Vol 19 No 2, p 269-275

CHARACTERISATION OF FOLLICULAR AND LUTEAL BLOOD FLOW IN FEMALE DROMEDARY CAMEL INDUCED TO OVULATE USING GnRH ANALOGUE

M.S. Rawy, D.R. Derar, T.M. El-Sherry and G.A. Megahed

Department of Theriogenology, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt

ABSTRACT

Present study investigated the changes in vascularity of preovulatory follicles and corpus luteum after induced ovulation. Three animals were examined ultrasonographically daily, using B-mode and colour Doppler for 12 follicular waves. Blood collected daily for progesterone assay. Blood area and blood area per cent were determined for the ovarian follicles. Single injection of Buserelin was used to induce ovulation for dominant follicles range from 9–18.9 mm. Ovulation rates of 100% was recorded. Ovulation occurred within 24–32 and 32–40 hours of treatment in 92.3% and 7.7% of trials, respectively. Blood area and blood area per cent were 19.18 ± 4.4 mm^2 and 9.8 ± 2.6%, 8 hours before ovulation, respectively. After ovulation, BA increased (P> 0.05) when the corpus luteum was 18.1 ± 0.4 mm in diameter (day 7). On day 10, both, the BA and BA% decreased (P> 0.05) concomitant with the beginning of luteolysis. In conclusion ovulation occurred within 24-32 hours in 92.3% and 32-40 in 7.7% of induced follicles.

Key words: Camel, GnRH, follicles, corpus luteum, Doppler

Efficient methods to induce ovulation in camel facilitate the use of reproductive technologies such as artificial insemination and embryo transfer in this species (Cooper *et al*, 1992). Ovulation can be induced in dromedary and bactrian camel by a single injection of LH, GnRH and hCG (Chen *et al*, 1985; Marie and Anouassi, 1987; Anouassi *et al*, 1992; Sheldrick *et al*, 1992 and Skidmore *et al*, 1996). Colour-Doppler ultrasound has been used for studying vascular perfusion of the dominant follicle in mares (Gastal and Gastal, 2011), superovulated follicles in cow (El-Sherry *et al*, 2010) and ovarian and uterine blood flow in woman (Pellizzari *et al*, 2002). Recently, transrectal Doppler ultrasonography has been utilised increasingly for research and clinical studies of ovarian and follicle hemodynamics in large farm animals (Ginther and Utt, 2004, Miyamoto *et al*, 2006, Ginther *et al*, 2007 and Herzog and Bollwein, 2007). Dromedary camel received little attention from physiologists especially in applying these techniques to reproductive aspects of this animal. The purpose of this study was to evaluate the ovulatory response and luteal activity following the usage of GnRH analogue to induce ovulation in female dromedary camel. The present experiment also aimed to characterise the changes in the blood-flow of the preovulatory follicle and CL of this camels.

Materials and Methods

Animals

Twelve reproductively sound, non pregnant, non-lactating female dromedary camels (8-12 years, 450-500 kg BW) belong to Veterinary Teaching Hospital of the Faculty of Veterinary Medicine, Assiut University were used in the present study. The experiment was conducted during the breeding season from February to May, 2010. All animals were maintained in good body condition with suitable hygienic measures and kept under natural day light and local environmental condition. Animals were supplied with water and hay *ad libitum* and fed a diet of mixed concentrates once a day.

Experimental design

Animals were subjected to daily examination using ultrasonography and Doppler devices to monitor the changes in the blood flow of dominant follicles throughout a complete follicular wave. Then, examinations continued throughout a second wave from the recruitment phase of the follicular

