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A comparative study of follicular dynamics, hormonal profiles, ovarian measurements, and endometrial thickness between well-fed nulliparous and multiparous dromedary she-camels during the breeding season

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ABSTRACT

The current study sought to determine whether well-fed nulliparous she-camels (NP, n= 8) aged 2:3 years could have the same pattern of fertility status similar to multiparous she-camels (MP, n= 13; aged 8 to 13 years). Ovarian follicular dynamics and associated hormones, ovarian measures and endometrial thickness, and pregnancy rates were recorded during the period of 90 days (December–March). Every two days, the ovaries and uterus were scanned by ultrasound, and blood samples were taken. Results showed that the right ovaries were more active than the left ovaries. Ovarian length and width and endometrial thickness increased with follicles growth, and NP had longer ovaries ($P<0.05$) than MP she-camels and the opposite was found in the ovarian width, whereas endometrial thickness was less in NP than MP. MP had a higher mean number of emerged follicles (17.21±0.41 follicles) than NP (10.42±0.41 follicles). The mature phase duration was longer ($P<0.05$) in MP (11.89 days) than in NP (9.94 days). The duration of regression for DF and oversized follicles were 19.38 and 15.13 days in NP compared to 23.31 and 17.27 days in MP. Progesterone concentrations remained at the basal level throughout the follicular waves, while E2 and FSH concentrations were associated with the follicular growth, and the peak levels were recorded in the mature phase (56.88 and 7.766 vs. 62.58 and 9.017 pg/mL and mIU/mL in NP and MP, respectively). In conclusion, well-fed NP demonstrated fertility status comparable to multiparous she-camel.

Introduction

Camels are a promising animal species for withstanding desertification and global warming due to their adaptability and resistance to diseases resulting in increased interest in raising camels worldwide (Bouâouda *et al*., 2014; Tibary and El Allali, 2020). Furthermore, dromedary camels have unique physiological and reproductive characteristics as opposed to other livestock. Camels are induced ovulators that require exogenous stimulators to complete the ovulation process such as either coitus or hormonal induction (Skidmore, 2011). Also, they are polyestrous animals with a higher tendency for reproductive seasonality (Monaco *et al*., 2015). During the breeding season, the ovarian follicular dynamics in dromedary camels take place in overlapping wave-like patterns (Manjunatha *et al*., 2012; Rawy *et al*., 2014). Therefore, follicular growth and regression are occurring in repeated cycles in the absence of ovulation stimulators. Furthermore, the inverse relationship between the number and diameter of the largest follicle was a coincidence with the follicular development, which confirmed the follicle wave theory for camels (Skidmore, 2011; Padalino *et al*., 2016).

Basically, the follicular waves are regulated by FSH and LH stimulation as a response to GnRH (Bravo *et al*., 1992), and in the absence of ovulation, progesterone (P4) concentrations were low, remaining at the basal level (<1.0 ng/mL, Skidmore *et al*., 1996). However, high estradiol (E2) concentrations have been revealed during the mature phase of DFs (Atigui *et al*., 2013), and there is a positive correlation between the E2 concentration and the follicle diameter (Riveros *et al*., 2010). Hence, after the DFs reach 17 mm in diameter, E2 concentrations in dromedary camels begin to decline (Skidmore *et al*., 1996).

It has been reported that sexual activity initiated early in dromedary camels at the age of 2-3 years (Zarrouk *et al*., 2003), but they are typically not allowed to mate until they reach physical maturity (70 % of adult body weight) at the age of 4-6 years (Kaufmann, 2005). There are numerous factors that influence the onset of ovarian activity, and it is expected that the body condition score in camelids has a positive relationship with ovarian activity and, consequently, the age at puberty, as observed in cattle (Pryce *et al*., 2001). Previous research has shown that she-camel with a lower body condition score cannot continue to reproduce without increased feeding (Gherissi *et al*., 2018). In contrast, a she-camel with a high body condition score may exhibit sexual activity even during the summer months (Gherissi *et al*., 2020). Consequently, the suggested hypothesis refers to the intensive breeding system that results in a she-camel with a good body condition score being offered for breeding without any deleterious effects on their reproductive lives. As a result, the current study was carried out to compare fertility status (follicular dynamics and associated hormones) of the well-fed dromedary nulliparous and multiparous she-camels.

