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## Clinical application of a rapid and practical procedure of transabdominal ultrasonography for determination of pregnancy and fetal viability in cows

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## ABSTRACT

**Objective:** To apply a rapid and practical procedure of transabdominal ultrasonography for determination of pregnancy and fetal viability in cows. **Methods:** The study was carried out on 104 clinical cases of cows. The cows were examined by rectal palpation and transabdominal ultrasonography in a standing position. **Results:** Results showed that determination of pregnancy and fetal viability were obtained significantly faster using transabdominal ultrasonography than rectal palpation. The accuracy, sensitivity and specificity of rectal palpation were 67.4%, 84.8% and 48.8% for detection of pregnancy, and 33.3%, 90.4% and 1.9% for determination of fetal viability, respectively. While these three parameters for transabdominal ultrasonography were 100% for both pregnancy and fetal viability determination. The pregnancy diagnosis was very fast at five months of gestation, and a significant negative but rather good correlation ( $r = -0.707$ ) was observed between the gestation period and the required time for pregnancy diagnosis. The fetal viability was determined easily and fast at four months of gestation, and there was a significant positive correlation ( $r = 0.32$ ) between the gestation period and the required time for finding the fetal viability. **Conclusion:** Transabdominal ultrasonography is a reliable procedure for determination of pregnancy and fetal viability especially for cows between fourth and sixth months of gestation.

### 1. Introduction

Different methods have been used for pregnancy diagnosis in cows. These vary between laboratory analysis and clinical methods such as ultrasonography and transrectal palpation[1]. Rectal palpation has been used and still the simple and practical method for pregnancy diagnosis in cattle[2]. However, at occasions pregnancy diagnosis could be difficult or impossible by rectal palpation especially for large pluriparous cows with deep abdomens[1]. The size and weight of gravid uterus increased gradually and passing to the abdominal cavity below the pelvic brim and its palpation at five months of gestation may be not possible[3]. Ultrasonography is consider as a versatile technique for pregnancy diagnosis and observation of the fetal growth and has several advantages in comparison with conventional methods[4]. The advantage of the ultrasound over other methods of pregnancy diagnosis is the ability of determine the fetal viability by observation of the heart contractility

between the ribs[5].

Transrectal ultrasonography is widely used for early pregnancy diagnosis in cattle[6-8], but pregnancy diagnosis and monitoring of the fetus at different period of pregnancy are also important. The importance of bovine ultrasonographic examination at mid to late stage of pregnancy has been discussed in detail previously[9].

According to our knowledge and literature review, there are few studies about the application of transabdominal ultrasonography for pregnancy diagnosis in cows[9,10].

In Iraq as in many countries, high percentages of cows are bred individually or bred together with bulls, these types of breeding have inadequate reproductive information especially estrus and insemination dates also the pregnancy status. Therefore, many of cows are presented to the veterinary clinics for pregnancy diagnosis at different periods of gestation; furthermore many of cows are presented to the clinic for determination of fetal viability especially after systemic disease infections which cause fetal mortality such as foot and mouth disease.

According to the clinical cases, many of veterinarians have a difficulty or inability to diagnose the pregnancy and determine the fetal viability in cows. The cows are difficult to diagnose especially that at the period between fourth and sixth months of gestation. For these reasons, this study was designed to apply a rapid and practical procedure of transabdominal ultrasonography for determination of pregnancy and fetal viability in cows, and to evaluate the accuracy, sensitivity and specificity of this procedure.

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## 2. Materials and methods

### 2.1. Animals

The study was carried out on 104 clinical cases of cows which were presented to the Clinic of the Veterinary Medicine College, University of Mosul, Mosul, Iraq between October 2011 and August 2013. Forty cows had no information about the reproductive status and they were presented to the clinic for pregnancy diagnosis. Twenty nine cows were presented to the clinic for pregnancy diagnosis after an identified time of insemination (11 cows at three months, 7 cows at four months, 5 cows at five months and 6 cows at six months of gestation). Twenty cows were presented to the clinic due to inability or difficulty of pregnancy diagnosis by more than veterinarian. Fourteen cows were presented to the clinic after a systemic disease infection (mostly after foot and mouth disease infection) for determining the fetal viability. One cow at 6 months of gestation had severe purulent vaginal discharge and it was presented as an aborted case after foot and mouth disease infection.