SEND REPRINT REQUEST TO D.R. DERAR email: derar40@yahoo.com

wave till the induction of ovulation. Twenty µg of Buserelin (5 ml Receptal; Intervet International B.V. Boxmeer, Holland) injected intramuscularly to induce ovulation (dominant follicles were 9-18.9 mm). The time of GnRH injection was defined as Day 0. The ovaries were scanned every 8 hours after treatment to monitor ovulation. Ovulation was identified when a dominant follicle was no longer present in two successive examinations. In order to study the blood flow of the ovarian follicles and corpora lutea, colour-Doppler ultrasound instrument (ESAOTE, Pie Medical, MyLab30Vet device - Via di Caciolle, 15 - 50127 Firenze, Italy), equipped with endorectal linear probe was used. All scans were performed at a pulse repetition frequency of 7.5 MHz, and constant colour-gain settings. The entire follicle was scanned in a slow continuous motion several times. Images were captured with an on-line digital video-taping system and stored. Only the presence or absence of blood flow with a velocity higher than 2 mm/sec was assessed for each follicle. When a clearly visible red or blue spot (blood flow) was detected in the follicular wall, it was considered as a follicle with detectable blood flow. Images were used to calculate the maximum diameter of the follicle. BA (the maximum measured area in mm^2 of blood patches detected by Doppler within the follicular or the CL walls) and BA% (the maximum measured area in mm^2 of blood patches detected by Doppler within the follicular or the CL walls relative to the maximum diameter of these structures expressed in %) were measured in the stored images of each examination. The automatic measurement of the number of coloured pixels (Coloured Area) was performed by selecting coloured pixels using Adobe Photoshop (Adobe systems incorporated, San Jose, CA) and then counting the number of selected pixels with ImageJ version 1.33. (http://rsb.info.nbh.gov/nihimage). Transrectal colour Doppler ultrasound was also used for the non-invasive investigation of luteal blood flow after inducing ovulation.

Progesterone assay

Blood samples were collected daily from the jugular vein into heparinised tubes from all female camels throughout the studied period and centrifuged immediately after collection at 1700 xg. The harvested plasma was stored at -20 ^{o}C until hormonal assay. Progesterone concentration was determined with a commercially available Progesterone EIA kits provided by Biosewoom Inc. (Sungdong-gu, Seoul, Korea, catalogue no. BS1405). The range of the standards used was 0.2–40.0 ng mL^{-1}. Three quality controls for low (oestrus animals, 0.23 ng/ml), medium (early diestrus, 2.75 ng/ml) and high (pregnancy, 14.5 ng/ml) were used. The inter- and intra-run precision had a coefficient of variation of 4.8 and 2.4%, respectively.

Statistical analysis

The data of hormonal concentration, follicular sectional diameter, follicular sectional area, blood area (BA) and blood area percent (BA%) were expressed as mean ± SEM. Follicular, luteal, vascular and hormonal data were analysed using General linear model and repeated measure analysis of variance, with Fisher's protected least significant difference (LSD) as the post-ANOVA test. One way ANOVA was used to study the effect of day on serum progesterone concentration and the effect of individuality on the follicular wave and blood flow pattern (SPSS, 1999). The significance was set at $P < 0.05$.

Results

The blood flow of ovulatory follicles

Individual females had non significant effect on the pattern of the follicular wave or the blood flow to the follicles or the corpus luteum. The blood flow of the ovulatory follicles detected for the first time at a diameter of 6.9 ± 0.8 mm (4.0 ± 0.5 days after emergence) when BA and BA% were 2.1 ± 0.6 mm^2 and 5.3 ± 0.6%, respectively. The BA and BA% increased significantly ($P<0.05$) 2 days before ovulation. Eight hours before ovulation, the diameter of the ovulatory follicle was 15.8 ± 0.7 mm and had BA and BA% of 19.18 ± 4.4 mm^2 and 9.8 ± 2.6%, respectively.

