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OVARIAN FOLLICLE DYNAMICS IN BACTRIAN CAMEL (*Camelus bactrianus*)

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ABSTRACT

Precise definitions for follicle growth phases provide a better insight to manage the reproductive cycle of farm animals. The purpose of this study was to characterise ovarian follicle dynamics in bactrian camels prior to the breeding season until the completion of at least one complete follicle wave cycle. Ovarian follicular status of 5 non-pregnant mature bactrian camels was recorded using ultrasonography (5 MHz trans-rectal linear array probe) on alternate days for 82 days (30th November to 20th February). The difference in the number of ovarian follicles was used to determine different phases of follicle growth. During the time approaching the breeding season there were incomplete (transitional) follicle wave cycles (wave length: 20.2±2.25 days) during which the largest follicles reached 10.3±0.66 mm in diameter. Complete follicle wave cycles (wave length: 44.3±2.32 days) started after this transitional period, concurrent with winter solstices, and consisted of growing, mature and regressing phases with lengths of 10±0.68, 10±0.35 and 24.6±1.29 days, respectively. The interval between detection of mature follicles of two successive follicle waves (inter-wave interval) was 19.1±0.59 days and the minimum and maximum diameters and daily growth rate of mature follicle were 13.0±0.34 (11.7-15.8), 19.7±0.64 (17-25) mm and 0.73 mm, respectively.

Key words: Bactrian camel, follicle wave cycles, mature follicle

Ovarian follicular growth occurs in a wave-like pattern throughout the oestrous cycle and culminates in spontaneous ovulation in cattle (Savio *et al,* 1988; Sirois and Fortune, 1988), horse (Ginther, 2000), sheep (Ravindra *et al,* 1994), goat (Rubianes and Menchaca 2003) and buffalo (Baruselli *et al,* 1997). However, the term "oestrous cycle", as indicated for other domestic ruminants, may not be applicable to the family members of Camelidae as they are induced ovulators, ovulating only when mated. Therefore, ovarian follicles have periods of growth, maturity and regression in regular "ovarian follicle wave cycles" throughout the breeding season (Vaughan *et al,* 2004; Miragaya *et al,* 2004; Skidmore *et al,* 1995; Skidmore *et al,* 1996; Skidmore *et al,* 1997; Adams *et al,* 1990; Bravo *et al,* 1990; Musa and Abusineina, 1978; Nawito *et al,* 1967), and there is no corpus luteum (luteal phase) during the non-pregnant reproductive cycle (Skidmore *et al,* 1995; Skidmore *et al,* 1996; Skidmore *et al,* 1997; Adams *et al,* 1990; Bravo *et al,* 1990; Musa and Abusineina, 1978; Nawito *et al,* 1967; Chen *et al,* 1987; Fernandez-Baca *et al,* 1970; Marie and Anouassi, 1987; Novoa, 1970). In lamas and alpacas, the duration of the growth, mature and regression phases have been reported to be between 3-9, 2-8 and 3-8 days, respectively, and the diameter of the ovulatory dominant (mature) follicle was 7-12 mm (Vaughan *et al,* 2004; Adams *et al,* 1990; Bravo *et al,* 1990; Bravo and Sumar, 1989; Chaves *et al,* 2002), whereas in the vicuna these phases were 3, 1.4 and 2.9 days, respectively (Miragaya *et al,* 2004; Miragaya *et al,* 2006). Ultrasound examinations of the ovaries during the follicular wave cycle in dromedary camels resulted in growing, mature and regressing phases of 10.5, 7.6 and 11.9 days, respectively and the diameter of the mature follicle was 13-17 mm (Skidmore *et al,* 1995; Skidmore *et al,* 1997). However, relative findings in the bactrian camel are neither comprehensive nor elaborated by ultrasonography (Zhao, 2002; Chen *et al,* 1980a; Chen *et al,* 1980b). The purpose of this study was to characterise follicle wave cycle in order to provide fundamental information for further research on controlling reproductive cycles in bactrian camels.