Materials and methods

Ethical approval and consent to participate

All animals and sampling procedures in this experiment were supervised and approved by the Institutional Animal Care and Use Committee of Alexandria University under number 146-2022. Also, all procedures and experimental protocols were in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

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Animal, management, and experimental design

The study was conducted during the breeding season (December to March) on one-humped Rashidi's dromedary she-camels (*Camelus dromedarius*) at Ras-Sudr Research Station, Desert Research Center, South Sinai Governorate, Egypt, at longitude 32, 42, 20 E and latitude 29, 35, 30 N. Twenty-one she-camels aged between 2.8 and 13 years, with a normal reproductive tract, no reproductive disorders were found and body condition scores (4 – 4.5) were determined based on a 5-point scale (0= very thin and 5=very fat) according to Faye *et al*. (2001). All the females were kept in half-cover shelter pens away from the males. The animals were fed the concentrate supplement (0.7 % of body weight) and alfalfa hay (1.0%) of body weight) according to Askar *et al*. (2023), along with free access to drinking water. The concentrate supplement consisted of 55% corn, 15% soybean meal, 10% cottonseed extract, 15% wheat bran, 2.5% limestone, 1.5% salt, 0.5% sodium bicarbonate, 0.3% premix, 0.1 yeast, and 0.1 antitoxins. The enrolled animals were divided into two groups based on age; nulliparous (NP; n= 8) she-camels were aged 2:3 years, and multiparous (MP; n= 13) dry she-camels (parities 2–6) were aged between 8 and 13 years.

Ovarian ultrasonography and follicular wave

Transrectal ultrasound examination of uterus thickness, ovarian measurements (length and width), and ovarian follicular growth and dynamics were monitored every other day for 90 days using ultrasonography equipped with a 5-7.5 MHz linear array transrectal probe (real time, B-mode, Sonoscope®, China). Camels were restrained in a standing position within specially designed crates prior to examination with minimal strains on the examined animals. After the removal of fecal matter, the lubricated transrectal transducer was positioned dorsally, cranially, and laterally to scan the uterus and the ovaries. The diameter and position of all follicles ≥2mm in diameter were recorded and drawn on the ovarian charts. According to Skidmore *et al*. (1996), ovarian structures were classified into small follicles (<5 mm in diameter), growing follicles (5 to <10 mm), DFs (>10 to <20 mm), and oversize follicles (> 20 mm).

The emergence of follicular wave was distinguished with the first detection of a cohort of small follicles on ovaries, and some of these follicles continue to grow (dominant and co-dominant follicles) while others (subordinate follicles) gradually regress at different intervals. The phases of follicular wave (growth, maturation, and regression), duration of each phase, and the number and duration of intra-wave interval (IWI; the interval between recruitment of the two consequent follicular waves) of each studied animal, as well as the whole follicular wave duration were recorded according to Manjunatha *et al*. (2012). Moreover, the occurrence of oversized follicles proportion and codominant follicles were recorded. All she-camels were mated naturally when the DFs (11-17mm) were observed without using hormonal treatments. After about 45 days post-mating, the uterine contents were scanned to diagnose the pregnancy.

Hormonal assessment

Before feeding access, four blood samples (10 mL each) from each camel were obtained from the jugular vein prior to ultrasound examination. These samples were specifically taken when small, growing, dominant, and oversize follicles were monitored. Sera samples were harvested and stored at -20 °C until hormonal analysis. Serum E2 (pg/mL) and P4 (ng/mL) concentrations were measured using the enzyme-linked immunoassay kits (ELISA, Catalogue No. EU0390 (E2), ECM0014 (P4), Wuhan Fine Biotech Co., Ltd., China). The lower limits of detection for E2 and P4 were < 7.5 pg/mL and < 0.188 ng/mL, respectively, and the intra-assay and inter-assay CVs for both were < 8 % and < 10 %. Follicle-stimulating hormone (FSH; mIU/mL) concentration was measured using an ELISA kit (Catalog No. KIF 40570, Medix Biotech Inc., USA), with a sensitivity of < 0.5 mIU/mL.

