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Seasonal histomorphometric analysis of ovarian follicular dynamics in dromedary camel (*Camelus dromedarius*)

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Abstract

A sound knowledge of follicular dynamics needs to be better understood in any species. However, there remains a need for comprehensive histomorphometrical quantification across all four seasons, incorporating both ovaries in dromedary camel is crucial prerequisite. Present study aimed to quantify histomorphometrical variations in follicles across seasons of a year. For this purpose, from 36 (4-7 years old) apparently healthy and no pregnant dromedary female camels (three in each month of a year) right and left ovaries were collected, processed through paraffin embedding technique, semiserially cut at 5.00 - 7.00 µm and stained with Hematoxylin and Eosin method. Through all the seasons, the highest counts were consistently recorded for primordial follicles, particularly in the right ovary. Primary and secondary follicles were stand in second record. Graafian follicles were detected in some animals, likewise reaching their maximum frequency in the winter (January). Atretic follicles were observed through all seasons. No significant differences were observed between the left and right ovaries for these follicular categories. The results indicated a general increasing trend in follicle numbers with advancing age. At 4 and 6.5 years of age, the mean follicle counts were increased with less sharpness, but at 7 years of age, the highest value was recorded. In conclusion, our results, confirm that while early folliculogenesis in this animal appears season-invariant, late-stage maturation and follicular atresia are strongly modulated by environmental and seasonal factors. These findings contribute to the optimizing reproductive management strategies, particularly in aligning breeding efforts with periods of enhanced ovarian activity.

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Introduction

Camels have long been domesticated and, as a very useful livestock, they provide food (milk and meat) and textiles (fiber and felt from hair). There are three surviving species of camel. The dromedary makes up 94.00% of the world's camel population, and the two-humped Bactrian camel makes up 6.00%. Domesticated dromedaries mostly live in the Horn of Africa, the Sahel, Maghreb, Middle East and Asia. They provide nomadic people in Somalia and Ethiopia with milk, food, and transportation.[1,2]

Iran is one of few countries that home to both dromedaries being spread in central deserts and Bactrian camels inhabit northwest region (especially Ardabil province). Iranian camel population is 1648000, whereas the camel population in Iraq is 4387260 being mostly located in southern regions of this country. The camels are distributed in Anbar, Najaf, Muthanna, and Basra provinces of Iraq.[3]

Full reproductive capacity of the female camel is reached at 6 years.[4-6] The female camel would breed until 30 years of age.[7] The follicular cycle is divided into growth phase (10.50 ± 0.50 days), mature phase (7.60 ± 0.80 days) and regression (11.9 ± 0.8 days) phases.[8]

Corresponding follicular stages (non-growing, and growing) were recorded by Nawito *et al.*,[9] and duration of estrous cycle averaged 24.20 days in Egypt,[10] 23.40 days in India,[11] and 28.00 days in Sudan.[12] Estrous cycle duration of 4 - 6 and 16 - 30 and 11 - 27 days, has been observed.[13] In the absence of mating there is only a succession of follicular waves with highly variable rhythm.[14] Particularly, the females are nearly polyestrous and due to that, although it shows strong tendency to be regarded as a seasonal breeder, pregnancy can occur in any season of the year. In addition, they are considered as induced ovulators and ovulation occurs after mating.[15-17] The evidence of induced ovulation is the absence of a corpus luteum formation and serum progesterone concentration

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is low in unmated females clarifying that the estrous cycle in the dromedary is restricted only to a follicular development and absence of luteinization and ovulation is induced by copulation. Serum estradiol concentration reaches peak values when the dominant follicle measures 1.70 ± 0.10cm in diameter.[18]

Most of the ovarian activity occurs form December to March and during July and August (rainy season) in India,[19] and from December to May, in Egypt.[20] During the breeding season, follicular growth occurs constantly in both ovaries in regular waves.[21,22] Induction of ovulation could be achieved in the camel by mating with an intact or vasectomized male. The left and right ovaries function alternatively.[23] The pattern of ovarian follicular development has been documented and the occurrence of ovarian follicular waves was confirmed in dromedary and Bactrian camels, alpacas, llamas, guanacos and vicunas. [24]

The increased importance of camel breeding in Middle East is getting obvious day after day, especially in the deserts and newly reclaimed areas. This is mainly, due to its unique physiological characteristics, helping it to survive and perform under such harsh conditions.[25]

Despite the previous appreciated insights, these works have largely focused on descriptive histology or single-factor comparisons. There remains a need for comprehensive histomorphometrical quantification across all four seasons, incorporating both ovarian side and their interaction. Therefore, the present study aimed to quantify histomorphometric variations in counts of ovarian follicles (primordial, primary, secondary, Graafian, and atretic) across autumn, spring, summer, and winter, moreover these assessments were also directed for each month of a year.

