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Accuracy of subjective evaluation of luteal blood flow by color Doppler ultrasonography for early diagnosis of pregnancy in Egyptian buffalo

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ABSTRACT

The objective of the current study was to evaluate the accuracy of trans-rectal color Doppler ultrasonography for early diagnosis of pregnancy in Egyptian buffalo based on subjective assessment of luteal blood flow (LBF). After timed artificial insemination (TAI), a total of 112 pluriparous buffalo were subjectively evaluated for LBF and there were score scales or grades (I-IV) determined at different times (Days 6, 14, 17, 21) post-TAI. Another trans-rectal B-mode ultrasonography of the uterus was performed at Day 35 to confirm pregnancy diagnosis based on recognition of the positive signs of pregnancy. Retrospectively, the results of B-mode ultrasonography were compared to that of the subjective evaluations of LBF for determining accuracy values. Furthermore, serum progesterone (P4) concentrations were determined as an indicator of corpora lutea (CL) function. Results indicate 68 of 112 buffalo (60.7%) were diagnosed as pregnant. The accuracy value of the LBF subjective evaluation was significant (80.4%; $P < 0.05$) at Day 17, and there was the maximum accuracy (96.4%) at Day 21 post-TAI. The percentage of false diagnoses was less at Days 17 and 21 compared with Day 6 and 14 post-TAI. There was a significant matching value between P4 concentrations and scores for LBF on and after Day 17 post-insemination. In conclusion, subjective evaluation of LBF using color Doppler ultrasonography is considered an accurate technique for early detection of pregnancy in Egyptian buffalo especially the non-pregnant animals as early as Day 17, with maximum accuracy at Day 21 post-TAI.

1. Introduction

In farm animals, early and accurate pregnancy detection is an important technique for enhancing reproductive efficiency. It provides breeders the opportunity to cull non-pregnant animals in ways that enhances efficiency of cattle production enterprises (Nanda et al., 2003; Karen et al., 2014). In addition, it allows rapid rebreeding of non-pregnant animals, and consequently reduces inter-insemination intervals, number of days non-pregnant, and calving intervals (Fricke, 2002; Stevenson, 2005).

Currently, several methods have been used for pregnancy diagnosis in cattle such as rectal palpation, progesterone assay in blood or milk, and pregnancy-associated glycoprotein assessments. Nevertheless, there are some limitations related to invasiveness, time-consuming, cost, sensitivity, and specificity of the aforementioned methods that could lead to false and negative results. In addition, with most of these techniques there are more reliable results after the 21 days post-insemination (Franco et al., 1987; Nanda et al.,

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2003). Development of non-invasive and accurate biomarkers that could be used as indicators of early detection of pregnancy, therefore, is of great value and could be used as important alternative approaches, to enhance the use of reproductive technologies for pregnancy detection in farm animals.

Ever since its introduction into human obstetrics in the late 1950s and thereafter in veterinary field in 1980s, ultrasonic evaluations have been very important in the characterization of the normal embryo and fetal development, and detection of intrauterine fetal death (Kahn et al., 1990; Rajamahendran et al., 1994; Samir et al., 2016). The use of Grey B-mode ultrasonography is considered a reliable diagnostic technique for accurate pregnancy monitoring in farm animals (Kahn et al., 1990; Rajamahendran et al., 1994; Szenci et al., 1998; Nanda et al., 2003; Karen et al., 2007; Samir et al., 2016). The development of the color Doppler ultrasonography technique has resulted in tremendous use of this technique in both research and clinical practice for various animal reproduction practices (Bollwein et al., 2016; Samir et al., 2015, 2018) because it enabled for the evaluation of not only organ morphology but also organ functions, based on its vascular perfusion (Bollwein et al., 2016).