Statistical analysis

Data of body condition score, ovulatory follicles life span, the time elapsed between growing and ovulatory follicles, endometrial thickness, reproductive hormone concentration and durations of each IWI, follicular wave, DF, regression duration of the largest and oversized follicle, numbers of follicles per wave and follicular waves were analyzed using GLM procedure of SAS (2009) for testing the age effect. Also, data on ovarian size, all follicle diameters and numbers were analyzed using the GLM procedure of SAS (2009) with both age and ovary (right or left) as main effects. Besides, the significant differences between means were tested using the Duncan multiple range test (Duncan, 1955). Also, chi-square analysis was used to test the effect of age on the pregnancy rate at a 95% confidence interval.

Results

Ovarian measurements and uterine thickness

The ultrasonography of the right and left ovaries dimensions in nulliparous (NP) and multiparous (MP) she-camels revealed that the width and length of ovaries changed significantly according to observed follicles. The left ovary in NP, the right ovary in MP, and the left ovaries in both groups were the widest ($P < 0.05$), respectively compared to other groups (Table 1). Furthermore, the right ovary in the NP she-camel group was longer ($P < 0.05$) as opposed to the other groups when small, growing, and DFs were observed (Table1).

Concerning the endometrial thickness, it was significantly ($P<0.05$) greater in MP she-camel than in NP she-camel. Furthermore, the endometrial thickness increased ($P<0.05$) in tandem with the follicular growth and was thicker ($P<0.05$) when the DFs were observed on the ovaries. However, the endometrial thickness decreased in coincidence with the existence of the oversized follicles on the ovaries (Table 1).

Ovarian structures and follicular dynamics

The follicular growth and regression in all studied animals were observed as an overlapping wave-like pattern. In addition, six out of the 29 waves in the NP she-camels and eight out of the 38 waves in the MP she-camels had co-dominant follicles. Furthermore, about ten out of 29 (34.48%) waves in NP she-camels and 14 out of 38 (36.8%) waves in MP she-camels had DFs that continued to grow after losing their dominance and developed into oversized follicles.

The mean of small follicle numbers that emerged per wave were higher ($P < 0.05$) in MP than NP she-camels (17.21 vs. 10.42 follicles, Table 2). The mean growth phase durations of follicles in NP and MP she-camels did not differ and were 6.1 and 5.8 days, respectively. While the mean duration of DF and oversized follicle phases were substantially greater in MP than in NP she-camels (11.9 vs. 9.9 days and 10.9 vs.9.6 days, respectively; Table 2). The DF might lose its fertilizing capacity and completely regress within 19.38 days in NP versus 23.31 days in MP with a significant difference ($P<0.05$). On the other hand, DF might take another course and develop some structural changes that reduce its fertilization capacity when it reaches larger sizes than DF. The development into oversize follicles lasted for 9.61 and 10.85 days in NP and MP she-camels, respectively, which varied ($P<0.05$). It took the oversized follicles 15.13 days to regress in NP animals compared to 17.27 days in the MP animals, in which significant differences ($P<0.05$) between them were recorded (Table 2). It was observed that the new follicular wave begins to grow before the regression of the DF, and the mean maximum diameters of the DF before regression were 20 and 23 mm in the NP and MP she-camel, respectively (Fig. 1). The mean IWI in the MP was significantly higher ($P < 0.05$) than

that of the NP (14.73 vs. 12.38 days, respectively). The duration of completed follicular waves in both groups significantly differed; it was 44.31 days in MP and 40.88 days in NP she-camels, and each animal had mean IWIs of 3.92 in MP versus 2.38 IWIs in NP she-camels (Table 2). Furthermore, the number of follicular waves that were observed per animal did not differ between she-camel groups (2.50 vs. 2.77 follicles, respectively).

Table 3 shows the ovarian activities of NP and MP she-camel, such as the number and diameter of the different follicles and the activity of the right ovaries vs. the left ovaries. Furthermore, MP she-camel ovaries produce significantly more follicles than NP. The number of small folli-

Table 1. Ovaries measurements (width and length) and endometrial thickness (mm) at different stages of follicular growth in nulliparous and multiparous dromedary she-camels during the breeding season.

