

Improving Fertility of Maghrabian She-Camels Treated with Different Hormonal Treatments during Breeding Season in Egypt

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Abstract: To achieve estrous synchronization in 28 mature dromedary she-camels, four groups (7 in each), included animals i.m. injected with 250 µg GnRH analogue on day 0 and 5 ml PGF_{2α} on day 10 (G1); CIDR for 10 days and 1000 IU eCG on day 8 (G2); 1000 IU eCG on day 0 and 5 ml PGF_{2α} on day 10 (G3); control (G4). At the end of treatment, animals in heat were naturally mated. Results showed that follicular diameter (FD) decreased in G2 pre-treatment, increased in G2 and G3 and decreased in G1 at mating compared with G4 (P<0.05). FD was wider (P<0.05) in pregnant (P) than in non-pregnant (NP) animals. Estrus rate was 100% in all groups; estrus incidence and mating time were the longest in G4 (170.6 and 198.6 h) and the shortest in G2 (72.0 and 75.7 h), being shorter in P than in NP. (P<0.05). In all groups, P4 was similar pre-treatment, lower at mating and higher on day 70 post-mating in P than in NP (P<0.05). E2 was higher (P<0.05) in NP than in P on day 70 post-mating. Pregnancy rate was 57.14, 85.71, 71.43 and 42.86% in G1, G2, G3 and G4, respectively (P<0.05). Total number of CLs decreased in all treated groups, while CL diameter decreased in G1 as compared to G4 (P<0.05). Estrus/ovulation and higher pregnancy rate of camel could be hormonally induced by CIDR insertion for 10 days and eCG injection on day 8 of CIDR insertion.

Keywords: Camel, estrous synchronization, GnRH, CIDR, eCG, progesterone, estrogen, pregnancy rate.

I. Introduction

Dromedary camels (one-humped) have been an important role as a source of meat and milk in Egypt. Low reproductive efficiency of the camel had been reported (Kaufmann, 2005), due to the short breeding season, long lactation period and generally only one calf is produced every two years for each female (Minoia *et al.*, 1992).

Camels are seasonally polyestrous animals with estrous cycle differed from that in other farm animals. The estrous cycle in camel is characterized by three phases lasting 24-28 days (Shalash, 1987) with absence of the luteal phase (Al-Sobayil, 2003). These phases include follicular growth, existence of mature follicles (estrous period) and follicular atresia. The estrous period is longer up to up to 8 days (Tibary and Anouassi, 1996). Ovulation in camel actually occurs mechanically by natural mating or induces by hormonal treatments (Al-Sobayil, 2003). The estrous signs in a female camel are characterized by the sitting of the camel to be mounted by other females, restlessness, frequent salivation, swelling of the vulva, vaginal mucous discharge, frequent urination (especially when the male comes close to her), and finally the acceptance of mating by the male (Homeida *et al.*, 1988).

Estrous synchronization for improving the reproductive performance is used in reproductive programs of most farm animals. Great attention of several researchers has been focused on different regimens of estrous synchronization in farm animals (Dahir *et al.*, 1990; Elias *et al.*, 1985). The progress in the methods of hormone extraction, purification and measurements of their concentrations in blood circulation has risen understanding the mechanisms of regulating reproduction in farm animals (Combarous and Anouassi, 1994).

Understanding the hormonal changes throughout the estrous cycle of the she-camel extremely helped in the application of different hormonal combinations to induce and synchronize the estrus. As in other mammals, estrous synchronization in camels using hormonal treatments stimulates growth and development of the ovarian follicles and ovulation. In previous reports, estrous synchronization in the she-camel was based on the use of several hormones, including PMSG (Elias *et al.* 1985; Anouassi and Ali, 1991), hCG or LH (Shalash, 1987; Anouassi and Ali, 1991), GnRH with LH or hCG (Chen *et al.* 1985; Bono *et al.* 1991 a&b), or GnRH, LH or its analogue, and hCG (Skidmore *et al.*, 1996; Hafez and Hafez, 2001).

Induction of ovulation by GnRH or LH depends mainly on the dominant follicle size at the time of hormone injection. When she-camels with the existence of an ovarian dominant follicle (<10 mm in diameter) were given LH, ovulation response approached 90% (Anouassi *et al.*, 1994; Skidmore *et al.*, 1996) after 26-28 h of i.m. or i.v. GnRH (0.5-1 mg) injection (Musa *et al.*, 1993). However, eCG was used to stimulate follicular

growth in order to induce the estrus during out of breeding season at a range from 1500-7000 IU (Agarwal *et al.*, 1993; Al-Sobayil, 2003).

Estrus also could be synchronized in she-camel using hormonal combination including progesterone (P4) or its analogue either as daily injection or as an intra-vaginal releasing the device of PRID for a period of 10-20 days, followed by eCG injection (1500-2000 IU) 24 h before or on day of the device withdrawal (Tibary and Anouassi, 1997). However, estrous synchronization with timed artificial insemination (AI) in dromedary she-camels was carried out using hCG, Progesterone Release Intra-vaginal Device (PRID) for 17 days plus hCG or PRID plus eCG (Al-Sobayil, 2008). In addition, Controlled Intra-vaginal Drug Releasers (CIDR) proved to control ovarian activity in llama (Chaves *et al.*, 2002).

She-camel mostly does not ovulate without a natural mating or hormonal induction, and to reproductively manage a herd of she-camels, this requires laborious efforts of the owner and might result in incorrigible outcomes. Therefore, there existed a need for the estrus/ovulation synchronization of She-camels (Al-Sobayil, 2008). Most of the previous attempts are still missing a complete regimen for induction and synchronization of estrus in the she-camel, and of contraversing results.

