and drafting the manuscript. Abdul Quddus, Quanwei Wei, and Wael Ennab were instrumental in developing the methodology and conducting investigations. Sheeraz Mustafa and Jiahao Sun handled data curation. Rahmani Mohammad Malyar and Abdul Baseer took

on formal analysis and software tasks. Dagan Mao was involved in writing, reviewing, and formatting the manuscript. Fangxiong Shi was in charge of resource acquisition, supervision, and project administration. All authors have reviewed and approved the final manuscript for publication.

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