Follicular Wave Pattern, Folliculogenesis and Assisted Reproductive Techniques in the Non-pregnant Female Dromedary Camel (Camelus dromedarius): A Review

Ghazi F. Basiouni
Department of Animal and Fishery Production, College of Agricultural Sciences and Food,
King Faisal University, P.O. Box 35039, Al-Hofuf, 31982, Saudi Arabia

Abstract: The rapid increase in human population in the developing countries has led to a high demand for meat production. The one-humped camel most probably, is a better provider of food in desert and semi-desert areas compared to cattle's which are severely affected by heat and scarcity of water and feed. Efforts to improve the reproductive efficiency of the female camel are closely related to a better understanding of the follicular cycle. The exact mechanism regulating folliculogenesis in the female dromedary camel have only been partly unraveled. This review summarizes the valuable information and achievements obtained during the last years on follicular wave pattern, folliculogenesis and hormones secretion in the female dromedary camel. In addition, some Assisted Reproductive Techniques (ART) such as follicular wave synchronization, the induction of ovulation and superovulation were also reviewed.

Key words: Camel, induced ovulators, folliculogenesis, follicular wave, assisted reproduction

INTRODUCTION

The one-humped camel (Camelus dromedarius) is considered as an induced ovulator that ovulate only in response to mating stimuli (neuroendocrine reflex) in which the initiation of the preovulatory LH surge from the pituitary gland is delayed until coitus occurs (Musa, 1969; El Wishy, 1987). Therefore, if ovulation is prevented by the absence of mating, mature follicle(s) will regress and a new wave of follicles will start to grow again (El Wishy, 1987). Therefore, it is more appropriate to use the term follicular wave or follicular wave pattern rather than estrous cycle to describe this type of cycle (Cockrill, 1979; Skidmore et al., 1995).

Puberty: The onset of sexual activities in the female camel marks the beginning of puberty and it has been found to start as early as 2-3 years of age (Molash, 1990; Arthur et al., 1985; Yagil, 1985; Chen and Yuen, 1979). However, they are usually not bred until they reach their physical maturity at about 70% of their adult body weight at 3-4 years of age (Yasin and Abdul Wahid, 1957; Molash, 1990; Al-Flozab, 1999), otherwise abortion rate will increase. Factors such as adequate nutrition, body weight, photoperiod, temperature and water availability can influence the onset of sexual activity (Wilson, 1989).

Seasonality: Camels breed only during certain times of the year and, therefore, are considered as seasonally polyestrous animals (Musa and Abusineina, 1978; Arthur, 1992; Shulash, 1965). The natural mating season and most conceptions occur in all areas at time of the year when the follicular wave is longest (Wilson, 1998). Contradictory reports has been reported concerning the beginning and duration of the seasonal activity in the female dromedary camel. It has been reported to occur from December to March in Pakistan (Yasin and Abdul Wahid, 1957), December to April in Egypt (Shulash, 1987), March to August in Sudan (Musa and Abusineina, 1978), October to April in Saudi Arabia (Al-Eknah et al., 1997), November to April in Tunisia (Burgemeister, 1975), November to March in India (Matharu, 1966), April to May in Somalia (Mares, 1954), August to September and February to March in Mali (Swift, 1979) and January to February in Iran (Islamy, 1950). However, in the United Arab Emirates, well nourished and watered female camels show ovarian activities throughout the year (Tibary and Anouassi, 1997).

Follicular wave pattern: In spontaneous ovulators (sheep goat and cattle), the estrous cycle consist of four distinct phases known as pro-estrus (follicular growth period), estrus (when female accept coitus), met-estrus (when the corpus luteum is developing) and di-estrus (when the corpus luteum is developed, activated and finally degenerated) (Allen, 1923; Hafez, 1974). In another classification, these four phases were divided into two main phases: follicular phase (estrogenic phase) and luteal phase (progestational phase) (Van Teinhoven, 1968;
Hafez, 1974). However, in induced ovulators such as the camel, there are three phases of the follicular wave which could be categorized as the growth phase (10.9±3.0 days), the mature phase (7.6±4.2 days) and the regressing phase (11.9±4.2 days) (Skidmore et al., 1995). A fourth phase was suggested to precede the growth phase: the non-follicular phase (Cockrill, 1979). The duration of each phase and the total length of the follicular wave were found to vary considerably. The total duration of the follicular wave was found to be 17.2-23.4 days in India (Joshi et al., 1978), 24.2 days in Egypt (Nawito et al., 1967; Wilson, 1984) and 28 days in Sudan (Musa and Abusineina, 1978). The duration of the follicular wave was also found to be longer (19-22 days) at the start (December) and end of the breeding season (April) compared to the middle (end of January to mid of March) of the breeding season (12-15 days) (Yagil and Ettzion, 1984).