Induction of ovulation

Ovulation rate was 100% (12/12) in the present study. Treatment was noticed to shorten the interwave interval from 17.03 ± 0.8 to 11.7 ± 1.7 days. The mean number of ovulatory follicles (including twin ovulations) responded to treatment with GnRH analogue was 1.42 ± 0.2. Ovulation was identified within 24–32 hrs in 92.3% of trials (11⁄12) and 32–40 hrs in 7.7% of trials (1⁄12). Corpus hemorrhagicum (CH) was noticeable on the first day post-ovulation and had a diameter of 8.8 ± 0.2 mm. CL lasted for 24.2 ± 1.9 days. CL attained its maximum diameter of 22.08 ± 1.04 mm after ovulation by 9.9 ± 0.9 days. Double ovulation was recorded in 42.8% of follicular waves. The incidence of double ovulations was 50% on the left ovary, 33.3% on the right ovary and 16.7% bilateral. Changes in the diameter, BA and BA% of

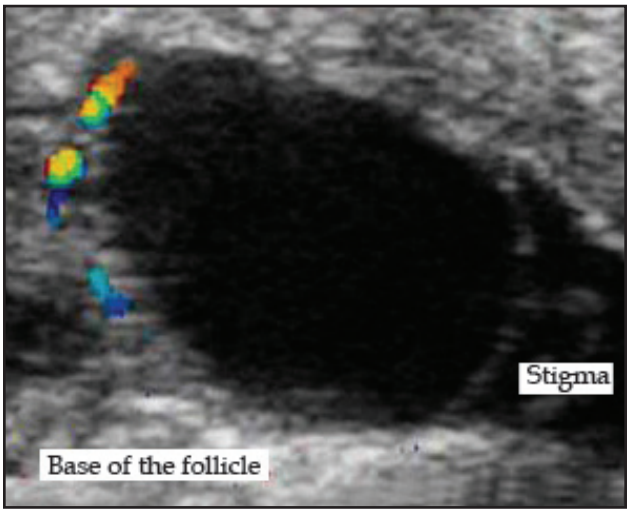

Fig 1. Changes in blood vasculature in the walls of ovulatory follicles 8 hrs before ovulation in She-camels; blood is not seen at the stigma (the side of the follicle contralateral to coloured areas and increased the blood areas at the base of the follicles (coloured areas).

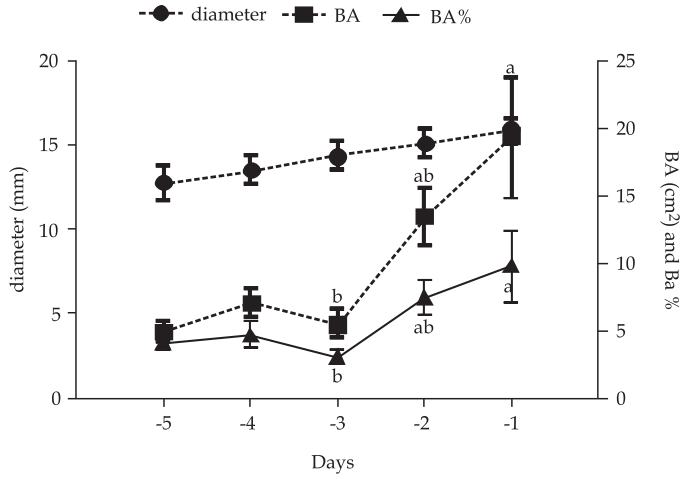

Fig 2. Changes in the diameter, BA and BA % of the ovulatory follicles 5 days before ovulation. (a) and (ab) meant that there was a significant increase in BA and BA% two days before ovulation.

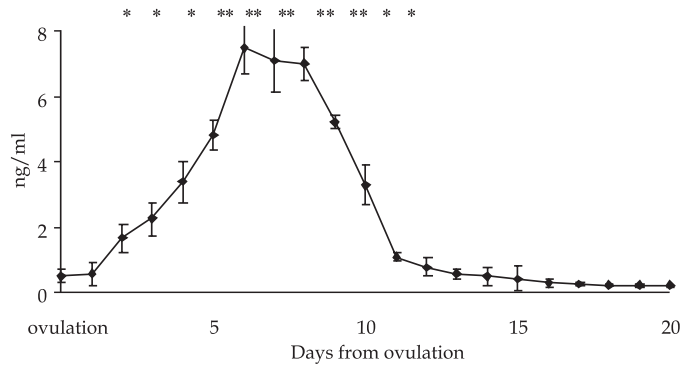

Fig 3. Progesterone profile (mean ± SEM) after GnRH treatment for induction of ovulation in female dromedary (n=12).
*significant at 0.05 **significant at 0.01

the dominant follicle were summarised in Fig 1 and 2.