Items	Nulliparous		Multiparous		SEM	*P*-Value
	Right Ovary	Left Ovary	Right Ovary	Left Ovary		
Width (cm)						
At small size follicles < 5mm.	2.87	2.53	2.78	2.65	0.09	0.63
At growing follicles (> 5mm and < 10mm).	3.036^{ab}	3.332^{a}	2.880^{b}	2.789^{b}	0.09	0.01
At dominant follicles (>10 mm to <20 mm).	3.136^{b}	3.044^{b}	3.438^{a}	3.023^{b}	0.07	0.01
At oversize follicles* > 20mm.	2.45^{b}	3.12^{a}	2.79^{b}	3.34^{a}	0.1	0.00
Length (cm)						
At small size follicles < 5mm.	3.592^{a}	3.186^{b}	3.238^{b}	2.965^{c}	0.07	0.02
At growing follicles (> 5mm and < 10mm).	3.791^{a}	3.312^{b}	3.399^{b}	3.156^{b}	0.06	0.01
At dominant follicles (>10 mm to <20 mm).	3.924^{a}	3.580^{b}	3.465^{b}	3.389^{b}	0.06	0.01
At oversize follicles* > 20mm.	3.11^{c}	3.70^{a}	2.63^{d}	3.52^{b}	0.11	> 0.001
Endometrial thickness (mm)						
At small follicles < 5mm	5.445^{bD}		6.075^{aD}		0.17	0.02
At growing follicles (> 5mm and < 10mm).	7.453^{bC}		8.418^{aC}		0.16	
At dominant follicles (>10 mm to <20 mm).	11.038^{bA}		12.401^{aA}		0.18	
At oversize follicles* > 20mm.	9.16^{bB}		9.73^{aB}		0.12	

[a-d] Means with different letters within the same row are significantly different (P < 0.05). [A-D] means with different letters at the same column (for endometrial thickness only) are significantly different (P<0.05). *Oversized follicle percentages were 34.48% in NP and 36.8% in MP she-camels.

Table 2. Follicular waves dynamics characteristics in nulliparous and multiparous dromedary she-camels during the breeding season.

Items	Nulliparous	Multiparous	*P*-Value
Number of follicles per wave	10.42^{b}±0.392	17.21^{a}±0.421	< 0.001
Number of follicular waves per animal	2.50±0.109	2.77±0.095	0.22
Number of IWIs per animal	2.38^{b}±0.212	3.92^{a}±0.199	< 0.001
IWI duration, days	12.38^{b}±0.311	14.73^{a}±0.276	< 0.001
Duration of follicular wave, days	40.88^{b}±0.462	44.31^{a}±0.434	< 0.001
Duration of Growth phase, days.	6.117±0.151	5.813±0.112	0.20
Duration of Ovulatory follicles (DF), days.	9.94^{b}±0.223	11.89^{a}±0.256	<0.0001
Duration of Oversize follicles phase*, days.	9.61^{b}±0.210	10.85^{a}±0.203	0.00
Regression duration of Follicle, day			
Ovulatory follicles (DF)	19.38^{b}±0.445	23.31^{a}±0.454	< 0.001
Oversized follicles*	15.13^{b}±0.291	17.27^{a}±0.272	< 0.001

[a- b] Means with different letters within the same row are significantly different (P < 0.05). *Oversized follicle percentages were 34.48% in NP and 36.8% in MP she-camels.

Table 3. The ovarian activity (numbers and diameters of follicles) at different stages of follicular growth in nulliparous and multiparous dromedary she-camels during the breeding season.