Color Doppler ultrasonography has been frequently used as early as Day 20 post- timed artificial insemination (TAI) for discriminating between non-pregnant and pregnant cows (Utt et al., 2009; Herzog et al., 2011; Siqueira et al., 2013; Pugliesi et al., 2014; Guimarães et al., 2015) and buffalo (Vecchio et al., 2012; Lasheen et al., 2018) based on assessment of ovarian and uterine vascularity. In some studies, there has been evaluation of the applicability of measuring luteal blood flow (LBF; colored pixel intensity) in cows and buffalo for early pregnancy diagnosis (Acosta et al., 2002; Vecchio et al., 2012; Lasheen et al., 2018). Greater blood flows to the corpora lutea (CL) were detected in animals that were pregnant, while in non-pregnant animals there was a lesser blood flow to the CL. Although these approaches resulted in objective measures (pixel analysis) of LBF, the necessity of post-acquisition image processing limits real-time decisions, and in turn, its application as a field method results in no real time diagnosis. Furthermore, determination of ovarian blood flow velocity (through pulsed Doppler mode) is somewhat difficult due to the small size and/or tortuosity of the blood vessels present in the female reproductive tract (Ginther, 2007).

With these considerations, some studies have been conducted based on the principle that there can be visual or subjective assessments of LBF with a rapid, reliable, easy-to-conduct, and consistent diagnostic approach for early diagnosis of pregnancy status in cows (Siqueira et al., 2013; Guimarães et al., 2015), ewes (Arashiro et al., 2018), and goat does (Balaro et al., 2017; Cosentino et al., 2018). The use of this method is dependent on visual assessment of LBF (without further processing) with a score scale (scale 1–4) being used for LBF classification based on the appearance of colored pixels in the area the CL is depicted (Siqueira et al., 2013; Arashiro et al., 2018). There have been strong correlations between the values obtained when using the objective (pixel analysis) and the subjective analysis of LBF throughout the entire luteal phase (Cosentino et al., 2018). The diagnostic criteria need to be clearly defined, straightforward and consistent for use by different evaluators and the outcomes with use of this technique were found similar to that with plasma P4 concentration assessments, as an indicator of CL function (Siqueira et al., 2013, Arashiro et al., 2018). To the best of our knowledge, the accuracy of use of the transrectal color Doppler ultrasonography technology for early pregnancy diagnosing based on the subjective score scale of LBF has not been evaluated in buffalo. This study, therefore, is important to be conducted in buffalo because of the limited capacity to detect estrus because of the lack of behavioural symptoms of estrus in buffalo, and prolonged calving intervals compared to that in cows (Nanda et al., 2003; Perera, 2011).

With these considerations, the aim of the present study was to evaluate the efficiency of trans-rectal color Doppler ultrasonography for early pregnancy detection in Egyptian buffalo based on visual evaluation of LBF at different time intervals post-insemination. Also, another aim of this study was ascertaining whether or not there is an existing relationship between serum progesterone (P4) and LBF scores at different time's post-TAI.

2. Materials and methods

2.1. Animals

This study was conducted at a dairy buffalo farm, located in the Kalubia governorate, Egypt during the period from early January to April 2017. A total 112 of pluriparous estrous cyclic buffalo cows were included in the study, weighing 450 to 560 kg that were 4.5 to 8 years of age, and maintained in a paddock where there was access to normal photoperiodic cues. Animals were fed daily a commercially prepared pelleted diet (16% crude protein/dry matter). Egyptian sweet clover (*Trifolium alexandrinum*), and wheat straw were also provided as bulk for the diet. Tap water and mineralized salt licks were available *ad libitum*. The animals were regularly prophylactically vaccinated for important infectious endemic diseases such as foot and mouth disease, Rift Valley Fever, and enterotoxemia. Other preventive treatments such as deworming were performed using appropriate broad-spectrum anthelmintic medications, and in none of the buffalo was there any evidence of disease upon physical examination prior to starting the study. All buffalo were monitored twice a day for behavioral estrus onset and examined ultrasonically twice a week to ensure there was estrous cyclicity (presence of a corpus luteum) for at least two consecutive estrous cycles prior to the beginning of the study. This study was performed in accordance with the Use and Animal Care Guidelines of the Cairo and Benha Universities.