Progesterone concentrations

Day of sampling after ovulation had a significant effect on the concentration of progesterone in the studied animals ($P<0.05$). Concentrations of progesterone increased from basal levels of 0.32 ± 0.05 ng/ml (at the day of ovulation) to reach a peak concentration of 7.15 ± 0.97 ng/ml (by day 7 post ovulation). Progesterone level decreased to 0.91 ± 0.38 ng/ml after ovulation by 11 days. On Day 16 after ovulation progesterone level reached a nadir of 0.31 ± 0.12 ng/ml and remained around this level until the regression of the CL (Fig 3).

The blood flow of the CL

Blood flow of the CH and subsequently CL was detected, in all trials, one day after ovulation when the CH was 8.8 ± 0.3 mm in diameter. Maximum BA% of 33.2 ± 9.01% for the CL was noticed 5 days post ovulation. While, maximum BA of 53.7 ± 11.5 mm^2 was detected 7 days after ovulation. The BA decreased to 32.7 ± 6.1 mm^2 after ovulation by 11 days. Both, BA and BA% decreased gradually till reached 1.6 ± 0.1 mm^2 and 2.9 ± 0.4%, before completely disappeared 22 days after ovulation (Figs 4 and 5).

Discussion

All follicles targeted in the current study had been ovulated. A previous study (Skidmore *et al*, 1996) designed to compare the efficacy of natural mating with a single injection of either 20 µg of the GnRH analogue or 3000 IU human chorionic gonadotropin (hCG) for inducing ovulation in female dromedary indicated that the optimum time to mate or attempt to induce ovulation in the female dromedary is when the growing follicle measures 0.9-1.9 cm in diameter in accordance with the present results. However, the stage of the cycle when mating or treatment occurred was important. Accordingly, the ultrasonographic examination revealed that ovulation could be induced in 100% of the camels by administration of 3000 IU hCG or 20 µg GnRH analogue (Vyas *et al*, 2004 and Nagy *et al*, 2005). Present results supported these findings although, in a similar study an ovulation rate of approximately 80% was achieved when the dominant follicle measured between 1–1.9 cm in diameter, but it was dramatically reduced

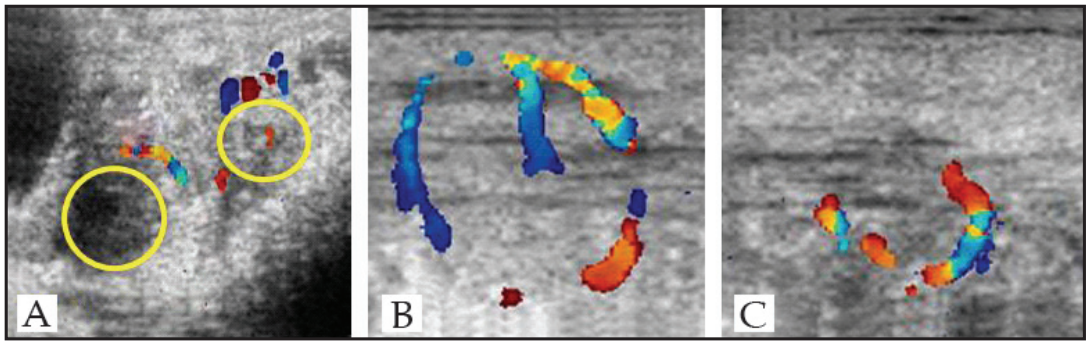

Fig 4. Changes in the blood vasculature during the different developmental stages of the CL in female dromedary **A)** one day, yellow circles pointed to the site of ovulation and coloured areas were indicatives to blood vasculature to these areas, **B)** 7 and **C)** days post ovulation.

