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Variation of Blood Prolactin Concentration at Different Reproductive Stages in the Maghrebi She-Camel.

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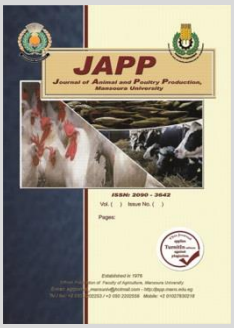

ABSTRACT

During breeding season, plasma prolactin concentration levels were measured pre-mating and monthly up to the 12th months post-mating in all animals (n=20), and post-calving only in pregnant animals (n=17) of 20 Maghrebi she-camels. The experimental she-camels were divided according to their parity order (1st to 6th parity). Concentration of prolactin was determined of all parities at different reproductive stages (Pre-mating, pregnancy stages, and post-calving). Results showed insignificant (P≥0.05) effect of camel parity on prolactin concentration at each reproductive stage, but prolactin showed similar trend of changes within each parity by advancing reproductive stage. Plasma prolactin nearly maintained its levels during the period from pre-mating up to the 9th month post-mating, then started to increase (P<0.05) up to the 12th months post mating. Prolactin showed similarity in pregnant and non-pregnant during the 1st month post-mating and insignificant differences from the 3rd month up to the 9th month post-mating, thereafter showed significant increase in pregnant than in non-pregnant up to the 12th months post-mating.From the results of the study, it is clear that the effect of camel parity order/age on blood prolactin levels is limited. However, blood prolactin level was pronouncedly affected by reproductive status of she-camels such as pre-mating estrous activity, pregnancy stage, and calving and lactation.

***Keywords**: She-camel, plasma prolactin, reproductive status, pregnancy.*

INTRODUCTION

With the rising importance of camels and the increase in research conducted on them, information about their endocrine glandular activity and hormonal performance is still limited and shrouded in a lot of ambiguity, especially the hormone of prolactin (PRL). This hormone is the most multitasking hormone among pituitary hormones, which plays prominent roles that may have more than a hundred effects (DeVlaming, 1979). The functions and roles of PRL vary according to the diversity of the ranks and families of the animal kingdom from the lowest to the highest in development (mammals), starting with its role in the process of osmotic regulation of fish, growth and moulting in crustaceans, amphibians and reptiles, through the phenomenon of fungal nesting in birds, up to its role in the development of milk gland and the initiation of lactation in mammals. It is well known that prolactin is a protein hormone with a molecular weight of more than 20,000 Da and is secreted by the pituitary gland. The rate of prolactin secretion varies according to a variety of environmental, physiological and psychological conditions. The undergo levels of blood plasma prolactin are subject to daily periodic variations, with their highest levels being during the darkness of the light-dark cycle (Ronnekleiv *et al*., 1973; Munro *et al*., 1980). Prolactin plays a prominent role in mammalian general health and as an indicator of it, as its concentration increases in the blood coinciding with an increase in cortisone concentration in affected and stressed animals, where it is a clear documented marker of stress in animals (Kataria *et al*., 2000).

The concentration of PRL and cortisone in the serum of infected animals is more than four times greater than their concentration in the blood of healthy animals (Nalini and Anil, 2010). Researches indicate the suitability (appropriateness) of PRL to be a sensitive marker on both physical and psychological stress in camels (Gala, 1990; Nalini and Anil, 2010). Researches on increased levels of PRL and cortisol in affected camels was in agreement with early reports of significant increases in PRL and cortisol concentrations in stressed cows (Ahmadzadeh *et al*., 2006) and stressed rats (Deis *et al*., 1989; Jean Kant *et al*., 2002). It is possible that increased prolactin in affected animals is a mechanism for increasing threshold limits of pain (Ramaswamy *et al*., 1983) and a protective behavior (Drago *et al*., 1982).

