

## REGULAR ARTICLE

# Ovarian monitoring and effects of Controlled Intravaginal Drug Releaser (CIDR) on vaginal environment and follicular activity in dromedary camels, during non-breeding season in Egypt

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## Abstract

The study was carried out to monitor ovarian activity in female dromedary camels reared in semi-intensive system in Egypt, during non-breeding season, and to assess the effects of Controlled Intravaginal Drug Releaser (CIDR 1,38g) on vaginal environment and on follicular number and diameters. Twenty females were monitored through vaginal inspection and ultrasonographic examination. Group I (n=10) was monitored in July, while Group II (n=10) in September 2010. Follicle number and diameter were recorded before CIDR's insertion (T0). In Group I CIDR's were inserted after cleaning of perineum with dry paper while, in Group II, after washing of perineum and vaginal flushing with water-5% iodopovidone solution. After 10 days CIDR's were removed, the vaginal status observed and follicles again counted and measured (T1). Results revealed that ovaries were active in July and even if in less measure, in September, which are considered non breeding season month in Egypt. CIDR's treatment caused vaginitis in almost all Group I camels but a better vaginal environment. On the day 10, CIDR were removed in Group II. Statistical analysis revealed that the CIDR's treatment significantly reduced mean follicular diameter in the two months ( $P<0.01$ ;  $P<0.05$  respectively) but did not affect follicular number, thus demonstrating its inefficacy in synchronize follicular wave in camels. Both ultrasonographic and hormonal studies will be necessary, simultaneously with CIDR treatment, for better understand effects of exogenous progesterone administration on ovarian activity and follicular wave pattern in female dromedary camels.

**Key words:** Dromedary, Semi-intensive, Season, CIDR, Vaginal environment, Ovary

## Introduction

Dromedary camels (*Camelus dromedarius*) are seasonal breeders and induced ovulators (Tibary and Anouassi, 1997) but factors that affect seasonality are not well documented and information about the breeding season are rather conflicting; in Egypt, it extends from December to May (Al-Eknaah, 2000; Shalash, 1987). Camels could be fertile as any other domestic species if appropriate reproductive management is applied (Nagy and Juhasz, 2008). Methods for follicular wave and A.I. synchronization could be very helpful for programming deliveries, for satisfying

milk market demand as well as to provide adequate assistance to dam and calves. Nevertheless, research and development in estrus synchronization and controlled breeding have been extensively achieved in ruminants but not enough in camels (Al-Eknaah, 2000). Progesterone Releasing Intravaginal Devices (PRID's) with 1.55 g of Progesterone have been tested, in camels, but seems to be of unsatisfactory use (Skidmore et al., 1992) causing vaginal discharges, and also a high proportion of recipients ovulated whilst carrying the device *in situ* (Cooper et al., 1992; Skidmore et al., 1992). Controlled Intravaginal Drug Releasers (CIDR, 330 mg) however, have been proved to control ovarian activity in the related species Lama (*Lama glama*) (Chaves et al., 2002). The present work was performed during two periods of the non-breeding season in Egypt, mid-late of July and mid-late of September, respectively, in female dromedary camels reared in semi intensive system. Aim of the study was to monitor ovarian activity and to understand if CIDR can be effective in

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control and synchronize follicular waves. In addition, to observe clinical effects of CIDR on vaginal environment trying to overcome, possible side effects.

## Materials and Methods

### Animals, management and feeding

The study was performed at Maryout Research Station of the Desert Research Center (34 Km North West of Alexandria, Egypt). Housing management and experimental procedures were carried out according to requirement of the Council for International Organization of Medical Sciences (CIOMS).

Twenty, healthy, non-pregnant and non-lactating dromedary, aged between 6 and 15 years, were selected. Mean animal weight was  $415\text{kg} \pm 55$  and body condition score, measured according to Faye et al. (2001), was  $2.5 \pm 0.5$ . Animals were daily watered and left free to graze from 10:00 a.m. to 16:00 p.m. After grazing they received supplement nutrition to cover their requirement as reported from Wilson (1989). Gynecological examination was performed for excluding disease or genital abnormalities, including anovulatory/hemorrhagic follicles and vaginal septum (Tinson and McKinnon 1992; Tibary and Anouassi, 1996).

### Ovarian monitoring, CIDR insertion and removal

Animals were randomly divided in two groups and monitored in different periods: Group I (n=10) from 15<sup>th</sup> to 26<sup>th</sup> of July; Group II (n=10) from 15<sup>th</sup> to 26<sup>th</sup> of September. The two periods were investigated as corresponding to the beginning and to the middle of non-breeding season, respectively.

Gynecological examination was performed, with camels in standing position, through vaginal inspection, transrectal palpation and ultrasonography. A Dynamic Imaging, concept MLV scanner ultrasound device (PieMedical 100, Falco) integrated with a 6.0 and 8.0 MHz linear dual frequency probe was used; follicles were counted and measured by the electronic caliper.

