PREGNANCY DETECTION OF IRAQI SHE-CAMELS (*Camelus dromedarius*) USING VARIOUS TECHNIQUES

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ABSTRACT

A study was conducted to investigate the most sensitive, early, and precise methods for detecting pregnancy in Iraqi She-camels using plasma progesterone levels, ultrasonography, and rectal palpation techniques. The study involved twelve multiparous, non-lactating one-humped She-camels.The sensitivity of plasma progesterone for diagnosing pregnant She-camels decreased ($P \leq 0.01$) from 100% at day 20 post-mating (PM) to 80% during subsequent periods. Conversely, the specificity for diagnosing non-pregnant She-camels increased ($P \leq 0.01$) from 71.4% at day 20 PM to 85.7% at day 30 PM, reaching 100% at days 40, 50, and 60 PM. The sensitivity for diagnosing pregnant she-camels using ultrasonography was 100% on day 20 post-mating (PM) and 80% during subsequent periods. The specificity for diagnosing non-pregnant she-camels increased significantly ($P \leq 0.01$), rising from 71.4% on day 20 PM to 85.7% on day 30 PM and reaching 100% by days 40, 50, and 60 PM. The sensitivity and specificity of rectal palpation achieved 100% on days 60 and 90 PM. In conclusion, we can effectively detect early and accurate pregnancy in Iraqi she-camels using progesterone levels and ultrasonography techniques as early as day 20 PM.

Keywords: Progesterone, ultrasonography, rectal palpation, camels.

تشخيص الحمل لإناث الإبل العراقية (*Camelus dromedarius*) باستعمال تقانات مختلفة

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المستخلص

أجريت هذه الدراسة لبيان أكثر الطرق حساسيةً وتبكيراً ودقةً في تشخيص الحمل لدى اناث الإبل العراقية باستعمال تركيز هرمون البروجستيرون في بلازما الدم والأمواج فوت الصوتية والجس عبر المستقيم. استعملت 12 من اناث الإبل العراقية ذات السنام الواحد. انخفضت ($P \leq 0.01$) حساسية طريقة حساسية تركيز هرمون البروجستيرون في بلازما الدم لتشخيص الاناث الحوامل من 100 % عند اليوم 20 بعد التلقيح الى 80 % عند بقية المدد. ازدادت ($P \leq 0.01$) خصوصية طريقة تركيز البروجستيرون في تشخيص الاناث غير الحوامل من 71.4 % عند اليوم 20 بعد التلقيح الى 85.7% عند اليوم 30 بعد التلقيح والى 100 % عند الأيام 40 و50 و60 بعد التلقيح. بلغت حساسية طريقة الأمواج فوت الصوتية لتشخيص اناث الإبل الحوامل 100 % عند اليوم 20 بعد التلقيح الى 80 % عند بقية المدد. ازدادت ($P \leq 0.01$) خصوصية طريقة الأمواج فوت الصوتية في تشخيص الاناث غير الحوامل من 71.4 % عند اليوم 20 بعد التلقيح الى 85.7% عند اليوم 30 بعد التلقيح والى 100 % عند الأيام 40 و50 و60 بعد التلقيح. من ناحية أخرى، بلغت حساسية وخصوصية طريقة الجس عبر المستقيم 100 % عند اليومين 60 و90 بعد التلقيح. يمكن الاستنتاج بان تشخيص الحمل بشكل دقيق ومبكر (20 يوم بعد التلقيح) لإناث الإبل العراقية تم اجراؤه باستعمال تقانتي مستوى هرمون البروجستيرون في بلازما الدم والأمواج فوت الصوتية.

الكلمات المفتاحية: البروجستيرون، الأمواج فوت الصوتية، الجس عبر المستقيم، الإبل.