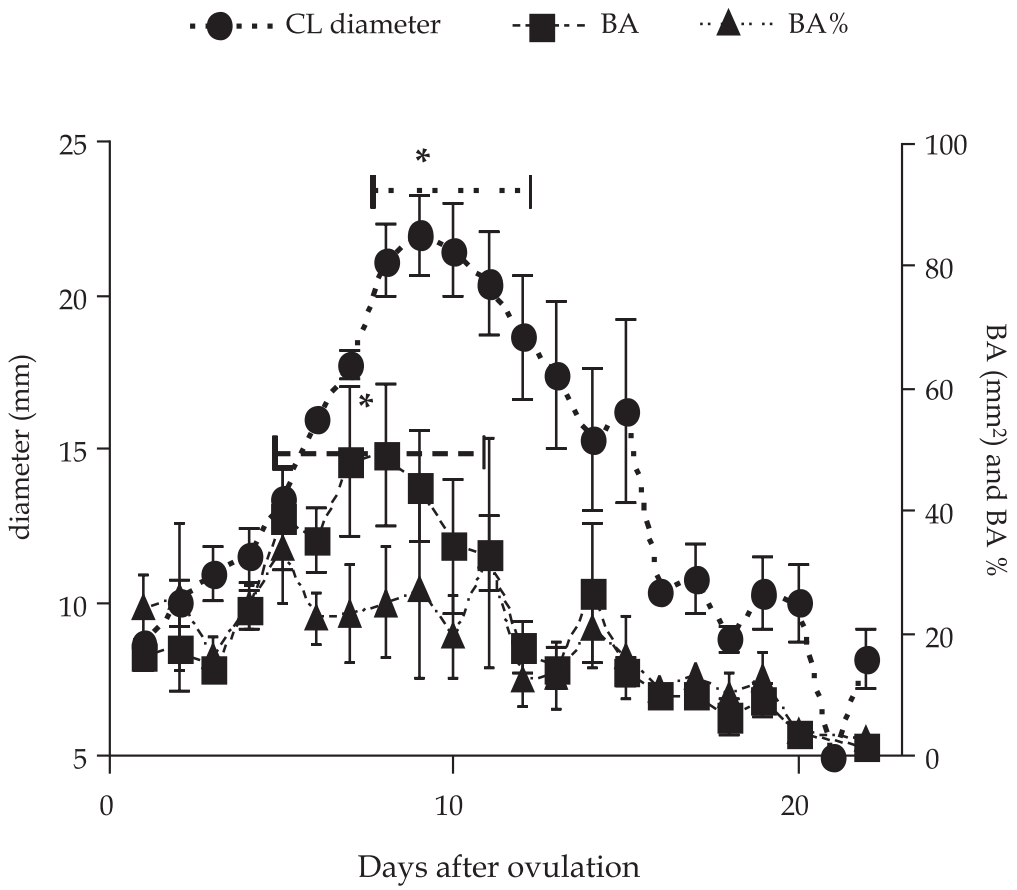

Fig 5. Changes in the diameter, BA and BA % of the CL.

to < 20% if it measured between 2.0-2.9 cm and no follicles more than 3.0 cm ovulated in response to any treatments (Skidmore *et al*, 1996). Regarding the ovulation rate and incidence of double ovulation, the discrepancy between the present study and previous studies may be attributed to breed difference, age, parity, months of the breeding season, management, lactation, nutrition, presence of male, work, environmental conditions and methodology used. We believed that the use of ultrasonography alone to detect ovulations and subsequent CLs is unreliable to visualise them during the first days after ovulation. In a previous study to compare the morphology and function of the CL after spontaneous and induced ovulations, it was emphasised that ultrasonographic morphology of the CL could be mistakenly interpreted as oversized, luteinised follicles (Nagy *et al*, 2005). In our study, Doppler

