

STUDIES ON PITUITARY-OVARIAN AXIS IN THE FEMALE CAMEL WITH SPECIAL REFERENCE TO CYSTIC AND INACTIVE OVARIES

By

A.A. Hegazy, A. Ali, M. Al-Eknah and S. Ismail

**College of Veterinary Medicine & Animal Resources,
King Faisal University, Al-Ahsa, Saudi Arabia.**

ABSTRACT

The present investigation was performed on 190 female camels slaughtered at Al-Ahsa Modern Slaughterhouse throughout one year period. Blood samples, pituitary glands and ovaries were collected from the animals at the time of the slaughter. Follicle stimulating hormone (FSH), luteonizing hormone (LH), oestradiol-17 β (E₂) and progesterone (P₄) hormones were determined in cases of cystic and inactive ovaries. The follicular ovarian wave was also studied. The ovarian examination revealed the increase incidence of inactive ovaries during summer and cystic ovaries during spring and autumn. Histological and histochemical pictures of pituitary gland during the inactive, cystic and ovarian follicular wave were described and discussed.

This study was financially supported by Deanship of Scientific Research, King Faisal University, Saudi Arabia.

INTRODUCTION

The female camel is a seasonally polyoestrus and induced ovulator. It has been found that the follicular growth occurs in regular waves during the breeding season (Musa et al, 1993), where waves of follicular growth, maturation and atresia occur constantly in both ovaries (Musa 1976, Elwishy and Hemeida 1984).

Al-EknaH et al (1993) recorded four distinct uterine phases corresponding to ovarian activity (follicular, atretic follicular, non-follicular and growing follicular stages). FSH and LH control growth and reproductive activities of the gonadal tissue (Franchimont, 1973 and Daughadny, 1985). The gonadotrophic cells of the pituitary gland secrete both FSH and LH in response to gonadotrophic releasing hormone LHRH or GnRH) from the medial basal hypothalamus. The release of both FSH and LH from the pituitary gland is under negative feedback control by the gonads (Bonnar 1973).

The incidence of cystic and inactive ovaries among Saudi female camels increases in summer (Hegazy et al, 2001). The actual cause has not been elucidated. Ovarian cysts are believed to be due to deficient LH surge (Jubb and Kennedy, 1993). However, inactive ovaries are attributed to the adverse body condition (Tibary and Anouassi, 1997).

To the best of our knowledge no previous study has been conducted to investigate the pituitary-ovarian axis in the female camel. Therefore, the present study aimed to investigate the cellular activity of the pituitary gland in cases of active, inactive and cystic ovaries and to determine FSH, LH, E₂ and P₄ concentrations in relation to cellular activity of par distalis and ovarian changes.

MATERIALS AND METHODS

This study was performed on 190 female camels slaughtered in Al-Ahsa Modern Slaughterhouse throughout one year period (January to December 2001).

10 ml blood samples were collected from each animal before slaughter in silicon-coated tubes. Sera were separated and marked according to the ovarian picture and stored at -40 °C for hormonal analysis.

The ovaries of each animal were grossly examined and ovarian structures were recorded.

A total of 100 pituitary glands were collected randomly representing the different ovarian cases. The glands were immediately dissected from the animals just after slaughter and fixed directly in 10% neutral formalin. Tissue samples were collected and processed by paraffin embedding method. Serial sections 4 μ in thickness were performed and stained by H&E stain (Harris, 1898), Orange-Fuchsin Green (Slidder, 1961), Halmi (1952), PFA, AB, PAS, Orange G (Adams, 1956).

The cell counting of anterior pituitary glands was performed using the technique adopted by Kandil (1975). Three fields in the anterior pituitary were chosen. The first field was adjacent to the cleft, the second field in the core and the third one was in the posterior part.

The analysis of FSH, LH, E₂ and P₄ was performed on 51 serum samples (20 active ovaries, 16 inactive ovaries and 15 cystic ovaries (11 follicular and 4 luteal). Hormonal evaluation was performed using ELIZA method and kits of Abbot Laboratories, U.S.A.

RESULTS

A. Ovaries

The incidence of ovarian activity, inactive and cystic ovaries in the female camels during the different seasons of the year is presented in table (1).