To offer a new insight into estrous synchronization in she-camel during breeding season, the current study aimed to compare three hormonal protocols (GnRH+PGF₂α, CIDR+eCG and eCG+PGF₂α) for estrous synchronization in dromedary she-camel and consequently their effects on pregnancy rate by applying the natural insemination at the synchronized estrus.

II. Materials and Methods

This study was carried out at Center of Studies and Development of Camel Production, belonging to Animal Production Research Institute, Agricultural Research Center, Marsa Matrouh Governorate, Egypt, during the breeding season.

Animals:

Twenty eight sexually mature dromedary she-camels having 460-550 kg live body weight, 8-13 years old and 2-5 parities were used in this study. Animals were randomly divided into four similar groups, 7 animals in each. All animals were fed a daily basal diet consisting of 3.5 kg concentrate feed mixture (CFM), 2.5 kg clover hay (CH) and 2 kg rice straw (RS) per animal. Chemical analysis of CFM, BH and RS are shown in Table 1. Ingredient of the CFM used in feeding all the experimental animals was composed of 25% wheat bran, 25% yellow corn, 9% un-corticated cotton seed meal, 20% barely, 15% rice brain, 3% molasses, 2% premix and 1% common salt.

Table 1. Chemical analysis of different feedstuffs in the basal diet of she-camels in all groups.

<u>Item</u>	<u>Concentrate mixture</u>	<u>Clover hay</u>	<u>Rice straw</u>
Dry matter (%)	89.44	89.00	88.46
<u>Chemical analysis (on dry matter basis, %):</u>			
Organic matter	92.43	87.70	82.24
Crude fiber	08.85	30.50	35.69
Crude protein	12.24	11.30	02.53
Ether extract	04.64	03.20	01.52
Nitrogen free extract	66.70	37.70	40.50
Ash	07.57	12.30	19.76

Experimental Groups:

In this study, four similar experimental groups were used as the following:

G1: Each animal was i.m. injected with 250 µg GnRH analogue (buserelin acetate; Receptal, Intervet International, Germany) on day 0, followed by 5 ml PGF₂α (Intervet, B.V., Boxmer, Holland) on day 10, and animals in heat within 72 h of PGF₂α injection were naturally inseminated.

G2: Controlled Internal Drug Release (CIDR) containing 1.9 g of progesterone (EAZI-BREED CIDRTM, Interag, Hamilton, New Zealand) was intravaginally inserted in each animal on day 0, followed by injection of 1000 IU eCG (Synncropart, Ceva Sante Animal, France) on day 8. CIDR was removed on day 10 and then animals in heat were naturally inseminated within 72-120 h of CIDR withdrawal. The CIDR was inserted by restraining the female camel in a regular chute and the vulva was cleaned, disinfected and lubricated. The device was loaded into the applicator and gently was inserted into the vagina leaving a free portion of the string outside the vulva to facilitate its withdrawal.

G3: Each animal was i.m. injected with 1000 IU eCG on day 0, followed by 5 ml PGF₂α injection on day 10 and natural insemination of animals in heat within 72 h of PGF₂α injection.

G4: Animals were left without hormonal treatment and saved as control.

All she-camels observed in heat following each protocol or in control group were naturally inseminated with fertile camel bull

Ultrasonography Examination:

She-camels were examined by ultrasound scanner Vetson-color machine (Kontron Medical, France) with endo-rectal ultrasound multi-frequency probe 5 LV (2-7 MHZ). Animals were scanned through the rectal mucosa to identify diameter of follicles at different sizes pre-treatment and at mating as well as number and diameter of corpora lutea (CLs) on day 70 post-mating on the right and left ovaries of each animal. Follicles and CLs were measured through a built-in caliper system inside the machine.

Blood Sampling:

Blood samples were collected from all animals in each group on days 0 (pre-treatment), at mating and on day 70 post-mating. Bleeding was done before morning feeding from the jugular vein-puncture into clean test-tubes. The test-tubes and their contents were allowed to stand for about six hours, and blood serum was separated carefully into vials. Serum samples were stored in deep freezer (-20°C) before being analyzed for progesterone (P4) and estradiol-17 β (E2). Both P4 and E2 assays were determined using chemical commercial kits and performed by RIA.

Estrus and Conception:

Animals in heat relative to total animals in each group were recorded to determine estrus rate. Also, time elapsed from the PGF2 injection (G1 and G3), CIDR withdrawal (G2) or start of treatment (G4) to estrus and mating was calculated. All inseminated animals were diagnosed for pregnancy on day 20-40 of mating using ultrasonographic examination to determine pregnancy rate based on total number of animals or number of inseminated animals in each group.

Statistical Analysis

Data were collected and statically analyzed by using the General Linear Model (GLM) procedures of the statistical Analysis Systems (SAS, 2004). Data obtained were tested by analysis of variance with one way design to test the group differences according to the following model: $Y_{ij} = \mu + P_i + e_{ij}$; where: Y_{ij} = observed values, μ = overall mean, P_i = experimental group and e_{ij} = Random error. Values were given as mean \pm standard error. All statements of significance were based on $P < 0.05$ using Duncan's Multiple Range (**Duncan, 1955**).

III. Results And Discussion

Ovarian Follicles:

Pre-treatment (Day 0), follicular diameter was higher ($P < 0.05$) in G4 and G3 than in G2, which showed the lowest diameter, but these groups did not differ from that in G1 (Table 2). This means that camel in CIDR group showed the lowest diameter for all follicles and the largest follicles on both ovarian sides as compared other groups before treatment.

