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Ovarian Kinetics and Control of Ovulation (17 June 2000)

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Introduction

Female camels are seasonal breeders with a relatively short breeding period during which ovarian activity is increased [1]. They are also 'induced ovulators' and therefore normally only ovulate in response to mating [2,3]. In these animals therefore the neuro-endocrine reflex involving initiation of the release of Luteinizing hormone (LH) from the pituitary gland must be delayed until coitus occurs. For this reason follicles tend to grow, have a period of maturity during which time they are capable of being ovulated, and then regress again if ovulation is not induced [3]. It is therefore more accurate to describe the changes in the ovarian follicular dynamics as a 'follicular wave pattern' rather than an oestrous cycle.

Earlier studies on the follicular wave pattern in dromedaries were based on post mortem examinations and on serial palpations of the ovaries per rectum in small numbers of camels. The results of these studies reported the duration of the follicular wave to range from 17 - 23 days in India [4], 24 days in Egypt [5] and 28 days in the Sudan [6], but they tend to be longer in at the beginning and end of the season (19-22 days) than during the middle of the season (12 - 15 days) [7]. For the Bactrian camel the average follicle wave length has been reported to be 19 days (14 - 21 days) [8].

However, more recent studies used realtime ultrasonography to monitor day-to-day ovarian follicular changes much more accurately [9,10]. The reproductive tract of the female camels can easily be examined using ultrasonography. The camel is restrained in stocks or in the sitting position with a kinch rope tied up around her hind legs (Fig. 1). The rectum is cleaned of faeces and the transducer of the scanner introduced carefully into the rectum. The probe is passed over the uterus and the ovaries and the fluid filled follicles are readily distinguishable as spherical, non-echogenic 'black holes' developing in the ovary (Fig. 2a and Fig. 2b: Ultrasonographs of the ovaries).



Figure 1. A female camel undergoing transrectal ultrasonographic examination of her ovaries. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 2a. Ultrasonographs showing the development of a mature follicle. An ovary with three small follicles (arrow) of approximately 0.8 cm in diameter. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 2b. Ultrasonographs showing the development of a dominant mature follicle of 1.8 cm in diameter. - To view this image in full size go to the IVIS website at www.ivis.org . -

These ultrasonographic studies have shown that the follicular wave pattern varies considerable between camels, but can be divided into four phases: namely: follicular recruitment, the growth phase, the mature

phase and the regression phase.

Recruitment Phase - The follicular recruitment phase is the time lapse between an examination which does not show any follicular activity, and the emergence of several follicles (2 - 3 mm) on the surface of the ovary. Not much is known about the mechanisms of recruitment of each follicular wave but it is possible that it constitutes a response to an increase in FSH, however, it can only be thoroughly investigated by histological techniques. In the dromedary, this follicular recruitment phase takes between 2 - 4 days [11]. **Growth Phase** - The period of follicular recruitment is followed by a period of follicular growth of 3 - 6 follicles until the establishment of one or two dominant follicles. In the dromedary, these follicles can grow at a rate of about 0.5 - 1.0 mm per day until they reach approximately 1.0 cm in diameter and then one or two follicles become dominant and continue to grow. This growth phase generally lasts between 6 - 10 days [9-11]. In about 50% of the cases, the dominant follicle grows to a mean maximum diameter of 2.0 ± 0.1 cm (range 1.5 - 2.5 cm) whilst the others regress, whereas in the other 50% of the cases the dominant follicle continues to grow to a mean maximum diameter of 4.2 ± 0.2 cm (range 4.0-6.4 cm) before it starts to regress, taking on average 18.4 ± 0.8 days to reach its maximum diameter. There is a strong relationship between the number of follicles present and the diameter of the largest follicle, which is consistent with the follicle wave theory (Fig. 3) [9,10].