Materials and Methods

Sampling. Collection of ovaries were taking place from September 2023 to August 2024 in Najaf, Iraq. The study involved collecting three samples for each month of the year, comprising 36 adults (4 - 7 years old) apparently healthy dromedary camels, slaughtered in an Al-Najaf slaughterhouse. The specimens were collected and placed in storage containers encompassing 10.00% formal saline solution for fixation and transferred to the histology laboratory for further processing.[26-28]

For histomorphometry, specimens underwent histo-technique processing through paraffin embedding, cut at 5.00 - 7.00 µm and stained through Hematoxylin and Eosin (H&E) method.[29-31] Every fifth section from the serially sliced ovarian tissue was selected to ensure representative sampling of follicular development. In both ovaries, follicles were identified and recorded in the sections, and categorized into distinct primordial, primary, secondary, Graafian and atretic follicles.[28,32,33] This study was conducted in accordance with the laws and methods approved by the Animal Welfare Committee of the Ministry of Health, overseeing the monitoring of the slaughter process, health inspections, and conditions of confinement. The study was carried out in collaboration between the College of Education in Qurna University of Basra, and the Model Slaughterhouse in Najaf, to facilitate the execution of the task. (Approval No. 7/35/1797, August 2023).

Statistical analysis. The study data were analyzed using SPSS Software (version 26.0; IBM Corp., Armonk, USA). To determine significant differences between the studied groups, a one-way ANOVA was performed. Duncan's post hoc analysis was used to identify which specific groups differed significantly. A p value of ≤ 0.05 was used to determine statistical significance.

Results

The histomorphology of ovaries on different categories of ovarian follicles in all of animal groups and in all of seasons of a year revealed different growing and non-growing healthy and atretic follicles (Fig. 1).

Primordial follicles were the most abundant in both ovaries, peaking in the left ovary during summer and in the right ovary during winter. Primary follicles showed seasonal and inter-ovarian differences, being higher in the left ovary in summer and in the right ovary in winter. Secondary follicles remained low and uniform across seasons. Graafian follicles were scarce but showed seasonal variation in the left ovary, with increased numbers in summer. Atretic follicles were present year-round with no significant seasonal changes, indicating stable follicular atresia (Fig. 2).

This study compared the average numbers of different types of ovarian follicles between the left and right ovaries. The right ovary contained significantly more primordial follicles than the left ovary. Although the number of primary follicles was slightly higher in the right ovary, this difference was not statistically significant. In contrast, secondary follicles were significantly more numerous in the left ovary. No statistically significant differences were observed in the numbers of Graafian or atretic follicles between the two ovaries. These findings are presented in greater detail below.

Across all seasons, primordial follicles were the most abundant follicle type in both ovaries. In the left ovary, their numbers peaked in summer, while in the right ovary they were the highest in winter (Fig. 3A). Primary follicle counts displayed seasonal and inter-ovarian alterations, accordingly the left ovary had significantly more in summer, whereas the right ovary had the highest numbers in winter (Fig. 3B). Graafian follicles were consistently scarce, though the left ovary showed seasonal variation, with summer having significantly more than spring (Fig. 3C).