At the end of treatment (at mating), follicular diameter (for all follicles or largest follicles) was the highest ($P < 0.05$) in G2 and G3, moderate in G4 and the lowest in G1 (Table 3). Such trend of change in follicular diameter at mating in comparing with at 0 day may indicate pronounced effect of CIDR or eCG treatment on follicular growth and development more than of OvSynch protocol.

Pre-treatment (0 day), there were insignificant differences in the follicular diameter between pregnant and non-pregnant camels in each group or overall for all groups. However, at mating, diameter of largest or all visible follicles on both ovarian sides was wider ($P < 0.05$) wider in pregnant than in non-pregnant camels in all groups. This trend was nearly observed within each group (Table 3).

It is of interest to note that follicular growth was evident on the ovaries of untreated camels (G1) on 0 day or at mating in most camels with clearly mature follicles. Similar findings were observed in control camels during breeding season in September and October (**Dholpuria et al., 2012**). The season affects the growth of dominant follicle during its dominance period but the other characteristics of the follicular wave are not affected (**Manjunatha et al., 2012**). No ovarian cysts were observed in CIDR or eCG group. In both groups, camels were injected with eCG at a level of 1000 IU. **Quzy et al. (2013)** detected ovarian cysts developed with doses of 2000 IU of eCG which could be due to prolonged half life of 72 h seen with eCG. However, lower doses as 1000 IU given by **Agarwal et al. (1997)** for 2 consecutive days after P_4 injection resulted in ovulation in 50-75% camels only without ovarian cyst. The observed highest diameter of the largest follicles in CIDR group may suggest that P_4 priming before eCG would be beneficial although this delays the appearance of ovulatory size mature follicle (**Monaco et al., 2012**).

Table 2. Follicular diameter pre-treatment and at mating on the left and right side as affected by hormonal protocol.

Group (Protocol)	Follicular diameter (mm)				
	Left ovary		Right ovary		Overall mean/animal
	Largest follicle	All follicles	Largest follicle	All follicles	
Day 0 (pre-treatment):					
G1 (OvSynch)	8.00±0.833	7.00±0.636	4.86±0.375	4.36±0.445	5.68±0.509 ^{ab}
G2 (CIDR)	7.86±1.952	5.43±1.170	4.14±1.069	4.07±1.018	4.75±0.219 ^b
G3 (eCG)	8.00±0.537	7.57±0.487	5.29±0.749	4.93±0.406	6.25±0.380 ^a
G4 (Control)	8.14±0.560	7.86±0.476	6.86±0.560	6.50±0.433	7.18±0.445 ^a
At mating:					
G1 (OvSynch)	8.00±1.272	5.57±0.783 ^b	5.29±0.315 ^b	4.71±0.312 ^b	4.54±0.417 ^c
G2 (CIDR)	10.90±1.030	7.93±0.424 ^{ab}	9.71±0.857 ^a	7.93±0.424 ^a	7.20±0.164 ^a
G3 (eCG)	10.29±0.668	8.50±0.360 ^a	8.57±0.715 ^a	7.57±0.487 ^a	7.84±0.354 ^a
G4 (Control)	10.00±1.077	8.50±0.360 ^a	5.86±0.698 ^b	5.21±0.562 ^b	6.82±0.421 ^b

Means denoted within the same column at each sampling time with different superscripts are significantly different at P<0.05.

Table 3. Follicular diameter pre-treatment and at mating on the left and right side of pregnant and non-pregnant camels within each hormonal protocol.

Group (Protocol)	Pregnancy status	Follicular diameter (mm)				Overall mean per animal
		Left ovary		Right ovary		
		Largest follicle	All follicles	Largest follicle	All follicles	
Day 0 (pre-treatment):						
G1 (OvSynch)	Non-preg.	7.33±0.733	6.33±0.604	4.66±0.273	4.17±0.227	5.50±0.432
	Pregnant	8.50±0.859	7.12±0.681	5.00±0.416	4.50±0.510	5.81±0.557
G2 (CIDR)	Non-preg.	8.00±0.000	5.00±0.000	4.00±0.000	4.00±0.000	4.50±0.000
	Pregnant	7.83±0.890	5.50±0.527	4.16±0.487	4.08±0.164	4.79±0.232
G3 (eCG)	Non-preg.	7.07±1.834	5.25±1.343	3.67±0.991	3.81±0.981	4.50±1.081
	Pregnant	8.20±0.553	7.90±0.464	6.00±0.589	5.10±0.425	6.50±0.367
G4 (Control)	Non-preg.	7.52±1.430	7.22±1.350	5.78±1.173	5.14±1.030	6.17±1.157
	Pregnant	8.00±0.961	7.83±0.843	7.00±0.961	6.67±0.843	7.25±0.841
Overall mean	Non-preg.	7.80±0.316	7.05±0.356	5.20±0.474	5.10±0.351	6.08±0.332
	Pregnant	8.11±0.437	6.92±0.409	5.33±0.398	4.89±0.353	5.91±0.332
At mating:						
G1 (OvSynch)	Pregnant	9.00±0.480	6.16±0.500	5.00±0.000	4.50±0.000	4.63±0.301
	Non-preg.	7.25±1.454	5.13±0.863	5.50±0.368	4.87±0.401	4.47±0.475
G2 (CIDR)	Pregnant	7.00±0.000	7.00±0.000	7.00±0.000	6.00±0.000	6.50±0.000
	Non-preg.	11.50±0.941	8.08±0.425	10.17±0.764	6.33±0.569	7.31±0.113
G3 (eCG)	Pregnant	9.84±2.595	7.20±1.709	8.69±2.284	7.15±1.740	6.51±1.538
	Non-preg.	9.60±0.565	8.90±0.204	9.00±0.589	8.23±0.224	7.10±0.425
G4 (Control)	Pregnant	9.02±1.697	7.96±1.487	7.14±1.709	6.26±1.459	6.84±1.390
	Non-preg.	10.00±0.480	8.33±0.139	5.67±0.555	5.17±0.555	7.33±0.284
Overall mean	Pregnant	9.20±0.451	7.45±0.391	6.10±0.529 ^b	5.40±0.407 ^b	5.99±0.373 ^b
	Non-preg.	10.11±0.684	8.03±0.505	8.07±0.595 ^a	6.89±0.425 ^a	6.94±0.371 ^a

Means denoted within the same column with different superscripts are significantly different at P<0.05.

