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Female Camel Hormonal profile during the Estrous cycle in the sahelian zone of Mali

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Abstract

The dromedary The dromedary is the only animal ensuring important productions under Malian northern harsh livestock raising conditions. Camel products constitute the main sources of income of the northen Mali nomadic populations. In the pastoral system, the low fecundity of the dromedary is one of the main obstacles to its reproduction. Among the main research topics on dromedary, mastering its reproduction was indicated. The objective of this study was to understand hormonal profile of female camel for improving reproduction. This study was conducted in Niono, Mali on 10 females, 5.5 ± 0.76 years old with average weight of 355.25 ± 25 kg. Two hundred and sixty serum samples were collected for 55 days. Blood samples were taken once a day outside heat period during one week and 3 times a day (6:00 AM, 2:00 PM and 10:00 PM) during heat periods to analyze estrogen, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and progesterone. Hormone were analyzed using Elisa specific camel kit. Results obtained showed an estrus cycle duration of 28.5 ±1.27 days and heat duration of 5.6 ± 1.43 days. The mean rate of follicle stimulating hormone was 1.19 ± 0.72mlU / ml outside heat and 4.17±1.2 mlU/ml during heat period. The mean level of luteinizing hormone was 1.72 ± 0.6 mlU /ml outside heat and during heat was 5,0 ± 1.99 mlU / ml. Estrogens had an average rate of 6.25 ± 0.46 µg/ml outside heat and 22.58 ± 3.37 µg/ml during the heat period. The mean rate of progesterone was 6.25± 0.46 ng/ml outside heat and 2.68 ± 2.31 ng/ml during heat period. Knowledge of these reproductive parameters is an important step in the control and implementation of a camel artificial insemination program in Mali.

Keywords: Dromedaries, Hormones, Heat, Mali, She-Camel

Résumé

Le dromadaire est le seul animal assurant des productions importantes dans les conditions difficiles d'élevage du nord

malien. Les produits de chameaux constituent la principale source de revenus des populations nomades du nord du Mali. Dans le système pastoral, la faible fécondité du dromadaire est l'un des principaux obstacles à sa reproduction. Parmi les principaux sujets de recherche sur le dromadaire, la maîtrise de sa reproduction a été indiquée. L'objectif de cette étude était de comprendre le profil hormonal de la chamelle pour améliorer la reproduction. Cette étude a été menée à Niono, au Mali, chez 10 femmes de 5,5 ± 0,76 ans d'un poids moyen de 355,25 ± 25 kg. Deux cent soixante échantillons de sérum ont été prélevés pendant 55 jours. Des échantillons de sang ont été prélevés une fois par jour en dehors de la période de chaleur pendant une semaine et 3 fois par jour (6h00, 14h00 et 22h00) pendant les périodes de chaleur afin d'analyser l'oestrogène, l'hormone lutéinisante (LH), l'hormone stimulant le follicule (FSH) et de la progestérone. Les hormones ont été analysées à l'aide du kit Elisa spécifique au chameau. Les résultats obtenus ont montré une durée du cycle de l'oestrus de 28,5 ± 1,27 jours et une durée de chaleur de 5,6 ± 1,43 jours. Le taux moyen d'hormone folliculo-stimulante était de 1,19 ± 0,72 mlU / ml en dehors de la chaleur et de 4,17 ± 1,2 mlU / ml pendant la période de chauffage. Le taux moyen d'hormone lutéinisante était de 1,72 ± 0,6 mlU / ml à l'extérieur de la chaleur et de 5,0 ± 1,99 mlU / ml pendant le chauffage. Les œstrogènes avaient un taux moyen de 6,25 ± 0,46 µg / ml en dehors de la chaleur et de 22,58 ± 3,37 µg / ml pendant la période de chaleur. Le taux moyen de progestérone était de 6,25 ± 0,46 ng / ml en dehors de la chaleur et de 2,68 ± 2,31 ng / ml pendant la période de chaleur. La connaissance de ces paramètres de reproduction est une étape importante dans le contrôle et la mise en œuvre d'un programme d'insémination artificielle par chameau au Mali.

