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Effect of Nutrition on Reproductive Performance During the Peri-Partum Period of Female Camel (*Camelus dromedarius*) in Algeria

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Abstract: Ten pregnant Saharawi camel breed were used to determine the effect of diet on the profile of blood parameters (biochemical and hormonal) in peri-partum and the kinetics of follicular development combined with ostrogen 17β and progesterone assays after calving. The animals were divided into two groups of five camels: the "S" group who received food from concentrate, hay and forage and the "NS" group not supplemented with green forage. Blood samples were collected at 30, 15 and 2 days before birth and on a weekly basis from the 2nd to the 40th day after parturition. The resumption of ovarian activity was monitored by ultrasonography from 10th-40th day after parturition. With the approach of parturition, protein and energy, lipid and mineral parameters increased in all camels ($p<0.05$). After parturition, protein-energy factors, minerals and progesterone decreased ($p<0.05$) gradually from day 2 until a physiological level at day 40. The gestational state had a significant effect on glucose ($R^2 = 0.79$), progesterone ($R^2 = 0.93$) and oestradiol 17β ($R^2 = 0.95$) in plasma. The supplemented camels showed a higher biochemical and mineral status ($p<0.001$) while triglycerides strongly decreased ($p<0.001$). The study showed a positive correlation between the size of the follicles and the kinetics of oestrogen 17β ($r = 0.718$ to 0.762). A negative correlation was observed between the values of oestrogen 17β and progesterone in plasma ($r = 0.11$ to 0.43).

Key words: Camel, peri-partum, blood parameters, ultrasonography, follicles

INTRODUCTION

Camels have contributed since antiquity, to sustain human populations living in arid and semi-arid (Ahmad *et al*., 2010), salinization, water scarcity and desertification of pasture exacerbated by the changes and warming. These natural threats prompted humans to search for new resources for animal and human nutrition. Camels are best suited for harsh environment (Ahmad *et al*., 2010) and are used for transportation, farming, milk production, wool and meat (Skidmore, 2011).

In Algeria, three quarters of the total country is represented by the arid and semi-arid areas. In the latter, cattle and sheep are dependent on pastures and streams. However, camel breeding is the best source of income for pastoralists and agro-pastoralists. Algeria is among the countries with recent rapid increase in camel population. The latter was estimated at 315,000 heads in 2011. However, it is still below expectations of national production. The increase in camel's population for a better farm profitability requires a thorough knowledge of the reproductive physiology of these animals. The camel is a seasonal polyoestrien species induced ovulation (Skidmore *et al*., 2013; Fatnassi *et al*., 2014). Puberty and reproductive development are between the age of 36-48 months with a gestation period that lasts from 360-390 days (Skidmore, 2011). However, this species has a short postpartum anoestrus associated with early resumption of ovarian activity which takes place between 15th and 20th day after parturition. In contrast, Derar *et al*. (2014) reported a longer postpartum anoestrus between 30-40 days.

A better understanding of the mechanisms involved in the resumption of ovarian activity remains of considerable economic interest. To date, investigations concerning the monitoring of ovarian follicular dynamics in indigenous camel remain scarce.

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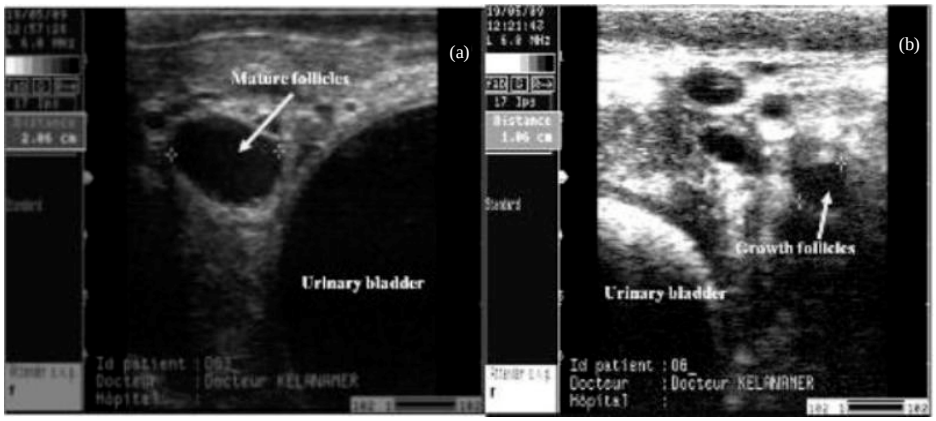