### 2.2. Pregnancy diagnosis

The cows were examined by rectal palpation and transabdominal ultrasonography for detection of pregnancy and fetal viability.

Ultrasonographic examination was performed according to the method published by Hunnam *et al.* [10], with minor modification on the area of examination. The examination was done using a real-time ultrasound scanner equipped with a 3.5 MHz sectorial convex probe (KAI XIN, KX5100, Zhou Kaixin Electronic Instrument Co. Ltd, China). The cows were examined transabdominally in standing position. The probe was applied on the area of examination after adding the gel without clipping or shaving of the abdominal wall. The area of examination is located on the right side of the abdominal wall around the base of the udder ventrally and the area between the stifle fold and abdominal wall dorsally. The examination began by applying the probe vertically on the abdominal wall between the base of the udder and stifle fold, then the probe was moved (dorsocaudally in the direction of the pelvic cavity or ventrocranially in the direction of the umbilicus) to identify the fluid filled uterus, placentomes and fetus.

Observation of the placentomes and anechogenic areas of the fluid filled uterus was depended upon for pregnancy diagnosis, and determination of fetal viability depended mainly on the detection of the heartbeat or fetal movement.

The accuracy of pregnancy diagnosis was calculated as the proportion of pregnant and nonpregnant cows that were correctly identified by the diagnosis method [(number of true positive results + number of true negative results) / (number of true positive results + number of true negative results + number of false positive results + number of false negative results)]. The sensitivity of pregnancy diagnosis was calculated as the proportion of pregnant cows with a positive result [number of true positive results / (number of true positive results + number of false negative results)]. The specificity of pregnancy diagnosis was calculated as the proportion of nonpregnant cows with a negative result [number of true negative results / (number of true negative results + number of false positive results)] [11].

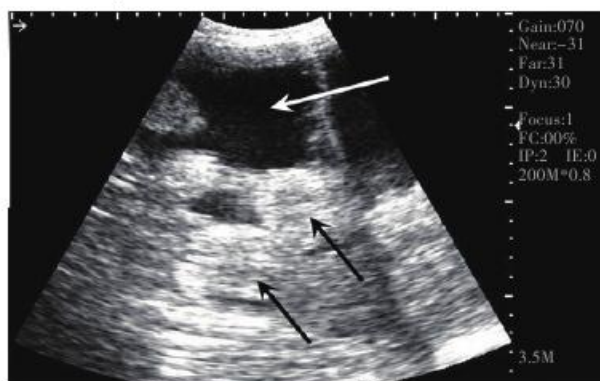
### 2.3. Statistical analysis

The times were required for determination of pregnancy and fetal viability by rectal palpation and transabdominal ultrasonography were compared by Student's *t*-test. The times were required for determination of pregnancy and fetal viability at different gestation periods were compared by One Way Analysis of Variance, and significant differences were determined by Duncan's Multiple Range Test. Pearson correlation coefficients was used to analyze the relationship between the time and gestation period. All statistical

analyses were performed by SigmaStat (Jandel scientific software V3.1).  $P < 0.05$  was considered as statistically significant.