After ultrasonographic examination (T0), Controlled Intra-vaginal Drug Releasers (CIDR 1.38 g, Pfizer®, Italy), were inserted. The stage of the follicle development at the time of CIDR insertion was random. In Group I, CIDR were inserted after cleaning the perineum with dry paper. In Group II, instead, after washing the perineum with water and povidone-iodine-based detergent solution (Betadine®, Italy); moreover a vaginal flushing with 20 ml of water and 5% Betadine solution, was performed through a syringe and an insemination catheter. Particular care was taken at

the moment of insertion, in both group, gently but firmly pushing the applicator against the cervix during pressing its plug for releasing the device; polyester tails were cut to avoid biting from other females. Devices were left in vagina for 10 days and every three days animals were controlled in case of CIDR lost. On day 9, 500 µg of PgF<sub>2α</sub> (Estrumate®, Ontario, Canada), were administered (Skidmore et al., 1998). On the day 10, CIDR were removed, vaginal cavity observed through a speculum and the vaginal discharge evaluated. According to the amount of the debris associated with the device and in the vagina a score was assigned: 0, no debris; 1, small flecks of purulent debris in the vagina and on the device; 2, copious amount of purulent debris in the vagina and on the device (Walsh et al., 2008). Moreover follicles were again counted and measured, by ultrasonographic scanning, the day of CIDR removal (T1).

### Statistical analysis

Number and follicle dimensions were subjected to a repeated measures analysis of variance (ANOVA) using the general linear model procedure (SAS, 1999). Independent variables were the period of the year (July and September) the time (T0 and T1) and the interaction between those variables. Data were normally distributed. Turkey's post hoc test was used to perform statistical multiple comparison. P level was set at 0.5. All data were expressed as quadratic mean and mean standard error (SEM).

### Results

Mean follicle number was found not to be statistically different between July and September in T0 (Table 1). Mean follicle diameter was found to be higher in July rather than September: 1.05 Vs. 0.61 cm (P<0.05) respectively, at T0 (Table 2). None of the animal lost the CIDR except one in group I where the tip of the device was observed out of the vagina after six days; CIDR was then removed, washed and reinserted. At the time of CIDR removal 6 females of Group I revealed vaginitis vaginal score 2 and 4 females had vaginal score 1; foul smell of vaginal discharge, was noticed in some of those animals but any sign of metritis was detected by ultrasonography. All Group II animals, instead, presented an overall vaginal score of 0-1. In those females, a small amount of white creamy mucus was found on CIDR surface and in vaginal cavity but such white mucus lacked of purulent and foul smell and vaginal walls were not hyperaemic. In two animals of Group I ultrasonography at the time of CIDR

removal revealed round body hyperechoic structures, embedded in ovaries and differentiated from ovarian stroma. Those animals were supposed to spontaneously ovulated while retaining the CIDR, and excluded from statistical analysis regarding comparison in T1. Progesterone treatment did not affect follicle number (T1, Table 1) in the periods of the study but, on the other side, statistically affected follicle diameters that regressed from 1.05 cm to 0.51 cm ( $P<0.01$ ) in July and from 0.61 cm to 0.41 cm ( $P<0.05$ ) in September, respectively (T1 Table 2).

Table 1. Mean follicle numbers (n) in female camels during July and September before and after CIDR treatment.

Period	T0 <sup>1</sup>	T1 <sup>2</sup>	SE
July	2.50	2.30	0.42
September	2.70	1.70	

<sup>1</sup>CIDR insertion <sup>2</sup>CIDR removal

Table 2. Mean follicle diameters (cm) in female camels during July and September before and after CIDR treatment.

Period	T0 <sup>1</sup>	T1 <sup>2</sup>	SE
July	1.05 <sup>A,x</sup>	0.51 <sup>B</sup>	0.10
September	0.61 <sup>a,y</sup>	0.41 <sup>b</sup>	

<sup>1</sup>CIDR insertion <sup>2</sup>CIDR removal

Different letters in the same line show statistical differences (A, B:  $**P<0.01$ ; a, b:  $*P<0.05$ )

Different letters in the same column show statistical difference (x, y:  $*P<0.05$ ).

## Discussion

Results revealed that, camel bred in semi-intensive system and in optimal condition, showed prolonged ovarian activity in July as well as, although with lower intensity, in September. This is contrast with Shalash (1987) that defined camel's seasonal anoestrus from June to November. Indeed, references concerning camel breeding season in Egypt are scanty and there aren't specific data concerning seasonality in extensive, semi-intensive or intensive breeding system. Arthur et al. (1985) claimed that camels can be truly polyestrous with a continuous supply of sufficient food. Adequate management and nutrition of the camel reared in Maryout Research Station, probably helped in overriding environmental effect on ovarian activity extending ovarian activity until July-September. Similar observations reported in India by Vyas et al. (2004), who found pre-ovulatory follicles  $\geq 1.0$  cm during non-breeding season (June–August), and obtained pregnancy with programmed mating in