Fig. 1: Ultrasonography of follicles in female camel; a) mature follicular and b) growth follicular

MATERIALS AND METHODS

Animals: The investigation was conducted during the peri-partum period. Ten Saharawi camel breed in their last third of gestation, aged 10-12 years, clinically healthy were selected among camel herds. Such females were drenched, before introduction into the experiment. They were divided into two groups: the S group (n = 5) supplemented with hay, green forage and concentrate) and the NS group (n = 5) not supplemented with green forage.

Biochemical and hormonal parameters: The assays for biochemical parameters (glucose, total protein, urea, triglycerides, cholesterol, calcium, phosphorus and magnesium) and hormones (oestrogen and progesterone) were performed using samples from the 10 camels. Blood samples on dry tubes were made at 30, 15 and 2 days before calving and on a weekly basis from the 2nd to 40th day after parturition. The resumption of ovarian activity was monitored by ultrasonography (Pie Medical, Falco, integrated with a 6.0 and 8.0 MHz linear dual frequency probe was used), 10th-40th day after parturition, at an interval of three days, according to the preparation of camels described by Vyas *et al.* (2008) and Purohit (2012). At ultrasonography, follicles were distinguished after observing the bladder as a benchmark which appeared black and spherical. Follicles were spherical more or less bounded and anechoic juxtaposed to the bladder (Fig. 1). We conducted 110 investigations during 40 days after calving.

The assay for biochemical parameters was carried out by the enzymatic and colorimetric methods using kits (Biomaghreb) while progesterone and oestradiol 17β were Measured by Enzyme Microparticulate (MEIA) by AxSYM.

Statistical analysis: Statistical analysis was performed using SAS Software. The Generalized Linear Model (GLM) has been used to perform an analysis of variance of each parameter to determine the differences between them (reproductive status, food and follicular phase of the cycle) and their statistical significance. For each parameter the mean square and standard error were calculated. The correlations between them were also studied.

RESULTS AND DISCUSSION

Depending on the day approaching parturition, protein-energy, lipid and mineral parameters increased significantly ($p<0.05$) in all camels (Table 1). However, progesterone has halved at 15 days and then to one third at 2 days from the initial rate ($p<0.01$). In contrast, oestrogen significantly increased at 15 days before parturition and this rate had almost doubled at 2 days before parturition ($p<0.01$). After parturition, the values of plasma protein-energy factors, minerals and progesterone decreased ($p<0.05$) gradually from day 2 until reaching a physiological level at day 40 post-partum (Table 1). However, the 2nd day after parturition, we recorded a sharp drop in oestradiol 17β plasma (Table 1), then followed by a gradual increase to reach a high level associated with follicular activity (Table 1).

Glucose, total protein, urea, magnesium, progesterone and oestrogen plasma data were significantly higher before parturition ($p<0.05$-0.001) compared to the period after (Table 1). Our results show that the gestational state had a significant effect ($p<0.001$) on these parameters.

We noted that supplemented animals showed a higher plasma sheet ($p<0.001$) for biochemical parameters and minerals (Table 2) compared to non-supplemented animals. In contrast, plasma levels of triglycerides

Table 1: Mean plasma levels of biochemical, mineral and hormonal parameters before and after the share at 10 Female camels (mean±SE)