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INTRODUCTION

The reproductive efficiency of she-camels under their natural bucolic conditions is minimal because of their late age at puberty (3-4 years old), short breeding season (3-5 months), and prolonged gestation period protracted to 13 months (5, 14, 21). Camel and livestock reproduction and genetic improvement depend on developments in reproductive techniques, the current assisted reproductive technologies used, and their future horizons. These techniques involve artificial insemination, early pregnancy diagnosis, *in vitro* fertilization, embryo transfer, sexing, gamete, and embryo micromanipulation (1, 6, 7, 12). Different pregnancy detection methods were investigated in she-camels, namely tail cocking of pregnant She camel when close adjacent to a male at 15 days post-mating, PM), changes in cervical mucus (16), Rectal palpation by 60 days PM (15), ultrasonography as early as 17 days PM (21) and steroid hormones concentrations throughout pregnancy, such as progesterone (from day 3 post ovulation), estradiol-17β (increase around day 20- 25 post-ovulation) and estrone sulfate (the first peak occurs around day 26 PM) (23). Early pregnancy in female camels has been diagnosed using real-time b-mode ultrasonography as early as 20 days post-mating (10, 30). However, previous studies did not pinpoint the specific period in pregnancy that offers the highest accuracy for detecting and determining accuracy parameters in female dromedary camels. Additionally, early pregnancy detection in she-dromedary camels using current precise equations has not been thoroughly investigated. This study aimed to evaluate early pregnancy detection in she-camels through three techniques: measuring plasma progesterone levels, ultrasonography, and rectal palpation.

MATERIALS AND METHODS

Experimental animals: At the University of Fallujah, in western Iraq, an experiment was conducted at the Animal Farm of the College of Veterinary Medicine. The study included twelve multiparous Iraqi she-camels (*Camelus dromedarius*), aged 7 to 8 years and weighing between 450 and 500 kg. A daily diet of 4 kg of green fodder (including alfalfa, barley, and sorghum), 10 kg of alfalfa hay, and 0.5 kg of barley grains per animal was fed to the camels. Water and mineral blocks were provided *ad libitum* (3, 4). From June to July, animals were treated with an anti-tick local skin solution (Actobor, 50 ml per animal). Camels were housed in semi-closed pens with appropriate areas for walking smoothly. Estrus and mating signs were monitored regularly for all animals. Estrus signs, like hind legs and tail raising, restlessness, recurrent salivation, and urination, were observed when the male came close to she-camel. Females mated naturally with fertile males.

Pregnancy detection

Plasma progesterone assay: Five blood samples were collected for each animal via jugular venipuncture on 20, 30, 40, 50, and 60 days post-mating (PM) for early pregnancy detection using plasma progesterone diagnosis (ng/ ml). The blood sample (10 ml) was taken, and the plasma was harvested. The samples were then centrifuged (3000 RPM for 15 minutes) and stored under -20°C until analyzed (1, 3). Radioimmunoassay (RIA) was employed to measure the plasma progesterone concentration (ng/ml). The kit was supplied by Immunotech, a Beckman Coulter Company, de Lattre de Tassigny, Marseille, France (3). The hormone assay was conducted at Al-Nadhaer Clinical Laboratory, Baghdad. The assay's sensitivity was 0.05 ng/ ml, whereas the intra-assay and inter-assay coefficients of variation (CV) were 5.8 and 9 %, respectively. The cross-reactivity with several endogenous and pharmaceutically steroids was (100, 20.18, 7.31, 2.38, and 0.22) % with progesterone, 5bpregnanedione, 5a- pregnanedione, 6b-hydroxyprogesterone and 17ahydroxyprogesterone, respectively (2). The cut-off value of progesterone level used to diagnose pregnant buffalo cows was one ng/ml (2).=

Ultrasonography: Female camels were early pregnancy detected using scanned ultrasonographically of the genital tract at days 20, 30, 40, 50, and 60 PM. In a sitting position, the ultrasound examination of internal genitalia was performed (28). The She camels were restrained in sternal recumbent posture on the ground with four legs steadily fastened using ropes (10). Xylazine (Kepro, Holland),

80-120 mg, administered intravenously for sedation. The current ultrasound scanner was My Lab Five VET Esaote, the Netherlands, equipped with a 5-7.5 MHz linear-array intra-rectal transducer (10).

Rectal palpation: A professional veterinarian performed rectal palpation on days 60 and 90 PM. He palpated the existence of the conceptus by feeling the "fetal slip," which refers to the placental membranes. Sensitivity, specificity, positive predictive values, and negative predictive values of each pregnancy-detecting method are estimated according to the equations reported by (9, 25).

Statistical analyses: Chi-square was used to compare the variation among sensitivity, specificity, and positive and negative predictive values (3, 24).

