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Research article

Testicular Morphology and Spermatogenesis in Pubertal Dromedary Camels during the Breeding Season

Aymen Z. Elzawam[1]*, Ismail M. Hdud[2], Mohamed M. Zurghani[3] and Fathi M. Abousaq[4]

[1]*Department of Surgery and Theriogenology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya*
[2]*Department of Pathology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya*
[3]*Meat Inspection Department, Tripoli City, Tripoli, Libya*
[4]*Department of Animal Science, College of Agriculture, University of Tripoli, Tripoli, Libya*

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Abstract
This study investigates the morphological and histological characteristics of the testes in pubertal one-humped camels during the rutting season. Thirty-five male camels aged 3 to 4 years were examined to assess testicular development, histological changes and their relationship with the rutting season. Testes were collected monthly and parameters such as testicular weight, seminiferous tubules development and Leydig and Sertoli cells density were evaluated. The Results showed significant monthly variations in testicular weight and histology, with a peak activity observed in December and January. Leydig cell numbers strongly correlated with testis weight and spermatogenesis productivity. These findings contribute to a better understanding of reproductive development in dromedary camels and the influence of seasonal changes on spermatogenesis.

Keywords: dromedary camel, puberty, testicle, histology, spermatogenesis

Introduction

Limited information on male camelid reproductive development indicates that camels reach puberty later than other domestic large mammals (Novoa, 1970; Arthur et al., 1985; Tibary et al., 2014). Reports suggest that male camels begin showing signs of puberty at about three years and attain sexual maturity at six to seven years (Novoa, 1970; Arthur et al., 1985; Tibary et al., 2014). Testicular weight and spermatogenesis studies are critical for evaluating the onset of puberty (Singh & Bharadwaj, 1978; Abdel-Rahim, 1997).

The initiation of puberty and spermatogenesis is a complex process involving the coordinated interaction of somatic cells, Leydig cells, and germ cells, regulated by the hypothalamic-pituitary-gonadal axis (Tibary & Anouassi, 1997). Sertoli cells play a vital role in supporting germ cell development, while Leydig cells are essential for testosterone production, a hormone critical for spermatogenesis (Tibary et al., 2008; El-Harairy et al., 2010).

Camels are seasonal breeders, with testicular histological activity peaking during the rutting season. However, determining a specific rut season is challenging due to regional variability (Shalash, 1965; Minoia et al., 1992; Tibary et al., 2008). Seasonal variations in semen characteristics, testicular histology, and hormonal changes during the rutting season have been well-documented (Skidmore et al., 1995; El-Harairy et al., 2012). Recent histochemical evidence shows that lectin-binding patterns in dromedary testes vary significantly with season and are tightly coupled with spermatogenic activation during rutting (Gewaily et al., 2023). Seasonal changes during the rutting season significantly affect the morphological and histological development of the testes in pubertal one-humped camels. This study aims to examine histological changes in camel testes during puberty and explore their relationship with the rutting season.

Materials and methods

Sample collection

The methodology aligns with similar approaches in camel reproductive studies (Tibary et al., 2014; Al-Qarawi et al., 2000). The testes were collected from 35 one-humped male camels aged 3 to 4 years at a recognized slaughterhouse in Tripoli, Libya. These animals originated in the southern region of Libya, representing a sample of the regional camel population. Testes were collected during the rutting season (November to March). Each month, 6 to 8 pairs of testes were randomly collected during the first week. The samples from each month were analyzed as a group to evaluate monthly variations and were later pooled for an overall analysis.

Testis processing

Immediately after collection, one testis from each pair was dissected, and a cubic piece of testicular parenchyma (approximately 5–7 mm^3) was excised, ensuring that no tunica tissue was included. The tissue samples were fixed in 4% paraformaldehyde overnight and then transferred to 70% ethanol for histological processing. The second testis was placed in a plastic bag and transported to the laboratory for weighing and dimensional measurements. Testicular volume (Ellipsoid) (cm^3) = Length x width x height x 0.5236.