Regarding the contributions of PRL to reproduction, as it is believed to interfere in the production of ovarian steroids, especially estrogen, by inhibiting FSH-induced aromatase activity, suppressing the manifestations of behavioral lust and ovulation, and reducing the pituitary gland release of gonadotropins in mammals (Tindal, 1974; Karg and Schams, 1974). The secretion cycle of PRL has been observed in many mammals including dairy cows (Gustafson, 1994), buffaloes (Singh and Madan, 1993), Asian pigs (Gromadzka *et al*., 1999), rats (Clark and Baker, 1964), and humans (Nokin *et al*., 1972; Stern and Reichlin, 1990). However, the information on PRL profile in she-camels in the literature are scare.

In addition, PRL has an influence and participation in many activities related to reproduction, such as stimulating the development of the milk gland during pregnancy and regulating lactation after parturition, and also has an effect on ductal glands such as the prostate gland and the lacrimal gland (Rossi, *et al*., 2002). Just as PRL stimulates the production of

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milk in the post-pregnancy period, it stimulates the growth, development and metabolism of the embryo, and this with its importance in the decay of the corpus luteum and the reduction of sexual steroids levels during the cycle of estrus. In addition to that it stimulates the process of ovulation, implantation and development of the placenta (Perks *et al.*, 2003).

The present study aimed to determine PRL profile in the blood of the Maghrebi she- camel with different ages and parities at various reproductive stages.

MATERIALS AND METHODS

The current study was performed in the Department of Animal Biotechnology, Animal Production Research Institute, Egypt. The experimental work was carried out in a Private Camel Farm, Marsa Matrouh Governorate, located in the North of Egypt, closest area to the western border of Egypt. This study lasted from November 2018 to June 2020.

Animals:

Twenty healthy Maghrebi she-camels (*Camelus dromedarius*) at different ages (3-20 year), parities (1-6 parities), and live body weight (395-563 kg) were used in this study. They were kept in sand bedded stockyard discrete from male's refuges, and wide paddock for exercising throughout the experimental period.

Feeding system:

The ration was consisted of 7 kg of a forage mixture barley straw *(Hordeum vulgare)* and 3-4 kg of a commercial feed concentrate mixture (12% CP). Ration per animal was offered twice daily at 8 a.m and 6 p.m. The rations were augmented 1-2 kg of a commercial feed concentrate mixture daily for the pregnant she-camel. The additions were given as of the ninth month from the gestation. This process was submitted to farm customary regime without any methodological interfering in it.

Experimental design:

All the experimental camels (n=20) were divided according to their ages and parities into 6 parities including 1st parity (3-5 y, n=5), 2nd parity (>5-10 y, n=3), 3rd parity (>5-10, n=3), 4th parity (>10-20 y, n=3), 5th parity (>10-20 y, n=3), and 6th parity (>10-20 y, n=3). Live body weight was 395-465, 460-515, and 517-563 kg for she-camels at ages 3-5, >5-10, and >10-20 y, respectively. She-camels were mated during one cycle of estrus (Breeding season). They were prepared for pollination during a mating season that extended from January to April, and guided by the abundant information recorded on the length of the estrus cycle in one-humped camels, which are abbreviated in the following: estrous cycle, it long ranged from 25-30 days and the duration of estrus was around 4-5 days with absence of the luteal phase (Al-Sobayil, 2003). Non-pregnant she-camels were retained until the next breeding season. The distribution of 20 mated she-camels was 17 pregnant as well as 2 non-pregnant she-camels at the first parity and one non-pregnant she-camel at the 6th parity.

Blood samples:

The blood samples were collected from the Jugular vein of three camels in each parity morning before feeding. Blood samples were taken pre-mating, then monthly post-mating up to the 13 months post-mating. During the breeding season that was targeted by the study, any she-camel return to estrus after a mating, blood samples were excluded and other blood samples were collected pre the next mating. Blood samples were started to collect in at end January 2019 and continued to April 2020.

Blood samples were collected in tubes containing EDTA, centrifuged at 3000 rpm for 15 min, then clear plasma were carefully drawn into micro covets and stored at -20 ºC until the time of PRL analysis.