2.2. Estrous synchronization and animal breeding

Animals were estrous synchronized using the Ovsynch protocol concurrent with use of an intravaginal controlled internal drug release (CIDR) insert for 7 days as described by Kelley et al. (2016). Briefly, the CIDR insert (EAZIBREED, inter Age, Hamilton, New Zealand) containing 1.38 g progesterone was placed in the vagina for 7 days. There were two intramuscular administrations of 20 μg of gonadotropin-releasing hormone analog (GnRH; 5.0 ml Receptal, MSD Animal Health, Germany) 7 days before (at Day 0) and 2

Table 1
Subjective criteria used as standards for determining whether the classification of an animal was positive or negative based on the luteal blood flow (LBF) as assessed by color Doppler flow ultrasonography for pregnancy diagnosis.

LBF Score	LBF Grade	Criteria
I	Relatively Less	No or a few colored pixels on the CL surface without any colored pixels within or penetrating the CL
II	Moderate	Some colored pixels on the CL surface and presence of only a few colored pixels in small parts within the CL
III	Relatively Greater	Colored pixels mostly cover the edge of the CL with some colored pixels within the luteal tissues
IV	Greatest	Colored pixels mostly or completely cover the edge of the CL and most of the luteal tissues toward the center

days after (at Day 9) a single dose of PGF2α analog (500 μg cloprostenol sodium; equivalent to 2.0 ml) administration (at Day 7). Regardless of whether there was detection of estrus, all buffalo were inseminated 20 h after the second GnRH administration (Neglia et al., 2015).

2.3. Ultrasonic examinations

All ultrasonic examinations in the current study were performed using a B-mode/ color Doppler diagnostic ultrasonography device (SonoAce R3, Samsung, Medison, South Korea) equipped with a multi-frequency (8–12 MHz) linear endorectal transducer.

Monitoring and characterization of ovarian structures were performed on Days 6, 14, 17, and 21 post-TAI by B-mode ultrasonography, and then the changes in LBF were monitored using color Doppler mode as previously described in cows (Herzog and Bollwein, 2007). Briefly, after introducing the endorectal transducer into the rectum, and after the ovary was visualized using B-mode ultrasonography, the image was adjusted to produce the more precise definition of the CL. Subsequently, the color Doppler mode was activated to display signals for subjective assessment of the blood perfusion in the CL. All Doppler examinations were performed at certain times (8.00 A.M.) by the same operator in slow, continuous motion; constant color gain, velocity, and with color-flow filter settings (Pulse repetition frequency (PRF):1.5 MHz; and color Doppler wall filter (WF): Middle 1).

2.4. Prediction of the pregnancy based on subjective assessment of LBF

For predicting early pregnancy in buffalo after TAI, the magnitude of LBF was subjectively evaluated based on the intensity, and arrangement of color Doppler signals in the CL on a 4 score scale or grade as utilized for classifications of LBF in previous studies in cows (Siqueira et al., 2013; Guimarães et al., 2015) with a few modifications (Table 1; Figs. 1 and 2). In brief, when there was a relatively greater and greatest LBF (Grade III and IV) these LBF grades were considered to be a positive indicator of pregnancy, whereas when there was no or a relatively lesser than average LBF (Grade I and II) these Grades were considered to be a negative pregnancy diagnosis. Only colored pixels within or in the immediate vicinity of the CL tissue were considered indicative of LBF. Blood supply to follicles near the CL was not considered as indicative of LBF and was carefully avoided. The predictive pregnancy diagnosis was then recorded by the ultrasonography operator (the first author of present manuscript) based only on subjective evaluation of LBF (classified as positive or negative).