***Corresponding Author:** Aymen Z. Elzawam. Department of Surgery and Theriogenology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya. Mobile: +218931352138. Email: a.elzawam@uot.edu.ly

Histological preparation

Histological preparation followed standard protocols similar to those described in Goyal et al. (1988) and Zaher et al. (2003). Fixed tissue samples were dehydrated through a series of graded ethanol, cleared in xylene, and embedded in paraffin. Sections of 4µm thickness were prepared and stained with hematoxylin and eosin for microscopic examination.

Evaluation of testicular histology

Histological analysis focused on cells within the seminiferous tubules and intertubular spaces. Ten seminiferous tubules cross-sections were examined per sample to assess the most advanced germ cell stage. The analysis included the identification and counting of gonocytes, spermatogonia, primary spermatocytes, elongating spermatids and mature sperm cells.

Leydig and Sertoli cells counts were performed to evaluate their roles in spermatogenesis. Immature and mature Leydig cells were counted in relation to the germ cell stages. The distribution and structural characteristics of Sertoli cells were evaluated in cross sections.

Morphometric analysis

The number of seminiferous tubules within a given area and their lumen formation were recorded to determine stages of development.

Statistical analysis

Statistical analysis was conducted using the SAS software suite (SAS Institute, 2002). Descriptive statistics for linear metric measurements were calculated using the means procedures. PROC CORR procedure was used to investigate the relationships between the study variables, which included testicular weight, testicular volume and different cell types. Specifically**,** Pearson's correlation coefficient was used for metric variables and Spearman's rank correlation coefficient was applied to qualitative variables. Difference was considered significant in P= <0.05.

Ethical approval

The samples of this study were collected from animals after slaughter in abattoirs, as per animals' welfare guidelines, and processed according to routine laboratory methods. Therefore, no special approval was requested for performing this study.

Results

Testicular weight

The findings showed significant variations with a strong correlation (r= 0.99, P= <0.0001) in testicular weight and volume across months (Figure 1). December exhibited the highest mean testis weight (20.8g), while January showed the lowest (6.5g), which reflects the seasonal influences on testicular function (Table 1). The monthly fluctuations aligned with the biological changes of the rutting season.

Seminiferous tubules features and histology

Histological evaluation revealed progressive testicular development across samples, with mature Leydig cells correlated with spermatogenesis productivity at 100X magnification (Figure 2). Formation of Seminiferous tubule lumens was observed in 80% of the samples, with a corresponding increase in tubule diameter and a reduction in tubular density under low magnification (Figure 2). Subsequently, average numbers of tubules that counted under low (10X) magnification within a similar area were decreased as the tubule diameter expanded (Figure 3). The number of mature Leydig cells increased with advancing germ cell stages (Figure 4), peaking in samples containing mature spermatozoa (r= 0.555, p= 0.0005).



Figure 1. Gross morphology of testes collected from pubertal dromedary camels. Variation in testicular size reflects differences in individual developmental stages. A millimeter scale is included for size reference.

Table 1. Monthly variation in testicular weight and volume in pubertal dromedary camels during the rutting season.

Month	No.	Testis weight range (g) (mean)	Testis volume range (cm^3) (mean)
November	7	5.9- 43.7 (13.6)	3.76 – 29 (11.88)
December	7	3.8 – 37.1 (20.8)	2.22 - 22.4 (12.12)
January	8	3.1 – 7.7 (6.5)	1.6 - 4.0 (2.95)
February	6	2.2 – 28-8 (10.9)	1.88 - 39.2 (13.06)
March	7	6.4 – 20.8 (10.3)	1.98 - 13.7 (7.19)

g: gram. cm: centimeter.

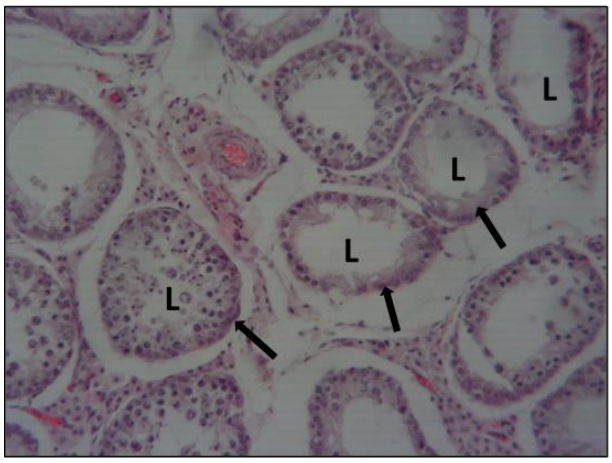


Figure 2. Histological features of seminiferous tubules in pubertal dromedary camel testes during the rutting season. A photomicrograph showing seminiferous tubules (arrows) with visible lumens (L). Tissue demonstrates early spermatogenic activity. Hematoxylin and eosin stain, 100X.

