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EVALUATION OF TAIL CURLING TEST FOR DIAGNOSIS OF PREGNANCY IN FEMALE CAMELS THROUGH SCIENTIFIC MEANS

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ABSTRACT

The tail curling behaviour of pregnant females in the presence of a rutting male was evaluated for its accuracy to diagnose pregnancy in camels through serum progesterone (P_4) concentration on day 14-15 after mating and recto-genital palpations. Of the 89 observations for females that did not curl their tails (assumed non-pregnant), 86 (96.6 %) were confirmed to be non-pregnant with P_4 <1 ng/ml on day 15. The remaining 3 cases were found to be false negative as they were pregnant with P_4 >1 ng/ml. Of the 66 females that curled their tails (assumed pregnant), only 45 (68.1%) were actually pregnant and had P_4 >1 ng/ml and 18 (27.2%) were false positive (non-pregnant with P_4 <1 ng/ml). Three (4.5%) pregnant females correctly diagnosed through the tail test were diagnosed false negative through P_4 concentration as P_4 <1 ng/ml. An intriguing aspect was that repeating the tail curling test at weekly intervals could detect non-pregnant status in false positive cases, as well as those pregnant females that lost their pregnancy due to early embryonic deaths. It was concluded that the tail-curling test can be effective in the early detection of non-pregnant females within the breeding herd. A single observation on day 15 could also detect pregnant females with an accuracy of 70%, but the high rate of false positives is a major problem. Repeated weekly tests are necessary to identify those false positive cases and those females that lose their pregnancy due to early embryonic deaths. However, this delayed detection might reduce the overall herd conception significantly if the tail test alone is adopted, as the breeding season is short in camels, thus the simultaneous P_4 concentration test on day 15 can detect pregnancy more accurately and would improve reproductive efficiency.

Key words: Camel, evaluation, pregnancy, tail curling

Pregnancy diagnosis is an integral part of reproductive management and is particularly important in camels as they have a short breeding season of only 4 – 5 months. Low conception rates could be reduced to a great extent if females that failed to conceive could be identified as early as possible after breeding. Pregnancy diagnosis in nomadic, as well as in managed camel herds, is based on tail curling as a pregnant female will curl her tail upwards when approached by a rutting male (Yagil, 2006). However, in no part of the world has this test been validated with other scientific methods. The opinions of veterinarians regarding this sign differ; some consider it to be true (Abdel Rahim and Al Nazier, 1992), while others regard it as unreliable (Agarwal and Khanna, 1998). Skidmore (2000) pointed out difficulties such as curling of tail by unmated females that have been treated with exogenous progesterone and younger animals alarmed by the approach of a male. It would be useful if this test could be evaluated through other scientific methods for its accuracy to detect non-pregnant and pregnant females. Previous studies have shown that camels have a very short luteal life span of only 8 – 9 days thus in non-pregnant animals P_4 concentrations decrease dramatically to basal concentrations of <1 ng/ml by 10–11 days after mating (Skidmore *et al,* 1995), whereas in pregnant females it remains elevated (Aminu Deen *et al,* 2007). Based on these facts, this study on pregnancy diagnosis in camels was conducted to evaluate and validate the tail curling test for pregnancy diagnosis with serum progesterone concentrations measured on day 14-15 after mating using radioimmunoassay and recto-genital palpation at the appropriate stages (Day 60 after mating).

Materials and Methods

Animals

The study was conducted at the National Research Centre on Camel, Bikaner using a total of 70 female camels (*Camelus dromedarius*) of 4 different breeds (Jaisalmeri n=18, Bikaneri n=32, Kachchi n=17 and Mewari n=3), which were bred during the breeding season 2007-2008.

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Recto-genital examinations and breeding

The reproductive tracts of the females were examined by transrectal palpation whilst they were in sternal recumbency and after sedation with Xylazine hydrochloride 70 mg (Xylaxin, Indian Immunologicals Limited, Rakshapuram, Gachbowli, Hyderabad, India) administered intravenously (i.v.). Those females, which exhibited a mature follicle >12 mm in diameter in their ovaries, were bred with stud males of the same breed, while females in nonfollicular phases were re-examined after intervals of 4-5 days and bred when mature follicles were present.

Tail curling test and blood sample harvesting

On day 14-15 after mating (Day 1 = Day of mating), the females were observed for their tail curling behaviour by parading past a rutting stud in the sheds. The positive or negative behaviour was recorded and blood samples were collected by jugular vene-puncture and serum harvested for progesterone concentration.

Analysis for serum progesterone

Progesterone was measured by radio-immunoassay using anti-progesterone coated tubes and I^{125} labeled progesterone as tracer procured from IMMUNOTECH SAS-130 av. De Lattre de Tassigny-B.P. 177-13276 Marseille Cedex 9 France.