With this consideration, another examination of the uterus using transrectal B-mode ultrasonography was performed at Day 35 post TAI to confirm pregnancy diagnosis based on the discernment of anechoic fluid and the embryo proper with a beating heart as positive signs for pregnancy (Szenci et al., 1998). Each female was then classified as pregnant or non-pregnant. Retrospectively, the results of B-mode ultrasonography were compared to that with the subjective evaluations of LBF for determining accuracy of values for pregnancy diagnosis.

2.5. Blood samplings

On the day of ultrasonic assessments, blood samples (10 mL) were collected from the jugular vein, and the harvested sera were then, stored at −20 °C until hormonal assay.

2.6. Hormonal measurements

Concentrations of progesterone (P4) in the sera were quantified using ELISA commercial kits (Equipar S.r.I Saronno, VA, Italy; Check et al., 1995). The sensitivity for P4-EIA (Cat. No. 74,020) was 2 pg/ml, and the intra-and inter-assay coefficients of variation were 6.1% and 11.4%; respectively.

2.7. Analyses of data

Based on the results of trans-rectal ultrasonic assessments of the uterus at Days 35 post-TAI, the results of subjective assessment of LBF at different times were arranged as follows: correct positive diagnoses (a), incorrect positive diagnoses (b), correct negative diagnoses (c), and incorrect negative diagnoses (d). From these values, the sensitivity ($Se = a/(a + d) \times 100$), specificity ($Sp = c/(c + b) \times 100$), positive predictive ($PPV = a/(a + b) \times 100$) and negative predictive ($NPV = c/(c + d) \times 100$) values were