Items	Nulliparous		Multiparous		SEM	*P*-Value
	Right ovary	Left ovary	Right ovary	Left ovary		
Number						
Small size follicles < 5mm.	13.23^{b}	7.602^{c}	26.067^{a}	8.343^{c}	2.92	0.01
Growing follicles (> 5mm and < 10mm).	8.282^{b}	4.613^{bc}	17.703^{a}	3.524^{c}	0.18	0.03
Dominant follicles (>10 mm to <20 mm).	1.250^{b}	0.846^{c}	1.615^{a}	0.625^{c}	0.13	0.02
Oversize follicles* > 20mm.	0.54	0.38	1.25	1.08	0.13	0.56
Diameter, mm						
Small size follicles < 5mm.	2.38	2.13	3.77	2.23	0.19	0.22
Growing follicles (> 5mm and < 10mm).	7.155^{b}	6.461^{c}	9.625^{a}	6.25^{c}	1.13	0.01
Dominant Follicles (>10 mm to <20 mm).	16.520^{b}	13.795^{c}	19.903^{a}	11.650^{c}	0.94	0.02
Oversize follicles* > 20mm.	27.14^{ab}	29.16^{a}	28.00^{a}	26.83^{b}	0.43	0.00

[a- c] Means with different letters within the same row are significantly different (P < 0.05).
*Oversized follicle percentages were 34.48% in NP and 36.8% in MP she-camels.

cles and the number and diameter of growing and DFs in the right ovary in MP she-camel were significantly higher in comparison to NP animals (Table 3). It was clear that there was a negative relationship between the number and diameter of follicles with the number decreasing while the diameter increasing. Regarding the diameter of the oversized follicles, it has been observed that the mean maximum diameter of oversized follicles (29.16 and 28.00 mm) was observed on the left ovaries of NP and the right ovaries of MP, respectively with no difference between them being observed (Table 3).

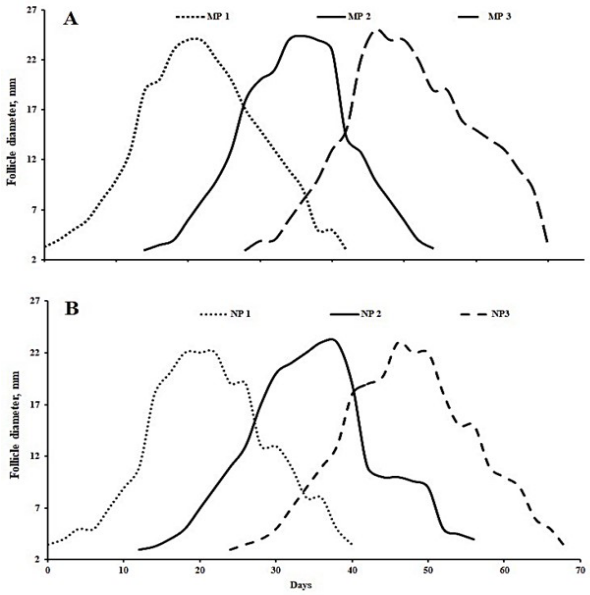

Fig. 1. The profiles of growth and regression of a dominant follicle in three consecutive follicular waves in an individual multiparous (MP, A) and nulliparous (NP, B) dromedary she-camels. The mean of IWI in NP was 12.38 days, and 14.73 days in MP.

Hormonal assay and pregnancy rate

Serum progesterone (P4, ng/mL) concentration did not differ between NP and MP she-camels concurrent with the follicular growth, and its concentrations remained at the basal level (<0.5 ng/mL) throughout the follicular waves. However, a slight increase in concentrations of P4 was observed concurrently with the presence of oversized follicles (Table 4). However, serum estradiol concentration increased with small follicle recruitment and was higher (P<0.05) in MP than in NP she-camel, peaking with ovulatory follicles, which no significant differences were observed between groups, while E2 declined steadily when oversized follicles were observed in both groups. Furthermore, serum FSH concentrations increased concurrently with the follicle's growth. When growing follicles were monitored, the mean FSH concentrations in MP were significantly higher than in NP she-camel (5.899 vs. 5.452 mIU/mL, respectively) and reached a peak of 9.017 mIU/mL in MP versus 7.766 mIU/mL in NP (P<0.05) during the mature phase and before ovulation. When the oversized follicles were observed on the ovaries, the FSH concentrations in both groups decreased steadily, with no significant difference between groups (Table 4).

No significant difference (P<0.05) was found in incidence of pregnancy in MP she-camels (five out of eight, 62.50%) compared to 11 out of 13 (84.6%) in NP she-camels.