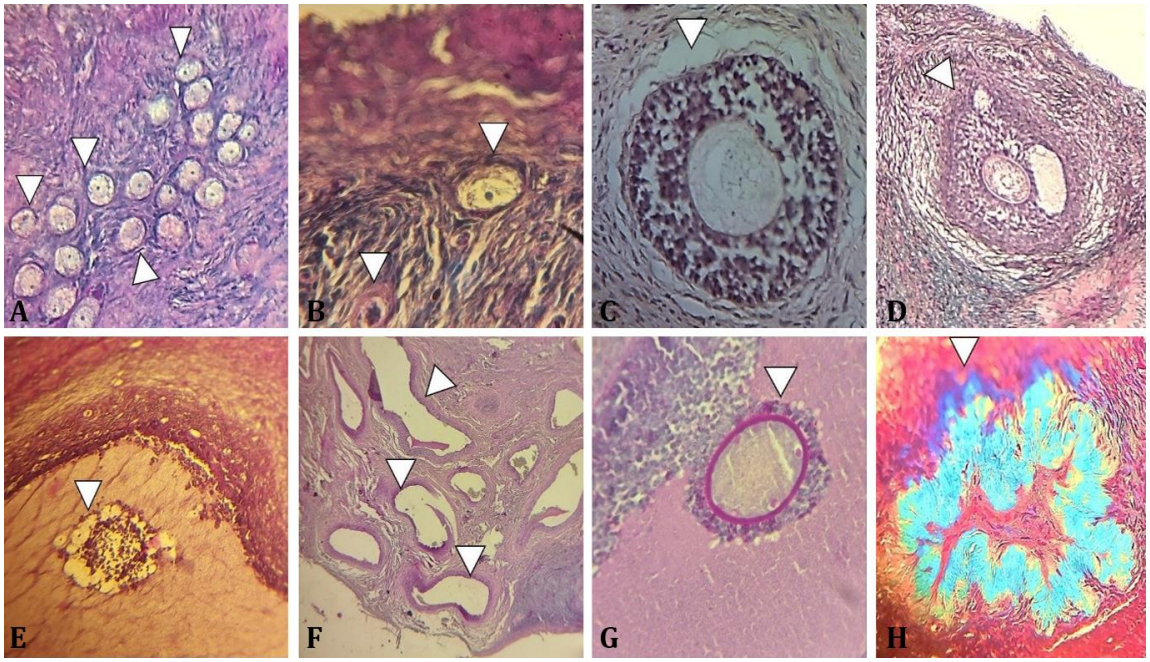
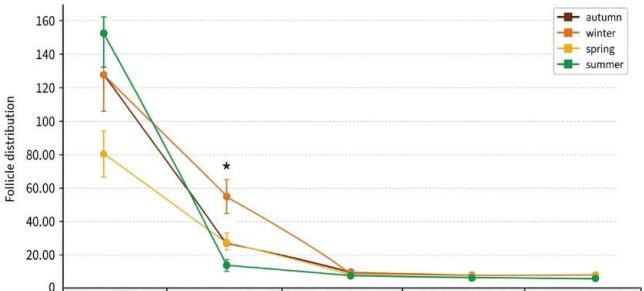


Fig. 1. Different categories of ovarian follicles observed in camels during this study. **A)** Group of healthy primordial follicles in the ovarian cortex (H&E, 100×). **B)** Two primordial follicles; the upper follicle is healthy, whereas the lower follicle shows a pyknotic nucleus, indicating atresia (H&E, 250×). **C)** Multilayered primary follicle with a high pyknotic index in the granulosa cells (H&E, 400×). **D)** Healthy secondary (vesicular) follicle (H&E, 250×). **E)** Atretic tertiary follicle showing advanced oocyte degeneration and a high pyknotic index in the granulosa cells (H&E, 250×). **F)** Numerous atretic tertiary follicles in the deeper region of the ovarian cortex (H&E, 250×). **G)** Portion of a mature healthy Graafian follicle with a prominent zona pellucida (PAS, 250×). **H)** Atretic follicle at the final stage of degeneration (Masson's Trichrome, 100×). Arrowheads indicate ovarian follicles.



	Primordial	Primary	Secondary	Graffian	Atretic
Winter	120.67	51.80	7.80	6.67	7.47
Spring	74.93	25.00	6.47	6.73	6.67
Summer	146.00	11.80	6.40	5.60	5.33
Autumn	121.20	24.60	8.27	7.07	6.80

Fig. 2. Seasonal distribution of ovarian follicle types (primordial, primary, secondary, Graafian, and atretic) pooled from both ovaries. The x-axis represents the different follicle types, and the figure compares their mean counts across seasons. The asterisk (*) indicates a statistically significant difference in the mean number of primary follicles, with the highest mean observed in winter and the lowest in summer ($p < 0.05$).

Secondary follicles remained low and relatively uniform across seasons in both ovaries (Fig. 3D). Atretic follicles were present year-round in both ovaries, with no significant seasonal differences (Fig. 3E).