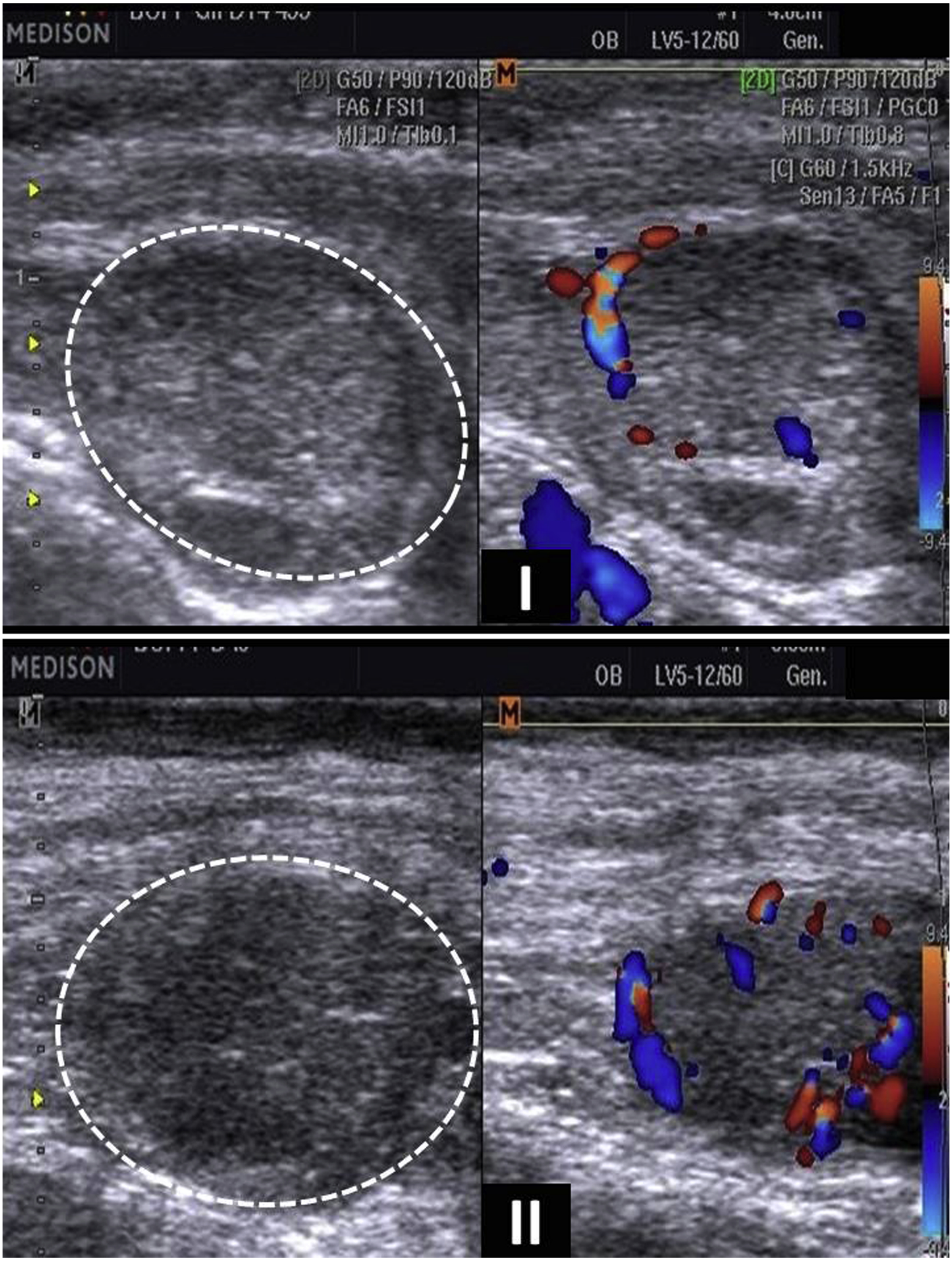

Fig. 1. Subjective assessment of luteal blood flow by color Doppler imaging for demonstrating only the score scale (I, II) of luteal vascularization in non-pregnant buffalo.
The left panel is CL imaged by B-mode ultrasonography.

calculated at each time point. Furthermore, the overall accuracy that equals $[(a + c)/ (a + b + c + d)] \times 100$ of the test was calculated (Martin et al., 1997). The Se, Sp, PPV, and NPV with trans-rectal color Doppler assessments of LBF for pregnancy diagnosis were compared among different days of examinations (scanning time points) using the Fisher's exact test followed by use of the