Discussion

The present study sought to determine whether well-fed nulliparous she-camels could enter the breeding season early, at ages 2 to 3, and have comparable reproductive performance compared to older multiparous camels. Ultrasonography was used to study follicular dynamics, ovaries measurements, endometrial thickness, and diagnose pregnancy in NP and MP she-camels.

The present result revealed that the ovaries' measurements (width and length) increased concurrently with the growth and development of follicles. The right ovaries were more active than the left ovaries in terms of ovarian structures. These changes in the ovarian dimension were mainly due to the main relevant physiological events and hormonal changes that occur during different stages of follicular growth. The ovarian dimensions were greatly altered with age and the different stages of follicular growth (Ali *et al*., 2007). Further, the NP had longer ovaries than MP she-camels, consistent with Ali *et al*. (2007). Contrariwise, Awad *et al*. (2018) reported that non-pregnant camels did not differ in weight, length, or width between the right and left ovaries. However, Usman *et al*. (2022) found that the left ovary weighed significantly more than the right ovary during the breeding season.

The endometrial thickness gradually increased with follicular growth and development and was less in the NP than in the MP. These disparities could be attributed to the number of births, which causes an increase in the thickness of the entire uterine layer in MP she-camels. Greater changes in uterine echotexture are correlated with ovarian activity and subse-

Table 4. Reproductive hormones at different stages of follicular growth (mm) as well as pregnancy rate in nulliparous and multiparous dromedary she-camels during the breeding season.

Items	NP	MP	SEM	*P*-Value
E_2 (pg/mL)				
At small follicles (<5 mm).	27.87 $^{b\,B}$	29.14 $^{a\,B}$	0.49	0.00
At growing follicles (>5 mm and < 10mm).	31.65^{B}	33.88^{B}	0.66	0.22
At ovulatory or DF (>10mm to 20mm).	56.88^{A}	62.58^{A}	1.04	0.1
At oversized follicles* (> 20mm).	30.13^{B}	30.46^{B}	0.7	0.82
P_4 (ng/mL)				
At small follicles (<5 mm).	0.318^{B}	0.308^{B}	0.01	0.86
At growing follicles (>5 mm and < 10mm).	0.425^{B}	0.386^{B}	0.01	0.57
At ovulatory or DF (>10mm to 20mm).	0.382^{B}	0.378^{B}	0.01	0.09
At oversized follicles* (> 20mm).	0.71^{A}	0.77^{A}	0.07	0.66
FSH (mIU/mL)				
At small follicles (<5 mm).	5.161^{B}	5.340^{B}	0.20	0.14
At growing follicles (>5 mm and < 10mm).	5.452 $^{b\,B}$	5.899 $^{a\,B}$	0.15	<0.0001
At ovulatory or DF (>10mm to 20mm).	7.766 $^{b\,A}$	9.017 $^{a\,A}$	0.18	0.00
At oversized follicles* (> 20mm).	5.34^{B}	5.59^{B}	2.36	0.42
Pregnancy Rate (%)	62.50 (5/8)	84.6 (11/13)	-	0.59

$^{a-b}$ Means with different letters within the same row are significantly different (P < 0.05). $^{A-D}$ means with different letters at the same column are significantly different (P<0.05).
*Oversized follicle percentages were 34.48% in NP and 36.8% in MP she-camels.

quent follicular growth (Derar, 2003). The greatest increase in endometrial thickness was found concurrent with the presence of DF, confirming a previous study that the uterus was toned and the edema was observed at the peak of follicular development (Tibary, 2018). The increase in uterine thickness may be due to hormonal and physiological changes in preparation for receiving embryos, as well as vascular changes due to an augmented metabolic rate (Usman *et al*., 2022).

Follicular activities in NP and MP groups occurred in wave-like patterns, where one cohort of follicles grew continuously to a DF while the other follicles regressed, and these findings agreed with previous studies by Skidmore *et al*. (1996); Manjunatha *et al*. (2012); Padalino *et al*. (2016). The ovaries of a she-camel contain a larger number of primordial follicles, which are released sequentially in different numbers (Ashour *et al*. 2017). Moreover, follicular growth occurs alternately in both ovaries during the breeding season (Tibary *et al*. 2007; Rawy *et al*. 2014). Contrary to these studies, in our study, the right ovaries were found to be more active than the left ovaries as reflected in the increased number and diameter of follicles.