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Materials and Methods

Experimental location

The investigation was conducted at the Bactrian Camel Research Centre, Jahadabad, Meshkinshahr, Ardabil province, Iran (latitude: 38′ 23″ N; longitude: 47′ 40″ E; altitude: 1568.5m) between 30th November (approaching breeding season) and 20th February (82 days).

Experimental animals

Five multiparous bactrian camels, 8-12 (9.6±0.75) years old and 535.8±59.26 kg (at the initiation of experiment; in the morning before food and water intake), free from any uterine and ovarian abnormalities detectable by rectal palpation and/or ultrasound examination, were used in this experiment. Females had a normal parturition with healthy calves in the last breeding season. The females were kept in the barn during the day, inside the stable at night and kept separately from male camels during the experiment. The distance between them was about 20 m; however, they could see the males and hear their sound. Each camel received alfalfa hay (*ad lib*) and wheat straw (1.5 kg) mixed with concentrate (2.5 kg) including barley (67.8%), cotton seed meal (12%), wheat barn (17.1%), molasses (1.9%), vitamins and minerals (1.2%) on a daily basis and had free access to water. The live weight of females at the termination of experiment was 547.1±57.16 kg.

Ovarian Ultrasonography

Ovarian ultrasound examination was carried out on alternate days for 82 days, starting from 30th of November, using a real time scanner (Aloka 500; Japan) equipped with a 5 MHz linear array trans-rectal transducer. Each complete follicle wave cycle consisted of growing, mature and regressing phases and the location and diameter of all follicles, e.g. 4 mm, were recorded using the ultrasound internal electronic caliper. The initiation of the growing phase was defined as the day in which the dominant follicle was, retrospectively, identified at a diameter of 4-5 mm. The initiation of the mature phase (dominant phase) was considered to be when there was a sudden significant decrease in the number of follicles, e.g. 4 mm in diameter. The period before a sudden significant increase in the number of follicles, e.g. 4 mm in diameter, was considered to be the termination of the mature phase. This was concurrent with the initiation of the regressing phase and the emergence of a new follicle wave. The regressing phase was defined from the initiation of follicle regression until the time that the regressing follicle had reduced to 7 mm in diameter. As there is gradual fibrin organisation in regressing follicles, small regressing follicles are irregular in shape (Tinson and McKinnon, 1992) and therefore, in the present study, follicle regression was only monitored up to 7 mm in diameter, when the spherical shape of the follicle was still in place. A follicle wave cycle, without a mature phase, was defined as a transitional (incomplete; 15) wave and the interval between the detection of mature follicles of the two successive follicle waves were considered as the inter-wave interval.

Statistical analysis

In the complete follicle wave, during which the ovarian follicle increased in size, the effect of time on the number of ovarian follicles was analysed using Mixed Models procedure of SAS (Little, 1996), considering individual camels as random. The mathematical model examined the effect of time (independent variable) on the changes in the number of follicle (dependent variable). In order to normalise the data, they were subjected to logarithmic transformation. Follicle growth rate was estimated using Proc Rsreg including Lackfit in the model. In the case that the lack of fit was not significant, and the linear relationship between time and follicle size was significant, linear regression was used to estimate follicle growth rate. All statistical analyses were conducted using SAS/ STAT (SAS, 2001). Data were expressed as means ± standard error of mean and range.

Results

This experiment was started, prior to the breeding season (November 30th), when there were no follicles greater than 6 mm in the ovaries. At this time, females did not have complete follicle wave cycles, but all females displayed at least one transitional (incomplete) follicle wave; three camels showed one (Fig 1) and two camels had three transitional waves (Fig 2). Transitional follicle waves lasted for 20.2±2.25 (12-32) days, during which time the ovarian follicle reached a maximum diameter of 10.3±0.66 (8.3-13.1) mm. During a transitional wave, there was no relationship between the number of follicles and follicle growth.