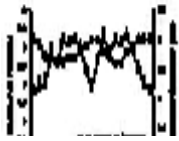


Figure 3. Inverse relationship between the number of follicles and follicle diameter (cm) in unmated camels. (Day 0 is taken as the day on which the largest follicle reaches its maximum diameter). - To view this image in full size go to the IVIS website at www.ivis.org . -

Mature Phase - This phase encompasses the time when the follicle has reached maximum diameter and is capable of ovulating. It lasts on average 7.6 ± 0.8 days if the mature follicle is between 1.5-2.5 cm in diameter and 4.6 ± 0.5 days if the follicle has grown to as large as 4.0-6.4 cm. In the latter case the follicles are unable to ovulate. The establishment of dominance and the regression of subordinate follicles is likely to be under the control of in-situ production of inhibin by the follicle. This is supported by the increased number of follicles from the same wave reaching sizes greater than 1.0 cm after immunisation of the dromedary female against inhibin [12].

Regression Phase - In the absence of mating or ovulation-inducing treatment the mature follicle starts to regress, taking an average of 11.9 ± 0.8 days if the mature follicle measures 1.5-2.5 cm, and 15.3 ± 1.1 days for the larger anovulatory follicles. During the regression period, the follicular fluid of these overlarge follicles, which is usually serous in the early stages, becomes more echogenic owing to the development of free floating echogenic strands which later becomes more organized into transecting strands of fibrin. These large follicles have been divided into 5 categories: 1) thin-walled, large follicular structure containing clear fluid, 2) thick-walled (2 - 4 mm) structure containing clear fluid, 3) thick-walled structure with some floating debris within its cavity, 4) thick-walled structure with blood clot and fibrin strands within the cavity (haemorrhagic follicle) and 5) luteinized follicle as some of these anovulatory follicles can go on to become luteinized and produce significant levels of progesterone similar to that seen in the presence of a CL. Nevertheless, these overlarge follicles do not inhibit the growth of other follicles in the same, or contra-lateral ovary, which mature and ovulate if the appropriate stimulus is applied [9-12]. In all cases the new follicles become visible and start to grow before the mature follicle has fully regressed to give an interwave interval of about 18.2 ± 1.0 days in dromedaries [9,10].

Control of Ovulation

As the commercial and scientific interest in camels increases, especially in the racing camels in Arabia, it has become desirable to develop methods for manipulating ovarian function in the females to maximise reproductive efficiency throughout the relatively short breeding season. Of fundamental importance is the ability to control ovulation in these induced ovulating species.

It is now well established that mating with an intact or vasectomized male will induce ovulation in camels but the detailed mechanism that controls it is not well understood. In Bactrian camels it is also said that ovulation

can be induced by either (i) deep intravaginal deposition of whole semen or sperm-free seminal plasma [13] or (ii) by intra muscular (im) injection of semen or seminal fluid [14]. This ovulating inducing effect of the seminal plasma is retained after mild heating and treatment with acid or alkali, but is destroyed by trypsin digestion, suggesting there is an active protein or polypeptide in camel semen which can express GnRH-like activity [15]. However, in dromedaries intrauterine injection of whole semen, seminal plasma, water or cloprostenol, does not stimulate the release of sufficient LH from the pituitary to cause ovulation [16]. Manual stimulation of the cervix with a finger for 2 - 15 minutes in dromedaries also failed to induce ovulation as did stimulation of the cervix with a rubber insemination tube in Bactrian camels [17].

Alternative methods for inducing ovulation had to be investigated because obviously, when animals are being prepared for artificial insemination or as recipients for embryo transfer, mating to a vasectomized male or insemination with camel seminal plasma would be impractical due to the risk of spreading venereal infections and the difficulty of collecting the seminal plasma from male camels [18]. In addition, due to the common occurrence of oversized unovulated follicles mentioned previously, it is important to determine the limits, in terms of follicular growth and maturation, as to when the dominant follicle is most responsive to such therapy. Studies were therefore carried out to investigate the reliability of treatment with Gonadotrophin Releasing Hormone (GnRH) or gonadotrophic hormones as a means of inducing ovulation in female camels exhibiting follicles of varying sizes. Camels were treated with either 20 µg of the GnRH analogue, buserelin (Receptal; Hoechst Animal Health, Milton Keynes, U.K.) or 3000 iu human Chorionic Gonadotrophin (hCG; Chorulon; Intervet Laboratories, Cambridge, U.K.) or were mated (controls) when the follicles were of different sizes. The results showed that ovulation did not occur if the follicle is <0.9 cm in diameter, but increased to 85% if the follicle grew to between 1.0 - 1.9 cm in diameter. It then decreased sharply to 12.5% if the follicle increased in size to between 2.0 - 2.9 cm but no follicles over 3.0 cm ovulated at all, nor did any follicle in the regression phase (Fig. 4, shows the ovulatory responses of camels with follicles of different sizes) [10].