Prolactin assay:

The plasma PRL concentrations were measured by specific radioimmunoassay using commercial kits of Diagnostic systems laboratories (DIA source Immuno Assays S.A.), according to the method previously described by Archer (1977) and Leong *et al*. (1983**)**.

Statistical analysis:

Data obtained were statistically computed by SAS (2002) version 9.0 to using one-way ANOVA to test the effect of parity order on PRL level of she-camels at each reproductive stage. T-test was used to determine the differences between pregnant and non-pregnant she-camels. Duncan's Multiple Range test (Duncan 1955) was set to detect the significant differences among means at $P<0.05$.

RESULTS AND DISCUSSION

Result

Prolactin profile as affected by camel parity order/age:

Plasma prolactin concentrations (ng/ml) of Maghrebi she–camels with different prairies (ages) at various reproductive stages are presented in Table 1. Statistical analysis revealed insignificant ($P\geq0.05$) effect of camel parity order on PRL concentration at each reproductive stage. Results showed slight increase in PRL level by advancing camel parity, but the differences among parities were not significant. On the other hand, PRL level showed similar trend of changes within each parity by advancing reproductive stage (Table 1).

Prolactin profile at different reproductive stages:

The trend of change in overall mean of PRL concentration of she-camels at different reproductive stages during the period from before mating up to 13 months post-mating (12 mo post mating for non-pregnant animals or post-calving of pregnant animals only) is illustrated in Figure 1. It is evident from the results that plasma prolactin nearly maintained its levels during the period from pre-mating up to the 9th month post-mating with insignificant decrease one-month post-mating. After the 9th month of mating, PRL level started to show significantly ($P<0.05$) a gradual increase up to the 12th months post mating for all animals and post-calving in pregnant animals only. This trend of change indicated that the increase in PRL level post-calving was higher by about 423.5and 209.7% as compared to its level pre-mating and one-month pre-calving, respectively (Fig. 1).

Table 1. Plasma prolactin concentrations (ng/ml) of Maghrebi she–camels at different prairies (ages)at various reproductive stages (mean±S.E).

Stage	Parity (Age, y)						P Value
	1 (3-5 y)	2 (>5-10 y)	3 (>5-10 y)	4 (>10-20 y)	5 (>10-20 y)	6 (>10-20 y)	
Pre-mating	3.28±0.233	3.35±0.307	3.42±0.181	3.56±0.141	3.56±0.265	3.35±0.076	0.9212
1 mo post	2.34±0.184	2.39±0.155	2.39±0.236	2.39±0.291	2.39±0.178	2.33±0.033	0.9997
2 mo post	2.32±0.106	2.39±0.156	2.40±0.147	2.31±0.181	2.27±0.135	2.26±0.066	0.9714
3 mo post	2.50±0.130	2.29±0.164	2.27±0.138	2.30±0.057	2.09±0.118	2.23±0.233	0.4977
4 mo post	1.74±0.241	2.12±0.141	2.13±0.124	2.20±0.023	2.13±0.075	1.70±0.300	0.3509
5 mo post	1.79±0.143	2.09±0.106	2.32±0.244	2.09±0.051	2.10±0.000	1.73±0.166	0.1104
6 mo post	1.81±0.069	1.96±0.088	2.03±0.037	2.05±0.02	2.01±0.049	1.87±0.086	0.1087
7 mo post	1.85±0.121	1.73±0.130	1.73±0.155	1.79±0.121	1.72±0.118	1.79±0.153	0.9665
8 mo post	1.99±0.176	1.70±0.146	1.75±0.141	1.77±0.075	1.72±0.072	1.70±0.000	0.6180
9 mo post	2.08±0.209	1.80±0.057	1.86±0.040	1.83±0.040	1.80±0.000	1.93±0.233	0.7537
10 mo post	3.34±0.653	4.31±0.594	4.90±0.692	4.39±0.28	4.26±0.092	3.46±0.733	0.4393
11 mo post	3.98±0.837	5.50±0.796	5.46±0.857	6.10±0.750	5.82±0.456	4.16±1.133	0.3777
12 mo post	7.78±2.489	12.33±2.339	11.70±1.443	12.40±1.270	12.58±0.993	8.41±3.206	0.4522
Post-calving	12.52±4.287	20.60±2.286	21.29±1.885	20.90±1.616	21.40±1.039	13.90±5.950	0.3049