Seasonal comparison showed that the right ovary had significantly ($p \leq 0.05$) more total follicles in winter, while the left ovary had significantly more in spring. In summer both ovaries were similar, and the right ovary was slightly higher but without statistical significance in autumn. Monthly analysis indicated notable left–right differences at several points of the year, including January, March, July, and October, with the right ovary often higher ($p \leq 0.05$), while other months showed little or no difference. Across age groups, the total number of growing follicles generally increased with age, reaching the highest level at seven years old (Figs. 4 and 5).

Monthly changes in follicle types differed between the left and right ovaries. Primordial follicles peaked in January in the right ovary and in August in the left one, with noticeable month-to-month fluctuations in both (Fig. 6A). Primary follicles showed a sharp, significant increase in February in both ovaries, with generally lower values during the rest of the year (Fig. 6B). Secondary follicles varied only slightly in the right ovary, but fluctuated more in the left one with some months showing significant left-right differences (Fig. 6C). Graafian follicles were generally few, but the left ovary showed greater variability and significantly higher values in several months (Fig. 6D). Atretic follicles changed only mildly across months, with February and November often showing higher values in the left ovary (Fig. 6E). Overall, some follicle categories showed strong seasonal or monthly peaks, and left-right differences appeared at specific times of the year.

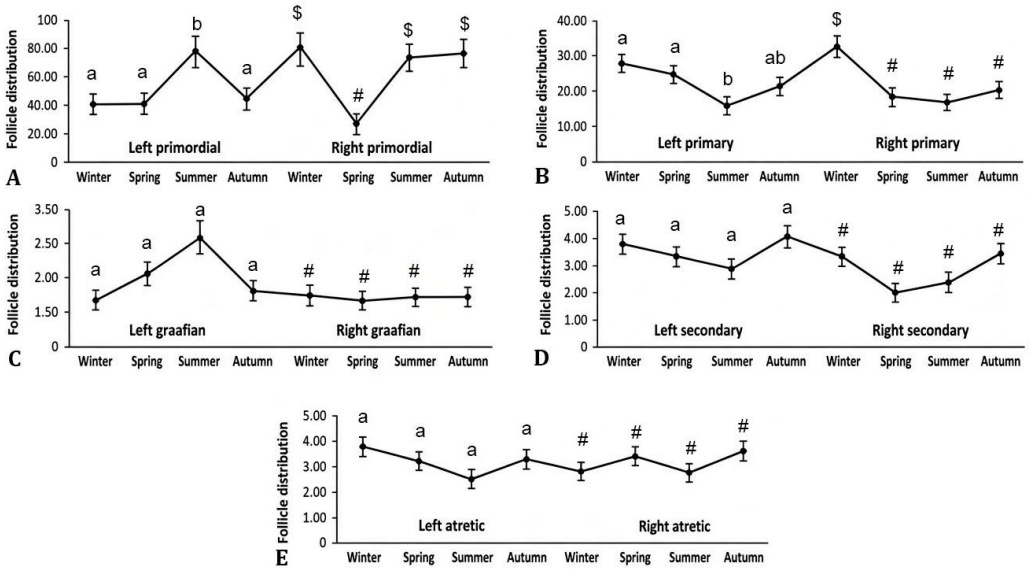


Fig. 3. Seasonal comparison of mean ovarian follicle numbers in the left and right ovaries. **A)** Primordial; **B)** Primary; **C)** Secondary; **D)** Graafian; **E)** Atretic. Dissimilar superscripts indicate significant differences ($p \leq 0.05$), while identical superscripts indicate no significant difference ($p \geq 0.05$).