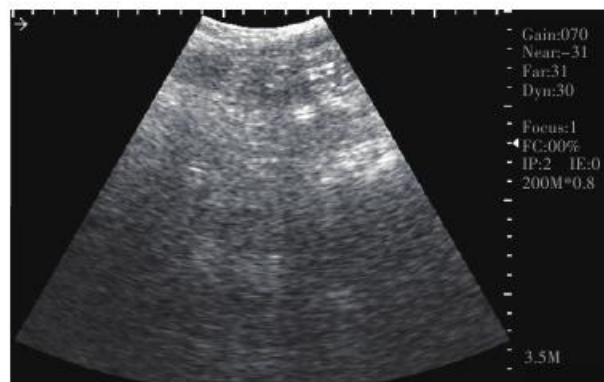
## 3. Results

Table 1 summarizes the results of rectal palpation and ultrasonographic examination of the cows that were presented to the clinic, sixty one cows were pregnant and the remaining twenty eight were non pregnant. The cows were diagnosed pregnant by observing of the fluid filled uterus and placentomes (Figure 1 & 2), and the fetal viability was determined by observation of fetal heartbeat or movement of the fetus (Figure 3&4).

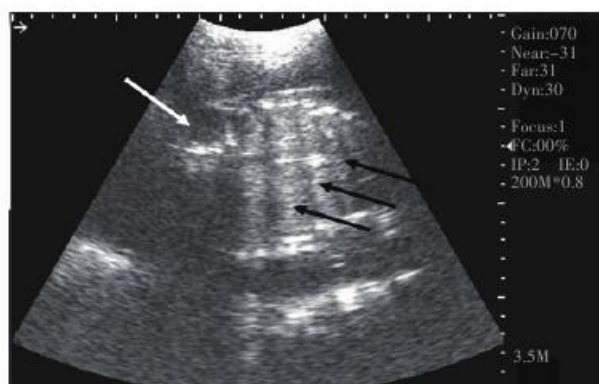


**Figure 1.** Transabdominal ultrasonographic image of cow at seventh month of gestation.

White arrow: anechogenic areas of the fluid filled uterus, black arrows: placentomes.

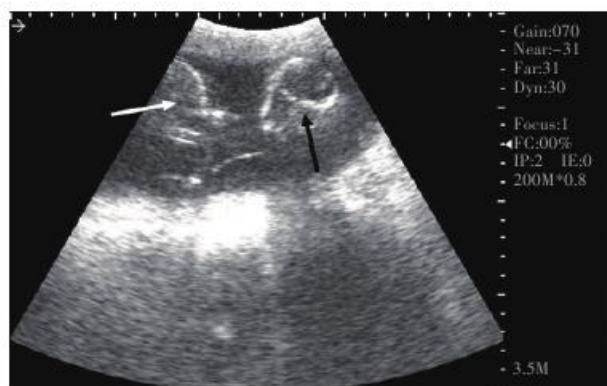


**Figure 2.** Transabdominal ultrasonographic image of non pregnant cow, there is no anechogenic areas of fetal fluid.



**Figure 3.** Transabdominal ultrasonographic image of cow at sixth month of gestation.

White arrow: the heart, black arrows: the ribs.



**Figure 4.** Transabdominal ultrasonographic image of cow at fifth month of gestation. White arrow: the heart, black arrows: head of the fetus.

Some of pregnant and non pregnant cows (especially that large pluriparous cows with deep abdomens and obese cows) were unable or difficult to diagnose, because the inability to palpate the structures that required for a positive diagnosis of pregnancy and fetal viability. The difficulties were observed mainly when the cows between fourth and sixth month of gestation. The detection of fetal viability was more difficult. The results of pregnancy diagnosis and fetal viability detection were obtained significantly

( $P < 0.001$ ) faster using transabdominal ultrasonography than rectal palpation ( $1.06 \pm 0.58$  vs.  $6.42 \pm 1.76$  and  $4.82 \pm 0.88$  vs.  $9.80 \pm 2.30$  min). The accuracy, sensitivity and specificity of rectal palpation were 67.4%, 84.8% and 48.8% for detection of pregnancy, and 33.3%, 90.4% and 1.9% for determination of fetal viability, respectively. While these three parameters for transabdominal ultrasonography were 100% for both pregnancy and fetal viability determination (Table 1 and 2).