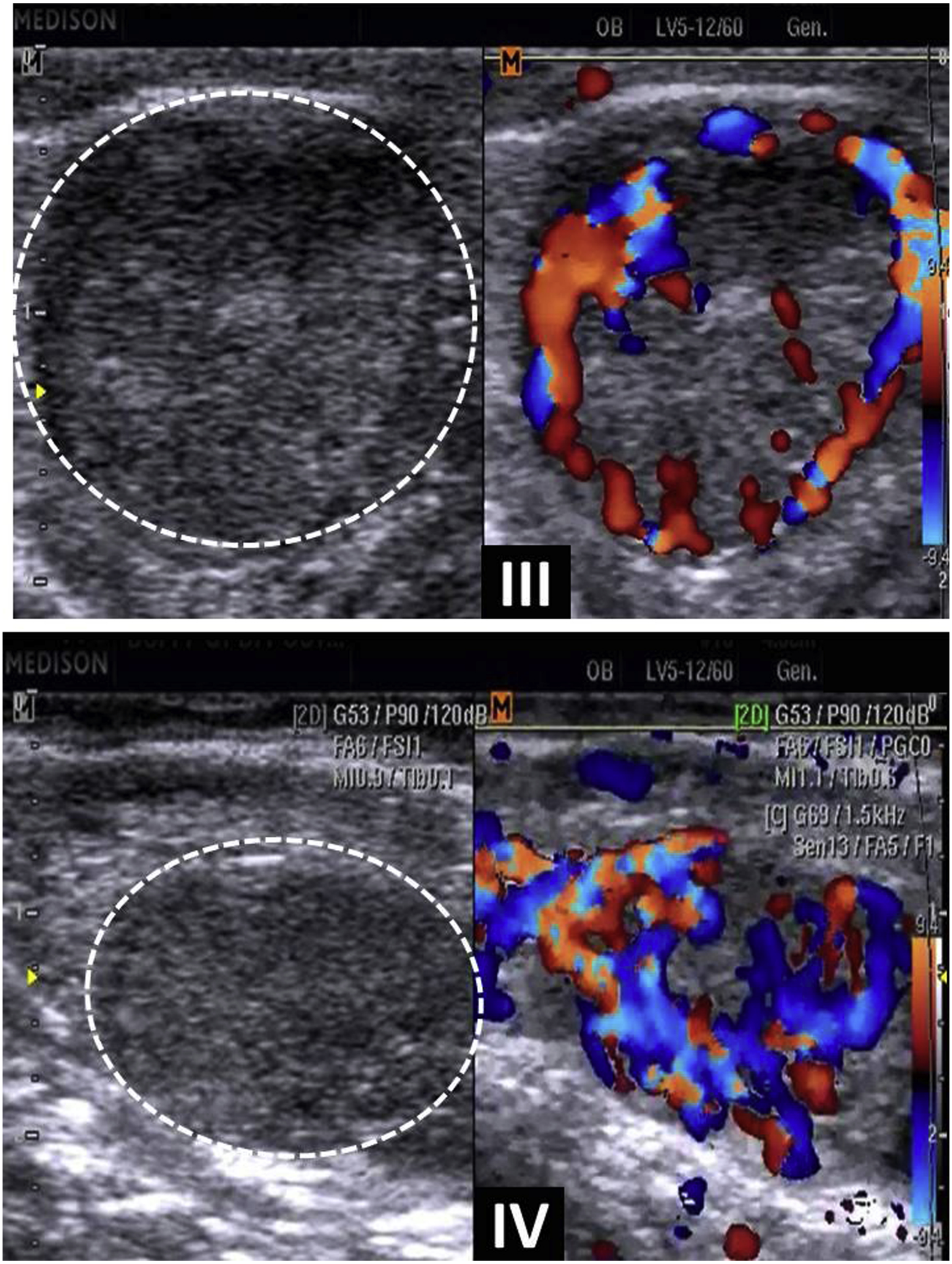

Fig. 2. Subjective assessment of luteal blood flow by color Doppler imaging for demonstrating only the score scale (III, IV) of luteal vascularization in pregnant buffalo.
The left panel is CL imaged by B-mode ultrasonography.

Bonferroni's test for multiple comparisons (Armitage et al., 2002). Based on the results of subjective assessment of LBF, when P4 concentrations in the buffalo were used to make the correct positive, incorrect positive, correct negative, and incorrect negative diagnoses at each time point were compared using an ANOVA. Data for circulating P4 concentrations are presented as means ±

Table 2
Sensitivity, specificity, and predictive values of assessment of LBF by color Doppler ultrasonography for early pregnancy detection in buffalo (n = 112).

Days	Correct + ve (a)	Incorrect + ve (b)	Correct -ve (c)	Incorrect -ve (d)	Se	Sp	PPV	NPV	Overall accuracy %
D6	28	34	10	40	41.2^{a}	22.7^{a}	45.2^{a}	20.0^{a}	33.9^{a}
D14	44	28	16	24	64.7^{b}	36.4^{a}	$61.1^{a,b}$	40.0^{b}	53.6^{b}
D17	62	16	28	6	91.2^{c}	63.6^{b}	$79.5^{b,c}$	82.4^{c}	80.4^{c}
D21	68	4	40	0	100.0^{c}	90.9^{c}	94.4^{c}	100.0^{d}	96.4^{d}

Abbreviations: Se Sensitivity; Sp Specificity; PPV Positive predictive value; NPV Negative predictive value; +ve positive; -ve negative.
[a, b, c, d]Values with different superscripts in the same column are different $P < 0.05$. (among the time points).

standard error of the mean (SEM). Statistical analyses of the data were performed using SPSS software version 16. The P values less than 0.05 were considered significant.