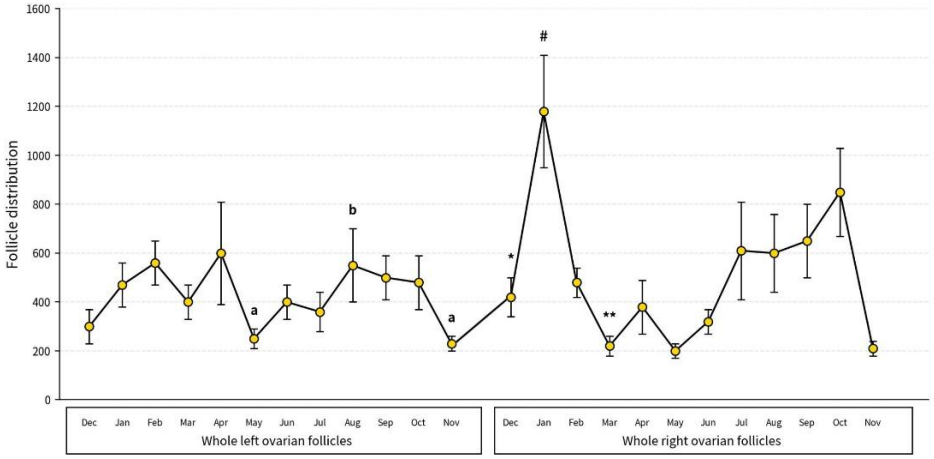


Fig. 4. Monthly total follicle counts in left and right ovaries show significant differences across some months ($p \leq 0.05$). The symbol # indicates that the right ovary exhibited a significant peak in January, whereas * and ** indicate low and the lowest values in December and March, respectively. In contrast, in the left ovary, a indicates the minimum values in May and November, while superscript b indicates the maximum value in August.

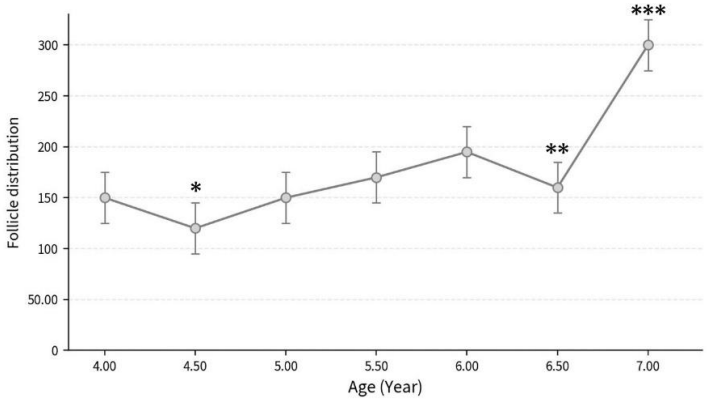


Fig. 5. Growing follicle distribution in camels aged 4-7 years shows significant age-related differences ($p \leq 0.05$), with non-superscripted values indicating no significant difference ($p \geq 0.05$).