ultrasonography was an accurate method to detect the CL as early as the first day after ovulation in all cases as we could visualise, not only the CL per se, but also the blood vasculature within its wall and consequently its outline. In contrast, previous ultrasonographic studies reported that the CL was only visualised 1-2 (Nagy *et al*, 2005) or 5–6 (Tibary and Anouassi, 1996) days after mating. CL could be visualised for over 20 days in this study although; progesterone concentrations fell dramatically on Day 11 post ovulation. In a previous study, luteal diameter declined 2–3 days later and at a slower rate than serum progesterone concentration (Nagy *et al*, 2005). It was reported that the corpus luteum can be palpated in 62.5% of cases and there is synchrony with plasma progesterone concentration, but the morphological luteal regression is prolonged (Marie and Anouassi, 1987). Our findings suggested that morphological visualization of CL does not necessarily indicate that it is functioning. This is similar to other species in which morphological regression of the CL occurs later than functional regression in these species including the dromedary camel (Nagy *et al*, 2005). Results of the present study suggested that, recently, Doppler ultrasonography is more accurate than ultrasonography and/or progesterone measurement to detect the presence of the CL in the first few days after ovulation in female dromedary. In the treated animals, ovulation was identified when a mature dominant follicle was no longer present on ultrasonographic examination within 24–32 hrs or 32–40 hrs of treatment in 92.3% (11⁄12) and 7.7% (1⁄12) of the trials, respectively. Accordingly, in the mated animal, ovulation is detected by the rapid disappearance of a mature follicle within 28–36 hrs of mating (Marie and Anouassi, 1987 and Skidmore *et al*, 1995). The incidence of double ovulation in the present result was 42.8%. Previous reports recorded the presence of more than one CL in 12.5 - 14.7% of ovulations in female dromedary (Musa and Abusineina, 1978). These findings are not fully comparable to previous studies because these studies were conducted at different locations, different times, management conditions, nutrition, breed of animals and methodology. The blood flow increased gradually with the growth of the follicles. In female dromedary, no available literatures about the study of the blood vasculature of the dominant follicles or the corpus luteum were found. In mare, the overall pattern of colored area of the ovulatory follicle showed a progressive increase from Day -4 to its maximum on the day before ovulation and the values on Day -1 were higher ($P < 0.05$) than on Day -2 (Palmer *et al*, 2006). Greater blood flow to the preovulatory follicle is associated with higher follicle maturation, oocyte recovery and quality and pregnancy rates (Gastal and Gastal, 2011). Similarly, in cow, an acute increase in the perfused area and blood flow velocity was observed 30 min after GnRH injection and synchronous with the initiation of the LH surge. The present data supported the concept that the complex structural and functional changes induced by the LH surge in a mature follicle are closely associated with a local increase in the blood flow within the wall of the pre-ovulatory follicle (Ginther and Utt, 2004). Degradation of collagen layers is accompanied by increased vascular dilatation and permeability, which are necessary for follicular rupture (Moor *et al*, 1975 and Murdoch *et al*, 1986). In accordance to our results showed in Fig 2, the blood flow at the apex of the ovulatory follicle decreases while it increases at the base of the follicle (Brannstorm *et al*, 1998), which facilitates follicular rupture. Several studies have reported that LH induces an increase in the ovarian blood flow in sheep (Niswender *et al*, 1976) and cow (Acosta *et al*, 2003). However, the mechanisms of LH-induced hyperemia remain unknown. FSH and estradiol stimulate follicular growth and vascular hypertrophy. The increase in blood flow into the theca layer of the dominant follicle probably results in increased supplies of gonadotropins, systemic biochemicals and factors necessary for complete maturation of the follicle (Acosta, 2007).

CH was first detected 24 hours after ovulation in the present study. This growing period is characterised by active angiogenesis and a parallel increase in progesterone secretion (Acosta *et al*, 2005). The blood flow to the CL rose distinctly after ovulation, reached their maximum 7 (BA) and 9 (BA%) days after ovulation and decreased thereafter, concomitant with the regression of the CL in the current study. We found that vascular changes during luteolysis included an initial acute increase in the blood flow of the CL by Day 11 post ovulation. In mare, the number of color pixels rose distinctly after ovulation until day 5 of the cycle and remained constant then declined (Bollwein *et al*, 2002). In the first days after ovulation the plasma progesterone concentration increased parallel to the number of color pixels. The morphological changes (size and form) are not as pronounced as the physiological changes (the luteal blood flow and the peripheral progesterone concentration) of the corpus luteum (Baumgartner, 1998). A temporary increase in blood