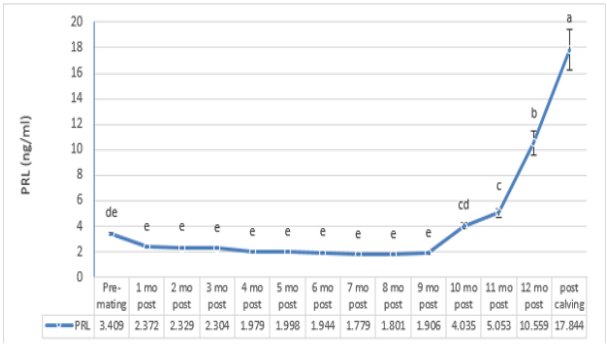

Fig. 1. Change in plasma prolactin concentration (ng/ml) of Maghrebi she–camels at different reproductive stages.

Prolactin profile in pregnant and non-pregnant she-camel:

The differences in plasma prolactin concentration (ng/ml) of pregnant and non-pregnant Maghrebi she–camels at different reproductive stages are shown in Fig. 2.

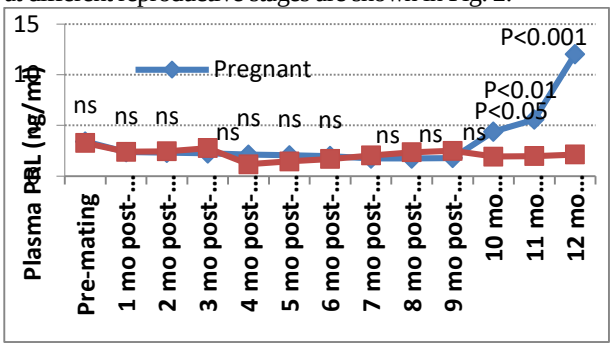

Fig. 2. Change in plasma prolactin concentration (ng/ml) of pregnant and non-pregnant Maghrebi she–camels at different reproductive stages.

Results cleared that PRL level showed similarity in pregnant and non-pregnant during the 1st month post-mating and insignificant differences from the 3rd month up to the 9th month post-mating, thereafter PRL level showed significantly remarkable increase in pregnant than in non-pregnant camels up to the 12th months post-mating (during the last three months of pregnancy in pregnant animals (Fig. 2).

Discussion

Based on the foregoing results, we found that pre-mating plasma PRL concentration of she-camels. Also, PRL concentration was low in non-pregnant than pregnant she-camels up to 12 months post-mating. The above-mentioned results, especially with regard to pre-mating or non-pregnant she-camels, it appears that the concentration of PRL in the blood of camels is generally low as reported by several authors (Marichatou *et al*., 1998; Al Qarawi and El Mougy, 2008; El Allali *et al*., 2018). The blood PRL concentration in camels is clearly lower than that in the blood of other ruminants, even those ruminants that are much smaller in size such as goats (Prandi *et al*., 1987) and sheep (Santiago-Moreno *et al*., 2000). It was observed the relative increase in the values of prolactin concentration in the blood samples of all she-camels that were taken in the day before pollination was ≥3 ng/ml compared to > 4 ng/ml in pregnant animals during the last three months of pregnancy and about 2 ng/ml in non-pregnant animals at the same interval. This finding may indicate the effect of reproductive status (pregnancy) on PRL profile in camels as found in other ruminants (El Allali *et al*., 2018). Concentration of PRL in reflect the general physiological conditions (pregnancy, parturition or lactation). An increase in blood PRL concentration is closely related to an increase in estrogen concentration. Estrogen stimulates the growth of the pituitary gland, promotes the synthesis and secretion of PRL, which increases its concentration systemically (Perez *et al*., 1986). Estrogen affects the release of PRL directly by acting on the pituitary gland or indirectly by affecting the dopamine cycle in the hypothalamus and pituitary (Maha *et al*, 2017). The concentration of estrogen in the blood gradually increases with the increase in ovarian activity and follicular growth during the estrous cycle, which leads to the evolution of estrous behavior and symptoms on the female and her acceptance of the male. It is known that an increase in the concentration of milk estrogen is an indicator of the occurrence of the estrous cycle (Lopez and Bunch, 2002), and there is a correlation and similarity between estrogen concentration in both milk and blood (Schams and Karg 1986). Progesterone is known to decrease the release of PRL (Neill and Smith, 1974). The decreases observed in PRL concentration of pregnant Maghrebi she-camels from the first months to the ninth month of pregnancy may be due to a