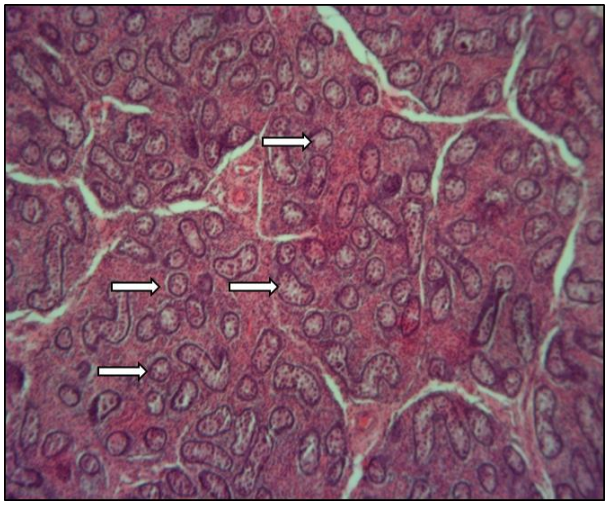


Figure 3. A cross section of the pubertal dromedary camel testis shows seminiferous cords (white arrows) and dense interstitial tissue. Hematoxylin and eosin stain, 10X.

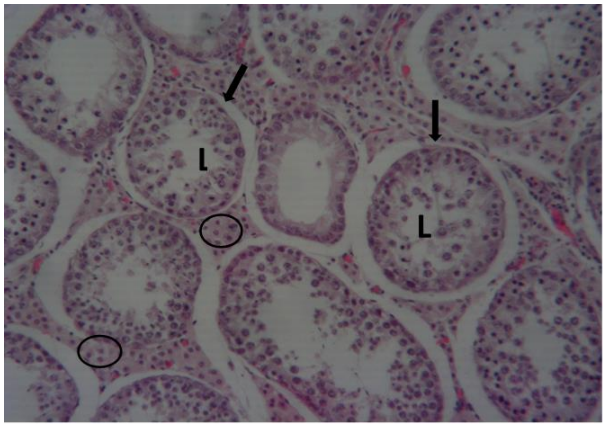


Figure 4. A photomicrograph of camel testicular tissue showing seminiferous tubules with developing germ cells, lumen (L), spermatogenic cell layers (arrows) and mature Leydig cells (circled) are visible. Hematoxylin and eosin stain, 100X.

Some of these gonocyte cells had migrated and relocated at basement membrane as spermatogonia and some remained in the central area of the tubule. Nine males (25.7%) exhibited only gonocytes in seminiferous cords, while 7 out of 35 males (20%) showed spermatogonia that were recognized at the basal lamina with their oval nucleus (Figure 5). Advanced stages, such as elongated spermatids and mature spermatozoa, were also observed. Seminiferous tubules of one male (2.8%) containing mature spermatozoa ready to be released in the lumen (Figure 6). Other eight males (22.8%) had only elongated spermatids as most advanced germ cells (Table 2). Sertoli cell nuclei had a variable shape and size between columnar to oval with a regular border. These cells were located at basement membrane of the cords and arranged at different heights. The average number of Sertoli cells per tubule in gonocyte stage group was 34 while in spermatogonia stage samples was 36.8. Sertoli cells in these groups were more consistently distributed and their nuclei located near the basal lamina. Males in these group had germ cell differentiation and thus had started spermatogenesis. Sertoli cell counts varied between stages, with a dramatic decrease observed in samples containing spermatids and mature spermatozoa ($r= 0.669$, $p= 0.0001$) (Table 3).