Mots clés: Dromadaires femelles - Hormones - chaleur - Mali

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Introduction

The recurrent droughts of the years 1972 to 1974 and 1984 have demonstrated the importance of dromedary in the arid areas of northern Mali. Camel milk and meat production and its use as a draught and saddle animal plays an indisputable role in the household economy and in the food security of pastoral populations in northern Mali. In addition to these important economic roles and nutritional contributions, dromedary is intimately associated with nomadic culture. However, dromedary farming encounters several constraints, including the low reproduction rate and a long interval between calvings (Ouologuem and al, 2016). In the pastoral system, the low fecundity of the dromedary is one of the main obstacles to its reproduction. Among the main research topics on dromedary, mastering its reproduction was indicated. This technique requires a perfect knowledge of its reproduction physiology whose parameters seems to variate according to the climatic zones. Indeed, studies by different authors showed that race, diet, climate, and ecology are factors of variation in data (Zarrouk et al., 2003; Pacholek et al., 2000; Nawito et al., 1967; Wiliamson and Payne, 1978). Zarrouk et al. (2003) reported that heats usually occur during

periods of low temperatures, heavy rains, and good quality pastures in the Maghreb. The sexual season runs from March to August in Sudan, from November to April in Arabia and all year round in the equator (Pacholek et al., 2000; Nawito et al., 1967; Wiliamson and Payne, 1978). Poly-oestrus seems to vary according to other hypotheses with the race, the altitude and the air humidity and the mode of breeding (Dahl, 1976; Novoa, 1970; Bosaev et al. 1985). Heat duration varies according to authors: It goes from 20 to 25 days with average of 23.4 days (Joshi et al., 1978) and 28 days (Musa and Abusineina, 1978). Estrus lasts on an average of 4-6 days (Joshi et al., 1978) and 6-8 days (Parkes, 1969; Williamson and Payne, 1978). Zarrouk et al. (2003) found that LH concentration begins to increase one hour after mating, reaches the maximum (3 to 19 ng / ml) in 2 to 4 hours after and begins to decline after 6-8 hours. Basal plasmatic concentrations of oestradiol increase with follicle size (1.3 and 2 cm) to reach 40 pg/ml. Progesterone, a product of the corpus luteum, plays an important role in fertilization and maintenance of gestation. In the case of non-fertilization, the concentration decreases from the 10th day (Zarrouk et al., 2003). In Mali, studies on the reproductive physiology of camels are still embryonic. It is to provide information on the physiological constants of the dromedary reproduction in Mali, that the present "Hormonal profile of the dromedary females during the estrous cycle in sahelian zone" was initiated and executed. This study will contribute to improve the knowledge on the estrous cycle of female's dromedaries on station breeding conditions in the sahelian zone of Mali.

Materials and Methods

1. Material

Description of the study area

The Agricultural Research Station of Niono (ARSN) is located 3 km northeast of the city of Niono in Ségou District. It has 12 000 ha fattening ranch located 20 km east of ARSN. This ranch is divided into three compartments. The second (an area of 4 000 ha) has served as an experimental site for this study. The ranch has a Sahelo-Sudanese climate (Boudet and al, 1970; Cissé, 1986; and Diarra, 1976). This specific climate is due to its border with the Sahel and the Office du Niger irrigation system that cover the entire zone. The average annual rainfall is 550 ± 121 mm and the temperature varies between 25.8°C (January) and 34.7°C (May).

Animals

The herd of camel had 17 female camels out of which 10 were adult females. The average female camel age was 5.5 ± 0.76 years. Females were selected based on two criteria; 1) the pursuit by the dominant male, that indicate that the female is in heat, 2) the female must have more than 2/3 of the weight of an adult female camel. The average weight of the selected females was 355.5 ± 25 kg versus for 380.5 ± 42 kg for adult female. Selected females haven't gate their thirst kid, it means they were nulliparous.