Set	Tube	Tracer	Sample	Standard	Control
Total count	Plain uncoated tube	1 ml	-	-	-
Non specific binding	Plain uncoated tube	1 ml	-	-	-
Standards	Antibody coated tube	1 ml	-	100 µl	-
Unknown	Antibody coated tube	1 ml	100 µl	-	-
Control	Antibody coated tube	1 ml	-	-	100 µl

Antibody coated tubes were appropriately marked for different standards and unknown samples. Plain tubes were used for total counts and non- specific bindings. Pipetting of samples, standards, controls and tracer were done as shown in tabular form. The contents of the tubes were then thoroughly mixed on a vortex mixer followed by incubation of 1 hour on a shaker at 350 rpm per minute. After incubation the supernatant was carefully aspirated and discarded appropriately except in total count set, which were not aspirated. Countings for g irradiations and assay report was obtained through automatic gamma counter.

- Sensitivity : 0.05 ng/ml of serum
- Intra assay co-efficient of variation: 5.8%
- Inter assay co-efficient of variation: 9.0%
- Percent binding for lowest calibrator has been 75%, while it was 8.5% for the highest calibrator.
- Serial dilutions (6) of high concentrated serum sample exhibited exactly similar displacement as with different calibrators.
- Recovery percentages varied between 85% and 110%.

Recto-genital palpation for pregnancy

Recto-genital palpation for confirming the absence of a corpus luteum in the ovaries of the female camels diagnosed non-pregnant by the tail curling test was carried out the day after negative behaviour was recorded. For those confirmed pregnant by the tail curling test, repeated monitoring for tail curling was done once weekly to detect if the behaviour persisted or reverted. Recto-genital palpation of pregnant camels with persistent tail curling was scheduled on day 50-60, while for those reverting it was carried out the day after detection of reverted behaviour.

The criteria adopted for pregnancy diagnosis using serum progesterone concentrations was that if the serum progesterone concentration was above 1 ng/ml the female was considered to be pregnant, while those below 1 ng/ml were considered non-pregnant.

Results

Results of tail curling and serum progesterone concentrations on day 14-15 after mating has been presented in table 1, which shows that out of a total of 89 observations on females not curling their tails in the presence of rutting males 86 (> 96%) observations were confirmed accurate with serum progesterone concentrations of <1 ng/ml. Progesterone concentration >1 ng/ml on day 15 was observed in the remaining 3 (4%) female camels (false negative detection in tail curling). But all these 3 females were found non-pregnant at later dates, which strengthens the efficiency of the tail curling test for detection of non-pregnant females on day 15 after mating. The absence of a corpus luteum through recto-genital palpation was also observed in all of these non-pregnant females.

Of a total of 66 observations of tail curling in the presence of a rutting male, 45 (68%) were confirmed accurate with serum progesterone concentration test results with P_4 >1 ng/ml. However, the results of the tail curling test did not match up with serum progesterone concentrations in the remaining 21 (32%)

cases as P_4 concentrations were <1 ng/ml. Of these, 18 females were found false positive, i.e. non-pregnant, as no corpus luteum was detected in the ovaries (Table 2) and the remaining 3 actually pregnant females, diagnosed accurately by the tail test, were diagnosed false negative by serum progesterone concentrations as P_4 <1 ng/ml test (Table 3). All these pregnant camels exhibited a corpus luteum in their ovaries and had a higher serum progesterone concentration in repeat samples taken between days 56-81. Pregnancy was confirmed in these animals by recto-genital palpation of the gravid horn.

The details of another 5 pregnant camels diagnosed accurately by both tail and serum progesterone concentration test have been presented in table 4. These animals reverted back to service between days 20 to 84 apparently due to early embryonic deaths. In the remaining 40 pregnant camels, pregnancy was confirmed by palpation of the gravid uterine horn in recto-genital palpation between days 50-60 of gestation.

Discussion

Pregnancy diagnosis in female camels has been determined through the tail curling test both in nomadic and managed herds all over the world for several decades. But the scientific basis of this behavioural change is unknown. Many veterinarians, particularly those least associated with breeding management of camels, are not fully aware of

Table 1. Curling of tail test and serum progesterone concentration for pregnancy diagnosis in female camels.

Breed	Non-pregnant			Pregnant				
	No. of observations with tail curling	No. of observations of serum P_4 concentration matching	No. of observationsv of serum P_4 concentration not matching	No. of observations with tail curling	No. of observations of serum P_4 concentration matching	No. of observations of serum P_4 concentration not matching	No. of false positive detection in tail test	No. of false negative detection in serum P_4 test
J*	26	25	1	16	12	4	3	1
B*	25	23	2	29	24	5	3	2
K*	38	38	0	21	9	12	12	-
T*	89	86	3	66	45	21	18	3

*J- Jaisalmeri, B- Bikaneri, K-Kachchi and T- Total

Note: Due to small number of Mewari females, observations on these animals were omitted.