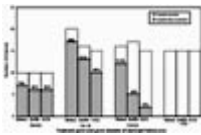


Figure 4. Ovulatory responses of camels with follicles of different sizes to three different ovulatory stimuli. As follicle size increases there is a significant decrease in ovulation rate. - To view this image in full size go to the IVIS website at www.ivis.org . -

Although follicles of only 1.0 cm in diameter will ovulate this is not really the pre-ovulatory size of a follicle, it is just the size at which a follicle acquires the ability to ovulate. In our experience the best ovulation results are obtained if the camel is induced to ovulate when the follicle measures between 1.3 - 1.8 cm in diameter.

Ovarian Side of Ovulation

The respective activity of the left and right ovary has attracted great interest because of the fact that the majority of pregnancies are established in the left horn of the uterus in all camelids. Many people have tried to explain the predominance of left horn pregnancies in the camelidae by a difference in follicular activity and incidence of ovulation between the left and right ovary, or by an increased incidence of embryo mortality for right horn pregnancies. It is now generally accepted that there is no real difference in activity between either ovary, and the pregnancy rate is similar whether ovulation occurs on the left or right ovary [12,19]. Although twin ovulations do occur, the recorded incidence of twin births is only around 0.4% and it is thought that this paucity of twins results for the death of the embryo that implants in the right horn when it reaches a crown-rump length of 2 - 3 cm [2].

Corpus Luteum (CL) and Luteolysis

The CL develops within a few days after ovulation, reaches a plateau and then regresses if a pregnancy is not present in the uterus. Endocrinological and clinical evidence suggest there are differences between the CL of camelids and that of other species. Firstly, it is slower to develop and secondly it has an early regression in the absence of a conceptus in the uterus. The CL can be indentified by ultrasonography on days 4 to 5 post mating and by rectal palpation on days 8 - 10. It tends to reach its maximum size by days 8-9 then regresses on days 9 - 10 post mating in the absence of a pregnancy [10,11]. In comparison with several other domestic species this luteal lifespan is relatively short, which means that the camel conceptus has to pass on its maternal recognition of pregnancy signal, or antiluteolytic signal to the maternal endometrium, by day 7 or 8 if the mother is to maintain the CL and thus stay pregnant. This is much earlier than in other species [20]. Luteolysis in ruminants, pigs, horses and other large mammals is brought about by the pulsatile release of

prostaglandin F₂α (PGF₂α) from the endometrium in late dioestrus. Current evidence in sheep and cattle suggest that progesterone from the CL and oestradiol from the developing follicles act in tandem to control the development and sensitivity of receptors for oxytocin in the endometrium [21,22]. In ruminants, oxytocin, primarily from the CL, but also from the pituitary, then interacts with its endometrial receptors to stimulate the spike-like pulses of PGF₂α required for luteolysis [23].

Studies in the dromedary camel have shown that there was a marked increase in basal concentrations of PGF₂α between days 8 and 10 in the cycling animal, that coincided closely with a decline in serum progesterone levels, thereby indicating that luteolysis was occurring. Both PGF₂α and progesterone concentrations had returned to basal levels by day 12 [24]. Further evidence for the involvement of PGF₂α in luteolysis in the dromedary was shown by the suppressive effect of meclofenamic acid. This compound is a prostaglandin synthetase inhibitor and when it is administered from day 6 onwards, it prevents luteolysis occurring and thus the CL is maintained. In sheep, cattle and horses, bolus injections of oxytocin given around the expected time of luteolysis stimulate a pronounced release of PGF₂α from the endometrium [25-27]. In contrast, in camels, injections of equivalent doses of oxytocin given on day 10 after ovulation, failed to stimulate any discernable increase in PGF₂α concentrations. This maybe due to a complete absence, or very low numbers, of oxytocin receptors in the endometrial epithelium in this species. These results suggest that PGF₂α plays an important role in luteolysis but there is no evidence to suggest that its release is controlled by oxytocin [24].

