

Comparative study on early pregnancy diagnosis by three different techniques in cows

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(Received 28/3/2016, Accepted 28/5/2017)

Abstract

The aim of this study was to determine the best method, most appropriate and high precision method between rectal palpation, ultrasonography and measuring level of progesterone for diagnosis of early pregnancy in cows. 20 cows were examined in Teaching Hospital / College of Veterinary Medicine / Mosul University, at the period between 1/11/2013 - 1/3/2014, cows undergone early pregnancy diagnosis using three different methods at 45, 60 and 90 days of pregnancy. Results of rectal palpation showed that the percentage of pregnancy was 0% 60% 85% at days 45, 60 and 90 of pregnancy, respectively, while results of ultrasonography showed that diagnosis early pregnancy was 30, 80 and 100 at days 45, 60 and 90 of pregnancy, respectively. Results of estimation of progesterone concentration by ELISA technique showed that pregnancy diagnosis was 60% 85% 100% at days 45, 60 and 90 of pregnancy, respectively. In conclusion, the best and fastest way to diagnose early pregnancy in cows was measure concentration of progesterone in blood serum of cows then followed by ultrasonography, while method of rectal palpation was inefficient in detecting early pregnancy in cows.

Keywords: early pregnancy, rectal palpation, ultrasonography, progesterone.

Introduction:

Livestock is the source of economic income after Iraq, especially cattle for their products included milk, meat and skin in addition to the newborn calves, also possibility of use of bones in production of animal protein that used in diets of other animals, also using their feces in the production of organic fertilizing elements (1). One of the main methods used for diagnosis of pregnancy in cows include administrative methods (2) vaginal smears (3), rectal palpation (4), measuring the level of some hormones such as estrogen and progesterone, in addition to measuring the concentration of certain specific proteins of pregnancy (5), finally the use of ultrasonography (6). Rectum palpation in cattle needs large scale also long time in diagnosis of pregnancy, but it considers a simple and easy method that doesn't require tools or laboratory materials just long clothes and lubricants (7). In past 50 years, a new method has been used known as ultrasonography, which considers a quick method gives accurate diagnosis also gives a good possibility to determining number and sex of fetus (8). A recent method of

pregnancy diagnosis is invented depending on measuring concentration of progesterone in the serum of suspected cows, which give a positive result can be up to 95% in the diagnosis of pregnancy, measure of progesterone level is done by several techniques like enzyme -linked immunosorbent enzyme (ELISA), Radio immune assay, Carbone absorbent assay (9). For the importance of early pregnancy diagnosis in cows, we decided to conduct this study to determine the best and most appropriate method from rectal palpation, ultrasonography, and measuring progesterone concentration for early pregnancy diagnosis between cows in Mosul city - Iraq.

Materials and Methods:

This study involved the examination of 20 local cows, which attended to teaching hospital of College of Veterinary Medicine at the period between 01/11/ 2013 – 01/03/2014, these cases were recorded within form which had been prepared in advance. The cows have been subject to early pregnancy diagnosis by using three

different techniques at 45 days, 60 and 90 days of pregnancy and these methods are:

1. Rectal Palpation; which done as described by (3).

2. Ultrasonography technique applied by using ultrasonography device of Chinese origin Real time ultrasound Scanner type supplied by KAL XIN Zhou klaxon Electronic instrument, and examination is done as described by (3) in which the tissue that reflect ultrasound wave like bones and gases appear white in color and called echogenic, while tissue that allow ultrasound wave to transmit through appear black in color like fluid which called anechogenic or anechoic, while tissue that allow to part of ultrasound wave to transmit or reflect appear in vary shadows of gray like soft tissue which the called echogenic or hypogenic (3) (Figure 1).

3. Estimation progesterone level in serum: This technique used to measure concentration of progesterone in the blood serum, by using ELISA Kits (manufactured by Monobind, USA) to measure

Results:

Rectal palpation: the result at 45 days of pregnancy shows no detection of early pregnancy, while at 60 days of pregnancy about 12 cows had signs of early pregnancy which represented about 60% (of 12 cows), which indicated by observed of asymmetry horns of uterus during palpation for presence of embryonic growth, in addition to appearance of thrilling in blood vessels. While in 90 days of pregnancy about 17 cows showed signs of pregnancy in percentage of 85% (of 17 cows), where it was noted by increase in size of uterus and presence of embryonic fluid in addition to sliding of embryonic membranes during palpation with possibility of uterine move down to abdominal cavity (table 1).

