Studies on the ovarian response to the season, local ecology and exogenous hormonal therapy in dromedary camels

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Abstract

To observe the seasonal reproductive pattern of female camels in response to hormonal therapy, 24 adult, active, non-pregnant and non-lactating female one-humped camels from three districts of Punjab (Faisalabad (n=12), Bhakkar (n=6) and Attock (n=6)) were selected. The blood samples were collected at one-day intervals for one month during each season for progesterone assessment. To induce ovulation, Buserlin® (5 ml IV) was given to camels in winter, and pregnant mare serum gonadotrophin (Folligon®) at 3 ml IM in summer.

Results revealed that the period of sexual activity started earlier in November and extended even up to April at Attock as compared to other zones. Breeding behaviour signs were more intense at Attock and Bhakkar as compared to Faisalabad in the autumn and spring seasons. Serum progesterone concentrations were significantly (P<0.05) higher in winter and spring in all-female camels. Ultrasonographic studies revealed that prominent follicles on both ovaries were present, their size was increased, and the uterine tone was also higher in winter and spring as compared to summer and autumn. Ultrastructural studies revealed a significantly higher value of the thickness of the granulosa cell layer of secondary and tertiary follicles in both left and right ovaries in winter as compared to summer. In summary, breeding activity in female camels extended from autumn till the end of spring as exhibited by behavioural signs, hormonal profile and microscopic studies.

Keywords: camel, ovulation, ecology, hormonal therapy, ultrasonography, ultrastructure,
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Introduction

Camels belong to the genus Camelus and have a hump on the back for discrete fat deposition which helps to survive in harsh conditions. Camels have three species: Camelus dromedarius commonly known as Arabian, dromedary or one humped camel which occupies Africa and Middle East, Camelus bactrianus locally named as Bactrians or two-humped camel which are found in Central Asia and the wild Bacterians (Camelus ferus). According to the Food and Agriculture Organization (2010), camel population in the world totals 24 million and Pakistan is numerically at the 8th position with 1.1 million camels (Economic Survey, 2016-17) which is about 23% of the Asian camel population (Aujla et al., 1998). Nomads in arid and semiarid areas of Pakistan are able to derive an income from camels due to the animals’ unique resilience qualities, which enable them to survive in harsh environmental conditions (Srikandakumar et al., 2001; Ahmad et al., 2010). Camels provide a good source of milk, meat and transport, especially in deserts, and can be a good replacement for ruminants (Skidmore, 2003). The reproductive performance of camels is regarded as very low under natural environmental conditions, which can be
related to lack of selective breeding, long gestation and calving periods, and improper interval housing and management (Skidmore, 2005; Ali et al., 2009).

Being a seasonal breeder, the camel’s reproductive performance is greatly influenced by ecological conditions. In Pakistan, the breeding season lasts from February to April in the mountains and from November to January in the plains. Oestrous duration is 3-4 days and the oestrous cycle lasts for 20-24 days (Qureshi, 1986). The seasonality of the breeding pattern may be revoked by adequate feeding and good housing condition. Camels that are well fed and well-watered may show polyoestrous sexual activity throughout the year (Arthur, 1992). Arabian camels under experimental conditions showed a correlation between ovarian morphological changes and ovarian steroidogenesis. As an induced ovulator, the female camel requires mating for ovulation when a fully matured follicle (0.9-1.9 cm diameter) is present in the ovary (Skidmore et al., 1996).

The objective of this project was to investigate the influence of climate change (Figure 1), micro-ecology and management on the reproduction of female camels in terms of gonads’ structure, their secretion and sexual behaviour.

Materials and methods

Animals and study design

A total of 24 adult, sexually mature, one humped female camel at Faisalabad (n=12), Attock (n=6) and Bhakkar (n=6) were selected for this study. Behavioural and physical observations were made for at least two hours from 10:00 am to 12:00 pm on each animal for a week at the beginning, mid and end of each season. Summary of the ecological conditions at all experimental zones; A: average temperature (°C), B: relative humidity (%), C: Total rainfall (mm) in different seasons is presented in Figure 1.

Ultrasonographic study

Animals were first physically examined, and examination of the external genitalia was performed followed by the ultrasonography via a Picker CS 9100 ultrasound (Picker International GmbH, Munich, Germany) using 5 MHz frequency for the transducer. Examinations of the camels were performed weekly. The frequency was increased to 2 - 3 times per week until ovulation once a follicle greater than 15 mm was detected in either of the ovaries.