2. Methods

Herd management

Dromedaries were grazed daily from 9 am to 5 pm. Back from pastures, they received an agro-industrial food supplement based on wheat flour at the rate of 3 kg per head. Herd spent the night in a metal park arranged for this purpose while drinking water was provided in a tank (Figure 1) from a drilling powered by a solar pump.

Figure 1: Dromedaries at the drinking point at the ranch of Niono Agricultural Research Station in 2018.

Sanitary treatments

Internal and external deworming was done. Camels were vaccinated against pasteurellosis, anthrax and symptomatic anthrax.

Collection, treatment of blood and preservation of serum

Serum samples were collected for 55 days. Blood samples were taken from jugular vein once a day outside heat periods and at 6:00 AM, 2:00 PM and 10:00 PM during heat periods. Two hundred sixty blood samples were taken from 10 female dromedaries and the serum obtained was stored at -20°C before Elisa method analysis.

Serological analysis

Elisa kits "my Biosource", specific to camels were used to determine female dromedary serum content in progesterone, estrogen, lutein hormone (LH) and follicle-stimulating hormone (FSH).

Principle of the specific Elisa analysis

It is intended to determine hormones concentration in serum, plasma and other body fluids of the dromedary. A purified camel hormone antibody was used to coat wells in ELISA microplates to obtain solid phase antibodies, and then the hormone and its

labeled antibody were added. Reagents become antibody-antigen-antibody-complex enzyme. After washing and addition of chromogens A and B, the enzymatic reaction was stopped by the addition of a sulfuric acid solution and the hormone level was measured spectrophotometrically at a wavelength of 450 nm. The interpretation was made from the standard curve of the specific kit camels of each hormone during the estrous cycle. This curve constitutes the reference with respect to each individual hormonal fluctuation. The micro plate reader in Excel format obtained results directly after reading and conversion of the well results.

Data analysis

Statistical calculations were done with Excel spreadsheet. Mean and standard deviation were determined for all samples for each hormone, and t-test was used to separate means.

Results

Duration of the oestrous cycle

Table I gives the cycle and heat duration per female. The average cycle time was 28.5 ± 1.27 days. The cycle varied from 31 days (F14) to 27 days (F11 and F12). Heat fluctuation duration was on average 5 days.

Table I: Duration of the oestrous cycle and heat in the dromedary female at the Niono ranch from June to December 2018

Number of identified female	Oestrous cycle duration (days)	Oestrus (heat) duration (days)
F03	28	5
F05	28	5
F06	28	6
F07	29	5
F11	27	4
F12	27	7
F14	31	5
F17	30	9
F18	28	5
F08	29	5
Mean ± standard deviation	**28,5±1,27**	**5,6±1,43**

Hormonal profile

Follicle-stimulating hormone (FSH)

Table II gives results obtained from females FSH during the cycle. Only four female samples out of 10 had positive result, because the concentration in other samples was below the threshold of positivity of the FSH camel specific kit (0.32 mlU / ml). Results from the 4 females showed an average significantly higher during heat than in the met-estrus period.

Table II: Concentration of follicle-stimulating hormone (FSH) in the serum samples of female dromedary in estrous and met-estrus periods

Female identification number	FSH fluctuation (mlU / ml)		
	Duration of heats (days)	Oestrus period	Met-estrus
F03	5	2,88	1,14
F05	5	3,52	0,82
F17	9	4,70	2,22
F18	6	5,58	0,58
Mean ± standard deviation	**6,25±1,90**	**4,17 ± 1,2**	**1,19±0,72**
P Value		**0.024**	

Concentration of luteinizing hormone (LH)

Table III gives the level of LH during the estrous cycle. The average level of LH during heat was very significantly higher than in the met-estrus period. The positivity threshold is 0.25 mlU / ml.