Ultrasonography: The result of current study (Table 1) showed that the percentage of diagnosis of early pregnancy in cows after examining 20 cows was 30% (6 cows) in 45 days of pregnancy, where it was noted pregnant uterus contain embryonic fluid which appeared black area represented embryonic fluid containing white area represented embryo (Figure 2-A). This

concentration of the progesterone. The blood samples were collected from each cow from jugular vein, and placed in a sterile test tube and left diagonally to get serum for purpose of measuring concentration of progesterone, then by using a centrifuge at 3000 rpm for 5 minutes, serum has been separated, then measuring procedure is done as described by (3).



Fig. 1: Ultrasonography device that used in this study

percentage increased up to 80% (16 cows) at 60 days of pregnancy, where it was observed an increase in size of black circles and the large size of embryos (white area) inside black areas, at this stage parts of fetus as the head and spinal cord can be observed (Figure 2-B). With time percentage of diagnosis of pregnancy increase to reach 100% (20 cows) at 90 days of pregnancy, it was noted the fetus parts like head and limbs with large size of uterine cotyledons can be seen (Figure 2-C), with listening to fetus heartbeat.

Measure concentration of progesterone in serum of cows: According to table (1) and Figure (3), results of current study after examined blood serum of 20 cows by using ELISA test to estimate progesterone level, showed that the percentage of early pregnancy diagnosis in 45 days of pregnancy was 60% (12 cows), where mean concentration of progesterone is 5.5 ng / ml of serum. The percentage of pregnancy diagnosis in cattle increased up to 85% (17 cows) at 60 days of pregnancy, and the mean of progesterone concentration reach

7.6 ng / ml of serum. The rate of pregnancy at 90 days of pregnancy reach up to 100% (20 cows), the mean concentration of progesterone reaches up to 9.3 ng / ml of serum.

Comparing three methods to diagnose early pregnancy in cows: As mentioned in statistical analysis (table 1), the result revealed presence of significant difference at 45 days of pregnancy between ultrasonography and estimate level of progesterone only, at $P < 0.05$. While at 60 days of pregnancy there is no significant

differences between ultrasonography and estimate concentration of progesterone, however; these previous two methods differed significantly from rectal palpation at 60 days of pregnancy, where they higher than rectal palpation at $P < 0.05$. At 90 days of pregnancy also there is no significant differences between ultrasonography and estimate concentration of progesterone, however; these previous two methods differed significantly from rectal palpation at 90 days of pregnancy, where they higher than rectal palpation at $P < 0.05$.

Table 1: Number of positive and percentage of diagnosis of early pregnancy in cows

Method	45 days of pregnancy		60 days of pregnancy		90 days of pregnancy	
	No. positive	%	No. positive	%	No. positive	%
Rectal palpation	0	0	12 ^b	60	17 ^b	85
Ultrasonography	6 ^b	30	16 ^a	80	20 ^a	100
Concentration of progesterone	12 ^a	60	17 ^a	85	20 ^a	100

Small litters that different in horizontal form are significant at $P < 0.05$



Fig. (2): Ultrasonography image showing uterus and fetus at 45 days (A), 60 days (B) and 90 days (C) of pregnancy in cows.

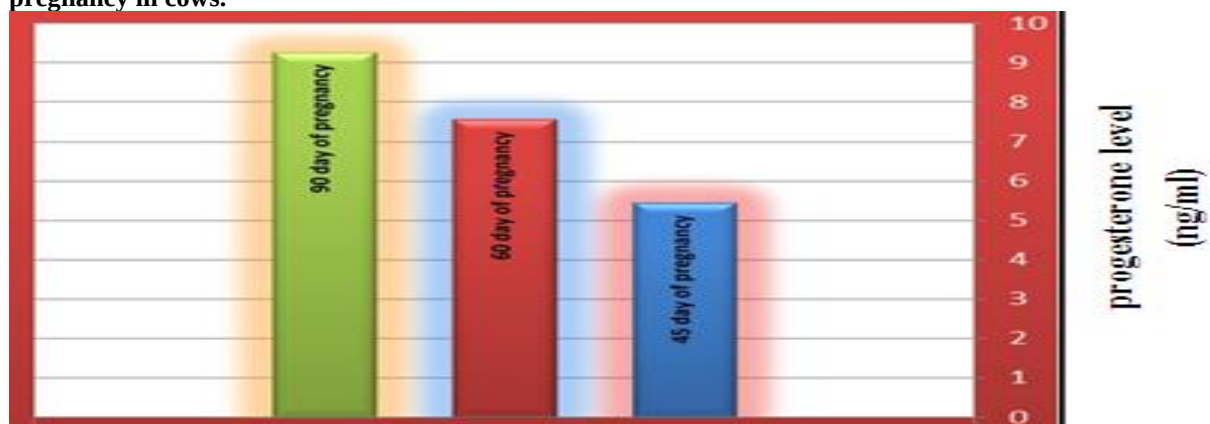


Fig. (3) The mean concentration of progesterone level (ng/ml) blood serum of cows at different days.