Females displayed variable numbers of complete follicle waves throughout the experimental period; two females showed two and three displayed one complete follicle wave (Fig 1 and 2). The first complete follicle wave cycle starts between 14th of

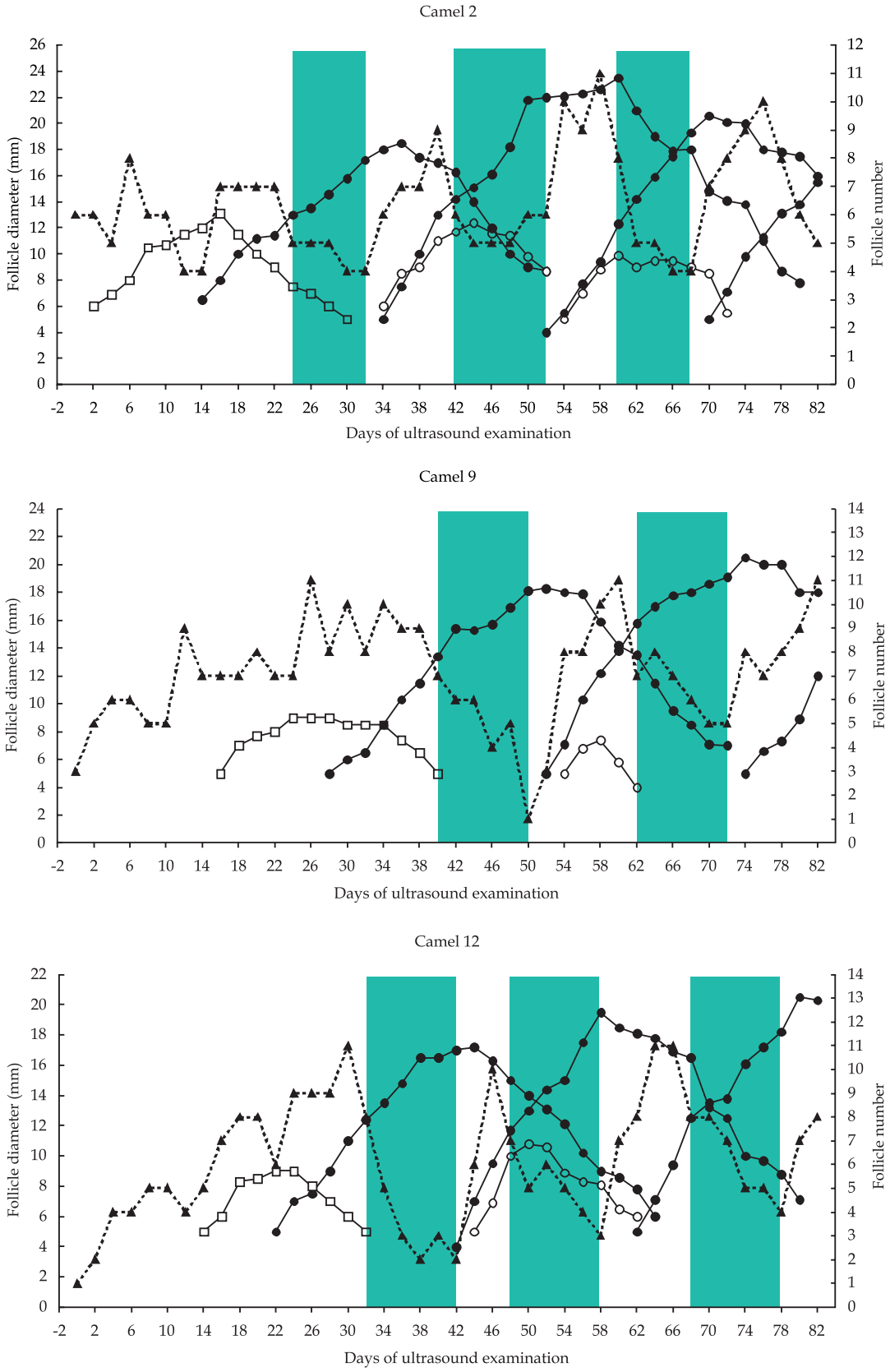

Fig 1. Follicular dynamics of bactrian camels that showed one transitional wave. Day 0 = Initiation of ultrasound examination (30th of November); Transitional (Incomplete) follicle wave (□); Mature follicle of the complete follicle wave (●); Subordinate follicle (○); Follicle number (▲); Gray bar = Mature phase.