Table III: Fluctuation of luteinizing hormone (LH) concentration in serum samples of female dromedary in estrus and met-estrus period (mlU / ml)

Female identification number	Heat duration (days)	LH fluctuation	
		Estrus (heats)	Met-estrus
F03	5	9,17	1,96
F05	5	4,03	0,79
F06	6	2,75	1,58
F07	5	5,00	2,34
F11	4	5,32	2,88
F12	7	1,84	1,42
F14	5	5,68	1,45
F17	9	5,33	1,39
F18	5	6,23	1,73

F08	5	4,60	1,87
Mean ± standard deviation	**5± 1,413**	**5 ± 1,99**	**1,74 ± 0,57**
P value		**0.000**	

Estrogen concentration

The mean rate of estrogens in the estrus period was significantly higher than in the met-estrus (heat) period (Table IV). The favorable positivity threshold for fertilization is 2.75 ng / ml.

Table IV: Estrogen Fluctuation (pg / ml) in the serum sample of female dromedary during the estrus and metestrus periods

Female identification number	Duration days)	Estrus (heats) period	Met-estrus period
F03	5	20,24	5,91
F05	5	15,97	5,41
F06	6	15,96	5,45
F07	5	23,53	6,51
F11	4	23,31	6,37
F14	5	20,74	5,80
Mean	**5 ± 1,41**	**19.96 ±3,37**	**5.91 ± 0,46**
P value		**0.000**	

Progesterone concentration

The average progesterone level was slightly higher in the met-estrus period than in the estrus period, but the difference was not statistically significant (Table V). The threshold of positivity is 1.25 pg / ml. Table V gives the level of progesterone per female at different periods of the estrous cycle.

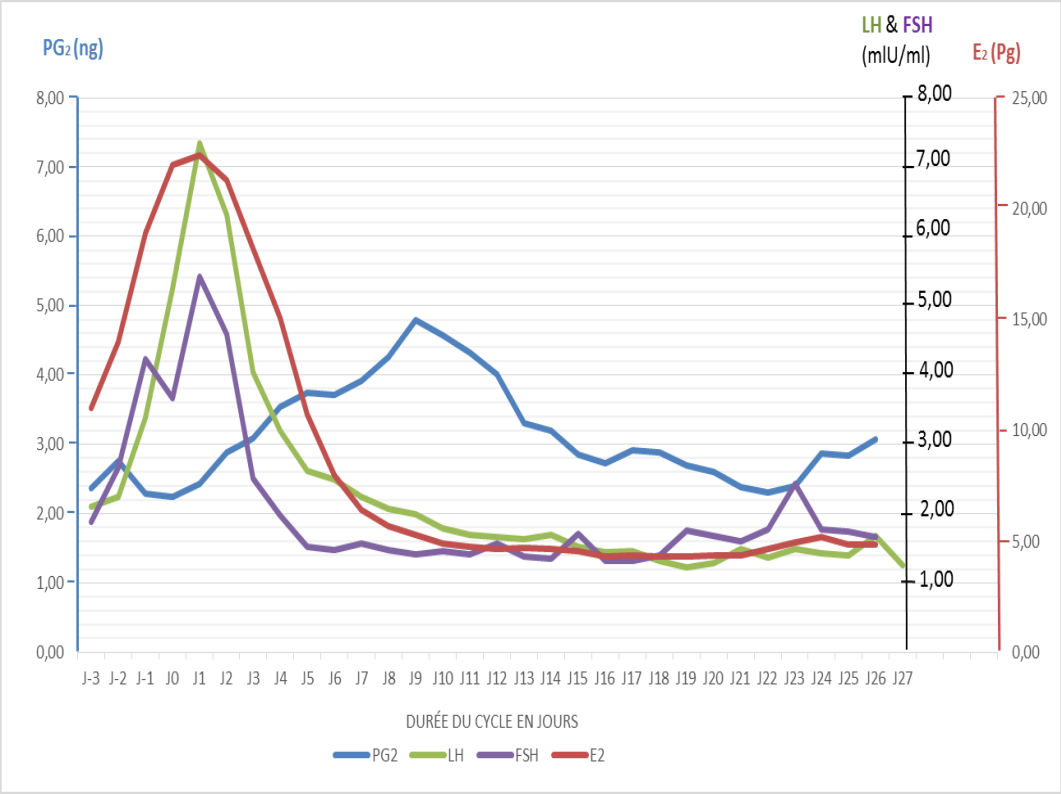
Table V: Hormonal Fluctuation of Progesterone (ng / ml) in serum samples of female dromedary