Table 1: The incidence of ovarian changes in the different seasons

Ovarian picture	Autumn No. P/S	Winter No. P/S	Spring No. P/S	Summer No. P/S
Follicular wave	28 56	34 85	46 70.76	10 28.57
Cystic ovaries				
A. Follicular	5 10	3 7.5	7 10.76	5 14.25
B. Luteal	3 6	1 2.50	5 7.69	1 2.85
Inactive ovaries	14 28	2 2.50	7 10.76	19 54.28
Total	50 100	40 100	65 100	35 100

P/S = Percentage per season.

The total incidence of the ovarian changes among examined cases per year is presented in table (2).

Table 2: The incidence of ovarian changes among examined cases per year

Ovarian picture	Autumn	Winter	Spring	Summer	Total	Percentage
Follicular wave	28	34	46	10	118	62.33
Cystic ovaries						
A. Follicular	5	3	7	5	20	10.46
B. Luteal	3	1	5	1	10	5.23
Inactive ovaries	14	2	7	19	42	21.98

The study on the ovarian changes revealed that the incidence of inactive ovaries occupied the highest percentage of ovarian abnormalities and reached its peak on summer season. However, the follicular cysts occupied the second place and reached its peak in summer followed by spring then autumn.

B. Hormonal Analysis

The mean values of FSH, LH, E₂ and P₄ in relation to the ovarian changes are illustrated in table (3).

Table 3: Mean values of FSH, LH, E₂ and P₄ in the different ovarian changes

Hormone	Active ovaries	Inactive ovaries	Follicular cystic ovaries	Luteal cyst
FSH	0.2135 0.0-1.2	0.1515 0.0-0.01	0.039 0.0-0.15	0.1175 0.01-0.22
LH	0.0643 0.0-0.32	0.065 0.0-0.55	0.0127 0.0-0.08	0.0975 0.3-0.19
E ₂	14.72 1.24-67.23	69.79 24.7-117.0	34.48 13.4-64.2	31.1 10.4-41.9
P ₄	1.1165 0.0-4.7	4.483 1.0-21.4	3.27 0.33-10.3	1.657 0.57-3.4

The hormonal analysis in case of cystic ovaries (follicular cysts) revealed the decrease of FSH and LH concomitant with the increase of E₂ and P₄ levels in comparison with that of active follicular wave.

In case of inactive ovaries, there was marked increase of E_2 and P_4 with decrease of FSH in comparison to the hormonal level in cases of active ovary.

In case of luteal cyst, there was an increase in the level of LH, E_2 and P_4 than in case of active ovaries with decreased FSH.

C. Pituitary glands

The histological study indicated that pituitary glands of the female camel are subdivided into adenohypophysis and neurohypophysis. The adenohypophysis consisted of three portions: pars distalis, pars tuberalis and pars intermedia.

The pars distalis and pars intermedia were separated from each other by interglandular cleft. A fibrous capsule of collagenous fibers continuous with the stromal fibers cover it. The parenchyma consisted of cords, clusters or pseudofollicles.

The cells of pars distalis were divided into acidophilic basophilic and chromophobe cells. The acidophilic cells were located at the central and posterior parts of the anterior pituitary glands. The types of acidophilic cells: the somatotrophic cells (STH) and lactotrophic cells (LTH) were recognised. The somatotrophic cells were large polyhedral and mostly localised in the posterior parts. The cells contained coarse intra cytoplasmic granules yellow in colour. The lactotrophic cells were mostly located in the centre of the gland. They were variable in shape, oval, round or elongated with eccentric vesicular nuclei and cytoplasmic granules stained orange red.

The number of basophilic cells are lesser than acidophilic one. The cells were located mostly in the peripheral parts of pars distalis, next to hypophysial cleft and the boundaries of blood vessels. The basophiles were differentiated into gonadotrophic cells, which were more abundant than thyrotrophic cells.

Thyrotrophic cells appeared polygonal containing coarse cytoplasmic granules stained magenta red by PAS after oxidation with performic acid and blue by Sleder stain. Gonadotrophic cells were arranged mostly at the boundaries and near by sinusoids. They were smaller in size and contain fine granules stained blue by alcian blue after oxidation with performic acid and red (LH) or purple (FSH) by PAS.

In case of cystic ovaries, the adnohypophysis revealed that great number of basophilic cells were stuffed with basophilic granules (purple or red by PAS). The cells appeared larger in size, swollen and its nucleus was vesicular in appearance. Degranulation and vacuolisation of some basophilic cells were observed. It was noticed that most of these cells were located near by blood vessels and faintly stained.