3. Results

There were 68 of 112 buffalo (60.7%) diagnosed as being pregnant at Day 35 post-TAI, as determined by B-mode ultrasonography. The data for subjective evaluation of LBF using trans-rectal color Doppler ultrasonography for early pregnancy detection are presented in Table 2. The accuracy value for pregnancy detection was significant (80.4%; $P < 0.05$) at Day 17, with the maximum accuracy was at Day 21 post-insemination (96.4%). The Se for early pregnancy detection increased ($P < 0.05$) from 41.2% at Day 6 to 91.2% at Day 17, while there was the maximum value (100.0%) at Day 21 post-TAI. The numbers of incorrect negative and incorrect positive diagnoses decreased ($P < 0.05$) as times of assessment advanced from Day 6 until Day 21 post-TAI. Furthermore, there were significant increases in the values of SP, NPV, and the overall accuracy at Day 21 compared to other time points when assessments occurred.

With these considerations, the buffalo with CL with a score of IV (greatest) of LBF at Day 6 were more accurately diagnosed as being pregnant, compared to those with a score of III for LBF. For buffalo with a LBF Score I (least grade), there was a much more accurate assessment for non-pregnancy diagnosis and the accuracy assessments were consistent from Day 14 and subsequently post-TAI (Table 3). There was also marked associations between the concentrations of P4 in the sera and scores of LBF, especially from Day 17 and subsequent to this day of assessment (Table 4). When there were relatively lesser P4 concentrations detected at Day 17 and 21 post-TAI, these were associated with a negative pregnancy diagnosis.

4. Discussion

Although in some recent studies there has been exploration of measuring LBF for early detection of pregnancy in buffalo (Neglia et al., 2015; Lasheen et al., 2018) however, in none of these studies was there consideration of the subjective assessment of LBF for the same purpose, especially from the accuracy of detection perspective. To our knowledge, the current study is the first where this technique was used for early determination of pregnancy status in buffalo. In this study, pregnancy was determined with high accuracy (80.4%) at Day 17, and maximum accuracy (96.4%) at Day 21 post-TAI. These findings may contribute to the development of different strategies for more intense reproductive management, such as early re-synchronization of estrus in non-pregnant buffalo, with there being a 2 week earlier diagnosis compared with early pregnancy diagnoses conducted using trans-rectal classical ultrasonic procedures (Szenci et al., 1998). Most importantly, this technique could be used more effectively in buffalo than in many other species because the signs of behavioral estrus are much less compared to that in cows (Perera, 2011).

Determination the earliest time at which the use of subjective evaluation of luteal vascularization is more efficient was mainly based on results of NPV and specificity (Arashiro et al., 2018). In this study, there was differentiation between pregnant and non-pregnant buffalo with use of subjective LBF assessment with lesser accuracy percentages when there were assessments at Day 6 and 14 post-insemination as compared with subsequent assessments. At Days 6 of the estrous cycle, the CL was considered inactive or immature (In the growing phase; (Kanai and Shimizu, 1983). The pregnant buffalo were, therefore, diagnosed as negative (incorrectly

Table 3
Comparing the results of the subjective evaluation of luteal blood flow(scores) as assessed using color Doppler ultrasonography to the findings of pregnancy diagnosis performed at Day 35 post-TAI by B-mode ultrasonography (as a reference) in buffalo (n = 112).

	Negative Findings						Positive findings					
	Score I			Score II			Score III			Score IV		
Days	n	Correct -ve	Incorrect -ve	n	Correct -ve	Incorrect -ve	n	Correct + ve	Incorrect + ve	n	Correct + ve	Incorrect + ve
D6	25	09	16	25	1	24	46	13	33	16	15	1
D14	16	14	2	24	2	22	42	18	24	30	26	4
D17	22	22	0	12	6	6	28	14	14	50	48	2
D21	30	30	0	10	10	0	18	14	4	54	54	0

Abbreviations: +ve: positive; -ve: negative; n = number of buffalo.

