

## The Early Pregnancy Diagnosis in She - Camel

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### ABSTRACT

The genital organs of forty-one she-camel, with unknown breeding history (13 empty and 28 gravid), were collected during one year period (August, 2003 to August, 2004) from Bilbis Abattoir in Sharkia Governorate, Egypt. The genitalia and uterine samples were immediately collected after evisceration and gently transferred to the laboratory. The ovaries were examined morphologically. The uterine horns were opened along their greater curvature to expose their contents. The feti were separated from the gravid horns to determine the crown rump length (CR) and curved vertebral rump length (CVR). The ages of the feti were estimated. Urine samples were collected to detect the estrone sulphate, using the Cuboni test.

The results revealed that the all the encountered pregnancies were located in the left uterine horn in the presence of functional right and left ovaries. The ages of the collected feti ranged from 30 to 370 days. The CR ranged from 1.1 cm to 1.6 cm and the respective CVR ranged from 2.2cm to 2.8cm., for 5 feti (gp.1) of 30 days age. The results of the Cuboni test revealed the presence of intense green fluorescent ring (+ + + +). Gp.(2) included 19 feti of 31-300 days age their CR ranged from 2.3 cm to 70.3cm., and the CVR ranged from 3.65 cm to 82.8 cm., The respective urine test revealed green fluorescent ring (+ to + +). Gp.(3) included 4 feti of 301-370 day age. Their CR ranged from 72.5 cm to 96.0 cm and CVR ranged from 83 cm to 117.5 cm. Their respective results of Cuboni test revealed intense green fluorescent ring (+ + + +). Gp.(4) included 13 non pregnant she- camel which were negative for the cuboni test.

It could be concluded that the application of the Cuboni test is a useful indicator for the early pregnancy-detection in the she-camel .

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### INTRODUCTION

The gestation period in the camel, ranges from 308 to 440 days, with an average of 375 days (*Noakes, 1*). The current methods of diagnosing pregnancy, in the dromedary camel, include recatal palpation (*Musa, 2*), determination of progesterone concentration, (*Foote, 3 and Adam, 4*), ultrasonography, (*Adams, 5*) and ballottement, (*Alarcon, 6*). The application and accuracy of these methods varies with time of gestation. For instance the rectal palpation is the most reliable method after 45 days of gestation (*Musa, 2*). The measurement of the plasma progesterone concentration, as a diagnostic tool for pregnancy, should be avoided until the 21<sup>st</sup> to the 30<sup>th</sup> days after breeding to prevent false-positive results attributed to early embryonic mortality (*Fernandez-Baca, 7*), and the inability to determine the viability of the fetus. Ultrasonography can detect pregnancy as early as 17 to 18 days after breeding (*Tinson, 8*). *El-Ghannam (9)* reported that pregnancy

diagnosis in the she- camel could be done by application of Cuboni's test for detection of estrone sulphate in urine. However, *Bravo (10)* reported that the demonstration of the concentration of estrone sulfate in the urine may be used as a diagnostic indicator of pregnancy in llamas and alpacas. Therefore, a significant increase in estrone sulfate was evident on the day 21<sup>st</sup> of gestation and the concentration remained high for 5 to 6 days. The early gestation was associated with 3 fold increase in estrone sulfate concentration compared with the concentration in the nonmated llamas (*Bravo, 11*) and alpaca (*Bravo, 12*) having typical follicular wave patterns. The source and physiologic importance of early gestation-increase in the estrone sulfate aren't known (*Bravo, 10*).

In this connection, no inordinate growth of fetal gonads during the 2<sup>nd</sup> half of pregnancy, like in the mare has been seen in the camel (*Noakes, 1*). Therefore, the early increase of estrogen-secretion in the pregnant

llamas and alpacas could reflect a direct continuation by the conceptus as in the case in sows (*Robertson, 13*). Estrone sulfate, in the urine and serum as a product of the fetal-placental is a good indicator of pregnancy in llamas and alpacas (*Bravo, 10*).

The purpose of the current study was to employ the estrone-sulfate, in the urine in Cuboni test for pregnancy diagnosis in the she-camel, compared with the non-pregnant ones.

