Studies on Pituitary-Ovarian Axis in the Female Camel with Special Reference to Cystic and Inactive Ovaries

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ABSTRACT

The present investigation was performed on 190 female camel slaughtered at Al-Ahsa modern slaughterhouse throughout one year. Blood samples, pituitary glands and ovaries were collected. FSH, LH, E_2 and progesterone hormones were determined in cases of cystic and inactive ovaries as well as follicular ovarian wave. 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 inactive, cystic and ovarian follicular wave were described and discussed.

(**Key Words:** Dromedary Camel, Pituitary Gland, Cystic Ovary, Reproductive Hormones).

Introduction

She-camel is seasonally polyoestrus and induced ovulators. 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 and Abu-Sineina, 1976; El-Wishy 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; Daughadny, 1985). The gonadotrophic cells of pituitary secrete both FSH and LH in response to gonadotrophic releasing hormone (LHRH or Gn-RH) from the medial basal hypothalamus. Release of both FSH and LH from the pituitary is under a negative feedback control by the gonads (Bonnar, 1973).

The incidence of cystic and inactive ovaries among Saudi camel females 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 were attributed to the adverse body condition (Tibary and Anouassi, 1997). No previous studies could be detected to explain and discuss this phenomenon, which initiated the idea of this work.

The present study aimed to investigate the cellular activity of pituitary gland in cases of

active, inactive and cystic ovaries and to determine FSH, LH, E_2 and progesterone concentrations in relation to cellular activity of pars distalis and ovarian changes.

Materials and Methods

This investigation was performed on 190 female camel slaughtered in Al-Ahsa modern slaughterhouse throughout one year (January – December 2001).

Blood Samples
10 ml blood was collected from each animal before slaughtering in silicon-coated tubes. Sera were separated and marked according to ovarian picture and stored at –40 ^{o}C for further hormonal analysis.

Ovaries
Ovaries of each animal were grossly examined and ovarian structure was recorded.

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

The Cell Count of Anterior Pituitary
The count of different cells of the anterior pituitary gland 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 while the third one was in the posterior part.

Hormonal Analysis

Evaluation of LH, FSH, E_2 , and progesterone were performed on 51 serum samples (20 active ovaries, 16 active ovaries and 15 cystic ovaries (11 follicular and 4 luteal).

Hormonal evaluation was performed using Enzyme Linked Immunosorbent Assay (ELISA) method using kits of Abbot laboratories (USA).

Results

Ovaries
The incidence of ovarian activity, inactivity and cystic ovaries in the 190 female camels during the different seasons of the year (January – December 2001) is presented in Table 1 and in Figures 1-3.

The total incidence of the ovarian changes among examined cases per year (January – December 2001) is presented in Table 2.

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

Hormonal Analysis
The mean values of FSH, LH, E_2 and progesterone in relation to ovarian changes are illustrated in Table 3.

The hormonal analysis in case of cystic ovaries (follicular cysts) revealed decrease of FSH and LH concomitant with increase of E_2 and progesterone levels in comparison with that of active follicular wave.

In case of inactive ovaries, there was a marked increase in E_2, progesterone and decrease in FSH in comparison to the hormonal level in cases of active ovary (follicular wave), while the level of LH was comparable to that in case of active ovary.

In case of luteal cyst, there was an increase in the levels of LH progesterone and E_2 levels than in case of active ovaries with a decrease in FSH level.

Pituitary Gland

The histological study indicated that pituitary glands of the female camels were subdivided into adenohypophysis and neurohypophysis.

The adenohypophysis consisted of three portions, pars distalis, pars tuberales 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 covers 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 are localized at the central and posterior parts of anterior pituitary glands. Two types of acidophilic cells, the somatotrophic cells (STH) and lactotrophic cells (LTH) could be recognized. The somatotrophic cells were large polyhedral and mostly localized in posterior parts. The cells contained coarse intra cytoplasmic granules yellow in colour. The lactotrophic cells were mostly located in the center of the gland. They are variable in shape, oval, rounded or elongated with eccentric vesicular nuclei and cytoplasmic glands stained orange red.