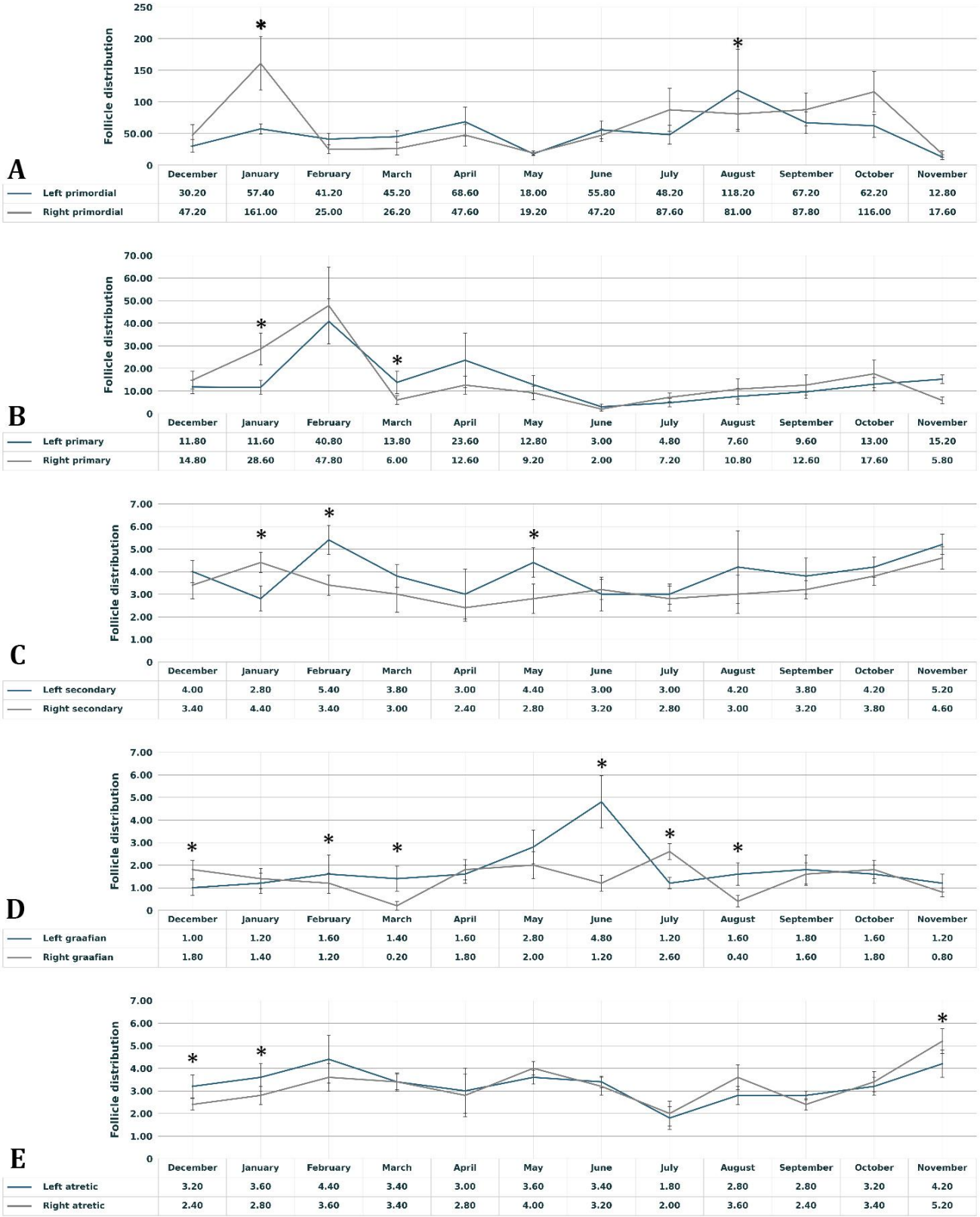


Fig. 6. Monthly distribution of follicle types in the left and right ovaries. **A)** Primordial; **B)** Primary; **C)** Secondary; **D)** Graafian; **E)** Atretic. Asterisks (*) indicate significant differences between the left and right ovaries ($p \leq 0.05$), whereas the absence of asterisks indicates no significant difference ($p \geq 0.05$).

Discussion

In dromedary camels, ovarian follicle dynamics vary with environmental conditions. Studies report increased numbers and sizes of mature follicles during the breeding season, with higher ovarian activity and corpora lutea in cooler, wetter months, and significantly more follicles in winter.[33,34] Moreover, in a report of investigated histo-morphometrical parameters of the female reproductive tract across four seasons, significant enhancements in follicle number were found during winter.[35]

According to the results of our study, in winter, dromedary camels show peak ovarian activity, with a predominance of primordial follicles—particularly in the right ovary-alongside appreciable numbers of primary and secondary follicles. The presence of Graafian follicles in January indicates mid-winter ovulatory readiness, while atretic follicles persist year-round. Taken together, these findings indicate that winter represents a peak period of reproductive activity in this animal. This seasonal pattern aligns with previous reports describing the influence of environmental factors such as photoperiod, ambient temperature, and nutritional status on ovarian function in camelids. [33-35] Moreover, these findings highlight winter as a peak reproductive season, influenced by environmental factors such as photoperiod, temperature, and nutrition.

In spring, dromedary camels show a transitional ovarian phase, with persistent primordial follicles and gradual growth of primary and secondary follicles, peaking in May. Graafian follicles remain low, and atretic follicles occur consistently. The right ovary still has slightly more primordial follicles, but asymmetry is reduced compared to the winter. This pattern is consistent with previous studies regarding the seasonal nature of camel reproduction.[36,37]

In summer, dromedary camels show reduced ovarian activity. Primordial follicles peak in July but represent only the reserve, with little advanced follicle growth. Graafian follicles are nearly absent, and atretic follicles remain low and stable, indicating minimal reproductive activity during this season.

In autumn, dromedary camels exhibit a transitional ovarian phase, with gradual growth of advanced follicles. Graafian follicles appear occasionally but not enough to indicate ovulation, possibly signaling a shift from low summer activity toward the reproductive peak in winter. This seasonal trend is consistent with established patterns of camel reproduction.[28]

Seasonal patterns reveal functional differences between the ovaries in dromedary camels. The left ovary shows a significant summer increase in primordial follicles, likely due to the follicular reserve accumulation and reduced recruitment, while the right ovary remains more stable, peaking in winter. Graafian follicles in the right ovary stay nearly constant across seasons, indicating functional stability and less seasonal influence on ovulatory readiness.