It is worth noting that mature follicles were seen on day 0 (the largest diameter of about 8 mm) and at mating (the largest diameter of 8-10.9 mm), particularly on the left ovaries in all groups. In camel administrated with 2000 IU of eCG or an OvSynch protocol (GnRH+PGF2α+GnRH on 0, 7 and 9 days), a high proportion of eCG treated and OvSynch treated camels evidenced a mature follicle on day 8 and 12 of treatment, respectively (Quzy *et al.*, 2013). In this respect, Skidmore *et al.* (2009) observed mature follicles on the ovaries of camels treated with GnRH (Day 0), followed by PGF2α on day 7. Also, Al-Sobayil (2008) reported that mature follicles were visible on the camel ovaries on day 12 of the OvSynch protocol. Similar findings were also recorded previously by Bono *et al.* (1991a).

Generally, eCG was used as a hormonal treatment for inducing ovarian follicle growth in camels during the non-breeding season (Al-Sobayil, 2003 and 2008) with high success rates and evidence of ovulatory sized follicles on day 13 when eCG was used in succession to vaginal implants of progesterone releasing intra-vaginal device used for cattle; CIDR-B (Al-Sobayil, 2008; Monaco *et al.*, 2012).

Estrus Rate:

All treated and control camels exhibited estrous activity at different times after the end of treatment for each group. Time of estrus incidence and subsequently of mating after the end of each treatment was longer (P<0.05) in G4 (control) than in treated groups, being the shortest in G2 as compared to G1 and G3. Overall mean of Time of estrus/mating was shorter (P<0.05) shorter in pregnant than in non-pregnant of all groups and this trend was recorded within each group (Tables 4 and 5).

Table 4. Estrus rate and time of estrus incidence and mating after the end of hormonal treatment.

Group (Protocol)	Estrus rate (%)	Estrus incidence after the end of treatment (h)		Mating incidence after the end of treatment (h)	
		Range	Mean±SE	Range	Mean±SE
G1 (OvSynch)	100	72-120	106.3±17.57 ^b	73-132	111.3±21.47 ^b
G2 (CIDR)	100	24-120	72.0±13.94 ^b	27-122	75.7±24.02 ^b
G3 (eCG)	100	48-120	92.6±15.04 ^b	56-134	101.1±26.66 ^b
G4 (Control)*	100	144-192	170.6±20.74 ^a	176-222	198.6±18.96 ^a

Means denoted within the same column with different superscripts are significantly different at P<0.05.

* At the same time of starting other hormonal treatments.

Table 5. Estrus and mating incidence after the end of hormonal treatment in pregnant and non-pregnant camels within each hormonal protocol.

Group (Protocol)	Pregnancy status	Estrus incidence after the end of treatment (h)	Mating incidence after the end of treatment (h)
G1 (OvSynch)	Non-pregnant	120.0±0.00	130.0±4.27
	Pregnant	96.0±7.35	97.3±7.45
G2 (CIDR)	Non-pregnant	82.0±0.00	94.0±0.00
	Pregnant	76.6±15.16	78.3±15.20
G3 (eCG)	Non-pregnant	120.0±0.00	131.0±2.50
	Pregnant	74.7±14.59	77.9±14.76
G4 (control)	Non-pregnant	137.3±30.10	154.2±34.30
	Pregnant	81.9±32.22	88.5±37.55
Overall mean	Non-pregnant	141.0±12.46 ^a	157.4±14.70 ^a
	Pregnant	93.3±8.86 ^b	101.8±10.70 ^b

Overall means denoted within the same column with different superscripts are significantly different at P<0.05.

In comparable with the present results, different rates and times of estrus incidence were recorded by Al-Sobayil (2008) for she-camel treated with eCG+hCG (T1); and PRID for 17 days and hCG (G2) or eCG (G3) at the time of device removal. Estrus rate was 20, 40 and 100% during breeding season, respectively. The estrous exhibition started 24 hours after the device removal and after the hCG injection. In accordance with the present results, the later author indicated that synchronizing female camels with progestagen in addition to the administration of an ovulatory hormone may result in a better estrous exhibition within a narrower period. Also, Ismail *et al.* (1998) offered a possibility to prolong the limited breeding season of the she-camel by a single injection with hCG followed by a single injection with PGF2α (Anouassi and Ali, 1991; Minoia *et al.* 1992).

Generally, eCG is the most widely used treatment for inducing high estrus rate in camels (Al-Sobayil, 2008; Monaco *et al.*, 2012). Also, the use of exogenous P4 for controlling the ovulation has been documented in dromedary camel (McKinnon *et al.*, 1994), Bactrian camels (Nikjou *et al.*, 2008) and llama (Chaves *et al.*, 2002). Although the OvSynch protocol is widely used for timed insemination in cattle, it less frequent for camel, but the use of GnRH alone (Ismail *et al.*, 1998) or in combination with P4 implants (Monaco *et al.*, 2012) has been suggested for estrus and ovulation induction in camels.