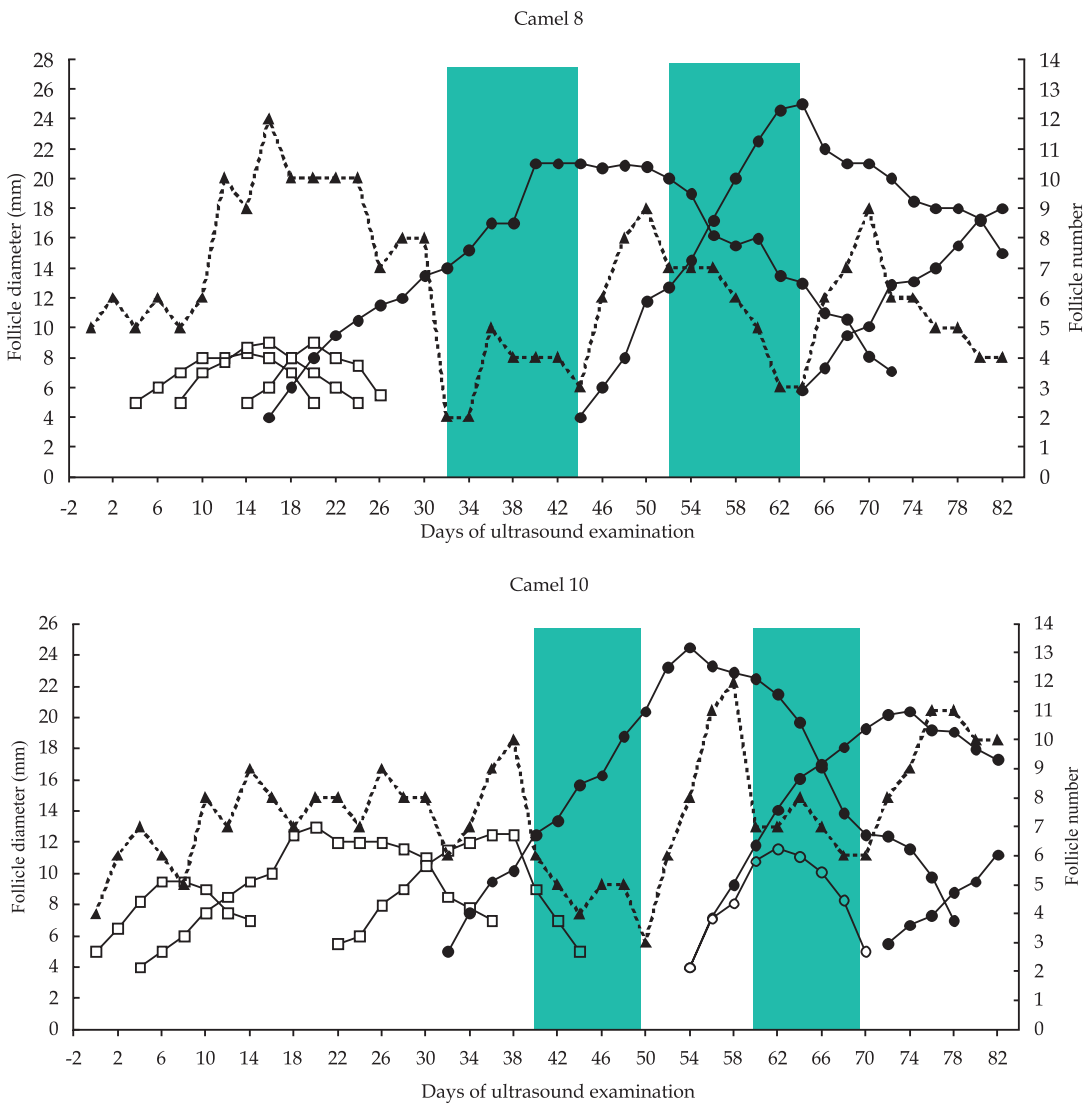

Fig 2. Follicular dynamics of Bactrian camels that showed three transitional waves. Day 0 = Initiation of ultrasound examination (30th of November); Transitional (Incomplete) follicle wave (□); Mature follicle of the complete follicle wave (●); Subordinate follicle (○); Follicle number (▲); Gray bar = Mature phase.