Folliculogenesis and hormones secretion: The ovaries of the non-pregnant camels are oval, flattened and relatively small (18-25 mm) (Novoa, 1970). Each ovary weighs 3-4 g (El Wisly, 1992), while ovaries containing graffian follicles may weigh 5.5 g (Shalash and Nawito, 1964). All follicles grow peripherally and randomly distributed over the surface of the ovary and therefore, can be detected easily when they are 3 mm or more in diameter (Tinson and McKinnon, 1992). The dominant follicle continues to enlarge and can ovulate after reaching 10-20 mm in diameter (Tinson and McKinnon, 1992). If ovulation does not occur, the dominant follicle starts to regress. However, some non-mated camels develop follicles > 25 mm in diameter (cyst-like follicles). These non-ovulatory cyst-like follicles do not appear to affect fertility and other smaller follicles may continue to grow normally (Tinson and McKinnon, 1992).

Luteinizing hormone (LH) secretion: The concentrations of Luteinizing Hormone (LH) were found to be higher during the breeding season compared to the non-breeding season (Bono et al., 1990). These variations in LH concentration levels throughout the year may explain why female dromedary camel in Egypt was found to conceive at any time of the year with a considerable variation between seasons (Nawito, 1967). Similar results were also found in Sudan confirming that maximum ovarian activities and conception occurred from January-August and minimum activity occurred from October-December (Khalil, 1989). Another reason for these variations could be the higher sensitivity of the pituitary to Gonadotrophin Releasing Hormone (GnRH) and consequently their higher secretion of LH during the breeding season compared to the non-breeding season (Bono et al., 1985). As induced ovulators, mating in camels is the stimulus for an LH surge needed for the completion of the final stages of follicular maturation and subsequent ovulation. In the dromedary female camel plasma LH concentrations were found to increase gradually to reach a maximum concentrations of 3-19 ng mL⁻¹ at about 2-3 h after mating and then start to decrease 3-4 h later (Marie and Anouassi, 1986, 1987).

Follicle stimulating hormone (FSH) secretion: The main role of FSH in the estrous cycle of female farm animals is to stimulate the early stages of follicular development. Along with low levels of estradiol, FSH plays a part in the development of follicular LH receptors allowing follicles to become more responsive to the follicular increase in tonic LH secretion and thereby preparing them for ovulation and luteinization (Haresign, 1985). Follicle stimulating hormone in the female dromedary camel tend to increase 3-4 days after mating compared to pre-mating values. However, this increase is not significant (Anouassi et al., 1987). It is possible that this little rise in FSH secretion maybe needed for the development of the next wave of follicles if the previous mating did not end up with conception.

Estradiol and testosterone secretion: Both testosterone and estradiol are synthesized from cholesterol. In both, sheep and cattle, ovarian follicles thecal cells are believed to provide granulosa cells with an androgen precursor (androstenedione and testosterone) for aromatization since granulosa cells can not synthesize androgens due to their lack of the specific enzymes involved in this process (Baird, 1977; Armstrong et al., 1981). Evidence supports the presence of a local feedback loop within ovarian follicles, where androgens produced by theca cells are used as a substrate for granulosa cell aromatization into estrogen, which in turn may feedback to stimulate thecal cell production of more androgens (Fortune, 1986; Roberts and Skinner, 1990). Gonadotrophins (LH and FSH) exert their major effects on steroidogenesis in both granulosa and thecal cells at least in part through the activation of membrane-bound adenylate cyclase, thereby increasing the rate of synthesis of cAMP (Weiss et al., 1978). Furthermore, as the follicles increase in size, cAMP content and estradiol production increases (McNatty et al., 1986).