Female identification number	Duration of oestrus(day)	Progesterone (ng / ml)	
		Estrus period	Met-estrus period
F03	5	7,96	5,20
F06	6	4,95	1,15
F07	5	1,33	5,91
F11	4	1,33	3,12
F12	7	2,17	1,25

F14	5	2,20	7,02
F17	9	1,90	4,63
F18	5	1,25	1,65
F08	5	1,02	3,08
Moyenne	**5,67 ± 1,50**	**2,68 ± 2,31**	**3,67±2,13**
P value		**0,355**	

Summary of hormonal profiles and estrous cycle duration

The synthesis of the hormonal profiles during the duration of the estrous cycle made it possible to obtain the results (Figure 2). This figure indicates that sampling began 3 days before ride when the male became interested in the female by brief pursuits and sniffing of the female's vulva and urines. The peak is the ride day (pro rude). It is found that the peak of LH and FSH is obtained shortly after ride. But, the progesterone level began to rise from the second day to a peak on the eighth day of the cycle, which corresponds to two days after the end of the heat.



PG (ng) = progesterone, LH (mlU / ml) = Luteinizing hormone, FSH (mlU / ml) = Follicle-stimulating Hormone, E2 (pg) = Estrogens.

Figure 2: Progesterone, LH, FSH and estrogen profile during the estrous cycle of female dromedaries in sahelian zone

Discussion

Estrous cycle duration

The average cycle duration is 28.50 ± 1.27 days on average for all 10 females. This 28-day duration was observed by Musa and Abusineina (1978). The average duration of heat (5.6 ± 1.43 days) in our study is similar to the results obtained by Joshi et al. (1978) with an interval of 4 to 6 days and by Musa et al. (1978) with 5 to 6 days. This cycle length can be linked to an individual factor and varies according to breed, breeding system, season and diet.

Hormonal profile

Several authors addressed hormonal profiles (Zarrouk, 2003; Homeida et al., 1988; Musa et al., 1978; Marie, 1987; Adamou and Baira, 2004) and found results similar to those reported from the current study at the Niono station.

Follicle Stimulating Hormone (FSH)

Four females gave values greater than or equal to the positivity threshold (0.27 mlU / ml). In females with a positive threshold, the low rate does not affect the cycle because the follicles are extremely sensitive to the stimulating hormone of the follicle. Therefore, the amount secreted by these females was sufficient to ensure the normal follicular cycle. In contrast, 6 females gave results less than one. This low rate, not specified by the ELIZA chain from the camel kit, affects female fertility by calving spacing.Fluctuations in follicle-stimulating hormone levels during heat and post-heat weather, observed at the Niono station, are consistent with observations of Zarrouk (2003) who concluded that a small increase in follicle stimulating hormone levels was observed 3 to 4 days after mating. This translates into a follicular propel.