Follicular Activity During the Luteal Phase

Follicular activity continues even in the presence of a CL [11]. In mated camels a new follicular wave starts to develop 4 to 6 days after ovulation, and gives rise to a mature follicle just after CL regression. These new dominant follicles can emerge equally on the ovary bearing the CL or on the contralateral ovary. This coincides with the timing of a new pre-ovulatory follicle in females mated by a vasectomized male or in which ovulation was induced by GnRH or hCG treatment [11]. Therefore, on average, the interwave interval is only 13 - 14 days in ovulating non-pregnant females [11,28]. In the Bactrian camel, a new follicular wave starts to develop 4 to 6 days after ovulation and gives rise to a mature follicle just after CL regression [8].

Hormone Profiles in the Non-Mated Female Camels

Gonadotrophins

Luteinizing Hormone - As camels are induced ovulators it is mating that induces an LH surge. In the dromedary camel LH plasma levels increase within one hour of mating and reach a maximum (3 - 19 ng/ml) 2 - 3 hours later, then start to decrease 6 hours following mating. The LH surge is thought to elicit the last stages of follicle development and subsequent ovulation [28,29]. In the Bactrian camel LH peaks (6.9 ± 1.0 ng/ml) 4 hours after insemination, then decreases 8 hours later [30].

Follicle Stimulating Hormone - In the dromedary, FSH levels tend to increase 3 to 4 days after mating, but this increase is of small amplitude [31].

Steroids Hormones

Oestradiol - Oestradiol levels are highly variable between camels, but do not show tremendous variations within each camel. In general they tend to follow follicular development i.e. as the follicle diameter increase, oestradiol concentrations increase from basal levels of 25.0 ± 0.4 pg/ml to 39.0 ± 1.8 pg/ml until the follicle reaches 1.7 cm in diameter. However, even though the follicle may continue to grow to >2.0 cm over succeeding days, mean oestradiol levels tend to decline to basal levels of 25.0 pg/ml where they remain until the next wave of follicles grow (Fig. 5). This may well be the reason why these overlarge follicles do not ovulate, as once they are over 2.0 cm they start to undergo atresia [10]. The fluctuations in the oestradiol concentrations are not great due to the fact that the unmated camel does not have a luteal phase and the ovaries are continually producing new waves of follicles [10,32].

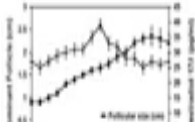


Figure 5. Mean (\pm s.e.m.) serum oestradiol-17 β concentrations (triangle) related to follicle diameter (square) in non-pregnant camels. - To view this image in full size go to the IVIS website at www.ivis.org . -

Testosterone - Plasma testosterone levels were found to follow the same variations as oestrogen. Increased size of the follicle is accompanied by an increase in testosterone levels. Plasma testosterone levels increase from 50 pg/ml, then declines with the regression of the follicle [33].

Progesterone - The main source of progesterone is the CL, therefore in the absence of mating and ovulation, progesterone concentrations remain low (<1ng/ml). After mating concentrations remain low for the first 3 - 4 days after ovulation, but then start to rise steadily to a peak of around 3 ng/ml by day 8 or 9, before falling sharply on days 10 and 11 to basal values of <1ng/ml again by days 11 or 12 in the non-pregnant camel (Fig. 6) [9,10]. Progesterone levels are low in comparison to other animals but are a good indicator of the occurrence of ovulation and the formation of a CL.

In the Bactrian camel, a rise of plasma progesterone levels to 1.73 ± 0.74 ng/ml was observed 3 days following ovulation. Progesterone continues to rise until day 7 post-ovulation when it reaches a plateau at 2.4 ± 0.86 ng/ml before it declines again in the non-pregnant animal.