RESULTS AND DISCUSSION

Plasma progesterone concentrations: The average progesterone concentrations in pregnant She camels were 3.97 ± 1.38 ng/ ml at day 20 PM to 4.78 ± 1.55 ng/ ml at day 30 PM. The concentration reached 7.16 ± 2.09 ng/ ml at day 60 PM (Table 1). The sensitivity of the progesterone assay for diagnosing pregnant She camels declined (P ≤0.01) from 100 to 80% during the remaining period (Table 1). The sensitivity of the progesterone assay for diagnosing pregnant She camels was highly precise (P ≤0.01, 100%) on day 20 PM and moderately exact (80%) during the other rest periods (Table 2). The specificity of the progesterone assay for diagnosing non-pregnant She camels decreased obviously (P ≤0.01) from 85.7% on days 20 and 30 PM to 71.4% on day 40 PM and 57.1 % on days 50 PM, at a time of increased again at day 60 PM to 85.7% (Table 2). The positive predictive values showed a progressive decline (P ≤ 0.01), decreasing from 83.3% on day 20 PM to 80% on day 30 PM. This trend continued, with values of 66.6% and 57.1% on days 40 and 50 PM, respectively. However, there was an increase on day 60 PM, returning to 80% (Table 1). In contrast, the negative predictive value of this assay for detecting non-pregnant subjects declined (P ≤ 0.01) from 100% on day 20 PM to a range of 80% to 85.7% during the subsequent periods (Table 2).

Ultrasonography: The sensitivity for diagnosing pregnant She camels was highly precise (P≤0.01, 100%) on day 20 PM and moderately precise (P≤ 0.01, 80%) on the rest of the experimental periods (Table 2). The specificity for detecting non-pregnant Iraqi She camel increased (P≤ 0.01) from 71.4% on day 20 PM to 85.7 on day 30 PM and 100% on days 40, 50, and 60 PM. The positive predictive value for detecting pregnant She camels was lesser (P≤ 0.01) on day 20 PM (71.4%), increased (P≤0.01, 80%) at day 30 PM to reach 100% on days 40, 50, and 60 PM (Table 2). In contrast, negative predicted values were higher (P≤0.01, 100%) on day 20 PM and slightly decreased (P≤ 0.05) to 87.5% throughout the remaining periods (Table 2). On the day 20 PM, the conceptuses appeared as an accumulation of an embryonic fluid, regular and round, with a diameter of 15-18 mm (Figure 1A). On day 30 PM, the diameter of the embryonic vesicle is about 38 mm, lying ventrally and attached to the uterine wall. The cardiac region appears as an echoic spot within the embryo (Figure 1B). At the third ultrasound examination (day 40 PM), the head, trunk, and limbs were visible, and also the eyeball appeared as an anechoic spot within the head. The crown-rump length was about 27 mm, and the head length was about 8 mm (Figure 1C). Fifty and sixty days PM, the fetal membrane was apparent, the endometrium was increased irregularly (folds) throughout its length, and fetal parts identification and measurement were tricky (Figure 1D).

Table 1. Plasma progesterone (ng/ml) of Iraqi She camels during various gestation periods (Mean ± SE).

Gestation period (days post-mating)	Plasma Progesterone concentration (ng /ml)
20	3.97 ± 1.38 b
30	4.78 ± 1.55 b
40	5.06 ± 1.85 b
50	7.16 ± 2.09 a
60	5.54 ± 1.88 ab