The number of basophilic cells were lesser than acidophilic ones. The cells were located mostly in the peripheral parts of pars distalis, next to hypophysical cleft and the boundaries of blood vessels. The basophiles were differentiated into gonadotrophic cells, which were more abundant than thyrotrophic cells (Table 4).

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 ovary the adenohypophysis 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 their nucleous were vesicular in appearance. Degranulation and vacuolization 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 showed no significant variation in number. However many cells show homogenous eosinophilic cytoplasm and pyknotic nuclei. These cells were identified as lactotrophic cells. Adenolypophysis in case of inactive ovaries showed that the basophilic cells were smaller in size with lesser amount of basophilic granules, which was clearly seen 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 by acidic dye. Excessive amounts 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.

Concerning the count of different pituitary cells, the results are presented in Table 4.

It is clear from Table 4, that the number of gonadotrophic and thyrotrophic cells decreased in case of inactive ovaries concomitant with increase in the number of acidophils in comparison to that observed in case of active

ovaries. In luteal cysts the number of gonadotrophs and thyrotrophic and lactotrophic cells increased in comparison with that of active ovaries. In case of follicular cyst the number of gonadotrophs and thyrotrophs were comparable to that of active ovaries with minimum increase in number of lactotrophic cells.

Discussion

The available literature indicates the absence of any previous study on pituitary ovarian axis in female camels. However the pituitary gland of male camel and the effect of seasonal variation on pituitary -testicular axis were studied by few authors (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), Ismail (1987) and Akral et al (1995) reported similar results in Egypt and Pakistan, respectively. In Saudi Arabia, Arthur and Al-Rahim (1982) and Arthur et al (1985) reported that breeding of she camel occurs throughout the year provided a good nutritional condition.

The incidence of inactive ovaries in the present study reached its peak in the summer, which may be responsible for the failure of conception during May, August and October 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 lesser in comparison with that of active ovaries. Moreover, the FSH level in the plasma was lower than that of active ovaries which 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 were obtained in Saudi Arabia (Hegazy et al. 2001). It seems that the incidence of cystic ovaries increase in summer and autumn. It is believed that the problem of cystic ovaries is the deficiency of LH surge, which confirmed by the present results of hormonal analysis, which indicated the low level of LH in cases of cystic ovaries in comparison with that of normal cyclic ovaries.

No base line data could be traced for FSH and LH levels, while few studies were performed to determine E_2 and progesterone in pregnant and non-pregnant she-camel.

The hormonal analysis of E_2 , progesterone, LH and FSH in case of follicular ovarian wave indicate a large individual variation. Such variation has been observed in E_2 to be between 9 and 110 pg/ml during the different follicular ovarian waves (Elias, 1984a; Xu et al. 1985). In case of pregnancy, oestrogen concentration increased progressively from basal level of 20 pg/ml at early stages of gestation to about 450 pg/ml at advanced gestation (Agarwal et al. 1987). Our results revealed that the level of E_2 . varied from 1.24 to 67.23 pg/ml with a mean value of 14.7 pg/ml.

In the present study, the progesterone level in non-pregnant female camel was between 0.0 and 4.7 ng/ml (Table 3), with a mean value of 1.11 ng/ml. Similar results were reported by Elias et al (1984); Xu et al (1985); Agarwal et al (1992), where the level of progesterone in non-pregnant female camel varied between 0.28 and 1.73 ng/ml. During pregnancy, the progesterone level fluctuated between 2 and 5 ng/ml (Hassan et al. 1996).

Also progesterone levels ranged between 1.01 and 6.34 ng/ml on day one post parturition (Zhao and Chen, 1995) then gradually decreased to reach undetectable level after 12 days.

However a sharp decline in progesterone level during postpartum to reach the non-pregnant values within 2 weeks has been reported by Agarwal et al (1992) and Hassan et al (1996).
No available data could be detected for FSH and LH levels. It was noticed that these hormones could cross-react with those of human being using ELISA technique, however, the results needs further investigation.

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 E_2 and progesterone levels is considered a sequel of cyst, which may secrete progesterone or E_2 depending on the degree of granulose cells luteinization (Jubb and Kennedy, 1993).