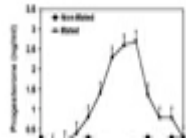


Figure 6. Mean (\pm s.e.m.) serum progesterone concentrations in non-mated (square) and mated, non-pregnant camels (triangle). - To view this image in full size go to the IVIS website at www.ivis.org . -

References

1. Novoa C. Reproduction in the Camelidae: A review. *J Reprod Fert* 1970; 22:3-20.
2. Musa BE. A Study of some aspects of reproduction in the female camel (*Camelus dromedarius*). MVSc Thesis University of Khartoum, 1969.
3. El Wishy AB. Reproduction in the female dromedary (*Camelus dromedarius*): A review. *Anim Reprod Sci* 1987; 82:587-593.
4. Joshi CK, Vyas KK, Pareek PK. Studies on the oestrus cycle in Bikaneri she-camels. *Indian J Anim Sci* 1978; 48:141-145.
5. Nawito MF, Shalash MR, Hoppe R, Rakha AM. Reproduction in the female camel. *Bull Anim Sci Res Inst Cairo* 1967; 2: pp. 82.
6. Musa BE, Abusineina ME. The oestrous cycle of the camel (*Camelus dromedarius*.) *Vet Rec* 1978; 103:556-557. - PubMed -
7. Elias E, Bedrak E and Yagil R. Estradiol concentration in the serum of the one humped camel (*Camelus dromedarius*) during the various reproductive stages. *Gen Comp Endocrinol* 1984; 56:258-264. - PubMed -
8. Chen BX and Yuen ZX. Reproductive pattern of the Bactrian camel. In: Cockrill WR, ed. *The Camelid. an all Purpose Animal*. Uppsala: Scandinavian Institute for African Studies, 1979; 1:364-396
9. Skidmore JA, Billah M, Allen WR. The ovarian follicular wave pattern in the mated and non-mated dromedary camel (*Camelus dromedarius*). *J Reprod Fert* 1995; 49:545-548

