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(RESEARCH ARTICLE)

APPLICATION OF SERUM PROGESTERONE ELISA VERSUS ULTRASONOGRAPHY FOR CAMEL PREGNANCY DIAGNOSIS UNDER FIELD CONDITIONS

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ABSTRACT

Early and accurate pregnancy diagnosis is essential for efficient reproductive management in camels. Traditional diagnostic methods are often limited under field conditions, creating a need for reliable, rapid, and practical alternatives. This study evaluates the effectiveness of a serum progesterone enzyme-linked immunosorbent assay (ELISA) for pregnancy diagnosis in camels under field conditions during the non-pregnancy period, as well as for the first and second pregnancy trimesters versus Ultrasonography. Combining serum progesterone ELISA and Ultrasonography for the detection of pregnancy provides an effective field strategy for early and accurate pregnancy diagnosis in female camels.

Key words: Progesterone, Elisa, Ultrasonography, Pregnancy Trimesters.

1 INTRODUCTION

Camels play a vital role in the economy and food security of arid and semi-arid regions, particularly within Saudi Arabia and across the Middle East and North Africa. Efficient reproductive management is essential to maximize herd productivity; however, reproductive inefficiency remains a major constraint in camel production systems. Camels are seasonal breeders, exhibiting distinct reproductive behaviors compared to other livestock, as both males and females display estrus simultaneously during the breeding season, which typically spans from November to March. Female

camels reach sexual maturity at 3 to 4 years of age, whereas males mature at 4 to 5 years. Females are induced ovulates, with ovulation triggered by mating, and approximately 99% of pregnancies are established in the left uterine horn (1)

The gestation period of a camel lasts approximately 13 to 14 months (385 to 410 days) and is divided into three equal trimesters of roughly 130 days each:

First Trimester (Days 1 to 130)**:** The embryo forms and migrates into the left uterine horn. The corpus luteum (CL) secretes high levels of progesterone to maintain the pregnancy [2-6].

Second Trimester (Days 131 to 260): Major organ systems undergo histological specialization. Compartment 1 of the stomach differentiates into distinct glandular and non-glandular regions. Lymphoid organs, such as the spleen, begin developing distinct red and white pulp, and the myocardium exhibits advanced structural fiber organization [2-4]. During this stage, the fetus rapidly gains weight, external anatomical features become fully defined, and the risk of embryonic loss significantly decreases [2-5].

Third Trimester (Days 261 to 390+): The fetus acquires most of its birth weight, averaging 30 to 35 kg. Maternal physiological preparation for parturition occurs, characterized by mammary gland development and colostrum accumulation toward the end of gestation. Delivery typically occurs between days 390 and 410, frequently during daylight hours [2-5].

Accurate and early pregnancy diagnosis is a cornerstone of efficient reproductive management. Conventional methods, including transrectal palpation and ultrasonography, are highly effective but require skilled personnel, specialized equipment, and stable handling conditions, which are often unavailable or impractical in nomadic or field environments. Consequently, there is a critical need for simple, reliable, and cost-effective diagnostic tools suitable for field conditions.

Progesterone is a crucial steroid hormone required for the maintenance of mammalian pregnancy. Monitoring serum progesterone levels serves as a reliable indicator of pregnancy status in various livestock species. The enzyme-linked immunosorbent assay (ELISA) offers a highly sensitive, practical, and accessible technique for hormonal quantification, making it an ideal candidate for field-applicable diagnostics.

Following successful mating in the dromedary camel, serum progesterone concentrations rise from day 3 post-ovulation, reaching approximately 3.4 ng/mL by day 8. In non-pregnant camels, concentrations rapidly decline to basal levels (<1 ng/mL) by days 10 to 12. Conversely, if pregnancy is established, progesterone concentrations are maintained between 3 and 5 ng/mL for the first 90 to 100 days of gestation. Some literature indicates that progesterone levels subsequently decrease slightly to 2–4 ng/mL, remaining stable until day 300, followed by a minor decline over the next 70 to 80 days and a precipitous drop to <1 ng/mL immediately prior to or on the day of parturition (7) Alternatively, other studies report a more gradual, continuous decline in progesterone concentrations from the fifth month of gestation until calving (8)

In camelids, pregnancy diagnosis by ultrasonography is possible as early as 17 days of gestation. This diagnosis is based on two main criteria, the visualization of an embryonic vesicle and the presence of a corpus luteum. The corpus luteum must be present to confirm pregnancy status unless the female is getting exogenous progesterone. In the early stages of pregnancy, the embryonic vesicle is relatively difficult to visualize because it is elongated, the embryonic fluid is dispersed and the uterus is relaxed. The vesicle is, however, almost always in the left horn and is best visualized at the tip of the horn where it is likely to have accumulated most fluid [9]

2 MATERIALS AND METHODS

2.1 Study area and period

The study was conducted at different camel farms during breeding season in Saudi Arabia.