By application of the transabdominal ultrasonography, the pregnant cows were diagnosed significantly faster ( $P < 0.001$ ) than those which were non pregnant ( $0.72 \pm 0.25$  vs.  $1.65 \pm 0.49$  min) and in comparison to the pregnancy diagnosis, the determination of fetal viability required longer time period ( $1.06 \pm 0.58$  vs.  $4.82 \pm 0.88$  min).

Results of ultrasonographic examination of the cows which were presented to the clinic for pregnancy diagnosis after an identified time of insemination are shown in Table 3. The pregnancy diagnosis was very fast at five months of gestation ( $0.60 \pm 0.22$  min), and a significant ( $P < 0.001$ ) negative but rather good correlation ( $r = -0.707$ ) was observed between the gestation period and the required time for pregnancy diagnosis. The fetal viability was determined easily and fast at four months of gestation ( $3.86 \pm 0.90$  min), and there was a significant ( $P < 0.05$ ) positive correlation ( $r = 0.32$ ) between the gestation period and the required time for finding the fetal viability.

**Table 1**

Results of rectal palpation and transabdominal ultrasonography for pregnancy diagnosis in cows.

| Method                         | No. of pregnant cows<br>(n=61)   | No. of non-pregnant cows<br>(n=28) | Accuracy<br>(%) | Sensitivity<br>(%) | Specificity<br>(%) | Time required<br>(min) |
|--------------------------------|----------------------------------|------------------------------------|-----------------|--------------------|--------------------|------------------------|
| Rectal palpation               | 39 <sup>a</sup> /22 <sup>b</sup> | 21 <sup>c</sup> /7 <sup>d</sup>    | 67.4            | 84.8               | 48.8               | 6.42±1.76              |
| Transabdominal ultrasonography | 61 <sup>a</sup> /0 <sup>b</sup>  | 28 <sup>c</sup> /0 <sup>d</sup>    | 100.0           | 100.0              | 100.0              | 1.06±0.58 <sup>*</sup> |

<sup>a</sup> Number of cows were true positive diagnosed, <sup>b</sup> number of cows were false positive diagnosed, <sup>c</sup> number of cows were true negative diagnosed, <sup>d</sup> number of cows were false negative diagnosed, <sup>\*</sup> a significant variation between the two methods of pregnancy diagnosis ( $P < 0.001$ ).

**Table 2**

Results of rectal palpation and transabdominal ultrasonography for determination of fetal viability in cows.

| Method                         | Viable fetus (n=72)              | Dead fetus<br>(n=3)            | Accuracy<br>(%) | Sensitivity<br>(%) | Specificity<br>(%) | Time required<br>(min)   |
|--------------------------------|----------------------------------|--------------------------------|-----------------|--------------------|--------------------|--------------------------|
| Rectal palpation               | 19 <sup>a</sup> /53 <sup>b</sup> | 1 <sup>c</sup> /2 <sup>d</sup> | 33.3            | 90.4               | 1.9                | 9.80 ± 2.30              |
| Transabdominal ultrasonography | 72 <sup>a</sup> /0 <sup>b</sup>  | 3 <sup>c</sup> /0 <sup>d</sup> | 100.0           | 100.0              | 100                | 4.82 ± 0.88 <sup>*</sup> |

<sup>a</sup> number of cows were true positive diagnosed, <sup>b</sup> number of cows were false positive diagnosed, <sup>c</sup> number of cows were true negative diagnosed, <sup>d</sup> number of cows were false negative diagnosed, <sup>\*</sup> a significant variation between the two methods of fetal viability determination ( $P < 0.001$ ).