December and 1st of January. A particular trend in the number of follicles present during the growing phase was noticed during each complete follicle wave cycle where the follicle number decreased as the follicle diameter increased. Therefore, the initiation and termination of the mature phase were based on the significant decrease and increase ($P<0.001$) in the number of follicles (Fig 3 and 4). A significant decrease in the number of follicles during the growing phase was associated with the initiation of the mature phase (Fig 3) and the day before a significant increase in the number of follicles during the mature phase was considered as the termination of mature phase (Fig 4). Therefore, the average lengths of growing, mature and regressing phases were 10±0.68 (6-16), 10±0.35 (8-12) and 24.6±1.29 (20-28) days, respectively. In addition, the length of the complete follicle wave was 44.3±2.32 (38-56) days whereas the interval between the detection of two consecutive mature follicles (inter-wave interval) was 19.1±0.59 (16-22) days.

The first mature phase starts between 24th of December and 9th of January. During the mature phase, the minimum, mean and maximum follicle diameters were 13.0±0.34 (11.7-15.8), 16.35±0.78 (11.7-25) and 19.7±0.64 (17-25) mm, respectively but out of the 12 mature follicles monitored in this study, 9 follicles continued increasing in size and reached a maximum diameter of 20.8±0.70 (17.2-25) mm whereas the other 3 follicles regressed after termination of the mature phase.

During the growing phase, follicle growth rate followed a quadratic equation (y=2.04+2.92t-

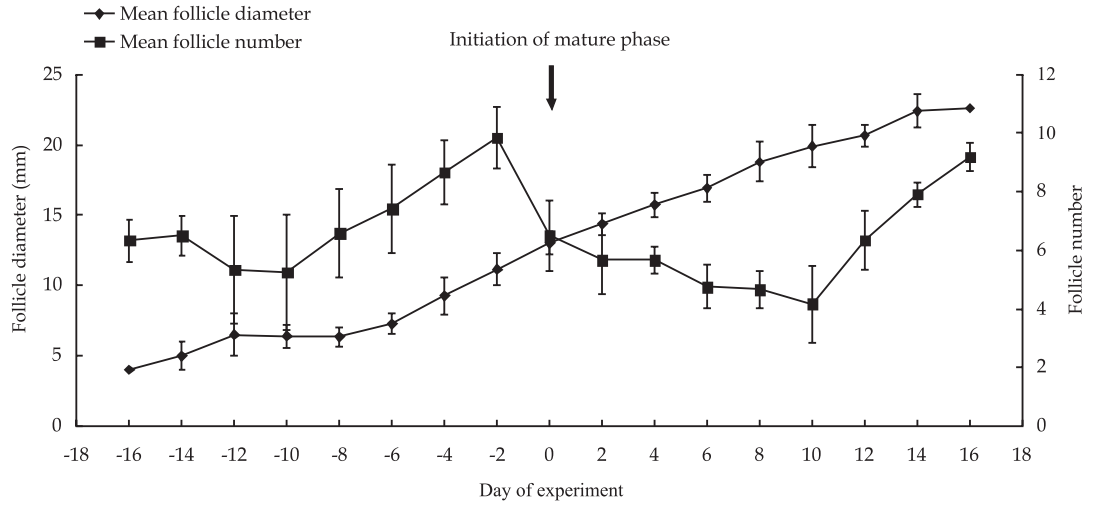

Fig 3. Relationship between the diameter of the largest follicle (mm, mean±S.E.M) and the number of follicles detected in both ovaries (mean±S.E.M.) during complete follicle wave cycle in bactrian camel. Day 0 (the initiation of the mature phase) is the day that significant decrease in follicle number occurred (P<0.001).