2.2 ELISA

A total of 65 female dromedary camels (*Camelus dromedarius*) were used in this study under practical field conditions. Prior to blood sampling, transrectal ultrasonography was performed on all camels to confirm their pregnancy status (representing non-pregnant, pregnancy period). and subsequently during the first and second trimesters post-mating.

2.3 Blood Collection and Serum Processing:

Blood samples were collected via jugular venipuncture into plain vacuum tubes. The samples were allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes to separate the serum. Serum aliquots were stored on ice on the field and then frozen at -20°C until hormonal analysis.

2.4 Progesterone Quantification via ELISA:

Serum progesterone concentrations were quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Demeditec Diagnostics GmbH, Kiel, Germany) following the manufacturer's instructions. Optical density (OD) values were measured at **450** nm using a microplate reader, and corresponding progesterone concentrations were calculated in nanograms per milliliter (ng/mL).

Based on the manufacturer's diagnostic thresholds, the classification criteria were defined as follows:

- Non-gestating status: Serum progesterone ≤ 0.52 ng/mL
- Gestating status: Serum progesterone ≥ 1.08 ng/mL
- *Values falling between 0.53 and 1.07 ng/mL were classified as inconclusive.*

2.5 Statistical Analysis

The overall diagnostic performance of the serum progesterone Elisa Kit and accuracy was evaluated in direct comparison with the ultrasonographic findings.

2.6 Ultrasonography for pregnancy evaluation

Transabdominal digital ultrasonic diagnostic imaging system iScan2 (Fig.1) from DRAMINISKI, POLAND with fixed convex rectal probe, 6.5 MHz and Gain1= 5, Gain2= 3, Depth= 12 & and Gamma = 4 was used.

The camel is kneeling on the ground without sedation to diagnose pregnancy and non-pregnancy. Pregnancy was confirmed by observing gestational sac, fetus, fetal part (s), placenta, etc. Figure (2,3,4)



Figure 1: iScan2 with fixed convex rectal probe.

Ultrasounds result which serve as gold standard test are compared with ELISA results which were illustrated in figures 2,3 ,4 and Table 1,

3 RESULT

Non -gestation progesterone level is found ≤ 0.52 ng/ml indicates a baseline, non-pregnant state typical of the early phase of a menstrual cycle (follicular phase).

Values fall between *0.53, and 1.07* ng/mL *were classified as* equivocal. An ambiguous or borderline range. This is too high for a strict baseline but insufficient to confirm ovulation or pregnancy.

Values ranging between 1.38-3.93ng/ml were in the first trimester and confirms an early pregnancy state, reflecting initial hormonal production by the ovaries.

Values between 4.76-11.29 ng/ml were in the second trimesters. Reflects a healthy upward trend as pregnancy advances, driven by the placenta taking over hormone production.

These findings confirm that serum progesterone ELISA is a highly practical tool for early pregnancy diagnosis in camels under field conditions, matching the diagnostic reliability of ultrasonography while offering distinct operational

advantages. The significantly higher progesterone levels observed in pregnant camels align with the physiological requirement of a functional corpus luteum for pregnancy maintenance. When compared to ultrasonography, which requires expensive portable equipment and specialized technical expertise to visually confirm the embryonic vesicle, the ELISA assay provides an objective, accessible alternative that can be easily executed in remote field setups. However, while the ELISA test demonstrates high sensitivity and specificity for early detection, it remains a biochemical indicator of luteal activity rather than direct proof of a viable fetus. Therefore, while ELISA is ideal for rapid, large-scale herd screening, integrating it with follow-up ultrasonography remains the gold standard to rule out false positives caused by persistent corpora lutea or early embryonic mortality.

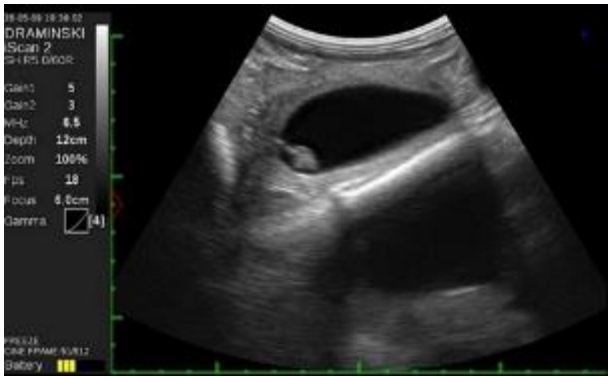


Figure 2: Camel Pregnancy at 30 days



Figure 3: Camel Pregnancy at 4 months



Figure 4: Camel Pregnancy at 7 months

Table 1: Progesterone ELISA and Ultrasound results.