Acidophilic cells show no significant variation in number. However, many cells show homogenous eosinophilic cytoplasm and pyknotic nuclei. These cells were identified as lactotrophic cells. The adenolypophysis in case of inactive ovaries showed smaller basophilic cells with fewer amount of basophilic granules, which were clearly observed by Slidder and PFAAB. PAS Orange G stains.

Many gonadotrophic cells (FSH and LH) were degranulated and vacuolated and some of them resemble chromophobe cells. Some of these cells appeared degenerated with pyknotic nuclei and vacuolated cytoplasm. Acidophils appeared also smaller in size with decrease in its cytoplasmic granules. The LTH cells were faint staining y acidic dye. Excessive amount of colloid substances were observed in the acinar like structure.

In case of luteal cyst, the gonadotrophic cells appeared large containing coarse basophilic granules stained purple or red. The LTH cells were also large with well distinct acidic cytoplasmic granules stained orange red, while the STH appeared large with eccentric nuclei and yellow cytoplasmic granules. The thyrotrophs appeared large with cytoplasmic bluish granules.

The amount of different pituitary cells are presented in table (4).

It is clear from table (4) that the number of gonadotrophic and thyrotrophic cells were decreased in case of inactive ovaries concometant with increase in number of acidophils in comparison to that observed in case of active ovaries. In luteal cysts the number of gonadotrophic, thyrotrophic and lactoptrophic cells were increased in comparison with that of active ovaries. In case of follicular cyst the number of gonadotrophs and thyrotrophs was comparable to that of active ovaries with minimum increase in number of lactotrophic cells.

Table 4: The percentage of different cells in the anterior pituitary in different ovarian status.

Ovarian Status	Acidophils			Basophils			Chromophobs
	STH	LTH	Total	GT	TH	Total	
Active Ovaries	30.1	33.2	63.3	24.3	10.28	34.58	2.19
Inactive Ovarie	36.2	34.1	70.3	18.7	8.26	26.96	2.74
Cystic Ovaries	28.4	34.3	62.7	24.35	10.4	34.45	2.93
Luteal Cyst	23.39	32.3	55.39	30.0	12.62	42.11	2.5

STH: Somatotrophic cells

LTH: Lactotrophic cells

GT : Gonadotrophic cells

TH : Thyrotrophic cells

DISCUSSION

The available literature indicates the absence of any previous study on pituitary ovarian axis in the female camel. However, the pituitary gland of male camel and the effect of seasonal variation on pituitary-testicular axis has been studied (Fahmy and Nasr, 1963; Ismail et al, 1985).

The present study revealed that the ovarian activity was observed throughout the different seasons with a maximum activity during winter, which corresponds to the breeding season. Shalash (1965) and Akral et al (1995) reported similar results in Egypt and Pakistan, respectively. In Saudi Arabia Arthur and Al-Rahim (1982) reported that breeding occurs throughout the year under good nutritional condition.

The incidence of inactive ovaries in the present study reached its peak summer, which may be responsible for the failure of conception during summer time as reported by Arthur et al (1985); Abdel Rahim and El Nazier (1992). This may be attributed to the higher temperature associated with adverse nutritional status of the animals during the summer season (Tibary and Anouassi, 1997).

In the present study, it was clear that in case of inactive ovaries the activity of pituitary gland was lower in comparison with that of active ovaries. Moreover, the FSH hormone in the plasma is fewer than that of

active ovaries. This may give an explanation for the increase of the ovarian inactivity in summer.

The cystic ovaries were observed throughout the whole year with variable percentage varied between 10.76% in summer and 5% in autumn. Similar results have been obtained by Hegazy et al (2001) in Saudi Arabia. It seems that the incidence of cystic ovaries increases in summer and autumn. It is believed that the problem of cystic ovaries is the deficiency of LH surge, which is confirmed by the present results of hormonal analysis indicating the low level of LH hormone in cases of cystic ovaries in comparison with that of normal cyclic ovaries.

The hormonal level in case of cystic ovaries revealed the decrease in LH level in comparison with that of normal cyclic animal. This may give an explanation for the cystic follicle formation. The increase of E2 and P4 levels is considered a sequel of cyst, which may secrete progesterone or oestrogen depending on the degree of granulosa cells luteinization (Jubb & Kennedy, 1993).