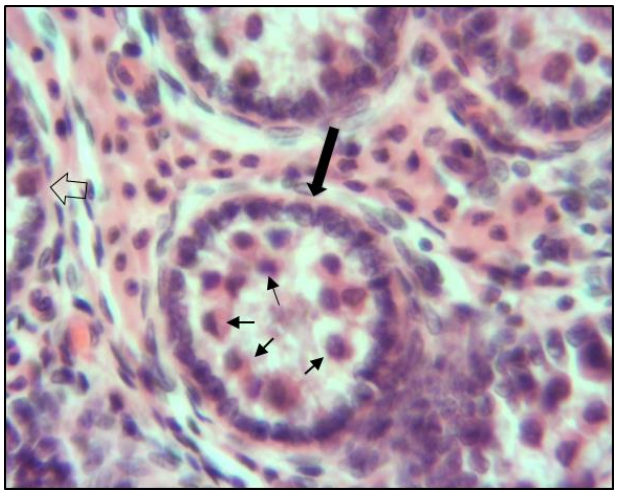


Figure 5. A photomicrograph of a cross section of pubertal camel testis showing a seminiferous cord (large black arrow) containing gonocytes (thin arrows) and one spermatogonia (open arrow) at the basement membrane. Hematoxylin and eosin stain, 40X.

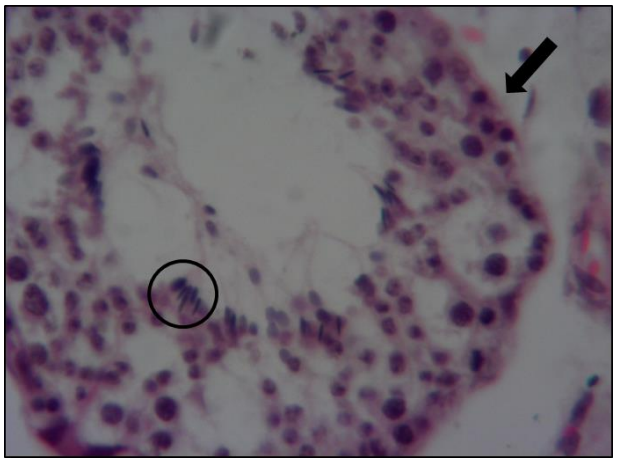


Figure 6. A photomicrograph showing advanced stages of germ cell maturation. Mature spermatozoa (circled) are aligned at the luminal border of the seminiferous tubule, indicating progression toward spermatogenesis. The thick black arrow indicates a seminiferous tubule. Hematoxylin and eosin stain, 100X.

Table 2. Determination of cellular components of the seminiferous epithelium.

	Nov	Dec	Jan	Feb	Mar	Total
Animals No.	7	7	8	6	7	35
Gonocyte per cord	4	1	3	1	0	9 (25.7%)
Spermatogonia	0	2	4	1	0	7 (20%)
Primary spermatocytes	0	1	1	3	5	10 (28.5%)
Elongating spermatids	2*	3	0	1	2	8 (22.8%)
Mature sperms	1	0	0	0	0	1 (2.8%)

*Sertoli-cell-one –layer tubules were seen.

Table 3. Number of Sertoli cells and most advanced cells per cross section.

Cell stage	G-gonia	S-gonia	PS	ES	MS
Animals' No.	9	7	10	8	1
Sertoli cells	34	36.8	35	5.7	4
No. of cells*	9.2	4.4	7.7	37.7	6

G-gonia: Gonocyte. S-gonia: Spermatogonia. PS: Pachytene spermatocytes. ES: Elongated spermatid. MS: Mature sperm.
* Number of cell type in the relevant column.

Cell composition

Table 4 presents the developmental parameters of the pubertal camel testes collected during the rutting season, categorized by distinct germ cell stages: gonocytes (G-gonia), spermatogonia (S-gonia), pachytene spermatocytes (PS), elongated spermatids (ES) and mature spermatozoa (MS). Each stage reflects progressive maturation within the seminiferous epithelium and is associated with changes in testicular characteristics. As germ cells progress through the spermatogenic cycle, a clear increase in testis weight was noticed, rising from 4.8g in the G-gonia stage to 43.7g in the sperm stage (Figure 7). This weight gain corresponds to increased tissue mass and cellular activity associated with advancing spermatogenesis. A notable decline was seen in the mean number of seminiferous tubules observed under 10X magnification, decreasing from 148 in G-gonia stage to 51 in the sperm stage. This reduction may reflect tubule expansion and structural reorganization as germ cell maturation progresses. In parallel, the mean number of mature Leydig cells was increased steadily from 30 in the G-gonia stage to 61 in the sperm stage, indicating an increase in androgenic support necessary for advancing testicular function and spermatogenesis.