In case of inactive ovaries, there was a decrease in FSH level, which may explain the failure of ovary to develop follicles. The decrease of FSH may be due to the increase of ovarian steroid hormones (E_2 and progesterone) as a consequence of the feedback mechanism. The increase of progesterone and E_2 levels in case of inactive ovaries can't be explained. However, it may denote exogenous source of secretion of these hormones other than ovaries, which needs further investigation.

In case of luteal cyst, there was increase in the levels of LH, progesterone and E_2 than in case of active ovaries. This was concomitant with decrease in FSH level. The increase of E_2 may be due to growing follicles.

Previous investigations on the histological and histochemical investigation of the pituitary gland of female camel could not be traced in the available literature. The present study indicates the decrease in number of gonadotrophic cells in addition to evidence of exhausted secretion characterized by degeneration and vacuolization of gonadotrophic cells in case if inactive ovaries. This picture was associated with decrease the FSH level in the blood as well as increase of progesterone and E_2. The low activity of the gonadotrophic cells may be due to the deficiency of gonadotrophic releasing hormone from the hypothalamus (Bonnar, 1973) and/or increase of the ovarian steroid hormones which leads to decrease the secretion of FSH by feedback mechanism. Somatotrophic cells showed no abnormalities.

In case of cystic ovaries, the gonadotrophic cells were similar in number to that of active ovaries, while the cells appeared overfilled with basophilic granules. The hormonal analysis revealed high E_2 and progesterone levels, which inhibit the secretion of FSH by feedback mechanism and in turn the accumulation of the granules in the gonadotrophic cells. The acidophilic cells either the lactotrophic or somatotrophic cells appeared comparable in number to active ovaries while increase accumulation of the acidic granules in LTH cells.

In case of the luteal cyst, the pituitary gland showed that the gonadotrophic cells as well as the lactotrophic cells were filled with basophilic and acidophilic granules, respectively.

Conclusions

It could be concluded that:

1. The maximum ovarian activity occurs in winter.
2. The maximum ovaries inactivity occurs in summer.
3. Cystic ovaries observed all over the year 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 showed inactivity in association with inactive ovary and it may be the cause of this condition during summer season.

6. Deficiency of LH surge may be considered the main cause of cystic ovaries and the changes of the pituitary are related to the feedback mechanism from the high levels of the ovarian steroids.

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Table 1. The incidence of ovarian changes in different seasons.

Ovarian Picture	Autumn		Summer		Spring		Winter	
	No	P/S	No	P/S	No	P/S	No	P/S
Follicular wave	28	56%	10	28.57%	46	70.76%	34	85.0%
Cystic ovary:								
A-Follicular	5	10%	5	14.25%	7	10.76%	3	7.5%
B-Luteal	3	6%	1	2.85%	5	7.69%	1	2.50%
Inactive ovaries	14	28%	19	54.28%	7	10.76%	2	5.0%
Total	50	100%	35	100%	65	100%	40	100%

P/S = Percentage per season.

Table 2. The incidence of ovarian changes among examined cases per year (January – December 2001).

Ovarian Picture	Autumn	Summer	Spring	Winter	Total	%
Ovarian follicular activity	28	10	46	34	118	62.33
Cystic ovary:						
A. Follicular	5	5	7	3	20	10.46
B. Luteal	3	1	5	1	10	5.23
Inactive ovary	14	19	7	2	42	21.98

Table 3. Mean values of the FSH, LH, E_2 and progesterone in 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
Progesterone	1.1165 0.0 – 4.7	4.483 1.0 – 21.4	3.27 0.33 – 10.3	1.657 0.57 – 3.4
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

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

Ovarian Status	Acidophils			Basophils		Chromo phobs	
	STH	LTH	Total	GT	TH	Total	
Active ovaries	30.1	33.2	63.3	24.3	10.28	34.58	2.19
Inactive ovaries	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	12.61	42.11	2.5

STH : Somatotrophic cells
LTH: Lactotrophic cells
GT: Gonadotrophic cells
TH: Thyrotrophic cells