Hormonal Profile:

The differences in overall mean of P₄ concentration between groups were not significant pre-treatment, at mating and on day 70 post-mating; however, there was a marked increase in P₄ concentration at mating

compared with pre-treatment and on day 70 post-mating in comparing with at mating in all groups (Table 6). In this respect, **Derara et al. (2014)** found that CL was detected by day 4.1 ± 1.6 after GnRH treatment and lasted for 6.0 ± 1.1 days. Thereafter, serum P_4 was at basal values and increased only after GnRH treatment. Also, **Chaves et al. (2002)** stated a rapid increase in plasma P_4 concentration in llama immediately after the insertion of intravaginal device, and then P_4 concentration sharply decreased until day 3, and slowly decreased until basal values were registered on day 11-13 post insertion.

Table 6. Mean and standard error of progesterone and estradiol-17-β concentrations in blood serum of She-camels as affected by hormonal protocol.

Protocol	Progesterone level (µg/ml)			Estrogen level (pg/ml)		
	Pre-treatment	At mating	70 d of mating	Pre-treatment	At mating	70 d of mating
G1 (OvSynch)	0.67±0.083	1.19±0.025	4.11±0.813	0.19±0.064 ^c	6.05±1.807 ^a	0.02±0.005 ^b
G2 (CIDR)	1.08±0.195	1.43±0.257	4.55±1.683	2.60±0.558 ^b	5.59±0.897 ^a	2.58±0.086 ^b
G3 (eCG)	0.30±0.065	1.06±0.370	5.06±1.845	6.23±0.425 ^a	2.18±0.360 ^b	1.61±0.519 ^b
G4 (Control)	1.02±0.790	1.92±0.447	3.90±0.525	2.98±0.959 ^b	4.49±1.637 ^{ab}	3.13±1.044 ^a

Means denoted within the same column with different superscripts are significantly different at $P < 0.05$.

Overall mean of E_2 concentration pre-treatment increased ($P < 0.05$) in G3 and decreased ($P < 0.05$) in G1, while did not differ in G2 as compared to G4. At mating, E_2 concentration increased ($P \leq 0.05$) in G1 and G2 and decreased ($P < 0.05$) in G3 as compared to G4. This means that OvSynch protocol and CIDR markedly increased E_2 concentration, while eCG decreased E_2 concentration at mating compared with pre-treatment (Table 6). Of course the trend of change in E_2 level is in relation with follicular size. In this respect, **Derara et al. (2014)** showed that serum E_2 peaked when a large follicle was detected on day 8.5 ± 2.8 post-GnRH treatment, concluding that dromedary camels are highly responsive to early GnRH treatment.

On day 70 post-mating, E_2 concentration was lower ($P < 0.05$) in all treated groups than in control one, showing inconsistent trend of changes at different sampling times (Table 7). The observed increase in overall E_2 concentration in OvSynch and CIDR after the end of treatment (at mating) may suggest initiation of new follicular wave after CL regression by PGF2α in OvSynch group and CIDR removal in CIDR group. In this respect, **Ismail et al. (1998)** found that PGF2α injection into a she-camel primed with hCG (having functioning CL) during the breeding season induced an increase in plasma estradiol-17β on day 5 post-injection as compared to that pre-treatment. Also, **Hussein et al. (2015)** reported that the overall E_2 profile after CIDR application for 10 days was higher than that after CIDR application for 7 and 14 days, respectively during breeding season.

Table 7. Mean and standard error of progesterone and estrogen concentrations in blood plasma of pregnant and non-pregnant camels within each hormonal protocol.

Group (Protocol)	Pregnancy status	Progesterone level (µg/ml)			Estrogen level (pg/ml)		
		Pre-treatment	At mating	70 d of mating	Pre-treatment	At mating	70 d of mating
G1 (OvSynch)	Non-preg.	0.29±0.092	2.19±0.062	3.59±0.908	0.25±0.081	6.64±1.821	0.01±0.001
	Pregnant	1.06±0.833	0.19±0.033	4.63±1.397	0.14±0.001	5.45±1.753	0.03±0.027
G2 (CIDR)	Non-preg.	1.36±0.000	2.61±0.000	0.38±0.000	0.00±0.000	4.48±0.000	5.07±0.000
	Pregnant	0.80±0.457	0.24±1.641	8.72±3.449	5.21±1.095	6.70±1.991	0.08±0.064
G3 (eCG)	Non-preg.	0.42±0.125	2.01±6.460	1.23±0.804	7.54±0.440	1.90±0.350	2.89±0.090
	Pregnant	0.17±0.022	0.10±2.108	8.89±1.574	4.93±1.804	2.45±1.500	0.32±0.292
G4 (control)	Non-preg.	0.85±0.354	3.17±1.264	3.43±1.169	5.97±1.764	4.12±0.999	4.45±0.900
	Pregnant	1.19±1.446	0.67±5.033	4.37±4.869	0.01±0.005	4.85±6.656	1.82±0.789
Overall mean	Non-preg.	0.73±0.411	2.50±0.258 ^a	2.16±0.102 ^b	3.44±0.865	4.28±1.623	3.10±0.807 ^a
	Pregnant	0.81±0.384	0.30±0.344 ^b	6.65±0.857 ^a	2.57±0.705	4.87±1.402	0.56±0.060 ^b

Overall means denoted within the same column with different superscripts are significantly different at $P < 0.05$.

On the other hand, **Chaves et al. (2002)** reported that E_2 concentration in llama decreased drastically by day one post-insertion of CIDR and remain low until day 6, when they started to increase again. In addition, these results suggested that the use of exogenous P_4 did not synchronize wave emergence in camels as follicular waves continued to emerge during the period of treatment. Similar results were reported by **Nikjou et al. (2008)**.