The ovaries were scanned to evaluate their follicular activity (ovarian dynamics) within each follicular wave and season. The number of follicles in each ovary was counted and recorded. The diameters of the two largest follicles in each ovary were measured; the morphology and the echogenicity of the follicular wall were assessed and recorded. The follicles were categorized into six differently sized groups (2–5 mm, 6-10 mm, 11-15 mm, 16-20 mm, 21-30 mm and >30 mm). The seasonal distribution of follicles was studied through the analysis of records of follicular numbers in each group and to measure diameter of the largest follicles in each ovary. Records on presence or absence, echo texture, and lifespan of corpus luteum (CL) were utilized to establish ovulation or stage in the ovarian cycle, and to characterize the ultrasonic morphology of the CL.
Figure 1. Summary of the ecological conditions at all experimental zones during year 2013; A: average temperature (°C), B: relative humidity (%); C: total rainfall (mm).
Hormonal therapy

Buserlin, (Receptal®) at 5 ml intravenous (IV) was used as hormonal therapy to induce ovulation during winter and spring. Following GnRH treatment, four animals on the 6th and one on the 8th day ovulated and started to develop the corpus luteum (CL). The corpora lutea persisted for 10-14 days, at which point it commenced to regress. Induction of oestrus during the non-breeding season was done with pregnant mare serum gonadotrophin (3000 IU) IM at Day 0 while ovulation was induced using GnRH analogue Buserlin, (Receptal®) at 5 ml IV at day 11.

Serum progesterone (P4)

Serum samples were analysed for progesterone by using a commercially available kit from Diagnostic Products Corporation (Los Angeles, Ca, USA) (Homeida et al., 1988). A total of 96 blood samples from 24 animals were collected from the jugular vein at one-day intervals for one month during each season.

Ultrastructural studies

Samples were collected via biopsy from animals kept at Faisalabad during the breeding and non-breeding seasons. A small piece of tissue, approximately 3 mm thick, was taken from the ovary and briefly perfused with normal saline and fixed with 2.5% glutaraldehyde, in 0.1 Macodylate buffer containing sucrose, at pH 7.4 (Sabatini et al., 1963). Small blocks of tissue were cut out, treated with osmium tetroxide and processed for Epon embedding (Luft, 1961). Thin sections were stained with uranyl acetate in ethanol (Watson, 1958) and lead acetate and examined under transmission electron microscope JEOL JEM 1010.

Statistical analysis

Microsoft Excel (Microsoft Office, 2016) was used for data computations. Serum progesterone concentration was analysed with General Linear Model (GLM) in IBM SPSS Statistics (New York, USA). Descriptive statistical data was used to describe seasonal, meteorological and reproductive parameters at 5 percent level of significance.

Results

Behavioural signs of female camels including interest and proximity to the male, vocalization, restlessness, biting, chasing and mounting the male were observed. Other physical signs included urination, winking, lowering the head and lowering the ear against the head, jawing, tail raising, swaying of the hip, positioning and standing to be mounted by the male were also recorded. All female dromedaries exhibited oestrous signs when they had growing, or mature follicles present in the ovaries during winter and spring (breeding season). Contrary to this observation, no oestrus signs were recorded in summer and autumn (non-breeding season).

Mean durations of the different phases of the follicular cycle in dromedaries were as follows: growth phase 10.8±1.0 days, maturation phase 7.5.0±0.6 days and regression phase 11.0±0.9 days. The oestrus cycle duration of 28 days was observed in all the zones (Table 1). Zones are not given in the table.

Table 1. Summary of observations of oestrous in dromedaries in different seasons.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Growth Phase</th>
<th>Maturation Phase</th>
<th>Regression Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average length of different phases of follicular cycle (days ± SE)</td>
<td>10-12 (10.8 ± 1.0)</td>
<td>7-8 (7.5 ± 0.6)</td>
<td>10-12 (11.0 ± 0.9)</td>
</tr>
</tbody>
</table>
Serum progesterone concentration (after hormonal therapy for ovulation) was significantly (P<0.05) higher in winter (Figure 2, 1.64 + 1.41 to 5.95 + 6.06 ng/ml, peak value: 13.2 ng/ml) and spring in all-female camels, while in summer, it was higher (Figure 3, 1.25-12.45 ng/ml) in a few camels only. On the other hand, no significant (P>0.05) increase in progesterone concentration was observed in autumn.

Figure 2. Serum Progesterone concentration following single dose (5ml IV) of buserlin (Receptal®) for induction of ovulation in female dromedaries (n=6) during Winter season (December-January 2013).

Figure 3. Serum progesterone concentration following single dose (3ml i.m.) of pregnant mare serum gonadotropin (Folligon®) for induction of ovulation in inactive and smooth ovaries of dromedaries (n=6) kept during summer season (June-July 2013).

Ultrasonographic evaluation of both ovaries revealed that prominent follicles were present and uterine tone was also high in winter and spring (Table 2). However, all female dromedaries showed inactive and smooth ovaries with no oestrous signs in summer and autumn (Table 2 & 3). The diameter of the ovaries was significantly (P<0.05) larger (mean=14.20 mm) in the breeding than in the non-breeding season (1.45 mm).