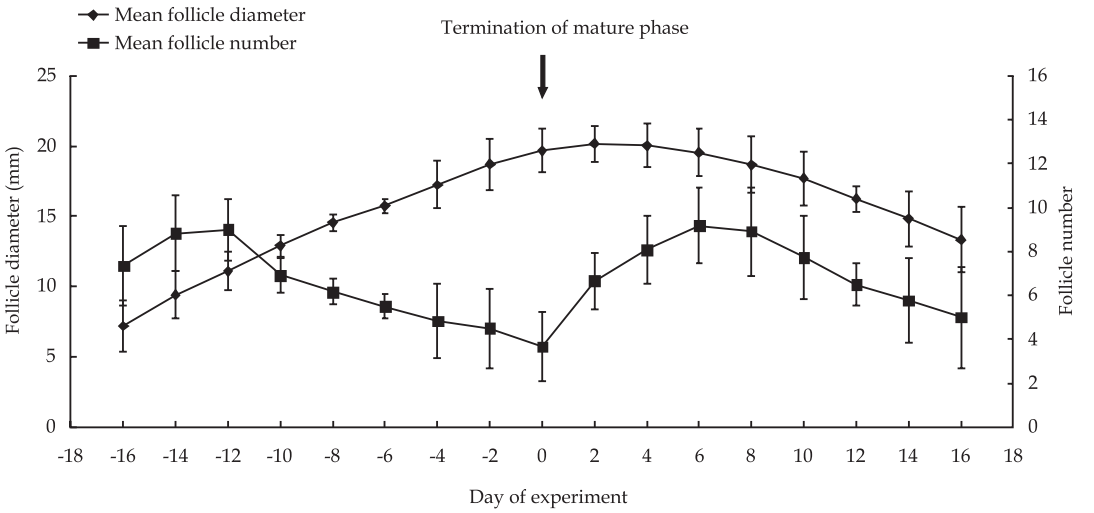

Fig 4. Relationship between the diameter of the largest follicle (mm, mean±S.E.M) and the number of follicles detected in both ovaries (mean±S.E.M.) during complete follicle wave cycle in bactrian camel. Time 0 (the termination of the mature phase) is the time after which the significant increase in follicle number occurred (P<0.001).

$0.19t^2$; r^2=0.82, P<0.0001) and was estimated to be 0.89 mm/day. During the mature phase, follicle growth rate followed a linear equation (y=11.4+1.46t; r^2=0.73, P<0.0001) and was estimated to be 0.73 mm/day. Follicle growth rate from emergence to the end of mature phase followed a quadratic equation (y=2.36+2.45t-$0.08t^2$; r^2=0.85, P<0.0001) and was estimated to be 0.785 mm/day.

Discussion

The present study characterised ovarian follicle wave cycles, prior to the breeding season and continued until completion of at least one complete follicle wave in bactrian camels. Before the start of the breeding season, there was at least one transitional follicle wave in which the follicle had a maximum diameter of 10.3±0.66 mm. This confirms a previous study carried out in bactrian camels before the onset of the breeding season (transitional period) that also indicated the presence of irregular patterns of follicular growth and regression. These follicles did not grow larger than 10 mm in diameter. In addition, the camels did not express estrous behavior during this period (Zhao, 2000). The same phenomenon occurs in mares (Ginther, 1992). The individual variation in the number of transitional waves is similar to that reported in mares (Newcombe, 2007). Not all mares, have a defined transitional phase. Some have several follicles, fluctuating in size between 20 and 25 mm, without an obvious follicular wave (Newcombe, 2007).