In the present study, MP she-camels had higher mean numbers of recruited follicles than NP she-camels. The variations in the cohort of follicles that emerged from the primordial follicles are associated with several factors. Follicle recruitment and selection of DF depend on gonadotrophins, especially plasma FSH concentrations, which regulate the follicular waves (Adams *et al*. 1992); thus, the follicle cohort emergence (>4 mm) at the beginning of the follicular wave requires FSH (Caixeta *et al*., 2009). However, the levels of FSH between the studied groups did not differ at this stage. Furthermore, age affected the existence of follicles in camels' ovaries, whereas camels aged 6-10 have the maximum number of follicles (Ashour *et al*., 2017). Ovary reserve size also affects the number of recruited follicles (Cushman *et al*. 2000). In addition, the number of recruited follicles in the successive waves in the present study was repeatable within individual animals (Manjunatha *et al*., 2012); however, great variations in the number of antral follicles during each follicular wave were observed in cattle (Burns *et al*., 2005).

The future DFs continued to grow while the subordinate follicles regressed, and the inverse relationship between the number and diameter of DFs was observed. These findings confirmed the concept that the DF restricts the growth of subordinate follicles in the current wave as well as the emergence of the following wave until it loses dominance, confirming the follicle waves theory for camels (Padalino *et al*., 2016; Skidmore, 2011). In the present study, the mean diameters of DFs varied from 11.65 to 19.90 mm, and the optimal ovulation rate occurs when the DF diameter is at least 11 mm (Manjunatha *et al*., 2015), indicating that the DF matured sufficiently to ovulate before reaching its maximum diameter (Skidmore *et al*., 1996). Furthermore, the mean diameter of DF in the NP she-camel was smaller than that of the MP she-camel, but both were larger than the diameter of the required follicles for ovulation (Manjunatha *et al*., 2015). Otherwise, Mohamed *et al*. (2021) reported that mean follicle diameters in both ovaries tended to be the same during the breeding season, but our study found that the DF diameter was greater in the right ovary in MP and NP she-camels than in the left ovaries. This discrepancy may be attributed to the nutritional status and BCS of the animals enrolled in this study, which were above grade 4 BCS.

There was no significant difference in the duration of the growth phase of follicles between MP and NP she-camels, which agreed with earlier studies in dromedary camels (Manjunatha *et al*., 2012; Qureshi *et al*., 2018; Mohamed *et al*., 2021); however, the length of the entire follicular wave was shorter in the NP than in the MP. The difference in follicular wave lengths was associated with the length of the mature phase of DF. The duration of the entire follicular wave in NP and MP (40.88 and 44.31 days, respectively) was similar to previous studies in dromedary camels, (Manjunatha *et al*., 2012; Rawy *et al*., 2014), which ranged between 35 and 47 days. This was lower than that reported by Mohamed *et al*. (2021), where the entire cycle was 25.41±1.36 days. Variations in sample size, age, season stage, geographic location, breeding, and nutrition systems may explain these discrepancies.

The duration of the dominance of DFs in MP she-camels was more prolonged compared to NP. However, in the absence of ovulation stimulants, DFs of both groups remained available to induce ovulation periods within a reference range of 5 to 10 days (Manjunatha *et al*., 2012; Qureshi *et al*., 2018; Mohamed *et al*., 2021). These DFs in NP she-camels may be less destined for follicular regression than in MP she-camels. In some cases, DF may increase in size and become oversized follicles with low ovulation and fertilizing capacity. (Manjunatha *et al*., 2012; Qureshi *et al*., 2018). The growth phase and the duration for complete regression of these oversized follicles were shorter in NP than in the MP she-camels, which were comparable with the earlier results (Skidmore *et al*., 1996; Skidmore, 2018). Further, the incidence of oversized follicles in our study was lower (34.48% in NP and 36.8% in MP) than those reported by Nagy *et al*. (2005) and Manjunatha *et al*. (2012) (47.3 % and 73.3%, respectively). The presence of oversized follicles is thought to be caused by the downregulation of LH receptors in granulosa or/and theca cells or by a sustained basal secretion of LH from the anterior pituitary, which causes follicle overgrowth and ovulation failure in the absence of an ovulatory LH surge.