Table 3. The ovarian response of dromedaries during autumn season before treatment.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Right ovary</th>
<th>Left ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Non-cyclic; Plain, no follicle</td>
<td>Non-cyclic; Plain, no follicle</td>
</tr>
<tr>
<td>2</td>
<td>Non-cyclic; Plain, no follicle</td>
<td>Non-cyclic; Plain, no follicle</td>
</tr>
<tr>
<td>3</td>
<td>Non-cyclic; Plain, no follicle</td>
<td>Non-cyclic; Plain, no follicle</td>
</tr>
<tr>
<td>4</td>
<td>Small-sized follicle (2-3mm)</td>
<td>Follicle present, hard in consistency</td>
</tr>
<tr>
<td>5</td>
<td>Non-cyclic; Plain, no follicle</td>
<td>Non-cyclic; Plain, no follicle</td>
</tr>
<tr>
<td>6</td>
<td>Plain</td>
<td>Three small-sized follicles</td>
</tr>
</tbody>
</table>
Table 2. Ovarian response of female camels in different seasons

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Winter season</th>
<th>Spring season</th>
<th>Summer season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right ovary</td>
<td>Left ovary</td>
<td>Right ovary</td>
</tr>
<tr>
<td></td>
<td>No. of follicles</td>
<td>Dia. largest follicle (mm)</td>
<td>No. of follicles</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>19.5</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.36±0.52</td>
<td>15.08±2.9</td>
<td>1.2±0.45</td>
</tr>
</tbody>
</table>
Ultrastructural studies revealed that the thickness of the granulosa cell layer of the secondary and tertiary follicles was bilaterally increased in winter when compared to summer in female one-humped camels (Figure 4).

**Figure 4.** Electron micrograph exhibited thicker granulosa cells in tertiary follicle during winter (1,3) than summer season (2,4) at 6000X, 3 and 4 at 8000X magnification. A: antrum, GC: granulosa cells, DGC: dividing granulosa cells, LGC: longitudinal granulosa cells, BM: basement membrane, N: Nucleus, M: mitochondria, LD: Lipid droplet, O: oocyte. (JEOL JEM 1010, Japan).

**Discussion**

The findings of the present study revealed that most of the breeding behaviour signs vary with the different seasons and climatic conditions of each area. All female dromedaries exhibited oestrus signs when they had growing, or mature follicles present in the ovaries during winter and spring (breeding season). In Faisalabad, the duration of the breeding season was from December to March, and during these months the average air
temperature is generally low (13.5 °C in winter and 24.1 °C in spring). These results confirmed the findings of Jasra and Isani (2000) who reported that the breeding season of camels in irrigated plains of Sindh and Punjab, Pakistan, begins in December and extends up to March, while in cooler areas it may continue to April.

The breeding behaviour of female camels was strongly related to the environmental temperature and the ovarian activity. Restlessness, swelling of and discharge from the vulva, bleating noises, attraction to male camels, and frequent urination were the oestrus signs observed in the camels. The experimental animals showed maximum ovarian activity in March, which is similar to Al-Eknah’s (2000) report of ovarian activity in dromedaries starting in December and ending in April. Skidmore (2004), and Cristofori et al. (1986) had also observed the same trend in the breeding activity of dromedaries when the air temperature was low.

The duration of different phases of follicular wave pattern (given in table 1) is comparable to observations by Skidmore et al. (1996). They used real-time ultrasonography to examine variation in ovarian follicles and have shown that the pattern of follicular wave differs significantly and is divided into three stages: a growing stage of 10.5 ± 0.5 days, a maturation stage of 7.6 ± 0.8 days, and a regression stage of 11.9 ± 0.8 days. Follicular wave development was reported to be longer (20-22 days) at the beginning and the end of breeding season than in the middle (14 days) (Skidmore, 2011).

A great variation is reported in the duration of camel’s oestrous cycle, with a minimum of 12 days and a maximum 28 days (Musa and Abu Sineina, 1978). The matured follicles retain their size for 13 days followed by regression. Variations in the oestrous cycle duration depends upon the breed, location, climate conditions, photoperiod and availability of fodder. The average duration of the oestrous cycle reported in India, Egypt and Israel is 21.3, 24.2 and 22 days, respectively (Joshi et al. 1978; Chen et al., 1980; Wilson, 1984). The duration of the oestrus cycle also relates directly to the breeding season; short (12-15 days) in the beginning, longer (19-22 days) in the mid breeding season (Nawito et al., 1967; Joshi et al., 1978; Meuli et al., 1982). The duration of heat and matured follicle on ovaries also varies; for instance, Bodenheimer (1954) reported 3-4 days and Elias et al. (1984) reported 6 days.