The developmental changes in testicular structure during different germ cell stages in pubertal camel testes collected in the rutting season are presented in figure 8. As spermatogenesis advanced, the mean number of seminiferous tubules (10X field) showed a marked decline, while the number of mature Leydig cells was increased steadily. These trends reflect the reorganization of testicular tissue and rising endocrine support as the testes prepare for active spermatogenesis.

Table 4. Testicular parameters across spermatogenic stages in pubertal dromedary camels.

Cell stage	G-gonia	S-gonia	PS	ES	MS
Animals' No.	9	7	10	8	1
Mean testis weight (g)	4.8	6.2	10.3	26.8	43.7
Mean ST No. (X10)	148	138.3	113.6	56.3	51
Mature Leydig cells (mean)	30	41.8	43.9	54.4	61

G-gonia: Gonocyte. S-gonia: Spermatogonia PS: Pachytene spermatocytes. ES: Elongated spermatid. MS: Mature sperm. ST: Seminiferous tubules. g: gram.

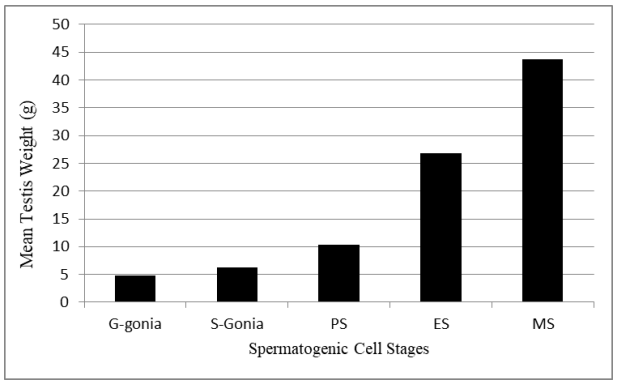


Figure 7. A bar graph illustrating the increase in mean testicular weight in grams across different spermatogenic stages in pubertal camels. A progressive rise in weight is observed from G-gonia to mature spermatozoa, indicating active testicular development and spermatogenic progression.
G-gonia: Gonocyte. S-gonia: Spermatogonia PS: Pachytene spermatocytes. ES: Elongated spermatid. MS: Mature sperm. g: gram.

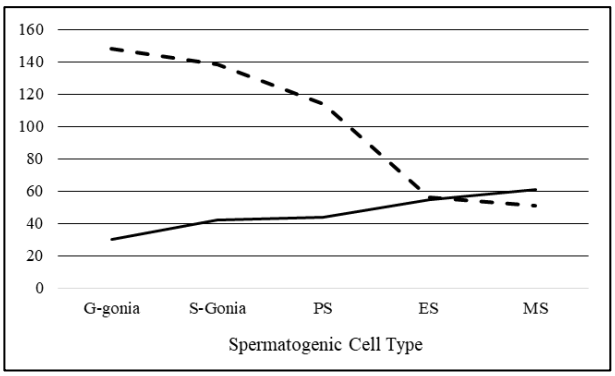


Figure 8. Variation in seminiferous tubule density (dotted line) and Leydig cell number (continuous line) across spermatogenic stages in pubertal dromedary camel testis.
G-gonia: Gonocyte. S-gonia: Spermatogonia PS: Pachytene spermatocytes. ES: Elongated spermatid. MS: Mature sperm.

Other observations

Myoid cells were elongated and sparsely distributed along the basal membrane of the tubules. Small blood vessels and lymphatic ducts were frequently observed, with occasional macrophages in the interstitial space.

Discussion

This study highlights significant monthly variations in testicular histology and spermatogenesis during the rutting season in one-humped camels. The observed peak in December aligns with prior findings on seasonal breeding activity (Shalash, 1965; Minoia et al., 1992; Tibary et al., 2008). The strong correlation between Leydig cell numbers, testicular weight and advanced germ cell stages is consistent with the functional roles of these cells in testosterone production and spermatogenesis, as reported by Tibary & Anouassi (1997). Our observation of increased Leydig cell numbers alongside spermatogenic progression aligns with melatonin and receptor localization studies during rutting (Doghbri et al., 2025).