During the regular cycles in the camelidae, the only hormone which would be be representative of the follicular cycle is oestradiol-17β (Thibault and Levassuer, 1991). High serum estrogen and testosterone concentrations during the 5 days follicular development
is probably the stimulus to behavioral estrus (Homeida et al., 1988). A high correlation ($r = 0.97$, $p<0.001$) between follicular size and plasma estrogen and testosterone concentrations was found. The concentration of these two hormones when follicles can be palpated was found to be 20 pg mL$^{-1}$ for estrogen and 50 pg mL$^{-1}$ for testosterone. As follicles grow in size, these concentrations increased to >80 pg mL$^{-1}$ for estrogen and >100 pg mL$^{-1}$ for testosterone. Regression of the follicles on the other hand was followed by low estrogen and testosterone concentrations (Khalil, 1989). Similar findings were obtained by Skidmore (1994) in which serum concentrations of estradiol-17β increased from basal concentrations of 25.0±0.4 pg mL$^{-1}$ to reach a peak at 39.0±1.8 pg mL$^{-1}$ as the follicle reach a diameter of 1.7±0.1 cm. However, if ovulation resulted from mating does not occur at this stage, estradiol-17β level decline to basal values of about 25.0±0.4 pg mL$^{-1}$ as follicles continue to grow to more than 2 cm in diameter and a new wave of follicles starts to grow. Measurement of estradiol concentrations in the follicular fluids also revealed that estradiol levels were found to be higher in large follicles and minimal in small sized, cystic and atretic follicles (Salem et al., 1997; Afaleq et al., 2003). However, follicular fluids estrogen and testosterone concentrations alone may be not a reliable indicator of the follicles estrogenic activity. It is the ability of granulosa cells to convert testosterone to estradiol under the aromatase system which determine whether such follicles is estrogenic (active) or non-estrogenic (non-active). A bi-modal distribution of follicles was observed when frequency (number of follicles) were plotted against estradiol: testosterone ratio (Basiouni, 1997). On this basis, individual follicles were classified as estrogenic or non-estrogenic if they had follicular estradiol: testosterone ratio of ≥6.1 or <6.1, respectively. As a result of this classification, no significant differences were found in the diameter of estrogenic and non-estrogenic follicles. These results indicate that the rate of converting testosterone to estradiol is more indicative of follicles estrogenic activity rather than the concentrations of these two hormones.

Progestosterone secretion: The primary source of progesterone in the female dromedary camel is the corpus luteum, therefore, in the absence of mating and ovulation, progesterone plasma level remain very low throughout the follicular wave (<1 ng mL$^{-1}$) (Homeida et al., 1988; Skidmore, 1994). However, after mating, progesterone concentrations start to rise reaching 3 ng mL$^{-1}$ by day 8-9 after mating (Skidmore et al., 1996). Follicular fluids progesterone concentration was also found to be low in follicles ranging from 0.5-3.0 cm in diameter (0.4-0.5 mg mL$^{-1}$). However, these concentrations were higher in atretic follicles ranging from 2-3 cm in diameter (1.3 mg mL$^{-1}$) (Khalil, 1989).

Ovulation: The first preovulatory LH surge as a result of mating leading to 1st ovulation (puberty) in the female dromedary camel was found to occur at 3-4 years of age (Williamson and Payne, 1978; Evans and Powys, 1979; Slivwart, 1992; Musa et al., 1993; Aboul-Ela, 1994) with an interval between mating and ovulation of about 12-24 h (Mulligan, 1982). Ovulation can be induced by the deposition of whole semen or sperm-free seminal plasma into the vagina of the bactrian camel (Chen et al., 1985) or by i.m. injection of semen or seminal fluid into the dromedary and bacterian camel (Zhao et al., 1990; Pan et al., 1992). It can also be induced in the dromedary camel by mating with either intact or vasectomized male (Marie and Anouassi, 1987). However, mechanical stimulation of the cervix, intrauterine injections of whole semen, seminal plasma, water, prostaglandin F analogue,
cloprostenol, was not successful in stimulating sufficient release of preovulatory LH surge to induce ovulation (Musa and Abusineira, 1978; Musa et al., 1990; Sheldrick et al., 1992; Elias et al., 1984).

An ovulation-inducing factor in the seminal plasma of the bactrian camel was isolated (Pan et al., 1997; Zhao et al., 2000). Blood plasma LH was found to increase significantly from 6.43±2.64 ng/mL−1·6 h after injection of L3 male camel semen fractioned by diethylaminoethylcellulose (DEAE-cellulose) chromatography, suggesting that the isolated L3 camel seminal plasma or one of its components might be the ovulation-inducing factor. Moreover, the insemination of the female bactrian camel with an ovulation inducing bioactive protein from the seminal plasma of a male was found to induce a preovulatory LH peak (Pan et al., 2003). These researchers also found that the main site for active absorption of the ovulation inducing factor was in the anterior vagina. As in the bactrian camel, further studies are also needed in the dromedary camel to obtain the full characterization of the pure ovulation-inducing factor.

Twin ovulations in camelds are around 14%. However, twin births are only 0.4%. Even though it is accepted that there is substantial differences in the activity between the right and left ovaries (Musa, 1979). Both ovaries appears to be equally capable of producing normal preovulatory follicles (Bassouni, 1997). However, in twin ovulation cases, the embryo in the right horn of the uterus dies during early pregnancy (Musa, 1969). Furthermore, 99.24% of pregnancies were found to be in the left horn of the uterus (Shalash, 1965).