Overall concentration of P_4 pre-treatment was similar in pregnant and non-pregnant camels, reflecting different reproductive statuses of camels within each group before treatment. At mating, overall P_4 concentration was lower ($P < 0.05$) in pregnant (≤ 0.05 ng/ml) than in non-pregnant (≥ 1 ng/ml) (Table 6). This trend was noticed

in all groups. Reducing P₄ concentration in pregnant animals below 1.0 µg/ml at mating may indicate complete regression of CL and incidence of estrus in pregnant animals at the time of mating. In this line, **Ismail et al. (1998)** found that PGF2α injection into a she-camel primed with hCG (having functioning CL) during the breeding season significantly decreased P₄ level on days 1 and 2 post-injection. Also, they observed increase in plasma P₄ to 1.0 µg/ml four days post-hCG injection, reaching its maximum level on day 8 post-injection.

On day 70 post-mating, overall mean of P₄ concentration was higher (P<0.05) in pregnant than in non-pregnant animals, ranging from 4.366 to 8.894 µg/ml in pregnant camels in all groups (Table 6). Several authors reported that Plasma P₄ concentrations recorded in pregnant camels varied from 1.40 to 6.45 ng/ml (**Agarwal et al., 1987 & 1997**). In pregnant camels, P₄ values are above 1 µg/ml on day 20 post-mating (**Dholpuria et al., 2012**) or varied from 1.40 to 6.45 ng/ml (**Agarwal et al., 1987 & 1997**). However, **Quzy et al. (2013)** considered camels with P₄ values between 1-2 ng/ml as doubtful because of the possibility of early embryonic deaths (**Dholpuria et al., 2012**). This trend was more pronounced in CIDR and eCG groups as a result of increasing pregnancy rate of these groups as compared to other groups.

On the other hand, overall concentration of E₂ was higher (P<0.05) in non-pregnant than in pregnant animals only on day 70 post-mating, reflecting higher ovarian activity in non-pregnant animals during this time in all groups, except for those in OvSynch group (Table 7).

Generally, there was a marked relationship between P₄ and E₂, in particular at mating, whereas P₄ showed the lowest levels versus the highest E₂ levels. The correlation between the plasma P₄ and E₂ levels augments the hypothesis that luteolysis can be induced within 2-4 days and mature follicles can be palpated 3-5 days posts PGF2α injection (**Ismail et al., 1998**). Some studies have shown that there is a sharp decline in P₄ after luteolysis in camel and mature ovulatory sized follicles are present on the ovaries within 4 to 5 days of treatment (**Ismail et al., 1998**).

Pregnancy Rate:

Pregnancy rate of synchronized camels was the highest (85.71%, P<0.05) in G2, followed by G3 (71.43%) and G1 (57.14%), while G1 showed the lowest pregnancy rate (42.86%, P<0.05). These results indicated significant improvement of camel fertility in all treated groups as compared to control one, being the best in camels treated with CIDR plus eCG, followed by those treated with eCG and PGF2α (Table 8). The physiological explanation for this increase may be attributed to that the eCG molecule has FSH-like activity which might have enhanced the follicular development accelerating the existence of the dominant follicle and subsequently causing ovulation via its LH-like action (**Al-Sobayil, 2008**).

Table 8. Pregnancy rate of camels as affected by hormonal protocol.

Protocol	Treated/inseminated animals (n)	Pregnant animals (n)	Pregnancy rate (%)
G1 (OvSynch)	7	4	57.14±12.3 ^c
G2 (CIDR)	7	6	85.71±15.8 ^a
G3 (eCG)	7	5	71.43±10.3 ^b
G4 (Control)	7	3	42.86±12.3 ^d

Overall means denoted within the same column with different superscripts are significantly different at P<0.05.

In accordance with the present results, **Al-Sobayil (2008)** found that the highest calving rate (80%) was achieved when PRID was followed by eCG. This result agreed with that obtained earlier by **Simpkin (1987)** when a 15-day progestogen treatment followed by the administration of 1500-2500 IU eCG. The later authors reported that an eCG dose guarantees the presence of mature follicle(s) and subsequent ovulation. Also, **Tibary and Anouassi (2000)** reported that the synchronization of follicular development and ovulation is improved by the administration of 1500-2000 IU eCG one day before the PRID removal, or the last day of progestogen treatment. In comparable with the present rates of pregnancy, **Quzy et al. (2013)** found that lower pregnancy rates than that reported in our study, being 50.0, 65.11 and 61.53% in control, eCG (2000 IU) and OvSynch treated camels, respectively.

It is of interest to note that the effect of hormonal treatment on camel fertility was associated with significant effect on number and diameter of CL of pregnant camels. Total number of CL per animal decreased (P<0.05) in all treated groups, while CL diameter decreased (P<0.05) decreased in OvSynch group only as compared to control one (Table 9). **Shalash (1965) and Elias et al. (1984)** reported that CL could be palpated 8-10 days post mating.

Table 9. Average of number and diameter of corpora lutea on the left and right ovary of pregnant camel on day 70 post-mating as affected by hormonal protocol.