Bloods parameter	Average values	Antepartum			Postpartum						Effect of time	R^2
		30 days	15 days	2 days	2 days	10 days	17 days	24 days	32 days	40 days		
Sugar (g L^{-1})	0.96±0.12	0.99±0.02^{b}	1.02±0.02^{b}	1.25±0.02^{a}	0.82±0.02^{d}	0.87±0.02^{dc}	0.88±0.02^{d}	0.92±0.02^{cd}	0.94±0.02^{c}	0.96±0.02^{bc}	***	0.73
Protein total (g L^{-1})	60±2.96	63.70±1.17^{a}	63.20±1.17^{ab}	60±1.17^{ab}	56.30±1.17^{d}	56±1.17^{d}	58.40±1.17^{cd}	60.10±1.17^{bc}	62.30±1.17^{ab}	64.20±1.17^{a}	***	0.41
Urea (mg L^{-1})	392.08±7.02	388.30±4.72^{bc}	393.40±4.72^{ab}	40.72±4.72^{a}	399.50±4.72^{ab}	395.1±4.72^{ab}	393±4.72^{ab}	389.40±4.72^{bc}	388.70±4.72^{bc}	360.00±4.72^{c}	**	0.18
Triglycerides (mg L^{-1})	385.57±5.40	382.10± 5.76^{a}	388.80±5.76^{a}	393.20±5.76^{a}	392.80±5.76^{a}	387.10±5.76^{a}	383.90±5.76^{a}	380.30±5.76^{a}	380.30±5.76^{a}	380.30±5.76^{a}	NS	0.08
Cholesterol (mg L^{-1})	243.91±6.81	242.40± 4.93^{bc}	246.40±4.93^{bc}	252.70±4.93^{ab}	255.50±4.93^{a}	245.40±4.93^{abc}	241.40±4.93^{bc}	238.90±4.93^{bc}	236.80±4.93^{c}	235.70±4.93^{c}	NS	0.16
Calcium (mg L^{-1})	82.86±5.57	74.80±4.78^{b}	78.30±4.78^{ab}	82.20±4.78^{ab}	85.20±4.78^{ab}	89.40±4.78^{a}	90±4.78^{a}	87.0±4.78^{ab}	81.50±4.78^{ab}	76.70±4.78^{ab}	NS	0.12
Phosphorus (mg L^{-1})	65.43±2.86	68.60±2.11^{a}	68.50±2.11^{a}	67.50±2.11^{ab}	67±2.11^{ab}	66.60±2.11^{abc}	64.60±2.11^{abc}	63.20±2.11^{abc}	62.00±2.11^{bc}	60.90±2.11^{c}	NS	0.15
Magnesium (mg L^{-1})	23.13±1.99	22.50±1.28^{b}	23.70±1.28^{a}	27.80±1.28^{a}	21.80±1.28^{b}	21.70±1.28^{b}	22.10±1.28^{b}	21.50±1.28^{b}	22.70±1.28^{b}	24.40±1.28^{ab}	*	0.19
Progesterone (ng mL^{-1})	***	6.02±0. 17^{a}	3.29±0.17^{b}	2.18±0.17^{c}	0.78±0.17^{d}	0.29±0.17^{d}	0.18±0.17^{d}	0.22±0.17^{d}	0.26±0.17^{d}	0.21±0.17^{d}	***	0.93
Oestrogen (pg mL^{-1})	***	106.60±7.24^{c}	154.00±7.24^{b}	330.00±7.24^{a}	27.20±7.24^{e}	34.34±7.24^{e}	43.20±7.24^{d}	43.15±7.24^{e}	33.60±7.24^{e}	36.15±7.24^{e}	***	0.95

a,bIndicates a significant difference at p<0.05 threshold, R^2 = Coefficient of determination, ***p<0.001, **p<0.01, *p<0.05, NS: >0.05

Table 2: Effect of diet on biochemical and mineral parameters in two groups of female camels

Variables	Groups		Significance	R^2
	Non supplemented	Supplemented		
Glucose (g L^{-1})	0.91±0.02^{a}	1.01±0.02^{b}	***	0.16
Total protein (g L^{-1})	57.84±0.46^{a}	62.98 ±0.56^{b}	***	0.32
Urea (mg L^{-1})	372.65±1.83^{a}	398.70±2.17^{b}	***	0.17
Triglycerides (mg L^{-1})	399.09±1.87^{a}	372.65±1.83^{b}	***	0.54
Cholesterol (mg L^{-1})	229.93±1.31^{a}	257.28±1.28^{b}	***	0.72
Calcium (mg L^{-1})	73.68±1.89^{a}	91.65±1.85^{b}	***	0.35
Phosphorus (mg L^{-1})	62.70±0.97^{a}	68.04±0.95^{b}	***	0.15
Magnesium (mg L^{-1})	20.77±0.55^{a}	25.39±0.54^{b}	***	0.29

a and b are significantly different at p<0.05, R^2 = coefficient of determination, S = Supplemented, NS = Not Supplemented, ***p<0.001, **p<0.01, *p<0.05, NS = p>0.05

Table 3: Effect of supplementation on the resumption of postpartum ovarian activity in she female camels

Groups	No. she camels	Days postpartum				
		10	12	15	18	21
Supplemented	5	4	1	/	/	/
Supplemented (%)		80	100	/	/	/
Non-supplemented	5	2	0	1	1	1
Non supplemented (%)		40	/	60	80	100

increased significantly in non-supplemented camels (p<0.001) compared to those supplemented (Table 2).