Atretic follicles, representing the natural elimination of non-dominant follicles, remain stable across seasons in both ovaries of dromedary camels. This suggests that follicular atresia is largely regulated by intrinsic ovarian and endocrine mechanisms, rather than by environmental factors, a pattern also observed in canines, ruminants, and humans.[36-38]

Follicular distribution between the left and right ovaries in dromedary camels is asymmetrical. The right ovary often has more primordial follicles, while differences in secondary follicles suggest variations in hormonal sensitivity, blood supply, or ovarian structure between the two sides.

Atretic follicles occur at similar rates in both ovaries, with minor variations in follicle regression. Primordial follicles dominate the right ovary, followed by primary follicles, indicating active but lower-level folliculogenesis compared to the ovarian reserve.

Secondary and atretic follicles are less abundant than primordial and primary follicles, while Graafian follicles are rare, highlighting limited maturation. Both ovaries emphasize primordial reserves with restricted development of mature follicles.

Monthly analysis shows primary follicles peak in February in both ovaries, indicating enhanced ovarian readiness, while levels are lower from March to November. Secondary follicles are more evenly distributed, with the left ovary showing higher activity in some months and the right ovary remaining stable. These patterns highlight seasonal folliculogenesis and inform ovulation timing and reproductive management in dromedary camels.

Graafian follicles, the final stage of follicular development, are scarce and show limited monthly variation. The left ovary peaks in May and June, while the right one peaks in July, indicating functional asymmetry in follicle maturation and ovulatory readiness, useful for reproductive management in dromedary camels.

Atretic follicles remain relatively stable throughout the year, with the right ovary showing higher activity in November and the left one in winter, reflecting functional differences in follicle elimination. Monthly monitoring and assessments of nutrition and metabolism are recommended to better understand seasonal and physiological influences on ovarian function in dromedary camels.

Follicular activity in dromedary camels is seasonally influenced. Primordial and primary follicles fluctuate most, especially in summer and winter, while secondary follicles remain stable. Graafian and atretic follicles also show seasonal changes, indicating environmental modulation of follicle maturation and atresia, important for reproductive timing and ovarian evaluation.

Total follicle counts in dromedary camels fluctuate seasonally. The right ovary is most active in winter, the left ovary peaks in spring, both are balanced in summer, and autumn shows no significant differences, indicating a seasonal convergence of ovarian activity.

These findings clarify seasonal ovarian dynamics in dromedary camels and inform reproductive management, including selection of the dominant ovary and optimal timing for interventions.

Monthly total follicle counts show functional fluctuations, with the right ovary peaking in January and October and the left one in August. Most months show similar activity between ovaries, indicating balance. Early folliculogenesis is seasonally stable, while late-stage maturation and atresia are influenced by environmental and seasonal factors, aiding reproductive management and breeding timing in dromedary camels.

According to the results of this investigation, ovarian activity in dromedary camels increases with age, peaking around 7 years. Lower activity at 4.50 and 6.50 years may reflect individual or environmental factors. Peak activity at 7 years indicates maximal reproductive capacity, informing breeding management, insemination timing, and selection of high-potential animals.

In the line with that, it can be suggested to collect data every month instead of every season to identify small seasonal changes, as recommended by Padalino *et al*.,[39] and measure nutrition and metabolism, such as feed intake and body condition score to determine whether changes are caused more by day length (photoperiod) or by the animals' metabolic condition. Our results, in accordance with reports of Vyas *et al*.,[40] and Nowshari and Ali,[41] Manjunatha *et al*.,[42] confirm that while early folliculogenesis in dromedaries appears season invariant, late-stage maturation and follicular atresia are strongly modulated by environmental and seasonal factors. These findings contribute to the optimizing reproductive management strategies, particularly in aligning breeding efforts with periods of enhanced ovarian activity.

In conclusion, our results confirm that, early folliculogenesis in dromedary camels is largely season-independent, whereas late-stage follicle maturation and atresia are strongly influenced by environmental and seasonal factors. This knowledge aids in optimizing reproductive management and timing breeding with peak ovarian activity.

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Conflict of interest

The authors declare no conflict of interest.

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