negative relationship between PRL and progesterone. In rats, the decrease in blood PRL concentration at early stages of pregnancy may be due to an elevated progesterone level (Dondr *et al*., 1991).

As such, we observed a significant increase in the concentration of PRL at the beginning of the last quarter of pregnancy, represented by the tenth month of pregnancy. This finding may be due to the demise of the negative effect of progesterone, which deteriorates the rate of its secretion during the last stage of pregnancy (Devorshak *et al*., 1987), accompanied by the positive retroactive effect of estrogen, its concentration gradually increased with pregnancy, clearly elevated in the later stages of pregnancy (Bridges, 1984; Devorshak *et al*., 1987; Dondr *et al*., 1991). The approaching date of labor was accompanied by a more significant increase in the level of blood PRL in pregnant she-camels. This increase in PRL concentration may be due to the aforementioned two effects of the deterioration in the rate of progesterone secretion and the growing rise in the concentration of estrogen in addition to the effect of stress that leads to an increase in the concentration of PRL. Increase in PRL levels was reported in stressed dromedaries (Kataria *et al*., 2000).

Prolactin could serve as a sensitive marker of both physical and psychological stress in camels (Gala, 1990). It is actually well known that, while progesterone depresses PRL secretion, estrogen and stresses of various nature facilitate the release of this pituitary hormone (Neill and Smith, 1974). In pregnant she-camels, blood levels of PRL increased after calving (before the period of colostrum milk passing) at very significant rates in comparison to before calving, and its concentrations doubled, the same behavior was recorded for PRL in other mammals such as humans. During fetal life and in newborns up to one week old, serum PRL levels are greater than 200 ng/mL (Parks, 2004). A similar behavior was recorded for PRL in rats (Linkie and Niswender, 1972; Dondr *et al*., 1991). The significant elevation of PRL may be attributed to the aforementioned factors of progesterone decay (Devorshak *et al*., 1987) and elevation of estrogen concentration. Estradiol increases PRL production, and is particularly important for females after labor (Maha *et al*., 2017) as well as the consequences of the effect of labor stress (Kataria *et al*., 2000), accompanied by the influence of other factors related to the postpartum period, including the effect of lactation, which PRL plays a prominent role in regulating it in all mammals, and effect of suckling.

The recorded elevation in PRL level at late pregnancy and early post-partum is paralleled with that PRL is characterized by influencing and particulating in reproductive activities such as stimulation of mammary gland development during pregnancy and regulation of postpartum lactation (Rossi *et al*., 2002). Also, PRL stimulates the production of milk during the postpartum period (Perks *et al*., 2003). In human, PRL levels during lactation should not exceed 200 ng/ml. If this occurs, other causes of hyperprolactinemia should be sought. These prolactin levels may remain high as long as the child is suckling (Melmed *et al*., 2003). Also, the levels of PRL secretion after birth are so high that there is a fear of hyperprolactinemia if the concentration of PRL in the blood exceeds (200 ng/ml), but in the she-camels, the level of PRL in the blood of she-camels is generally low (Al Qarawi and El Mougy, 2008; El Allali *et al.*, 2018).