Because of the large volume of the uterus, ultrasonography examination of the ovaries was not possible before 8 days post-partum. On the 10th day post-partum, ultrasonography of the ovaries revealed that 80% (n = 4) of the supplemented camels were in follicular activity state, at least one follicle with a diameter >3 mm being observed (Table 3). Contrarily, a rate of 40% (n = 2) had been indexed for non-supplemented females. Nonetheless, 100% of supplemented females (n = 5) were in follicular activity at 12 day post-partum. Conversely, the follicular activity of the totality of non-supplemented females was not observed until 21 days post-partum (Table 3).

We recorded a gradual increase in the kinetics of oestradiol 17β according to the increase in the size of the follicle in the recruitment phase (non-follicular phase) until maturation (Table 4). This relationship has been confirmed by the Pearson coefficient. Despite that, the increase in volume during the follicular phase of atresia is accompanied by a decrease in oestrogen in which the correlation coefficient is negative (r = -0.648). Similarly, a negative correlation was observed between the values of oestradiol 17β and progesterone in plasma (Table 4).

Despite the features that characterize the camel (*Camulus dromedarius*) breeding such as the polyoestrien seasonal cycle, the camels farming profitability is subject to weather difficulties and to the scarcity of pastures in local area (Fatnassi *et al*., 2014). The blood parameters as an indicator of the physiological statues of the animals (Al-Saiady *et al*., 2013). A food shortage causes negative effects on body growth and reproductive performance, including the delay of puberty and ovarian inactivity. According to Abdel-Rahim *et al*. (1994), optimization of reproductive faculties in the camel requires a high energy intake in protein and minerals. In view of the ration distributed to camels, we recorded a balance of average values of protein-energy, mineral and hormone parameters before and after parturition (Table 1), similar to that reported in the literature.

However, these values are high relative to those reported at Djibouti by Al-Ali *et al*. (1988) in Saudi Arabia. In the contrary, we saw (for most parameters) an increase of these values approaching parturition, indicating an increase in energy and mineral metabolism and oestradiol 17β secretion (p<0.01) which contributed to the preparation of the genital tract and the uterine contraction efforts in order to expel the offspring by the pregnant camel.

In camel production, infertility is attributed mainly to inadequate supply (El-Bahrawy *et al*., 2011) and injuries caused by handling during delivery (Ali *et al*., 2011). However, there was no evidence of trauma during birth in all camels.

Table 4: Plasma changes in Oestradiol 17β and progesterone in relation to follicular size (mean±SE)

Parameters	Recruitment phase (diameter ≤3 mm)	Growth phase (3<diameter ≤ 9 mm)	Maturation phase (9<diameter ≤ 21 mm)	Phase of atresia (diameter>21 mm)
Number of ultrasound operation in each phase	n = 10	n = 43	n = 42	n = 15
Average size of dominant follicles (in mm) (DFT)	2.53±0.3	5.61±0.20	14.54±0.33	24.12±0.22
Average value of oestradiol 17β (E2) in the blood (pg mL^{-1})	24.14±4.54	30.38±6.13	51.82±12.22	31.17±11.17
Average value of progesterone (P4) in blood (ng mL^{-1})	0.42±0.28	0.21±0.17	0.19±0.06	0.56±0.15
Coefficient (r) Pearson (values of the E2 and size of follicles)	-0.557^{NS}	0.718**	0.762**	-0.648**
R coefficient of Pearson (P4 rate and size of follicles)	-0.36^{NS}	-0.23^{NS}	-0.25^{NS}	-0.27^{NS}
Correlation r (E2/P4)	-0.36^{NS}	-0.11^{NS}	-0.22^{NS}	-0.43^{NS}

E2 = Ostrogen, P4 = Progesterone, FDT = Dominant follicle size, n = Number of ultrasound examinations, NS = p>0.05, **p<0.01

In addition, the values of protein-energy and minerals parameters decreased progressively from the 2nd day after birth to reach physiological concentrations. This situation calls for a return to the normal physiological state by the 35th day after parturition.