Means with different superscripts within each column are different at p<0.05

Rectal palpation: The sensitivity for diagnosing She camels was 100% at 60 and 90 days PM (Table 2). The specificity for diagnosing non-pregnant She camels was high (100 %) on days 60 and 90 PM. The positive predictive value of rectal palpation for diagnosing pregnant She camels was 100% of the above periods (Table 2). Greater negative predictive values (100%) for diagnosing non-pregnant camels were shown on days 60 and 90 PM. The sensitivity and specificity were high during days 60 and 90 PM (Table 2). Due to the lack of positive and negative false, positive and negative predictive values were also more significant (100%) during this gestation period (Table 2). Compared with the two techniques mentioned above, rectal palpation could be precise in detecting pregnant and non-pregnant She-camels from day 60 PM onwards. This technique has certain management constraints. Generally, the progesterone assay and ultrasonography tests demonstrated greater accuracy ($P \leq 0.01$; 100%) and earlier detection on day 20 PM for identifying pregnant female camels compared to the rectal palpation method. Conversely, the progesterone assay identified 85.7% of non-pregnant she-camels earlier (on day 20 PM) than the ultrasonography technique, which identified 71.4%. However, this trend reversed during days 40 to 60 PM, with the ultrasonography method, which ascertained higher accuracy (100%) compared with the progesterone assay method within similar periods (57.1-85.7%; Table 2). Moreover, ultrasonography and rectal palpation methods were superior ($P\leq 0.01$, 100%) in detecting pregnant and non-pregnant female camels on day 60 PM as compared with progesterone assay (80-85.7%; Table 2). The present data were consistent with Quzy *et al* (19), who noticed elevated serum progesterone concentrations of 1-2 ng/ml on day 20 PM and up to 2.5 ng/ml on day 30 PM. The main reasons behind decreasing sensitivity during days 30-60 PM to 80% may be erythrocyte metabolism (27), storage time, and blood temperature from collection to plasma separation (12). Notably, no previous reports were published investigating the sensitivity or precision data of plasma progesterone concentrations to express early pregnancy detection in She camels. The precision obtained for detecting non-pregnant She camels (specificity and positive predictive value) decreased obviously (-48%, $p \leq 0.01$) from 85.7 and 83.3% on day 20 to 57.1% on day 50 PM (Table 2). The reasonable reason may relate to the incidence of early embryonic mortality, which seems to be high in the camel (20). Early embryonic losses occur more frequently in the camel than in other species (8-32%, 24). 9.21% of early embryonic deaths occurred between day 20 and 30 PM in She camels during the natural breeding season in the United Arab Emirates, concurrent with a decline in serum progesterone levels (19). One incorrect non-pregnant female camel contributed to the 80-85.7% negative predictive value on days 30-60 PM (Table 2). The primary possible mechanism for this may be increased progesterone concentrations as a result of increasing feed intake of these female camels during the 2-3 hours pre-sampling period. Notably, the liver blood flow and metabolic clearance rate of progesterone reached a maximum of 2 hours post-feeding in lactating cows. They persisted longer in cows given more significant amounts of feed (28). The other reasons may relate to erythrocyte metabolism, storage time, and elevated blood temperature from collection to plasma separation, as discussed above. More work has been done on South American Camelids, like llamas and alpacas (11, 16), than on the dromedary camel (30, 31) using ultrasonography. The sensitivity of this assay for detecting pregnant She camels was high (100%) and earlier (day 20 PM; Table 2).

Table 2 Early pregnancy detection of Iraqi She camels using three various techniques.

Days post-mating Evaluation	Progesterone assay					Ultrasonography					Rectal palpation		Chi-square and level of significance
	20	30	40	50	60	20	30	40	50	60	60	90	
Sensitivity (%)	100	80	80	80	80	100	80	80	80	80	100	100	7.20 **
Specificity (%)	85.7	85.7	71.4	57.1	85.7	71.4	85.7	100	100	100	100	100	9.61 **
Positive predictive value (%)	83.3	80	66.6	57.1	80	71.4	80	100	100	100	100	100	9.27 **
Negative predictive value (%)	100	85.7	83.3	80	85.7	100	85.7	87.5	87.5	87.5	100	100	7.53 **