**Assisted reproductive techniques in the female dromedary camel:** The recent knowledge acquired in regard to follicular wave pattern in the female dromedary camel has led to the improvement of Assisted Reproductive Techniques (ART) such as follicular wave synchronization, induction of ovulation and superovulation. The recent wide use of ultrasonography techniques has made it easier to monitor ovarian follicular wave development and hence to use hormonal treatments at an appropriate stage of development to induce ovulation. As in farm animals, progesterone impregnated devices has been used to inhibit follicular growth, estrus behavior and ovulation in the female dromedary camels. Progesterone-Releasing Intravaginal Devices (PRIDs) were used to stimulate the camel natural luteal phase (Cooper et al., 1990; Skidmore et al., 1992) to insure that after the removal of the device all follicles start to grow from the same stage of development and hence to synchronize their follicular wave growth and ovulation. However, PRID alone did not seem to be a reliable method of synchronizization in the dromedary camel since the presence of the spring-loaded PRID in the vagina close up against the cervix for a period of 7 days may have initiated the release of LH triggering the necessary steps required for ovulation (Cooper et al., 1992).

The improvement of reproductive efficiency in the female dromedary camel are limited by the short breeding season, long gestation period and the continuous use of traditional reproductive management systems. The use of 3000 IU of hCG or 20 μg of buserelin (GnRH analogue) was found to be successful to induce ovulation (Cooper et al., 1992). The regular palpation of the ovaries (Musa, 1969) and the use of the ovarian ultrasonography techniques (Skidmore et al., 1996) have together indicated that during the breeding season, waves of follicular growth, maturation and atresia occur continuously in both ovaries. Treatment of the dromedary female camels with 20 μg of buserelin or 3000 i.u. of hCG when the follicles were at different sizes showed that ovulation did not occur if follicles was <0.9 cm in diameter. However, ovulation increased to 85% if follicles size was 1.0-1.9 cm in diameter. Furthermore, ovulation rate decreased to <30% if follicles size was 2.0-2.9 cm in diameter and none of the follicles had ovulated if their sizes was >3.0 cm in diameter. These results concluded that the optimum time for natural breeding or induced ovulation in the female camel is when follicles are 0.9-1.9 cm in diameter (Skidmore et al., 1996, Skidmore, 2003).

Techniques such as superovulation can also be used to stimulate the growth of a large number of follicles so they could be used for embryo transfer to produce multiple progeny of desirable genetic merits for both production and the high monetary value of racing camel. To stimulate the growth of multiple follicles, equine chorionic gonadotrophin (eCG), follicle stimulating hormone (FSH) and gonadotrophin releasing hormone (GnRH) has been used in camels at various doses which may or may not, be given after a period of progesterone priming. A single dose of eCG (1500-6000 iu) injected in a single dose either one day before, or on the day of ending a 5-15 days of progesterone priming was found to be reasonably successful (Cooper, et al., 1990; McKinnon and Tinson, 1992; Ismail et al., 2007a). Follicle stimulating hormone of porcine or ovine origin has been also used with a total dose of 18 mg of ovine FSH or 400 mg of porcine FSH given over a period of 4 days was also successful (Cooper et al., 1990; McKinnon et al., 1994). A regime combining both eCG and FSH achieved the best response using a 2500 iu of eCG given as a single injection on day 1 of treatment.
CONCLUSIONS

In spite of all of the recent advancement in the ART in camels, there is some problems associated with superovulation in the female dromedary camels due to the following reasons:

- Such techniques may never be used to breed camels commercially due to the costs involved and the lack of both infrastructure and technical support needed.
- Variability in ovulation response and number of embryos collected.
- High incidence of ovarian over stimulation.
- High incidence of non-responsive females.
- High incidence of luteinized follicles.
- Complete arrest variability of ovarian activity in some females as a result of a repeated superovulation over several years due to immunization of the female against the hormones used in superovulation.
- The response variability between different labs applying similar regimens.
- The variability between different labs in regard to follicular cycle length and hormones concentration.
- More researches are needed to understand the kinetics of folliculogenesis and ovulation in the female dromedary camel.

Improving the reproductive efficiency, therefore, still remains a challenging task under the widely used commercial production systems known for its long calving intervals. Consistent and accurate control of follicular wave growth cycles and atresia with the use of both hormonal treatments and ultrasonography scanning of the ovaries are urgently needed. Attempts also should be made to advance the onset of puberty, shortening the postpartum interval to conception and to induce ovulation outside the breeding season.

REFERENCES