Our observations revealed that oversized follicles do not hinder the emergence of a new follicular wave (Skidmore *et al*., 1996; Manjunatha *et al*., 2012; Rawy *et al*., 2014). However, the new follicular wave was visible before the complete DF regression (Rawy *et al*., 2014), giving the inter-wave intervals of 12.38 and 14.73 days in NP and PM, respectively, similar to a previous study in dromedary camels (Manjunatha *et al*., 2012) and shorter than other studies (Skidmore *et al*., 1996; Manjunatha *et al*., 2012; Rawy *et al*., 2014).

Serum E2 concentration associated with follicular development was higher in the MP than in the NP she-camels at the small follicle stage. Also, E2 concentrations were higher than expected in the growth phase and in the presence of oversized follicles, which were assumingly due to overlapping follicular waves. Further, the highest E2 concentration was recorded when the DFs were observed (56.88 and 62.58 pg/mL in NP and MP, respectively). These results agreed with those of Rawy *et al*. (2014) and Ghallab *et al*. (2022), who indicated that the highest E2 concentration was recorded at the same time as the largest follicle size. Furthermore, E2 concentrations have a positive correlation with follicular size (Atigui *et al*., 2013; Padalino *et al*., 2016). The E2 concentration declined when the oversized follicle was observed (Rawy *et al*., 2014) or the follicular size was over 1.7 cm (Skidmore *et al*., 1996), which suggested that the follicle atresia had already begun, besides the downregulation of LH receptors in the theca interna. Generally, the P4 source is primarily the luteal tissue of CL, and in the absence of ovulation stimulators, P4 concentrations remain at the basal level (<1 ng/mL) throughout the follicular waves (Skidmore *et al*., 1996; Rawy *et al*., 2014), which is consistent with our findings. When the DF was monitored in the ovaries, the serum FSH concentration increased in coincidence with follicular growth and peaked at 9.017 vs. 7.766 mIU/mL in MP and NP, respectively. Similarly, Kanitz (2003), Hussein *et al*. (2008), and Derar *et al*. (2014) reported that FSH surged before ovulation and decreased in the presence of oversized follicles (Hussein *et al*., 2008).

The pregnancy rate ranged between 60 and 85% and did not differ between NP and MP she-camel, and this is partially consistent with a previous study by Bakheit *et al*. (2016), who found that the semi-intensive housing system was more effective than the traditional system. Indeed, herd fertility is affected by the housing system, herd size, genital disorders, breeding season, bull fertility, hormonal treatments, and, most importantly, nutritional status. Therefore, the well-fed NP she-camels aged 2--3 years had similar reproductive performances that was comparable to those of the MP she-camels.

Obviously, the follicular dynamics of well-fed NP she-camels matched previous studies on dromedary camels. The authors suggest that an intensive feeding system that increases the body condition score of nulliparous she-camels can help them enter the breeding season early. Unfortunately, the interaction between BCS and ovarian activity is poorly understood in she-camels, but dromedary she-camels had active ovaries when their BCS was 3-3.5 and low ovarian activity when their BCS was less than 2.5 (Gherissi *et al*., 2020). Furthermore, both ovarian activity and BCS ameliorate during the breeding season (Gherissi *et al*., 2020), which proves the positive relationship between them. Therefore, camelids' reproductively can be improved by maintaining an intensive feeding system even during the non-breeding season (Tibary and Anouassi, 1997).

Conclusion

Well-fed nulliparous dromedary female camels can enter the breeding season early (2:3 years). Where follicular dynamics and associated hormones occur in a wave-like pattern. Also, ovarian and endometrial measures, as well as pregnancy rates, occur in NP to some extent, similar to what happened in MP female dromedary camels. However, further research is needed to understand the reproductive pattern of nulliparous dromedary camels, which could lead to improved reproduction and productivity through modern reproductive technologies.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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