Group (Protocol)	Number of CL			Diameter (mm) of CL		
	Right ovary	Left ovary	Total	Right ovary	Left1 ovary	Overall mean
G1 (OvSynch)	1.25±0.22 ^b	1.00±0.00 ^b	2.30±0.20 ^b	16.33±0.481 ^b	19.60±1.191 ^b	18.01±1.191 ^b
G2 (CIDR)	1.50±0.22 ^{ab}	1.00±0.00 ^b	2.50±0.20 ^b	18.33±1.734 ^{ab}	22.75±0.594 ^a	20.51±0.826 ^a
G3 (eCG)	1.20±0.41 ^b	1.40±0.20 ^{ab}	2.60±0.40 ^b	21.00±0.289 ^a	19.63±0.515 ^b	20.30±0.515 ^a
G4 (Control)	2.00±0.00 ^a	2.00±0.00 ^a	4.00±0.00 ^a	21.00±0.000 ^a	19.17±0.612 ^b	20.09±0.518 ^a

Means denoted within the same column with different superscripts are significantly different at P<0.05.

IV. Conclusion

Administration of eCG (1000 IU), as it contains mainly the FSH-like activity and partially the LH-like activity, 48 h prior to CIDR insertion for 10 days or 10 days prior to PGF2α injection, caused a better synchrony of estrus and ovulation, and improved pregnancy rate of camels. For achieving an appropriate estrous synchronization of dromedary camels during breeding season, the estrus and ovulation could be hormonally induced by CIDR insertion for 10 days and eCG injection on day 8 (48 h before CIDR withdrawal) with the highest pregnancy rate. The use of OvSynch did not exceed the pregnancy rate. Further studies are needed to monitor the failure of OvSynch protocol to improve fertility of she-camels during breeding season.

References

- [1] Agarwal, R., West, C., Conway, G.S., Page, M.L. and Jacobs, H.S. (1997). Pregnancy after treatment with three recombinant gonadotrophins. *Lancet*, 349, 29–30.
- [2] Agarwal, S.P., Khanna, N.D., Agarwal, V.K. and Dwaraknath, P.D. (1987). Circulating level of oestrogen and progesterone in female camels (*Camelus dromedarius*) during pregnancy. *Theriogenology*, 28, 849-859.
- [3] Agarwal, S.P.; Rai, A.K. and Khanna, N.D. (1993). Effect of low dose of FSH administration on ovarian activity during non-breeding season in the camels. *Indian J. Anim. Sci.*, Vol. 63:387-390.
- [4] Al-Sobayil, K.A. (2003). Hormonal treatments for inducing fertile estrus in young dromedary females at the end of their seasonal anestrus in Qassim region, Saudi Arabia. *J. Camel Practice and Research*, (December 2003): 179-181.
- [5] Al-Sobayil, K.A. (2008). The use of estrus synchronization and timed artificial insemination in dromedary she-camels in Saudi Arabia. *Journal of Agricultural and Veterinary Sciences*, Qassim University, Vol. 1, No. 1: 3-9.
- [6] Anouassi, A. and Ali, A. (1991). Embryo transfer in camel (*Camelus dromedarius*). *Camel Newsletter*, 8:20.
- [7] Anouassi, A., Tibary, A., Adnani, M. and Sghiri, A. (1994). Preovulatory phase characterization in *Camelus dromedarius* and induction of ovulation. *Proceedings of the Conference Organized by FIS. Niamey, Niger*. pp 159-167.
- [8] Bono, G., Dahir, A.M., Comin, A. and Jumale, A.M. (1991a). Seasonal effects on the endocrine reproductive activity of the dromedary camel. *Camel Newsletter*, 8:22.
- [9] Bono, G., Jumale, A.M. and Dahir, A.M. (1991b). Seasonal variations of LH response to GnRH treatment in camels (*Camelus dromedarius*). *Camel Newsletter*, 8:19.
- [10] Chaves, M.G., Aba, M.A., Agüero, A., Egey, J., Berestin, V. and Rutter, B. (2002). Ovarian follicular wave pattern and the effect of exogenous progesterone on follicular activity in non-mated llamas. *Anim. Reprod. Sci.*, 69, 37–46.
- [11] Chen, B.X.; Yuen, Z.X. and Pan, G.W. (1985). Semen induced ovulation in the Bactrian camel (*Camelus bactrinus*). *J. Reprod. Fertil.*, Vol. 74, 335-339.
- [12] Combarnous, Y. and Anouassi, A. (1994). Radioimmunoassay and enzyme-linked immuno-sorbent assay technique as aid to studying pituitary hormones and improving reproductive management of the one humped camel. *J. Arid. Enviro.*, Vol. 26, 15-20.
- [13] Dahir, M.A.; Jumale, A.M.; Halane, M.I. and Prandi, A. (1990). Effect of progestogen-PMSG treatment on estrus activity of she-camel dromedaries at the end of the dry season. *Proceedings of the Workshop "Is It Possible to Improve the Reproductive Performance of the Camel?"*, Paris, France, pp 189-195.
- [14] Derara, R.; Ali, A. and Al-Sobayil, F.A. (2014). The postpartum period in dromedary camels: Uterine involution, ovarian activity, hormonal changes, and response to GnRH treatment. *Animal Reproduction Science*, 151:186–193.
- [15] Dholpuria, S., Vyas S., Purohit G.N. and Pathak K.M.L. (2012). Sonographic monitoring of early follicle growth induced by melatonin implants in camel and the subsequent fertility. *J. Ultrasound.*, 15: 135-141.
- [16] Duncan, D.B. (1955). Multiple Range's and Multiple F-test. *Biometrics*, 11: 1-42.
- [17] Elias, E.; Bedrak, E. and Cohen, D. (1985). Induction of estrus in camel (*Camelus dromedaries*) during seasonal anestrus. *J. Reprod. Fert.*, Vol. 74: 519-525.
- [18] Elias, E.; Bedrak, E. and Yagil, R. (1984). Peripheral blood levels of progesterone in female camels during various reproductive stages. *General and Comparative Endocrinology*, Vol. 53: 235-240.
- [19] Hafez, E.S. and Hafez, B. (2001). A Review: Reproductive Parameters of Male Dromedary and Bactrian Camels. *Arch. Androl.*, Vol. 4: 85-98.
- [20] Homeida, A.M.; Khalil, M.G.R. and Taha, A.A.M. (1988). Plasma Concentration of Progesterone, Estrogen, Testosterone and LH-like Activity During the Estrus Cycle of the Camel (*Camelus dromedarius*). *J. Reprod. Fert.*, Vol. 83: 593-598.
- [21] Hussein, F.M., Metwelly, K.K., Mahmoud, Mona, A. and Ragab, Mohamed H. (2015). Effect of CIDR application duration (7-10-14 Days) on circulating estrogen and progesterone during breeding and non-breeding season in she-camels. *Alexandria Journal of Veterinary Sciences*, 44: 125-129.
- [22] Ismail, A.A.; Siam, A.A.; Amal El-Nahla and S.M.M. Abuzead (1998). Synchronization of estrus in the she-camel. *Proceedings of the Third Annual Meeting for Animal Production Under Arid Conditions*, Vol. 1: 96-107. United Arab Emirates University.
- [23] Kaufmann, B.A. (2005). Reproductive performance of camels (*Camelus dromedarius*) under pastoral management and its influence on herd development. *Livestock Production Science*, 92: 17-29.