#### MATERIALS AND METHODS

The genital system of forty-one she-camel (*Camelus dromedarius*) with unknown breeding history (13 empty and 28 gravid) were collected from Bilbis abattoir in Sharkia Governorate, Egypt, during the period from August, 2003 to August, 2004. The genitalia and urine samples were immediately collected after evisceration and gently transferred to the laboratory. The ovaries were free from extraneous tissues and were exposed to further examination, regarding their structure which presented. Graafian follicles (small = 0.2 to 0.4 cm., medium = 0.7 to 1.2 cm., large = 1.9 to 2.2 cm and supposed to be a case of follicular cysts because the dimensions of the follicle was larger than normal = 4.2 to 7 cm) corpora lutea (cyclic or of pregnancy) and corpora albicantia. These structures were recorded and measured to the nearest 0.1 cm by Avarnier Caliber. The uterine horns were opened along their greater curvature to determine whether the uterus is empty or gravid. The feti were separated from the gravid uteri to determine the crown rump length (CR) and the curved

vertebral rump length (CVR) to the nearest centimeter. The age of the fetus was estimated according to *El-Wishy (14)*.

The cuboni-test was carried out as described by *Cox (15)*. The results were read in reflected daylight. Results were expressed as negative (-ve), false ( $\pm$  ve) positive (+, ++, +++ and ++++) according to the developed intensity of green fluorescent colouration.

#### RESULTS

Examination of the uteri revealed that 28 out of 41 uteri were gravid in the left side, in the presence of functional right and left ovaries. The placentation, in the camel, is of the simple diffused type. The ages of the collected feti ranged from 30 to 370 days. Table (1) shows that the 5 feti with CR of 1.1cm to 1.6 cm and CVR of 2.2 cm to 2.8cm.(gp.1) were found to be around 30 days old. The results of the cuboni test revealed the presence of intense green fluorescent ring (++++). The 19 feti of (gp. 2) exhibited CR of 2.3cm to 70.3 cm and CVR of 3.65 cm to 82.8 cm were calculated for feti with ages which ranged from 31 to 300 days. The respective urine test revealed a green fluorescent ring (+ to ++). The 4 feti of (gp. 3), showed CR of 72.5 cm to 96 cm and CVR of 83 cm to 117.5 cm which were calculated for ages which ranged from 301 - 370 days. Their respective results of cuboni test revealed an intense green fluorescent ring (++++). Table (2) shows that the cuboni test on the urine of the 13 camels in (gp. 4) was negative. The morphological findings, in the ovaries, were also included in tables (1 and 2).

Table (1): The CR, CVR, expected fetal age in days, ovarian finding and cuboni test on urine of 28 pregnant she- camel.