She-camels are seasonally poly-oestrus (Nawito *et al,* 1967) like mares (Ginther, 1992) and ewes (Chemineau *et al* 2008). Although latitude, age, nutrition, breed, environmental temperature and management may play a rule in the resumption of ovarian activity (Ginther, 1992; Chemineau *et al* 2008; Allen, 1987), the annual photoperiodic changes provide the main cue for synchronizing the seasonal cycle of reproduction in seasonal breeders (Ortavant *et al,* 1985; Karsch *et al,* 1984). While resumption of cyclic activity is largely dependent on the increasing day length in mare (Burkhard, 1946), decreasing day length has a stimulatory effect on reproductive activity in ewe (Chemineau *et al,* 2008). The initiation of the breeding season in camels coincides with increasing day length (Chen and Yuen, 1984). Accordingly, the breeding season extends from December to March in different parts of the world (Islamy, 1950; Mcknight 1969; Khanna *et al,* 1990; Abdel Rahim and El-Nazir, 1990; Yagil, 1985). Melatonin, secreted by the pineal gland, is a key regulator of seasonality in mammals (Chemineau *et al* 2008) including camels (Vyas *et al,* 1997). In the present study, the mature phase was first detected on the 24th of December coinciding with winter solstice. Here the question might be raised as to whether the camel is a short or long-day breeder? In mares, there is a transitional period during the spring months with a peak of ovulation around about the summer solstice (Ginther, 1992). Therefore, mares are considered as long-day breeders; because, reproductive activity starts with increasing day length and becomes maximised during the period of maximum day length in the year. In ewes, as an accepted model for short-day breeders, resumption of ovarian activity starts about 2 months after summer solstice and become maximised around about the autumnal equinox (Chemineau *et al,* 2008). Therefore, the breeding season of the ewe is not during the short days of the year but lies in the decreasing trend of the day length. The breeding season of camels starts around the winter solstice when there is an increasing trend in day length, like the mare, but occurs during the short days of the year, unlike the ewe, and continues for short time after vernal (spring) equinox. As a result, using classical terminology, it seems that camel is neither a short-day nor long-day breeder. According to the present study, breeding season of bactrian camel starts just after winter solstice and continues along with increasing day length and ceases shortly after vernal (spring) equinox.

In the present study, a new criterion was defined to determine the mature phase using fluctuations in the number of follicles in relation to follicle diameter rather than using follicle diameter alone. The results showed that the sudden significant decrease and increase in the number of follicles is concurrent with the initiation and termination of the mature phase, respectively. This is in line with the common rationale in cattle that the loss of follicle domination is concurrent with the emergence of a new follicle wave (Fortune, 1994; Irland *et al,* 2000). An inverse relationship between dominant follicle size and follicle number in the ovaries has also been reported in the dromedary camel (Skidmore *et al,* 1995), llama (Adams *et al,* 1990), alpaca (Vaughan *et al,* 2004) and vicuna (Miragaya *et al,* 2004). In dromedary camels, the use of serial ultrasonography, hormone assays and oestrous detection, indicated that a mature follicle ranged from 13 to 17 mm in diameter (Skidmore *et al,* 1996). The results of the present study indicated a wider range of diameter for mature follicles (11.7-25 mm) in bactrian camels which is in agreement with a previous study using rectal palpation in this species (11-24 mm; Chen *et al,* 1985). However, due to the great variations among individuals, one has to be very cautious in using such ranges to define mature follicles as we have found the minimum (11.7-15.8; 13.0±0.34 mm) and maximum (17-25; 19.7±0.64 mm) ranges for mature follicles. This results in more conservative values and closer to the ones reported by Skidmore *et al*., 1996 for dromedary camels (Skidmore *et al,* 1996). In Bactrian camel, we did not observe any oversized follicles reported in dromedary camel (follicles >30 mm in diameter; Skidmore *et al,* 1996).

The duration of the follicular wave cycle in the present study was 44.3±2.32 (38-56) days and the average lengths of growing, mature and regressing phases were 10±0.68 (6-16), 10±0.35 (8-12) and 24.6±1.29 (20-28) days, respectively. These results agree with those reported by Zhao (2000), whose study was based on rectal palpation (Zhao, 2000). He reported that it takes 19 days from emergence of new follicular wave (5-6 mm in diameter) to the start of follicle regression. Also, he reported a growth phase of 9.27±4.7 days during which time a developing follicle of 5-6 mm reaches an ovulating size of 12 mm. This follicle either remained approximately this size or continued to grow for about 10 days before starting to regress (Zhao, 2000; Chen *et al,* 1980a; Chen *et al,* 1980b). In previous studies, based on rectal palpation of dromedary camels, the duration of the follicle wave cycle was reported to be 17.2-23.4 (Joshi *et al,* 1978), 24.2 (Nawito *et al,* 1967), 28 (Musa and Abusineina,