Discussion:

The result of current study showed that diagnosis of early pregnancy using rectal palpation at 45 days of pregnancy is difficult at this period due to inability to palpation fetus and placental cotyledons that

confirmed by (10), who noted the difficulties in early pregnancy diagnosis in this period. While at 60 days of the pregnancy it is possible to diagnosis pregnancy but at low percentage and this

done by identified absent of similarity of both horns of uterus and possibility of by detection thrill in blood vessels on the wall of the uterine tube, this was confirmed by (11). while in 90 days of pregnancy the result of this study were agreed with study conducted by (12), in which diagnostic accuracy is increased up to 85% and this is due to increase in size of uterus and completion of growth of uterine cotyledons and embryonic membranes in addition to possibility of palpation different parts of fetus during examination, while these result is not agreed with study done by (13) because the pregnancy diagnosis in these days may interfere with some pathological cases such as pyometra, early fetal death, mummification of embryo which all gives a false positive result as conducted by (2). The rectal palpation is a good method to diagnose pregnancy in last third part, but not accurate to diagnosis early pregnancy in cows because this method is difficult to apply by person with no experiences, so the examiner must be at high level of experience, plus this method need physiological changes in uterus of cow to be palpated or touched either fetus, it's membranes and uterine cotyledons. This method has positive or negative false result such as pathological occupying lesions in uterus like pyometra, masses of tumors, and mummification of fetus that could affect results of this method (14). The early pregnancy diagnosis using Ultrasonography at 45 days of pregnancy up to 30% by observing pregnant uterus filled with embryonic fluid, which appear black color as well as areas of white spot which represented embryos inside these black areas, these results was confirmed by (15) which they also recorded these white and black areas during the 45 days of pregnancy, while (8) reported that accuracy of the diagnosis in 60 days of pregnancy was 84% and this ratio was almost close to ratio obtained in this study which reach 80%, and this increase in diagnosis percentage due to increase in size of fetus, such as head and spinal cord. At 90 days of pregnancy the percentage reach 100%, where the examination showed an increase in size of

areas with the observation of fetus with hearing fetus heartbeat, which agreed with studies done by (16, 8) which they use linear transrectal probe, which gives a high accuracy during scan. This percentage was very high compared with the study conducted by (15) where its result reach to 76% using a convex probe through abdominal wall and this different in percentage might be related to breeds of cattle under investigation. Ultrasonography is considered a good method and favorite for a pregnancy test, especially at day 60 and upwards of pregnancy that can be done at field, as this method depend on evolution of embryonic membranes and fluid with development of uterine cotyledons and fetal. The type of probe used in ultrasonography either linear or convex probe, will give a different percentage of diagnosis, while false positive result in ultrasonography is less than other methods and it come from inability of examiner to explain the black and white areas (11). The results of the current study related to estimate mean concentration of progesterone in blood serum in 45 days of the pregnancy was reach up to 60% since level of progesterone in non- pregnant cows will be less than 2 ng / ml, while any increase above that level will be considered a pregnant cow, and this match to result recorded by (17). In 60 days of pregnancy, percentage of pregnancy diagnosis reach up to 85% and this rate was higher than that recorded by (18) this can be related to some pathological conditions lead to increase in level of progesterone, such as retained cystic luteal ovary that lead to false positive results. At 90 days of pregnancy percentage of early pregnancy diagnosis is reach 100% and this confirmed by some studies done by (19, 20). The measuring of progesterone concentration in serum of cows is laboratory method and depends on a set of biochemical reactions with present of considerable results of negative and positive false, and this come from some disease such as persist corpus luteum (3). This method is based on measuring level of progesterone in blood serum of pregnant females that secreted from the corpus luteal from the beginning of pregnancy without any need

for development of large size of uterus or embryo that depend by other methods (21). In statistical analysis comparison between three different methods, the results indicated that the best method to diagnose early pregnancy in cows were the biochemical test by measuring level of progesterone in blood serum of pregnant cows, then followed by ultrasonography and ranked at

last step rectal palpation, which these results has been confirmed by (22). In conclusion, the best and fastest technique to diagnose early pregnancy in cows was measure concentration of progesterone in blood serum of cows then followed by ultrasonography, while method of rectal palpation inefficient in detecting early pregnancy in cows.

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