**: P≤0.01

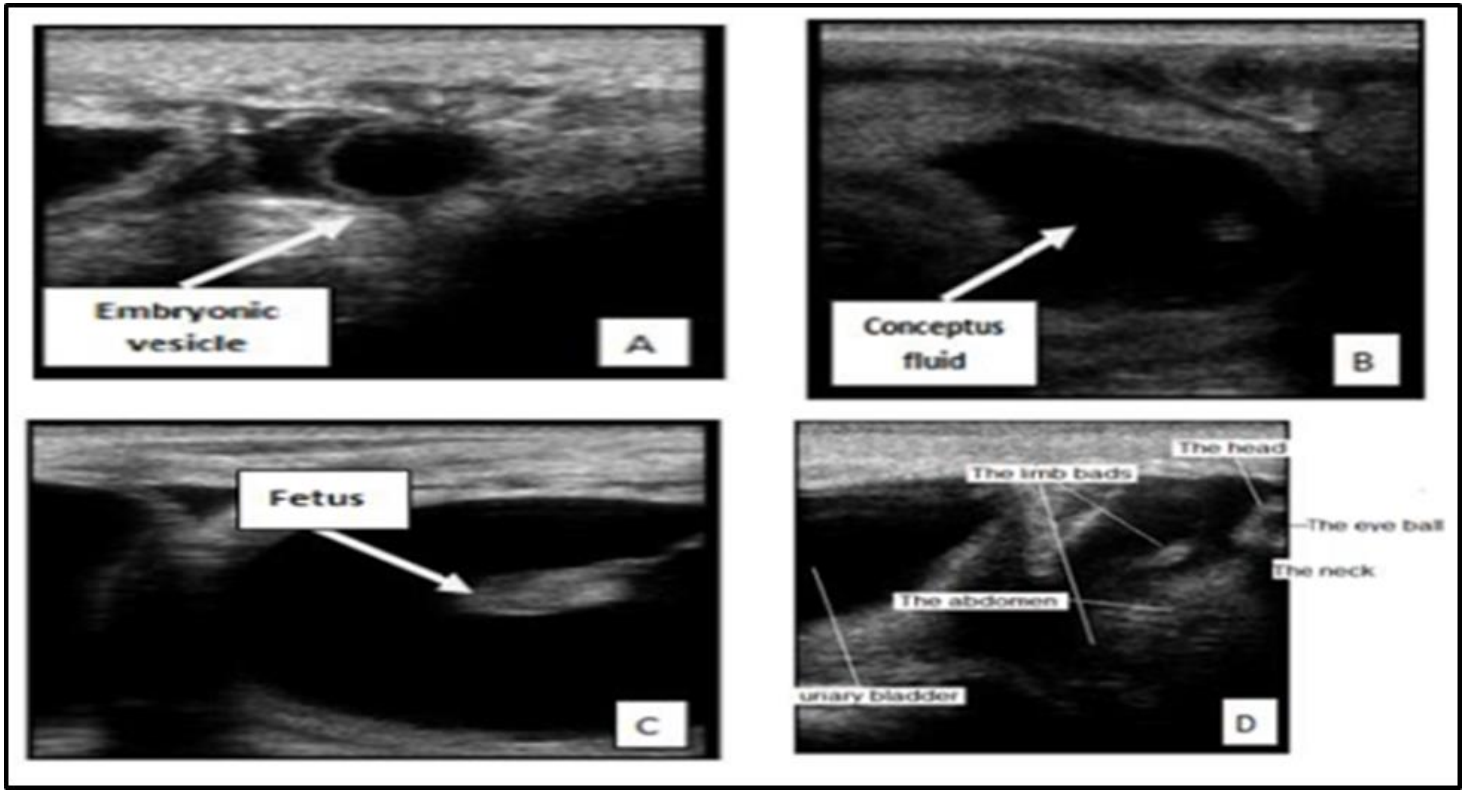

Figure 1. Ultrasonogram of Iraqi She camels at days 20 (A), 30 (B), 40 (C), and 50 (D) post-mating

These results concurred with those reported by Vyas et al (30) recognized the conceptus as a fluid accumulation in the lumen of the left uterine horn. However, they observed that definite pregnancy detection could be made based on the accumulated fetal fluids and echogenic embryo with its pulsatile heart by day 30 PM. Similar findings were reported by Ali et al. (8), who noted that intrauterine fluid accumulation was observed in 3 out of 7 cases by the second week of gestation and in all camels by the third week. Furthermore, the embryo was detected in 2 out of 7 cases by the third week and in all animals by the fourth week of pregnancy. The time between mating and ovulation in camels ranges from 24 to 36 hours (17). Therefore, there were no apparent variances in the days of the first embryo detection proper in the current study (day 20) compared to the earlier reports. On days 30 and 40PM, the present sensitivity (80%) is more significant than those obtained by Vyas et al (31), not exceeding 50%. It is worth mentioning that no previous study expressed the pregnancy detection values of female camels using current accuracy equations. Furthermore, the present study exhibited much higher (100%) specificity and positive predictive values for detecting non-pregnant females using ultrasonography on days 40 PM onwards (Table 2). This notion disagreed with Vyas et al (31) findings by identifying non-pregnant females as early as day 20 PM. However, good results were also obtained for detecting non-pregnant camels at days 20 (71.4%) and 30 (80-85.7%) PM currently. One incorrect non-pregnant female camel contributed to the 85.7% negative predictive value of the ultrasonography method on days 30-60 PM (Table 2). The reasonable explanation behind this negative false may be attributed to improper scanning, as the uterus is positioned higher than the pelvic inlet at this stage of gestation, leading to poor visualization during the procedure. This study presents the first accurate detection of early pregnancy in dromedary female camels using progesterone assays and ultrasonography on day 20 PM. These findings will improve reproductive and productive efficiency by implementing valuable management tools in Iraq and worldwide.

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