1978), 16-30 (Bakkar and Basmaeil, 1988), and 11-27 (Al-Eknah *et al,* 1993) days. However, more accurate studies based on ultrasonography of dromedary camel ovaries, indicated growing, mature and regressing phases of 10.5, 7.6 and 11.9 days respectively (Skidmore *et al,* 1995; Skidmore *et al,* 1996; Skidmore *et al,* 1997). Therefore, the duration of the growing phase in bactrian camels achieved in the present study (10±0.68 days) and dromedary camels (10.9±0.5 days; Skidmore *et al,* 1996) are comparable. However, it seems that the duration of the mature phase in Bactrian camels is longer than in dromedary camels (10±0.35 versus 7.6±0.8 days; Skidmore *et al,* 1996). The difference between mature phase durations could be due to the different criteria used to define a mature phase. In this study we used fluctuations in follicle number whereas Skidmore *et al* (1995, 1996) used follicle diameter (Skidmore *et al,* 1995; Skidmore *et al,* 1996).

In the present study, the duration of the regressing phase was 24.6±1.29 days, which is longer than that reported for dromedary camels (11.9±0.8 days; Skidmore *et al,* 1995; Skidmore *et al,* 1996; Skidmore *et al,* 1997). This again may be due, in part, to the different classifications used to report the data. The regression phase in the studies by Skidmore *et al* (1995, 1996) started when the mature follicle progressively reduced in diameter (Skidmore *et al,* 1995; Skidmore *et al,* 1996), whereas in this study it started as soon as the mature follicle had lost its dominance, which was concurrent with an increase in the number of follicles and emergence of a new follicle wave. Even if the follicle still increased in size (3/12), this time was considered as part of the regressing phase. The inter-wave interval reported in the present study (19.1±0.59 days) was similar to the one reported for dromedary camels (18.2±1.0 days; Skidmore *et al,* 1996) indicating that the different length of the regressing phase, reported in two studies, did not influence inter-wave intervals. This could be because regressing follicles are functionally inactive and do not have a suppressive effect on new follicle emergence (Vaughan *et al,* 2004; Miragaya *et al,* 2004; Skidmore *et al,* 1995; Skidmore *et al,* 1996; Skidmore *et al,* 1997; Chaves *et al,* 2002), thus the duration of growing and mature phases mostly affect inter-wave intervals.

In this study the follicle growth rate during growing and mature phases was 0.89 and 0.73 mm/day, respectively whereas Nikjou *et al,* (2008) reported follicle growth rates of 1 and 0.89 mm/day in follicular and luteal phases, respectively (Nikjou *et al,* 2008). In dromedary camels, Skidmore *et al* (1996) reported that a new follicular wave emerges 5 days after a sterile copulation and after a period of 8 days one of these follicles reaches a diameter of 13 mm (Skidmore *et al,* 1996). Therefore, the follicular growth rates during the luteal phase of the dromedary camel must be about 1 mm/day as well. Follicle growth rate in the growing phase of alpacas and vicunas were 0.43 and 1.8 mm/days, respectively (Vaughan *et al,* 2004; Miragaya *et al,* 2004).

In conclusion, prior to breeding season, there is a transitional follicle wave cycle followed by a complete follicle wave cycle in bactrian camels which starts just after winter solstice and follows the same principles and patterns reported in other camelids. Serial ultrasound examination, considering the relationship between the number and size of the follicles, might be the correct way to determine mature follicles in the future and therefore reliably predict the correct time for mating or insemination. However, further studies need to be carried out to ensure that the definition of a mature follicle presented in this study, i.e. the largest follicle present when there is a significant decrease in the number of follicles in the ovaries, is the one that is capable of ovulating a viable fertile oocyte.

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