sample	concentration	evaluation	Ultrasound Results
ID	ng/ml	result	
1	0.58	equivocal	non-pregnant
2	0.79	equivocal	non-pregnant
3	<0,13	non-gestating	non-pregnant
4	0.66	equivocal	non-pregnant
5	0.58	equivocal	non-pregnant
6	0.80	equivocal	non-pregnant
7	0.24	non-gestating	non-pregnant
8	<0,13	non-gestating	non-pregnant
9	0.16	non-gestating	non-pregnant
10	0.86	equivocal	non-pregnant
11	0.25	non-gestating	non-pregnant
12	0.56	equivocal	non-pregnant
13	0.59	equivocal	non-pregnant
14	0.58	equivocal	non-pregnant
15	0.26	non-gestating	non-pregnant
16	0.30	non-gestating	non-pregnant
17	0.19	non-gestating	non-pregnant
18	0.32	non-gestating	non-pregnant
19	0.94	equivocal	non-pregnant
20	<0,13	non-gestating	non-pregnant
21	0.29	non-gestating	non-pregnant
22	0.26	non-gestating	Non-pregnant
23	0.18	non-gestating	Non-pregnant
24	0.23	non-gestating	Non-pregnant
25	0.14	non-gestating	Non-pregnant
26	0.22	non-gestating	Non-pregnant
27	0.18	non-gestating	Non-pregnant
28	0.21	non-gestating	Non-pregnant
29	<0,13	non-gestating	Non-pregnant
30	<0,13	non-gestating	Non-pregnant
31	0.18	non-gestating	Non-pregnant
32	1.14	gestating	Pregnant
33	1.27	gestating	pregnant
34	1.38	gestating	Pregnant
35	1.54	gestating	Pregnant
36	1.58	gestating	Pregnant

37	1.64	gestating	pregnant
38	1.96	gestating	Pregnant
39	2.00	gestating	Pregnant
40	2.17	gestating	Pregnant
41	2.20	gestating	Pregnant
42	2.40	gestating	Pregnant
43	2.85	gestating	Pregnant
44	2.39	gestating	Pregnant
45	2.93	gestating	Pregnant
46	3.54	gestating	pregnant
47	3.82	gestating	Pregnant
48	3.93	gestating	Pregnant
49	4.76	gestating	Pregnant
50	5.08	gestating	Pregnant
51	5.61	gestating	Pregnant
52	6.56	gestating	Pregnant
53	7.14	gestating	Pregnant
54	7.17	gestating	Pregnant
55	7.32	gestating	Pregnant
56	7.32	gestating	Pregnant
57	7.38	gestating	Pregnant
58	7.47	gestating	Pregnant
59	7.47	gestating	Pregnant
60	7.72	gestating	Pregnant
61	7.90	gestating	Pregnant
62	8.13	gestating	Pregnant
63	8.69	gestating	Pregnant
64	10.82	gestating	Pregnant
65	11.29	gestating	Pregnant

4 DISCUSSION

Progesterone is a critical hormone for the maintenance of pregnancy in mammals. Measurement of serum progesterone levels has been widely used as an indicator of pregnancy status in several livestock species. The enzyme-linked immunosorbent assay (ELISA) is a sensitive and practical technique for hormone detection and has the potential to be used as a field-applicable diagnostic tool.

The results of this study demonstrate that serum progesterone ELISA is a highly effective and practical diagnostic tool for detecting early pregnancy in camels under field conditions. The significantly elevated progesterone levels observed in pregnant camels directly reflect the active role of the corpus luteum in maintaining pregnancy in camelids.

When evaluated alongside ultrasonography, each method presents distinct advantages and limitations for field veterinary medicine.

Ultrasonography allows for the direct visual confirmation of the embryonic vesicle and fetal heartbeat. However, the ELISA-based progesterone test offers a highly sensitive biochemical alternative that can detect luteal activity around the same critical early window (days 12–20 post-mating) without the need for high-end imaging equipment.

Implementing ultrasonography in remote field conditions is often constrained by the high cost of portable units and the requirement for highly specialized operator expertise. In contrast, the ELISA assay proved to be highly practical, reproducible, and accessible for large-scale herd screening where rapid, field-applicable processing is required.

The primary limitation of the progesterone ELISA is its reliance on a hormonal marker rather than direct fetal visualization. A high progesterone profile indicates a functional corpus luteum, which can occasionally lead to false positives in cases of early embryonic mortality or persistent corpora lutea. Ultrasonography remains superior in confirming fetal viability and ruling out these false positives. Therefore, utilizing ELISA as a primary screening tool followed by selective ultrasonography offers an optimal, cost-effective reproductive management strategy.

5 CONCLUSION

In conclusion, serum progesterone ELISA provides a reliable, accurate, and highly accessible method for early pregnancy diagnosis in camels under field conditions. The assay exhibits strong sensitivity and specificity, making it an excellent candidate for routine field use, particularly in resource-limited or remote environments where ultrasonography may not be readily available. For maximum diagnostic accuracy and herd efficiency, a dual-approach leveraging the speed of ELISA screening and the visual verification of ultrasonography is highly recommended.

Progesterone serum concentration in the third trimesters need to be investigated as well as during hormonal disturbances due to cystic ovaries, repat breeder and pseudopregnancy.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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