Table 2. False positive detection in tail curling test results.

No. of camels	Tail curling test result	Mean serum P_4 concentration on day 15 (range)	Average number of days for camel to revert to non pregnant by tail test (range)	Average no. of days lost in detecting as non-pregnant through tail test (range)
18	Pregnant	0.33 (0.049- 0.798)	39.5(23-72)	24.5 (8- 57)

Table 3. False negative detection in serum progesterone concentration test.

S. No.	Animal No.	Date of breeding	Date of tail cocking and progesterone concentration test	Result of tail curling	Serum progesterone concentration on day 15	Current serum progesterone concentration	Results of recto-genital palpation for pregnancy
1	J-405	31.01.08	14.02.08	Pregnant	0.010	5.789 (On day 56)	Pregnant
2	B-439	08.01.08	22.01.08	Pregnant	0.082	3.773 (On day 81)	Pregnant
3	B-465	11.01.08	25.01.08	Pregnant	0.483	4.774 (on day 78)	Pregnant

Table 4. Emptied pregnant females returned to breeding apparently due to early embryonic death.

S. No.	Animal No.	Date of breeding	Date of tail cocking and progesterone concentration test	Result of tail curling	Serum progesterone concentration on day 15	Date of detection as empty in tail test
1	J-139	21-1-08	05.02.08	Pregnant	2.788	18.02.08
2	J-141	5-1-08	19.01.08	Pregnant	1.146	25.01.08
3	B-493	10-1-08	24.01.08	Pregnant	2.031	19.02.08
4	K-159	24.12.07	07.01.08	Pregnant	1.227	14.02.08
5	B-573	20-12-07	04.01.08	Pregnant	7.725	14.03.08

this behavioural change and hence regard it as an unreliable test (Agarwal and Khanna, 1998; Al-Eknah, 2001). However, those actively associated with camel breeding regard this sign as true (Skidmore, 2000) but emphasise the unreliability of the test in that unmated female camels treated with exogenous progesterone and young animals alarmed by a male may also curl their tails. To date, no single study has been carried out to investigate the correlation between the tail curling test and serum progesterone concentrations in determining pregnancy. This study was therefore performed in a managed camel herd, where the females were subjected to pregnancy diagnosis on days 14-15 after mating by recording the number of curling tails and measuring serum progesterone concentration in peripheral blood. Pregnancy was later confirmed by recto-genital palpation at the appropriate stages of gestation. The results revealed that pregnant females on day 15 after mating will curl their tail's whilst standing quietly pulling their necks towards their bodies and typically raising their heads, when a rutting male is paraded nearby. In addition, the male does not pursue pregnant females any more or force them into mating. A non-pregnant female does not curl her tail and is frequently being chased and forced by the male to sit down on the ground for mating. These findings resemble to those of Abdel Rahim and Al Nazier (1992) who observed that tail curling was a constant reaction of pregnant camels to a male camel approach from 2 weeks after mating. The diagnosis of non-pregnancy by using the tail test was almost 100% accurate as revealed by low serum progesterone concentration and the absence of a corpus luteum in the ovary on day 15-16 after mating following recto-genital palpation. However, the efficiency of accurate detection of pregnancy on day 15 was slightly lower using the tail test as only approximately 70% of females were accurately diagnosed as pregnant whilst 27% of the females were diagnosed false positive. Around 4.5% females accurately diagnosed as pregnant in tail test were detected as false negative in serum progesterone concentration test. An intriguing aspect has been that by continuous parading of a male near the females all of these false positives on day 15 could be successfully identified as non-pregnant later in gestation by the tail test but as many as 20-45 days are lost before these were detected. If serum progesterone concentration test is performed simultaneously, the efficiency of detection can be tremendously improved. Similarly, pregnant females emptied due to early embryonic deaths can also be detected through repeated tail tests but the results of serum progesterone concentrations is much superior. It is concluded that the tail-curling test is highly effective in early detection of non-pregnant females, but efficiency to detect pregnancy accurately was lower (70%). High rate of false positive detection in a single tail curling observation was the major problem. As the scientific basis of tail curling behaviour is not known, it is very difficult to comment about false positive detection. But it can be speculated that the behaviour might be manifested due to unknown chemical produced after fertilisation and its effect might persist for few days/weeks after early demise of embryo. Serum progesterone concentration test on day 15 is highly efficacious tool, however insignificant number of females with low concentrations of serum progesterone were actually found pregnant, but these were speculated to be due to unavoidable human errors, it is concluded that this test can be utilised at organised research oriented herds to aid to breeding management as well as research on infertility investigation.

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