Pre-mating plasm PRL concentration increased insignificantly in she-camels during the day before pollination compared to its levels during the other post-mating months. These results reinforced that the increase in blood PRL concentration is closely related to the increase in estrogen concentration associated with the growth and development of follicles and an increase in ovarian activity during breeding season. The growth of the pituitary gland is stimulated and the synthesis and secretion of PRL is enhanced by the action of estrogen (Perez *et al*., 1986). In comparable with the present results, El Allali *et al*. (2018) found that blood PRL levels in she-camels at estrous period and three months after pollination decreased to averages of less than 3 ng/ml with an overall mean of (2.76 ± 0.06 ng/ml). In the fourth month after (during June), which coincided with the beginning of the summer season, the level of blood PRL decreased sharply (1.17 ± 0.16 ng/ml), rising gradually within this very low rate until the sixth month after mating (1.69 ± 0.06 ng/ml). In non-pregnant she-camels, this dramatic decrease in blood PRL levels coincided with the solstice of summer. The plasma PRL concentrations in guanacos (*Lama guanicoe*) were higher during short days than long days (Correa *et al*., 2020).

The great symmetry between the levels of blood PRL concentration in the monthly overall mean as affected by parity order (age) may be due to the close and logical relationship between the parity order and age advancing. The results may differ or change, if hypothetical conditions would be available that contradict this realistic relationship, such as if we get a she-camel that produced its first pregnancy after it exceeds the age of ten years (it gives first calving after it becomes in the age hierarchy, we may then find results different in value and significance level.

CONCLUSION

From the results of the study, it is clear that the effect of camel parity order/age on blood prolactin levels is limited. However, blood prolactin level was pronouncedly affected by reproductive status of she-camels such as pre-mating estrous activity, pregnancy stage, and calving and lactation.

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التغير في تركيز البرولاكتين في الدم خلال مراحل تناسلية مختلفة فى النوق المغربي

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تهدف الدراسة الى تقييم تركيز هرمون البرولاكتين فى بلازما دم النوق المغربي خلال المراحل التناسلية المختلفة ولذلك تم قياس مستويات البرولاكتين في بلازما الدم خلال موسم التربية قبل التزاوج وبشكل شهري حتى الشهر الثاني عشر بعد التزاوج في جميع الحيوانات (عدد 20 من النوق المغربي) ، و بعد الولادة فقط في الحيوانات الحامل (عدد 17) من 20 من النوق المغربي. ولذلك تم تقسيم النوق وفقًا لترتيب موسم الولادة (من الأول إلى السادس).و تم تقدير تركيز هرمون البرولاكتين في بلازما الدم لجميع مواسم الولادة في مراحل تناسلية مختلفة وهى (ما قبل التزاوج ، ومراحل الحمل ، و وبعد الولادة). وقد أظهرت النتائج: أن كان لتعدد مواسم الولادة للنوق تأثير غير معنوي ($P\geq0.05$) على تركيز البرولاكتين في كل المراحل التناسلية ، أظهر البرولاكتين تغيراً متشابه فى الاتجاه داخل كل موسم خلال المراحل التناسلية. حافظ برولاكتين البلازما تقريبًا على مستوياته خلال الفترة من قبل التزاوج و حتى الشهر التاسع بعد التزاوج ، ثم بدأ في الزيادة المعنوية ($P<0.05$) حتى الشهر الثاني عشر بعد التزاوج. أظهر مستويات تركيز البرولاكتين تشابهًا في النوق الحوامل وغير الحوامل خلال الشهر الأول بعد التزاوج واختلافات غير معنوية من الشهر الثالث حتى الشهر التاسع بعد التزاوج ، وبعد ذلك زاد تركيز مستوى هرمون البرولاكتين معنويا في النوق الحوامل مقارنة بغير الحوامل حتى الشهر الثاني عشر بعد التزاوج. نستنتج من هذه الدراسة : أن تأثير تعدد مواسم ولادة النوق / العمر على مستويات البرولاكتين في الدم محدود. بينما كان مستوى برولاكتين في الدم يتأثر بشكل واضح بالحالة التناسلية للنوق مثل النشاط الشبقي قبل التزاوج ، ومرحلة الحمل ، والولادة والحليب.