Being an induced ovulator, the camel requires coitus for ovulation. According to Skidmore et al. (2011), chances of inducing ovulation by hormonal therapy (GnRH analogue) are 80 to 85% in the presence of 10 to 20 mm diameter sized follicle only. The measured mean follicle diameters on the right and left ovaries during the breeding season are 15.08±2.9 and 15.3±6.74 mm, respectively (Table 2). The findings of the current study regarding induction of ovulation through hormonal therapy (4 out of 6 animals) and follicular size are in line with the reports of Skidmore et al. (2011).

Serum progesterone concentration was low before ovulation and started increasing after ovulation. The cyclic progesterone concentration trend documented by Xu et al. (1985) and Ismail et al. (2008) regarding concentration of progesterone in blood was less than 0.36 ±0.28 ng/ml, reaching 1.73± 0.74 and 2.4±0.86 ng/ml at 3rd and 7th day, respectively, after ovulation, which is compatible the findings of the current study.

The highest serum progesterone concentration measured in one-humped camels was 13.2 ng/ml, followed by 12.20 ng/ml. As described also by Skidmore et al. (1996), Tibary and Anouassi (1997) and Ismial et al. (2008), ovulation takes place after a GnRH injection administered to upsurge basal LH values to the
required level, and it consequently causes the elevation of progesterone due to the formation of the corpus luteum. Serum progesterone levels started to increase after GnRH injection and reached maximum level at day 12.

The statistical data showed that a higher concentration of serum progesterone was evident in the breeding season as compared to the non-breeding season. Tibary and Anouassi (1997) stated that the peripheral plasma concentration of progesterone remained low (<1ng/ml) throughout the follicular cycle in the absence of ovulation. The average plasma progesterone level was low during the first 5-7 days after mating, increased to 4-8 ng/ml between day 7 and 8, reached a peak at day 9, and then declined again to basal levels (<1 ng/ml) by day 15 and 17 of the cycle. In contrast, Chamany and Khazali (1998) reported that the mean plasma progesterone concentration in four-year-old camels was not different during the breeding (0.570 ± 0.004 ng/ml) and the non-breeding seasons (0.574 ± 0.003 ng/ml).

No follicles were present in autumn; therefore, no serum progesterone level was evaluated at any of the localities during autumn. In summer, only one animal developed follicles, and that camel showed a high progesterone concentration during the luteal phase.

In winter (breeding season), the mean follicular values on both right and left ovaries was 1.36±0.52 and 1.2±0.45, respectively, and these values in spring were 1.75±0.50 and 1.33±0.82, respectively. Ali (2006) reported that two or more follicles of various sizes were often found in the ovaries of unmated camels. The follicular numbers were higher in the breeding than in the non-breeding season due to ovarian cyclic activity. The same pattern was observed by Riveros et al. (2009), who reported that the total number of follicles present in ovary was higher (P<0.01) on-season than off-season.

During winter, the breeding season, the mean value for the largest follicular diameter was 15.08±2.9 mm for right ovaries and 15.3±6.74 mm for left ovaries. In spring, the mean largest follicular diameter on right ovaries was 13.90±2.66 mm and on the left ovaries it was 12.53±5.37 mm. The results of the current study agree with El-Wishy (1992), who described the size of mature follicles as ranging between 15 and 30 mm, with two or more follicles of different sizes (5 to 30 mm) often found in the ovaries of unmated camels. Riveros et al. (2009) reported that the maximum diameter of follicles in each wave of follicle was 10.2±2.1 mm, with a range from 7.2 to 16.1 mm. That variation in diameter was observed from the start of the breeding season to the end.

All female dromedaries showed inactive and smooth ovaries with no oestrous signs in summer and autumn. Camels showed breeding activity in the cold season, whereas during the remaining period of the year the ovaries remained non-functional and showed a restricted number of small follicles, if any.

According to our results, a significantly high value of the thickness of the granulosa cells layer of secondary and tertiary follicles in both left and right ovaries can be found during winter as compared to summer in female one-humped camels, which is also described by Davoodian et al. (2011) and Kafi et al. (2014).

Conclusion

The female camelid reproductive physiology is greatly influenced by the breeding and non-breeding seasons. Through the assessment of ovarian follicular dynamics, different hormonal treatments to improve reproductive efficiency can be used and regularly monitored through progesterone levels. On the basis of ultrasonography, electron-microscopy and general observation, it can be
concluded that the period of sexual activity started earlier in November and extended up to April.

The results of the present study indicated that the reproduction in camels in Pakistan is strongly under the influence of fluctuating seasonal climatic and management factors. Female dromedary camels exhibited oestrous signs during winter and spring, while these signs were absent in summer and autumn. The breeding season in camels starts in November and ends in March at the Faisalabad and Bhakkar districts of Punjab in Pakistan.

References