dromedary camels, bred in a semi-intensive system. CIDR have been well retained from almost all camels. Good results in term of PRID retention were reported also from Cooper et al. (1992), who observed only four devices expelled once, and one animal completely failing in retaining the device, out of 66 camels. PRID retention relies on the resistance associated with the broad flat surface of the device against the vaginal mucosa, due to its outside diameter (4.5 cm) while, CIDR fastening is due to the pressure of the 2 wings against the vaginal walls. Both mechanisms seem to be effective for retaining of such devices in dromedary camels vagina. CIDR treatment, however, caused bacterial vaginitis in almost camels of Group I. The distinction between of devices on the vaginal environment, and the introduction of bacteria during insertion is difficult, because in this study, we performed only a clinical evaluation of CIDR effects. However, as a significant improvement of the vaginal environment was obtained in group II with washing of perineum and vaginal flushing, we could reasonably suppose that the contamination of vagina at the time of insertion was the main responsible of the observed vaginitis and vaginal discharge. Al-Sobayil (2008) treated 10 female camels with PRID for estrus synchronization. Devices were inserted after washing, disinfection and lubrication of vulva, and left in vagina for 17 days, but no data about vaginal environment at the time of their removal was reported (Al-Sobayil, 2008). Walsh et al. (2008), observed an 11% of vaginitis in cows when Intravaginal devices (with or without progesterone impregnation) were inserted, after washing of perineum with water, and lubricated with 1% w/w chlorhexidine cream. Further analysis would be necessary for evaluating the effect of vaginal flushing, compared to clorexidine cream, on bacterial growth during CIDR retention as well as the effect of 5% water-betadine solution on vaginal mucosa. According to ultrasonography in T1, two spontaneous ovulations (10%), were supposed to happens. Cooper et al. (1992), by monitoring plasma progesterone after the retrieval of PRID devices, found 33% of spontaneous ovulation in 36 camels during the treatment. Marie and Anouassi (1987) reported some spontaneous ovulation in female camels exposed to a male after a prolonged period of separation and after the regression of an induced luteal phase. They also reported that, in one of four camels, progesterone secretion was observed after the removal of PRID device. Mechanism operating in spontaneous ovulators can be effective in certain

situations in dromedary camel (Marie and Anouassi, 1987; Nagy et al., 2005) but is not possible to distinguish such spontaneous ovulation from a follicular luteinization (Tibary and Anouassi, 1996). Thus, for clarifying the occurrence and mechanism of “spontaneous ovulation” and/or “follicular luteinization” induced by progesterone intravaginal devices (CIDR or PRID), it would be useful to monitor follicular dynamics by ovarian ultrasonography, estradiol and progesterone determination, during and after the treatment. It would be also worthwhile to examine mechanical effect of “unloaded” or progesterone free CIDR (or PRID) on ovarian activity, as well as on vaginal environment, as suggested from Cooper et al. (1992). The progesterone administered through CIDR was effective in reducing mean follicular diameter in both periods of this study. Statistical differences concerning the efficacy of the treatment in those periods, probably was related to the different diameters of follicles at the time of insertion in July and September. Nevertheless, the treatment did not affect mean follicular number, demonstrating its failure in controlling the emergence of new follicular waves. This result is in agreement with Skidmore et al. (2009) that, treating camels with a daily dose of 150 mg progesterone-in-oil for 14 days induced a reduction in follicular size at the end of treatment, but only a small reduction of its number. Latest authors stated that, although exogenous progesterone will hasten the regression of large follicles it does not completely inhibit follicular growth in camels (Skidmore et al., 2009). Indeed, endogenous progesterone does not completely control ovarian activity in dromedary camel females; it is reported that mature follicles (>10 mm) can be found until 105 days of pregnancy (El-Wishy et al., 1981; Musa and Abusineina 1978). However, in the related South American Camelids, Vaughan (2001) stated that 100 or 200 mg of progesterone, administered IM every two days, were effective in inducing regression of the existing dominant follicle and preventing new wave emergence. Whereas, Chaves et al. (2002), by evaluating follicular diameters and sexual hormones concentrations during treatment, stated that CIDR (0.33 g) could be effective in completely preventing follicular development for a period of up to 7 day (Chaves et al., 2002). Those result let us suppose that a higher dose of progesterone (CIDR 1.9 g), probably should be investigated as a mean for control and synchronize follicular waves in dromedary camel females.

## Conclusion

Ovarian activity in female dromedary camels, bred in semi intensive system could be found in July and, even if with lower intensity, in September, yet considered non breeding season periods in Egypt. Cleaning of perineum and flushing of vagina with a iodopovidone solution before CIDR insertion clinically improved vaginal environment at the end of the treatment. Progesterone administration through CIDR (1.38 g) for 10 days, was effective in reducing follicular diameter in the beginning and in the middle of the non-breeding season, but did not reduced follicular number, revealing its inefficacy for synchronizing follicular waves. More comprehensive studies will be necessary for better understand effects of exogenous progesterone administration through CIDR, on ovarian activity and follicular wave patterns in dromedary camels.

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