- [24] Manjunatha, B.M., David, C.G., Pratap, N., Al-Bulushi, S. and Hago, B.E. (2012). Effect of progesterone from induced corpus luteum on the characteristics of a dominant follicle in dromedary camels (*Camelus dromedarius*). Anim. Reprod. Sci., 132 (3-4): 231-236.
- [25] McKinnon, A.O.; Tinson, A.H. and Nation, G. (1994). Embryo transfer in dromedary camels. Theriogenology, Stoneham, Mass.: Butterworth-Heinemann, 41 (1): 145-150.
- [26] Minoia, P.; Moslah, M.; Lacalandra, G.M.; Khorchani, T. and Zarilla, A. (1992). Induction of estrus and management of reproduction in the female dromedary camel. Proceedings of the 1st International Camel Conference, 119-123. Edited by W.R. Allen, A.J. Higgins, I.G. Maybew, D.H. Snow and J.F. Wade. R & W Publications (Newmarket) Ltd.
- [27] Monaco, D.; Lacalandra, G.M.; El-Hassanein, E.E.; Rateb, S.; Salama, O. and El-Bahrawy, K.A. (2012). Effect of a controlled intravaginal drug releaser (CIDR) and GnRH administration on ovarian follicular dynamics of female dromedary camel during seasonal anestrus period. Proc 3rd Conf Int. Soc. Camelid Res. Dev. Jan., 29-Feb, Muscat, Sultanate of Oman, p 123-24.
- [28] Musa, B.H.; Sieme, H.; Hago, B.; Cooper, M. J.; Allen, W. R. and Jochle, W. (1993). Manipulation of Reproductive Function in Male and Female Camels. Anim. Repro. Sci., Vol. 33: 289-306.
- [29] Nikjou, D., Niasari-Naslaji, A., Skidmore, J.A., Mogheiseh, A., Razavi, K., Germai, A. and Ghanbari, A. (2008). Synchronization of follicular wave emergence prior to superovulation in Bactrian camel (*Camelus bactrianus*). Theriogenology, 69:491-500.
- [30] Quzy, I., Anwar, S. and Purohit, G.N. (2013). Hormonal management of ovarian activity in breeding camels two months ahead of the natural breeding season. Camel- International Journal of Veterinary Science, 1(1):37-49.
- [31] SAS (2004). SAS/STAT User's Guide: Volumes 1-7. SAS Institute Inc., Cary, North Carolina.
- [32] Shalash, M.R. (1987). Review Article: Reproduction in camels. Egypt J. Vet. Sci., Vol. 24: 1-25.
- [33] Shalash, M.R. (1965). Some reproductive aspect in the female camel." World Rev. Anim. Prod., Vol. 4: 103-108.
- [34] Simpkin, S.P. (1987). A summary of the increased productivity in camels as a result of application of a veterinary program. Proc. Workshop Camel Management and Ecology, Landskrona, Sweden.
- [35] Skidmore, J.A.; Billah, M. and Allen, W.R. (1996). The ovarian follicular wave pattern and induction of ovulation in the mated and non-mated one humped camel (*Camelus dromedarius*). J. Reprod. Fertil. 106:185-92.
- [36] Skidmore, J.A.; Adams, G.P. and Billah, M. (2009). Synchronization of ovarian follicular waves in the dromedary camel (*Camelus dromedarius*). Anim. Reprod. Sci., 114:249-255.
- [37] Tibary, A. and Anouassi, A. (1996). Ultrasonographic changes of the reproductive tract in the female camel (*Camelus dromedarius*) during the follicular cycle and pregnancy. J. of Camel Practice and Research, Vol. 3: 71-90.
- [38] Tibary, A. and Anouassi, A. (1997). Theriogenology in Camelidae: Anatomy, Physiology, Pathology, and Artificial Breeding. Mina, UAE: Abo Dhabi Printing and Publishing Company, pp. 51-61, 171-173.
- [39] Tibary, A. and Anouassi, A. (2000). Ultrasonography of the genital tract in camels (*Camelus dromedarius* and *Camelus bactrianus*). In: Gahlot, T.K. (Ed.), Selected Topics in Camelid Medicine. The Camelid Publishers, Bikaner, India, pp. 431-465.