The progression from gonocytes to mature spermatozoa underscores the gradual establishment of spermatogenesis, influenced by age and season. Sertoli cell dynamics also support earlier studies showing their essential role in early germ cell development but declining prominence during advanced spermatogenic stages (Skidmore et al., 1995). This reduction may reflect tubule expansion and structural reorganization as germ cell maturation progresses. In parallel, the mean number of mature Leydig cells increases steadily from 30 at the G-gonia stage to 61 at the sperm stage, indicating a rise in androgenic support necessary for advancing testicular function and spermatogenesis. These trends reflect the reorganization of testicular tissue and rising endocrine support as the testes prepare for active spermatogenesis. Siglec5 expression in the testes and epididymis during rutting suggests immunoregulatory roles in spermatogenesis, supporting our morphological findings (Al Khodair et al., 2023). Variability in testicular development among individuals may reflect environmental and nutritional factors affecting puberty, as noted by Singh & Bharadwaj (1978) and Abdel-Rahim (1997). The observed discrepancy between higher mean testicular weight in December and higher volume in November may reflect differences in tissue density, hormonal activity, and the histological composition of the testes. While November higher volume may exhibit early volumetric expansion due to increased blood flow or interstitial fluid accumulation, December higher weight likely represents a phase of increased cellular activity and tissue density, resulting in heavier but more compact testes. For instance, December could represent a period where spermatogenic activity is ramping up, increasing testicular mass (weight) due to cell proliferation, even before significant volume expansion occurs. November may precede this stage, showing early signs of activation such as swelling (volume) but with less sperm production or cellular content.

The observed variability in testicular development among individuals may be due to environmental and nutritional factors that affect the onset of puberty. The unusually low testicular weight and volume in January likely results from a combination of early or late sampling, developmental variation among individuals and environmental or hormonal modulation. This month might capture a transitional or less hormonally active window, explaining the observed dip. Overall, these findings underscore the importance of seasonal and age-related factors in camel reproduction. Future research could explore molecular markers and hormonal influences to provide deeper insights into camel spermatogenesis, as suggested by Tibary et al. (2014) and El-Harairy et al. (2012).

This study provides comprehensive insights into the testicular development and spermatogenic progression of pubertal dromedary camels during the rutting season. Significant monthly variations in testicular weight, volume, and histological structure reflect the strong influence of seasonality on reproductive activity. The clear correlation between increased testicular mass, rising numbers of mature Leydig ($r= 0.475$, $p= 0.004$) and Sertoli cells ($r= -0.774$, $p= 0.0001$), and the advancement of germ cell stages, from gonocytes to mature spermatozoa, highlights the coordinated physiological changes preparing the testes for active spermatogenesis. The observed decline in the number of seminiferous tubules per field, alongside increased tubule diameter and cellular differentiation, suggest structural remodeling as spermatogenesis progresses. These findings emphasize the critical roles of age, season, and endocrine regulation in the initiation of puberty and reproductive capability in camels. Further studies incorporating hormonal profiling and molecular markers are warranted to deepen our understanding of the mechanisms underlying camel reproductive physiology.

Conclusion

This study confirms that testicular development and spermatogenesis in pubertal dromedary camels are strongly influenced by seasonal changes during the rutting period. The coordinated increase in testicular mass, Leydig and Sertoli cell activity, and germ cell maturation underscores the physiological preparation for reproductive function. Structural changes within the seminiferous tubules further reflect active tissue remodeling. Future research should integrate hormonal and molecular analyses to better elucidate the mechanisms driving these seasonal reproductive patterns.

Conflict of interest

The authors declare no conflicts of interest with respect to the publication of this paper.

Authors' contribution

Aymen Z. Elzawam designed the study and supervised the project. Aymen Z. Elzawam and Ismail M. Hdud performed the experiments. Aymen Z. Elzawam and Mohamed M. Zurghani collected the samples. Fathi M. Abousaq analyzed the data. Aymen Z. Elzawam and Ismail M. Hdud interpreted the results. All authors contributed to writing and reviewing the manuscript.

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