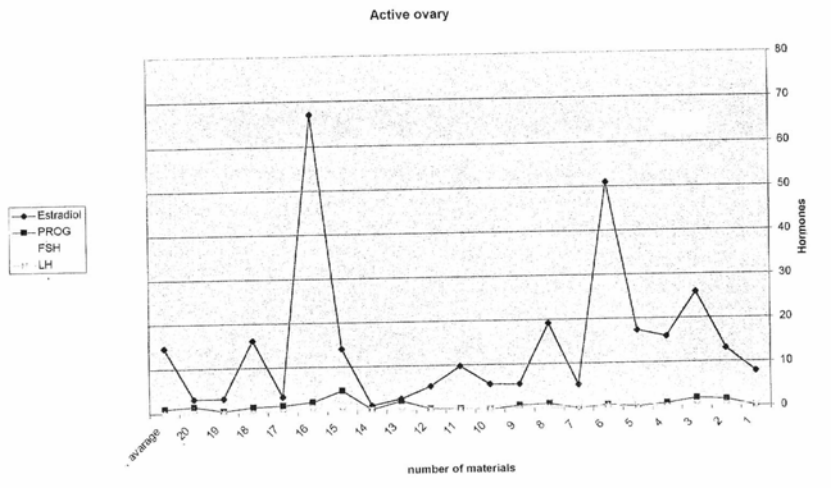

Fig. 1.

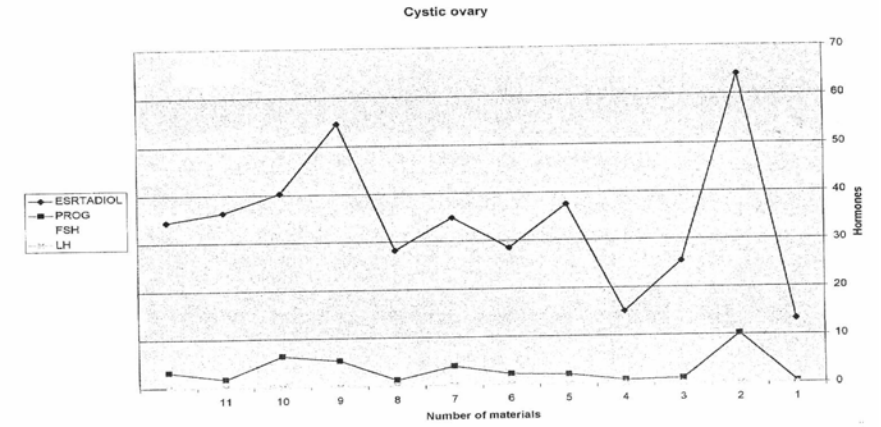

Fig. 2.

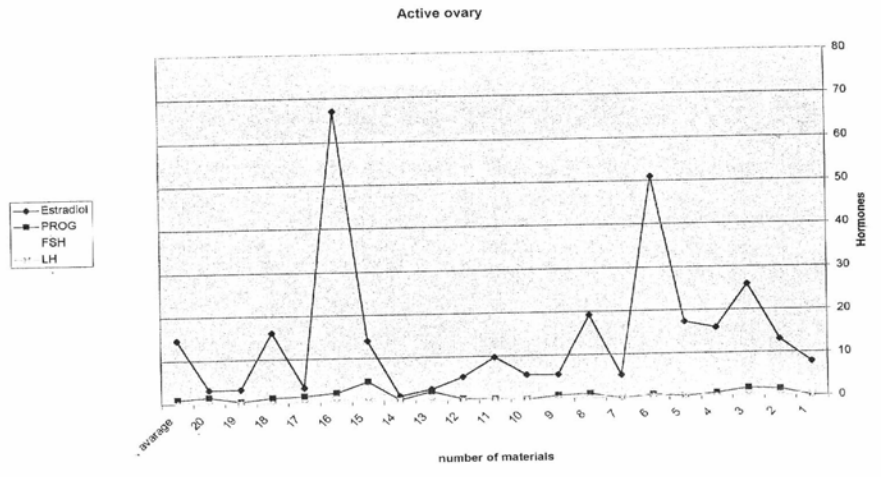

Fig. 3.