There was an inversely proportionality between plasma oestradiol 17β values (r = 0.95) and progesterone (r = 0.93) indicating a resumption of follicular activity similar to results reported by Skidmore (2011).

Food has an important influence on protein-energy and mineral parameters in camels. The energy balance was significantly higher among camels supplemented compared to non-supplemented (p<0.001). This improvement in the energy profile in animals is due to green forage (Table 2). Our results corroborate with those reported previously (Al-Harbi, 2012). However, Hussein *et al.* (2008) provided that food does not exert a significant effect on the secretion of oestradiol 17β and progesterone.

Ultrasonography allows close monitoring of follicular growth during the post-partum. Because of the importance of uterine volume, access to the ovaries to follicle observation was possible only from day 10 after birth. It is likely that the ovarian activity has an earlier onset. The different phases of follicular growth were identified by the diameter of the follicles during their development as set by Zerrouk. During the follicular growth, oestradiol 17β is the only representative hormone. Our study showed that the plasma concentration of oestradiol 17β was proportional to the follicular size. When the volume of the follicle increased, the concentration of oestradiol 17β rose from a basal value of 24.14±4.54 pg mL^{-1} to a concentration of 51.82±12.22 pg mL^{-1}. A positive correlation (r = 0.762) was observed between follicular size and plasma concentration of oestradiol 17β (Table 4). Our results are in agreement with those reported by Ghazi and Basiouni (2007), Hussein *et al.* (2008), El-Hariary *et al.* (2010), Riveros *et al.* (2010), Ali *et al.* (2011), Skidmore (2011) and Deen and Hassanein (2013). However, when follicle size continued to increase (ϕ>21 mm), the average concentration of oestradiol 17β tends to be reduced to a value of 31.17±11.17 pg mL^{-1}, indicating that these giant follicles stayed active until the next wave of follicular development (r = - 0.648). The latter evolved towards a total atresia as reported by Zarrouk and Skidmore (2011).

In the absence of ovulation in camels, progesterone remained <1 ng mL^{-1}. After ovulation, her concentration of this hormone remained low during the 1st 4 days and showed a peak of 4.6±2.6 ng mL^{-1} between days 7 and 9 then dropped between days 10 and 12 (Manjunatha *et al.*, 2012). However, after fertilization, it remained high throughout the period of pregnancy (Hussein *et al.*, 2008; Skidmore, 2011). This decrease over the last few days before calving is shown (Table 1). After parturition, concentration of the progesterone decreased gradually to a plasma concentration of 0.2±0.17 ng mL^{-1} (Table 1), similar to those reported by Hussein *et al.* (2008). During the different phases of the follicular cycle (Table 4), plasma progesterone was <0.6 ng mL^{-1} as reported by Ali *et al.* (2011) and Rawy *et al.* (2014). These results supported the absence of corpus luteum. In contrast, the progesterone concentration started to increase to reach 0.56 ng mL^{-1} during the stage of atresia. This increase was probably due to the luteinizing of older follicles. These observations line with those previously reported by Hussein *et al.* (2008) and Skidmore (2011).

Our research reports, the recovery and the monitoring of ovarian activity by ultrasonography in postpartum period in camels, coupled with the determination of oestradiol 17β and progesterone plasma. Our results, based on the use of a diet adapted to the needs of breeding camels have shown an excellent protein-energy and mineral balance before and after parturition. This food intake was followed by an indicator of early resumption of ovarian hormonal activity. The latter was confirmed by monitoring follicular development by ultrasonography including a positive correlation (r = 0.718-0.762) between follicle size and the plasma concentration of oestradiol 17β.

CONCLUSION

The objective of this research was to highlight the effect of diet on reproductive performance including the resumption of ovarian activity in post-partum period, the

determination of protein-energy parameters (glucose, protein total, urea, triglycerides, cholesterol), minerals (calcium, phosphorus, magnesium) and hormones (oestradiol 17β and progesterone) coupled with ultrasonography of ovarian follicular dynamics in post-partum.

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