In case of inactive ovaries, there was a decrease level of FSH which may explain the failure of the ovary to develop follicle. The decrease of FSH may be due to the increase of ovarian steroid hormones as a consequence of feedback mechanism. The increase of progesterone and oestrogen levels in case of inactive ovaries cannot be explained. However, it may denote to the exogenous source of secretion of these hormones other than ovaries, which needs further investigation.

In case of luteal cyst, there was an increase in the level of LH progesterone and oestrogen than the in case of active ovaries. This was concomitant with the decrease in FSH. The increase of oestrogen may be due to the growing follicle, which always detected in association of with the corpus luteum.

The previous investigation on the histological and histochemical study of the pituitary gland of the female camel cannot be traced in the available literature. The present study indicates that the decrease in the number of gonadotrophic cells in addition to evidence of exhausted secretion characterised by degeneration and vacuolisation of gonadotrophic cells in case of inactive ovaries.

CONCLUSION

It could be concluded that:

1. The maximum ovarian activity occurs in winter.
2. The maximum ovarian inactivity occurs in summer.
3. Cystic ovaries are observed all over the year round with tendency to be increased in summer and autumn.
4. There is a great demand to establish a base line data for the hormonal level during the different phases.
5. The pituitary glands show inactivity in association with inactive ovaries and it may be the cause of this condition during summer season.
6. Deficiency of LH surge is considered the main cause of cystic ovaries and the changes of the pituitary are related to the feedback mechanism from the higher levels of the ovarian steroids.

REFERENCES

- Abdel Rahim, S.E.A. and El Nazier, A.T. (1992). Study on the sexual behaviour of the dromedary camel. Proc. 1st Int. Camel Conf., February, 1992, Dubai, UAE.
- Adams, C.W.M. (1956). The PFAAB, PAS, Prange G method for the human hypophysis. Cited by Carleton's Histological Technique, 4th edition, Oxford Uni., Press. UK.
- Agarwal, S.P., Khanna, N.D., Agarwal, V.K. and Dwaraknath, P.K. (1987). Circulating level of oestrogen and progesterone in the female camel during pregnancy. *Theriogenology*, 28, 849-859.
- Agarwal, S.P. Rai, A.K. and Khanna, N.D. (1992). Hormonal studies in postpartum female camel and their neonates. *Theriogenology*, 38, 735-747.
- Akral, S.N. and Khanna, N.D. (1995). Ovarian activity during breeding season in Indian camel. *J. Anim. Sci.*, 65, 889-890.
- Al-EknaH, M, Dafalla, E, Homeida, A., Galil A. and Al-Taher, A. (1993). Spontaneous uterine activity during the oestrous cycle of the camel. *Ani. Reprod. Sci.*, 32, 91-97.

Arthur, G.H. and Rahim, A. T. (1982). Aspects of reproduction in the female camel in Saudi Arabia. *Vet. Med. Rev.*, 5, 83-88.

Arthur, G.H., Rahim, A. T. and El-Hindi, A. (1995). Reproduction and genital diseases of the camel. *Br. Vet. J.*, 141, 650-659.

Bonnar, J. (1973). The hypothalamus and reproductive function. In: Scott R. B. and Walker, R.M., Editors. *The Medical Annual*, Wright and Sons, Bristol, England.

Daughady, W.H. (1985). The adenohipophysis. In: Wiolso, J.D., Foster, D.W., Editors. *Williams Textbook of Endocrinolog*, Saunders, Philadelphia, USA.

Elias, E., Bedrak, E. and Yagil, R. (1984). Peripheral blood level of progesterone in female camel during various reproductive stages. *Gen. Comp. Endo.*, 53, 235-240.

Ismail, S.T., Hemeida, N. A., Kandil, M.H., El-Wishy, A. B. and Shahein, Y.M. (1985). Histology and hstochemistry of th eparis distalis of the pituitary of the one-humped male camel. *J. Egypt. Vet. Med. Ass.*, 45, 127-137.

Musa, B.E. and Abu-Sineina, M.E. (1976). Some observations on the reproduction in the female camel. *Acta Vet. Yugosl.*, 26, 63-69.

Xu, Y.S., Wang, H.Y., Zeng, G.Q., Jaing, G.T. and Gao, Y.H. (1985). Hormone concentration before and after semen induced ovulation in the bacterian camel. *J. Reprod. Fert.*, 74, 341-346.

Zhao, X.X. and Chen, B.X. (1995). Peripheral endocrine changes in camel. *J. Camel Practice and Research*. 2, 123-124.