10. Skidmore JA, Billah M, Allen WR. The ovarian follicular wave pattern and induction of ovulation in the mated and non-mated one-humped camel (*Camelus dromedarius*). *J. Reprod Fert* 1996; 106:185-192. - PubMed -
11. Tibary A, Anouassi A. Ultrasonographic changes of the reproductive tract in the female camel (*Camelus dromedarius*) during the follicular cycle and pregnancy. *J Camel Practice and Research* 1996; 3:71-90.
12. Tibary A, Anouassi A. Reproductive physiology in female camelidae. In: Institut Agronomique et Veterinaire Hassan II, ed. *Theriogenology in camelidae*. Rabat: Abu Dhabi Printing and Publishing Company, 1997; 169-241.
13. Chen BX, Yuen ZX and Pan GW. Semen-induced ovulation in the Bactrian camel (*Camelus bactrianus*). *J Reprod Fert* 1985; 74:335-339. - PubMed -
14. Pan GW, Zhao XX, Chen BX, Jiang S, Huang YM, Zu YS, Wang HY. The ovulation-inducing effect of seminal plasma in the Bactrian camel, in *Proceedings. 1st Int Camel Conf* Allen WR, Higgins AJ, Mayhew IG, Snow DH, Wade JF. Eds. Newmarket: R & W Publications 1992; 159-162.
15. Zhao XX, Huang YM, Chen BX. Biological activity of Gonadotrophin-releasing hormone-like factors in the seminal plasma of the Bactrian camel, in *Proceedings. 1st Int. Camel Conf* Allen WR, Higgins AJ, Mayhew IG, Snow DH, Wade JF. Eds. Newmarket: R & W Publications 1992; 163-168.
16. Sheldrick EL, Flick-Smith H, Skidmore JA, Wensvoort S, Billah M, Ali Chaudry M, Allen WR. LH release profiles in female dromedary camels following mechanical and hormonal stimuli to induce ovulation, in *Proceedings. 1st Int Camel Conf* Allen WR, Higgins AJ, Mayhew IG, Snow DH and Wade JF. Eds. Newmarket: R & W Publications 1992; 193-201.
17. Chen BX, Yuen ZX, Pan CW. Factors inducing ovulation in the Bactrian camel. In WR Cockrill, ed. *The Camelid. An all Purpose Animal*. Uppsala: Scandinavian Institute for African Studies, 1984; 387-398.
18. Billah M, Skidmore JA. The collection, evaluation and deep freezing of dromedary camel semen, in *Proceedings. 1st Int Camel Conf* Allen WR, Higgins AJ, Mayhew IG, Snow DH, Wade JF. Eds. Newmarket: R & W Publications 1992; pp.410 (abst.).
19. Musa BE. Studies on the ovary of the camel (*Camelus dromedarius*). *Sudan J Vet Sci and Anim Husb* 1979; 20:51-56.
20. Skidmore JA, Allen WR, Heap RB. Oestrogen synthesis by the peri-implantation conceptus of the one-humped camel (*Camelus dromedarius*). *J Reprod Fert* 1994; 101:363-367. - PubMed -
21. McCracken JA, Schramm W, Okulicz WC. Hormone receptor control of pulsatile secretion of PGF₂ α from the ovine uterus during luteolysis and its abrogation in early pregnancy. *Anim Reprod Sci* 1984; 7:31-56.
22. Vallet JL, Lamming GE, Batten M. Control of the endometrial oxytocin receptor and uterine response to oxytocin by progesterone and oestradiol in the ewe. *J Reprod Fert* 1990; 90:625-634. - PubMed -
23. Flint APF, Stewart HJ, Lamming GE, Payne JH. Role of the oxytocin receptor in the choice between cyclicity and gestation in ruminants. *J Reprod Fert* 1992; 44 (suppl):53-58. - PubMed -
24. Skidmore JA, Starbuck GR, Lamming GE, Allen WR. Control of luteolysis in the one-humped camel (*Camelus dromedarius*). *J Reprod Fert* 1998; 114:201-209. - PubMed -
25. Fairclough RJ, Moore LG, Peterson AJ, Watkins WB. Effect of oxytocin on plasma concentrations of 13,14-dihydro-15-keto prostaglandin F and the oxytocin-associated neurophysin during the oestrous cycle and

early pregnancy in the ewe. Biol Reprod 1984; 31:36-43. - PubMed -

26. LaFrance M, Goff AK. Effect of pregnancy on oxytocin-induced release of prostaglandin F₂ in heifers. Biol Reprod 1985; 33:1113-1119. - PubMed -

27. Goff AK, Pontbriand D, Sirois J. Oxytocin stimulation of plasma 15-keto-13,14-dihydro prostaglandin F₂α during the oestrous cycle and early pregnancy in the mare. J Reprod Fert 1987; 35:253-260. - PubMed -

28. Marie M, Anouassi A. Induction of luteal activity and progesterone secretion in the non-pregnant one-humped camel (Camelus dromedarius). J Reprod Fert 1987; 80:183-92. - PubMed -

29. Marie M, Anouassi A. Mating induced luteinizing hormone surge and ovulation in the female camel (Camelus dromedarius). Biol Reprod 1986; 35:792-798. - PubMed -

30. Xu YS, Wang HY, Zeng GQ, Jiang GT, Gao YH Hormone concentrations before and after semen-induced ovulation in the bactrian camel (Camelus bactrianus). J Reprod Fert 1985; 74:341-346. - PubMed -

31. Anouassi A, Combarous Y, LeCompte F, Cahoreau C, Guillou, F. Purification and characterization of luteinizing hormone from the dromedary (Camelus dromedarius). Biochimie 1987; 69:647-654.

32. Shalash MR. Some reproductive aspects in the female camel. World Rev Anim Prod 1965; 4:103-108.

33. Homeida AM, Khalil GR, Taha AAM. Plasma concentrations of progesterone, oestrogens, testosterone and LH-like activity during the oestrus cycle of the (Camelus dromedarius). J Reprod Fert 1988; 83:593-598. - PubMed -

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