Luteinizing hormone (LH)

The average level of luteinizing hormone obtained at the Niono station is 2.79 ± 0.63 mlU / ml. This rate varies from 5 ± 1.43mlU / ml (in heat periods) to 1.72 ± 0.6mlUml (outside heat periods), with a peak of 16.71mlU / ml. The average rate obtained converges with the results of Zarrouk, (2003), Xu (1985), Marie (1987), that ranged between 3 - 19 ng / ml, .7 ± 1.2 ng / ml and 2.7 ng / ml. The results obtained by Homeida et al., (1988) and Wael (2006) were 20 pg / ml and 13 -17.5 mIU / ml, respectively, higher than the averages obtained here. According to Homeida et al. (1988), this rate increases slightly and gradually during the first four months of gestation. The maximum concentration of luteinizing hormone (LH peak) that reflects ovulation lasts only 3 to 4 hours. This concentration regresses beyond 6 hours. This peak of LH occurs at the last stage of follicular development. As emphasized by Homeida et al. (1998), in camels, there is not necessarily a proper luteal phase because in the absence of induced ovulation, there is no cyclic corpus luteum. In this case, follicles that are too big or atresic are anovulatory.

Estrogen

The average rate of estrogen (12.31 ± 4.78 µg / ml) is lower than that obtained by Skidmore (1994) which is approximately 25 µg / ml. This difference in rates can be explained in part by the different methods of analysis and reagents used. Our results, although different, converge with those of Zarrouk (2003) who reports the existence of important individual variations of the estrogen concentration threshold (4 - 11 ng / ml). This is explained by the post-pubertal stage of females, during which low testosterone levels influence estrogen synthesis (Homeida et al., 1988; Musa et al., 1978). In camels of local breeds, entry into reproduction is late and hormonal production is minimal (Faye, 1997). This low rate may also be related to an individual factor. A large and steady increase in estrogen occurs after fertilization (Skidmore, 1994).

Progesterone

Results of this study showed an average rate of 3.08 ± 1.34 ng / ml of progesterone. This rate is similar to those obtained by Marie and Anouassi (1986) and Skidmore (1994). Zarrouk (2003) found this average rate in the range of 3-4 ng / ml between the 8th and 9th day of observation, which corresponds to the 3rd day after the LH peak. The progesterone levels of F11 (3.12ng / ml) and F08 (3.08ng / ml) experienced little fluctuation during the luteal phase. This confirms the protrusion followed by ovulation, because the progesterone is between the maximum fertility threshold (4.4 ng / ml) and the minimum fertility threshold (2.1 ng / ml). This range of progesterone values is favorable for conception because the anti-luteolytic signal is transmitted to the mother between day 7 and 8 (Zarrouk, 2003).
On the other hand, there is a probability of heat return in females F6, F12 and F18 which have a respective rate of 1.15 ng / ml, 1.25 ng / ml and 1.65 ng / ml. These levels of progesterone eventually cause a degeneration of the corpus luteum and the beginning of new follicular wave recruitment.
Females F03 and F14 have progesterone levels above the maximum threshold (4.4 ng / ml). Beyond this threshold, the follicles give eggs that are not fertilizing in camels, and turn into a corpus luteum (Adamou et al., 2014).
Results of heat periods (2.68 ± 2.31 ng / ml) and periods post-heat periods (3.67 ± 2.13 ng / ml) of this study, are close to those of Adamou and Baira (2014) who noted a rate of 2.1ng / ml during heat and a rate of 4 ng / ml outside heat. According to Skidmore (1994), progesterone is maximal between the 3rd and 4th day after ovulation, confirming our result. This is explained by the fact that this Metestrus period corresponds to a time when the follicles are in their luteal phase and the progesterone prepares the endometrium for the maintenance of gestation if fertilization occurs.

Conclusion

The mean values of the rates during the heat periods were: progesterone (2.68 ± 2.31ng / ml), estrogen (22.58 ± 3.87 µg / ml), LH (5 ± 1.99 mlU / ml) and FSH (4.17 ± 1.20).

mIU / ml). The mean cycle time was 28.50 ± 1.27 days and 5.6 ± 1.43 days of heat. The optimum timing of females protruding and duration of heat as well as the peak of LH were better known. This study has expanded our knowledge on female camel reproduction in Mali outside of their natural habitat. Studies such as early diagnosis of pregnancy must be carried out of the dromedary reproduction control with the ambition to establish a successful national program of artificial insemination in Mali.

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