Group	No.	CR in cm.	CVR in cm.	Expected fetal age in days	Right ovary						Left ovary						Cuboni test
					Follicular		CL		CA		Follicular		CL		CA		
					No.	Ø	No.	Ø	No.	Ø	No.	Ø	No.	Ø	No.	Ø	
1	1	1.1	2.2	30	-	-	-	-	2	0.4	-	-	1	2.2	2	0.4	++++
	2	1.2	2.4		1	0.6	-	-	2	0.6	-	-	1	2.3	1	0.5	++++
	3	1.3	2.5		1	0.8	1	2.4	3	0.5	-	-	-	-	1	0.8	++++
	4	1.5	2.7		-	-	-	-	3	0.4	1	0.8	1	2.3	3	0.4	++++
	5	1.6	2.8		1	0.5	1	3	1	0.5	1	0.9	1	2.2	2	0.4	++++
2	1	2.3	3.65	31-45	1	0.6	-	-	2	0.3	1	0.8	1	2.5	1	0.4	++
	2	2.4	3.75		1	0.6	-	-	3	0.4	-	-	1	2.3	1	0.5	++
	3	2.4	3.75		1	0.7	1	3	1	0.3	-	-	1	3	1	0.4	+
	4	2.5	3.85		1	0.7	-	-	1	0.5	-	-	1	2.4	2	0.5	++
	5	3.0	4.35		1	0.6	-	-	4	0.5	-	-	1	2.4	1	0.8	+
	1	3.1	4.4	46-120	1	0.6	-	-	3	0.6	1	0.5	1	3	2	0.5	+
	2	6.3	10.5		1	0.4	-	-	1	0.8	-	-	1	3	2	0.4	++
	3	10.1	14.9		-	-	-	-	4	0.4	1	0.5	1	2.5	2	0.6	+
	4	11.3	16.3		1	0.7	-	-	2	0.6	1	0.6	1	2.7	2	0.6	+
	5	14.5	20.1		-	-	-	-	3	0.7	-	-	1	2.8	2	0.5	+
	1	16.2	21.8	120-240	-	-	-	-	5	0.5	-	-	1	2.3	-	-	+
	2	17.5	22.6		-	-	-	-	3	0.5	-	-	1	2.5	2	0.8	+
	3	35.5	41.7		-	-	-	-	2	0.6	1	0.6	1	2.7	1	0.7	++
	4	42.5	45.8		-	-	-	-	3	0.5	-	-	1	2.6	3	0.6	++
	5	49.5	58.0		-	-	-	-	4	0.6	1	0.8	1	2.4	1	0.5	++
	1	53	66	240-300	-	-	-	-	3	0.4	-	-	1	2.7	2	0.3	++
	2	54	66.5		1	0.5	-	-	2	0.4	-	-	1	2.1	1	0.4	++
	3	55	70.0		1	0.3	-	-	4	0.5	-	-	1	2.8	-	-	++
	4	70.3	82.8		-	-	-	-	3	0.4	-	-	1	3	2	0.4	++
	3	1	72.5	83.0	301-370	1	0.6	-	-	2	0.3	1	0.6	1	2.2	3	0.4
2		84.3	95.4	-		-	-	-	1	0.4	-	-	1	3	2	0.4	++++
3		94.2	114.7	-		-	-	-	3	0.5	1	0.7	1	2.6	2	0.5	++++
4		96.0	117.5	-		-	-	-	1	0.7	-	-	1	2.6	1	0.5	++++

Ø = diameter

Table (2): Ovarian findings of group (4) and the cuboni test on urine of the non pregnant she-camels.(n=13)

Ovarian findings														
No.	Remark	Right ovary						Left ovary						Cuboni test
		Follicular		CL		CA		Follicular		CL		CA		
		No.	Ø	No.	Ø	No.	Ø	No.	Ø	No.	Ø	No.	Ø	
1	Supposed to be a case of follicular phase	1	1.2	1	0.8	2	0.4	1	2	1	0.9	2	0.4	Negative
2		2	1.5	1	0.8	1	0.4	1	0.5	1	0.8	1	0.3	
3		2	1.8	1	1	2	0.5	1	2.2	1	1	3	0.4	
4		2	2.5	1	1.4	2	0.6	1	2.5	-	-	3	0.5	
5		1	0.5	1	1.1	1	0.4	1	2.5	1	0.8	1	0.4	
1	Supposed to be a case of luteal phase	1	0.5	1	1.5	2	0.6	-	-	2	1.6	3	0.6	
2		2	0.7	2	2	2	0.3	1	0.9	2	2.2	3	0.6	
3		2	0.5	1	2.2	-	-	-	-	1	1.8	2	0.5	
4		-	-	1	1.8	1	0.7	1	0.5	1	1.5	1	0.8	
5		2	0.5	2	1.4	2	0.5	1	0.8	2	1.8	1	0.5	
1	Supposed to be a case of cystic ovary	1	6	1	0.6	1	0.4	1	2.5	1	0.6	3	0.4	
2		1	5	1	0.8	1	0.4	1	5.5	1	0.7	2	0.5	
3		1	7	1	1	1	0.5	1	3.5	-	-	3	0.6	