**Table 3**

Results of ultrasonographic examination of the cows that presented to the clinic for pregnancy diagnosis after identified time of insemination.

| Gestation period | No. of cows | Time required (min)<br>(Mean ± SD) |                            |
|------------------|-------------|------------------------------------|----------------------------|
|                  |             | Pregnancy diagnosis                | Fetal viability            |
| 3 months         | 11          | 2.18 ± 0.75 <sup>a</sup>           | 4.91 ± 0.83 <sup>a</sup>   |
| 4 months         | 7           | 0.93 ± 0.53 <sup>b</sup>           | 3.86 ± 0.90 <sup>b</sup>   |
| 5 months         | 5           | 0.60 ± 0.22 <sup>b</sup>           | 4.88 ± 0.76 <sup>a,b</sup> |
| 6 months         | 6           | 0.67 ± 0.26 <sup>b</sup>           | 5.83 ± 1.17 <sup>a</sup>   |

<sup>a,b</sup> The different letters at the columns refer to significant difference ( $P < 0.05$ ).

Ultrasonographic examination of the cow that presented to the clinic as an abortion case indicated that the animal had vaginitis and the fetus was viable and its heart contractility was seen between the ribs during the ultrasonographic examination.

#### 4. Discussion

Pregnancy diagnosis is very important to improve the reproductive efficiency of the cows. Rectal palpation is still the practical method for pregnancy diagnosis, but sometimes the diagnosis and monitoring of pregnancy becomes difficult or impossible especially between the fourth and sixth months of gestation. The pregnant uterus at this period of gestation becomes larger and heavier and

sinks down into the abdominal cavity[3]. Also, position of the fetus in the abdominal cavity and its size during the second half of gestation decreases the possibility of fetal monitoring by transrectal ultrasonography[12]. For these reasons, many of the veterinarians failed to get a correct diagnosis of pregnancy or give a fault diagnosis. Therefore, many of cows were presented to the clinic of Veterinary Medicine College for confirming the pregnancy diagnosis.

Few studies were published about the use of transabdominal ultrasonography for pregnancy diagnosis in cattle[9,10]. Therefore the current study was designed to apply a rapid and practical procedure of transabdominal ultrasonography clinically for determination of pregnancy and fetal viability in cows, and to evaluate the accuracy, sensitivity and specificity of this procedure.

The procedure of transabdominal ultrasonography which is applied in the present study for determination of pregnancy and fetal viability is practical, rapid and easier than rectal palpation. The accuracy, sensitivity and specificity of present examination were 100% and this procedure requires no shaving of the abdominal wall and the diagnosis can be done within five minutes.

The area of examination which was applied in the present study has short hair, therefore, no shaving was required. This area is located over the direction of the uterus when it sinks down from the pelvis to the abdominal cavity, therefore the diagnosis was rapid at different periods of gestation.

The pregnant cows were diagnosed faster than non pregnant ones. The placentomes and anechogenic areas of the fluid filled uterus can be seen easily and clearly in a short time (few seconds) after the application of the transabdominal probe. But the ultrasonographic images of non pregnant cows had no anechogenic area, therefore, the examination required a longer time period to confirm the diagnosis.

Examination of the cows with predetermined insemination date, indicates that the diagnosis of pregnancy becomes faster and easier by increasing of gestation period. The diagnosis can be done within less than one minute at fourth month of gestation and later, because the gravid uterus rests on the ventral abdominal wall[12].

Determination of the fetal viability in this study was also easy and fast and can be done within five minutes. At four months of gestation, fetal viability was determined faster than the other gestation periods because the fetus at this period is located directly under the area of examination. At third and fifth months of gestation, the fetal viability requires a longer time period because the fetuses are located not directly under the area of examination. At sixth months of gestation, the fetal viability required a longer time period for determination because the fetuses at this period of gestation resting on the ventral abdominal wall[12].

The procedure of transabdominal ultrasonography presented here has some advantages in comparison with rectal palpation for determination of pregnancy and fetal viability in cattle. These include no need for rectal evacuation, reduction the possibility of rectum injury, and

it can be used for small and large cows at different times of gestation. Further, the diagnosis can be done within a short time and a restriction of the animal is not required and the examination can be done in the field.

Transabdominal ultrasonography is a reliable procedure for determination of pregnancy and fetal viability especially for cows between fourth and sixth months of gestation.

#### Declare of interest statement

We declare that we have no conflict of interest statement.

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