flow is important for luteolysis (Acosta *et al*, 2002). These findings also rule out vasoconstriction as the primary cause of luteolysis in cows (Miyamoto *et al*, 2005). The increase in the follicular blood flow around the time of ovulation and within the CL at initial stage of regression is associated with a drastic increase in the local secretions of prostaglandins and vasoactive peptides. The acute change in the blood flow may trigger the cascades of the final step of ovulation and the initial step of CL regression, respectively (Acosta *et al*, 2005). It is concluded that ovarian follicles best respond to induction of ovulation using GnRH analogue when their size ranges from 9-18.9 mm. Ovulation occurred within 24-32 hrs in 92.3% and 32-40 hrs in 7.7% of induced follicles. Concentrations of progesterone peaked after ovulation by Day 7 and decreased thereafter. Transrectal colour Doppler ultrasonography is a useful and accurate diagnostic tool for the non-invasive evaluation of follicular and luteal vascularity in camels allowing visual observations of the blood flow within their walls.

References

Acosta TJ (2007). Studies of follicular vascularity associated with follicle selection and ovulation in cattle. Journal of Reproductive Development 53:39–44.

Acosta TJ, Hayashi KG, Matsui M and Miyamoto A (2005). Changes in follicular vascularity during the first follicular wave in lactating cows. Journal of Reproduction and Development 51:273–280.

Acosta TJ, Hayashi KG, Ohtani M and Miyamoto A (2003). Local changes in blood flow within the preovulatory follicle wall and early corpus luteum in cows. Reproduction 125:759–767.

Acosta TJ, Yoshizawa N, Ohtani M and Miyamoto A (2002). Local changes in blood flow within the early and midcycle corpus luteum after prostaglandin F (2 alpha) injection in the cow. Biology of Reproduction 66:651-658.

Anouassi A, Adnani M and El Raed E (1992). Artificial insemination in the camel requires induction of ovulation to acheive pregnancy. Proceedings of the First International Camel Conference, Dubai (UAE). pp 175-177.

Baumgartner U (1998). Farbdopplersonographische Untersuchung der Arteria uterina und des Corpus luteum beim Rind. Tierarztliche Fakultat der Ludwig-Maximilians-Universitat Munchen, Germany. pp 67-89.

Bollwein H, Stolla R, Rohr S, Welter H, Weber F and Einspanier R (2002). Relationships between uterine blood flow, estrogens and nitric oxide in cycling mares. Theriogenology 58:575-578.

Brannstorm M, Zackrisson U, Hagstorm HG, Josefsson B, Hellberg P, Granberg S, Collins WP and Bourne T (1998). Preovulatory changes of blood flow in different regions of the human follicle. Fertility & Sterility 69:435-442.

Chen BX, Yuen ZX and Pan GW (1985). Semen-induced ovulation in the bactrian camel. Journal of Reproduction and Fertility 4:335-339.

Cooper MJ, Skidmore JA, Allen WR, Wensvoort S, Billah M, Ali-Chaudry M and Billah AM (1992). Attempts to stimulate and synchronize ovulation and superovulation in dromedary camels for embryo transfer. In: Allen WR, Higgens AJ, Meyhew IG, Snow DH, Wade JF (Eds.), Proceedings of the First International Camel Conference. UK, R&W Publications, Newmarket. pp 187-191.

El-Sherry TM, Matsui M and Miyake YI (2010). Haemodynamic Changes of the Superovulated Follicle as a Cause of Superovulation Variability in Cattle. Journal of American Science 6(10):744–751.

Gastal EL and Gastal MO (2011). Equine preovulatory follicle: blood flow changes, prediction of ovulation and fertility. Animal Reproduction 35(2):239-252

Ginther OJ, Gastal EL, Gastal MO and Beg MA (2007). Incidence, Endocrinology, Vascularity, and Morphology of Hemorrhagic Anovulatory Follicles in Mares. Journal of Equine Veterinary Science 27(3):130-139.