Ø = diameter

### DISCUSSION

The results revealed that the CR of 1.1cm to 1.6cm and the respective CVR of 2.2 – 2.8 cm (gp. 1) were calculated for ages around 30 days of pregnancy. The cuboni test showed the presence of an intense green fluorescence (++++). On the other hand, *El-Ghannam (9)* found doubtful levels of urinary oestrogen (±) starting from a fetal CVR of 26 cm. Slightly positive green fluorescence (+ to +) was obtained when the fetal CVR ranged from 26 to 55cm. *Bravo (11)* and *Bravo (10)* reported a significantly increase in urine on the 21<sup>st</sup> days of gestation (basal 8 ng/ mg cr. in urine) raised on the 17<sup>th</sup> day of gestation and increased rapidly between the 21<sup>st</sup> to 25<sup>th</sup> days (75 ng / mg cr in urine), then the concentration remained high for 5 to 6 days. The 19 feti of (gp. 2) exhibited CR of 2.3 to 70.3 cm and CVR of 3.65 to 82.8 cm which were calculated to give age of fetu which ranged from 31 to 300 days. Their respective Cuboni-test for urine revealed a green fluorescent ring (+ to ++). The current results are in agreement with *El-Ghannam (9)*.

The 4 fetu of (gp.3) showed a CR of 72.5 to 96 cm and CVR of 83 to 117.5cm.

They were the longest fetu the calculated ages ranged from 301 to 370 days of gestation. Their respective results of of the Cuboni-test revealed intense green fluorescent ring (+++). The present results are in agreement with , *El-Ghannam (9)* recorded similar observation.

The obtained data indicated that the urinary oestrone seems to be at lower concentrations than those which show negative cuboni test in the urine of supposed non - pregnant camel despite having typical follicular waves (Gp.4).

The source and physiological importance of the increased estrone sulfate, during the early gestation are unknown (*Bravo 10*). The time of onset of such an increase may trigger the preexisting trophoblasts to be transferred into the maternal vascular compartment. Therefore, the early increase in the estrogen-secretion, in pregnant llamas and alpacas, could reflect a direct contribution by the conceptus, as is the case in sows, (*Lumaas, 16*).

No follicular activity of the maternal ovaries, throughout the stages of the gestation, was studied. Therefore, if a chorionic gonadotropins is involved as in mare (*Terqui,*

17and Daels, 18), its direct detection in blood could lead to specific markers in the maternal ovaries (Bravo, 10) and a specific marker for pregnancy in llamas and possibly other camel species (Noakes, 1).

A second increase in estrone sulfate concentration in the urine, which gave an intense green fluorescent ring (+ + + +), mostly came from the fetal placental unit and may be indicative of fetal health. The high concentrations of estrogens, near the end of gestation, has been, demonstrated in mares, (Nett, 19 and Lovell, 20), cows (Choi, 21and Robertson, 22), sows (Robertson, 23 and Choi, 21), ewes (Challis, 24) and llamas (Bravo, 25).

It could be concluded that the cuboni-test is the simplest and direct method for the she-camel early pregnancy diagnosis.

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## الملخص العربي

### التشخيص المبكر للحمل في النوق

#### توفيق بركات

قسم التوليد والتاسل والتلقيح الاصطناعي - كلية الطب البيطري - جامعة الزقازيق- مصر

تمت تلك الدراسة خلال عام ٢٠٠٤ على واحد واربعين رحم نوق غير معلوم تاريخ نسلها تم جمعها من مجزر بلبيس - محافظة الشرقية- مصر . وكان الغرض من الدراسة تقييم استخدام اختبار كوبوني في تشخيص الحمل في الناقة . وتم الاعتماد على الطول الجنيني CR و CVR لتحديد فترات العشر.

وأوضح من النتائج التي قسمت إلى أربع مجموعات أن المجموعة الأولى (N= 5) ظهر بها اللون الفلوروسنت الأخضر الشديد (++++) في بول النوق العشار في الشهر الأول . ثم انخفض اللون الفلوروسنت الأخضر (+ و ++ ) في المجموعة الثانية (n = 19) التي كان العشار يتراوح ما بين ٣١ إلى ٣٠٠ يوم . إما المجموعة الثالثة (n=4) التي تراوحت أعمار الأجنة فيها ما بين ٣٠١ إلى ٣٧٠ يوماً وهي أكبر الأعمار التي تم الحصول عليها فقد عاد الأستروجين إلى الزيادة مرة أخرى ليبين شدة في اللون الفلوروسنت الأخضر (++++) . أما المجموعة الرابعة (n=13) فكانت غير عشار ونتيجة فحص البول سالبة لاختبار كوبوني .

خلصت الدراسة إلى أن اختبار كوبوني يمكن استخدامه في تشخيص الحمل المبكر في الناقة.