Ginther OJ and Utt MD (2004). Doppler ultrasound in equine reproduction: principles, techniques, and potential. Journal of Equine Veterinary Science 24:516-526.

Herzog K and Bollwein H (2007). Application of Doppler Ultrasonography in Cattle Reproduction. Reproduction of Domestic Animals 42(2):51-58.

Marie M and Anouassi A (1987). Induction of luteal activity and progesterone secretion in the non-pregnant one-humped camel (*Camelus dromedarius*). Journal of Reproduction and Fertility 80(1):183-192.

Miyamoto A, Shirasuna K, Hayashi KG, Kamada D, Awashima C, Kaneko E, Acosta TJ and Matsui M (2006). A potential use of color ultrasound as a tool for reproductive management: new observations using color ultrasound scanning that were not possible with imaging only in black and white. Journal of Reproduction and Development 52:153-160.

Miyamoto A, Shirasuna K, Wijayagunawardane MP, Watanabe S, Hayashi M, Yamamoto D, Matsui M and Acosta TJ (2005). Blood flow: a key regulatory component of corpus luteum function in the cow. Domestic Animal Endocrinolology 29:329-339.

Moor R, Hay MF and Seamark RF(1975). The sheep ovary; regulation of steroidgenic, haemodynamic and structural changes in the largest follicle and adjacent tissue before ovulation. Journal of Reproductive Fertility 45:595-604

Murdoch WJ, Peterson TA, Van Rick EA, Vincent DL and Inskeep EK (1986). Interactive roles of progesterone, prostaglandins, and collaginase in ovulatory mechanism of the ewe. Biology of Reproduction 35:1187-1194.

Musa BE and Abusineina ME (1978). The oestrus cycle of the camel (*Camelus dromedarius*). Veterinary Records 103:556-557.

Nagy P, Juhasz J and Wernery U (2005). Incidence of spontaneous ovulation and development of the corpus luteum in non-mated dromedary camels (*Camelus dromedarius*). Theriogenology 64:292-304

Niswender G, Reimers TJ, Diekman MA and Nett TM (1976). Blood flow: a mediator of ovarian function. Biology of Reproduction 14:64-81.

Palmer E, Chavatte-Palmer P and Verdonck E (2006). Field trial of Doppler ultrasonography of the preovulatory follicle in the mare. Animal of Reproductive Science 94:182-185.

Pellizzari P, Esposito C, Siliotti F, Marchiori S and Gangemi M (2002). Colour Doppler analysis of ovarian and uterine arteries in women with hypoestrogenic amenorrhoea. Human Reproduction 17(12):3208-3212.

Sheldrick LE, Flick-Smith H, Skidmore JA, Wensvoort S, Billah M, Ali CM and Allen WR (1992). LH release profiles in female dromedary camels following mechanical and hormonal stimuli to induce ovulation. Proceedings of the First International Camel Conference, Dubai (UAE). pp 407.

Skidmore JA, Billah M and Allen WR (1995). The ovarian follicular wave pattern in the mated and non-mated dromedary camel (*Camelus dromedarius*). Journal of Reproductive Fertility (Suppl) 49:545-548.

Skidmore JA, Billah M and Allen WR (1996). The ovarian follicular wave pattern and induction of ovulation in the mated and non-mated one-humped camel (*Camelus dromedarius*). Journal of Reproduction and Fertility 106(2):185-192.

Tibary A and Anouassi A (1996). Ultrasonographic changes of the reproductive tract in the female camel (*Camelus dromedarius*) during the follicular cycle and pregnancy. Journal of Camel Practice and Research 3:71-90.

Vyas S, Rai AK, Goswami PK, Singh AK, Sahani MS and Khanna ND (2004). Superovulatory response and embryo recovery after treatment with different gonadotrophins during induced luteal phase in *Camelus dromedarius*. Tropical Animal Health Production 36:557-565