Table 4
Concentrations of P4 (mean ± SEM; ng/ml) in buffalo (n = 112) that were classified into four groups (Correct + ve, Incorrect + ve, Correct -ve, and Incorrect -ve) based on the results of subjective assessment of CL as measured by color Doppler ultrasonography during different times of scanning in the study.

Days	Positive Findings				Negative Findings			
	Correct + ve		Incorrect + ve		Correct -ve		Incorrect -ve	
	n	P4 (ng/ml)	*n*	P4 (ng/ml)	*n*	P4 (ng/ml)	*n*	P4 (ng/ml)
D6	28	$1.88 \pm 0.4^{a,A}$	34	$0.98 \pm 0.1^{a,B}$	10	$0.82 \pm 0.2^{a,B}$	40	$1.23 \pm 0.2^{a,A,B}$
D14	44	$2.67 \pm 0.3^{b,A}$	28	$2.09 \pm 0.2^{b,A}$	16	$1.75 \pm 0.4^{b,A}$	24	$2.06 \pm 0.3^{b,A}$
D17	62	$3.40 \pm 0.4^{b,A}$	16	$1.81 \pm 0.3^{b,B}$	28	$0.94 \pm 0.1^{a,C}$	6	$2.13 \pm 0.3^{b,B}$
D21	68	$3.89 \pm 0.3^{b,A}$	4	$1.85 \pm 0.1^{b,B}$	40	$0.71 \pm 0.2^{a,C}$	0	*ND*

Abbreviations: + ve: positive; -ve: negative; *n*: number of buffalo; *ND*: not detectable.
[a, b]Values with different superscripts in the same column are different $P < 0.05$ (among the time points).
[A, B, C]Values with different superscripts in the same row are different $P < 0.05$ (among the evaluation results).

so). At Days 14 of the estrous cycle, however, the CL were considered active (the pre luteolysis period; Kanai and Shimizu, 1983; Juengel et al., 1993) and, in turn, non-pregnant buffalo were wrongly identified as being pregnant (incorrect positive).

Compared to the accuracy values at Days 6 and 14, there was a lesser percentage of false diagnoses at Day 17 and subsequently post-TAI. This finding may be attributed, in part, to the occurrence of luteolysis (Juengel et al., 1993), and consequent decreasing of blood flow to the CL (Matsui and Miyamoto, 2009; Herzog et al., 2011), and a decrease of LBF scores as assessed using color Doppler ultrasonography. These decreases in LBF might result from one of the primary luteolytic actions of PGF2α as a result of its production and release from the endometrium during this time frame post-AI if there is no pregnancy (Figueira et al., 2015; Scully et al., 2015; Balaro et al., 2017). At Day 17 post-insemination, most animals diagnosed as non-pregnant using color Doppler ultrasonography in the present study were later confirmed as not being pregnant using B-Mode ultrasonography (incorrect negative = 6 and NPV = 82.4%). The specificity of this proposed methodology, however, was not maximal until Day 21 post insemination (90.9%), when the incorrect positive results were minimal. There were similar findings at Days 20 and 21 post-insemination in cows (Siqueira et al., 2013) and goats (Cosentino et al., 2018). In sheep (Arashiro et al., 2018) however, there was the maximum accuracy in pregnancy detection at Day 17 post-insemination. These inconsistent results may be related to the effect of species in terms of the length of the estrous cycle, and the timing of functional luteolysis. This method, therefore, can be reliably used as a pregnancy diagnostic assessment because of desirable accuracy, high sensitivity, and NPV but the operator's experience and clarity of criteria for positive and negative diagnoses are of great importance for successful outcomes (Siqueira et al., 2013).

Even though there were misdiagnoses of pregnancy status, it is proposed that incorrect positive results, as observed at each time point (especially at Day 21 post-TAI) of the current study, may be explained in two ways. Firstly, variability in the length of the estrous cycle among animals may contribute to a misdiagnosis of pregnancies (the occurrence of a longer than typical estrous cycle; > 21 days). The prolonged estrous cycle may be related to the persistence of an active CL which would likely result in variability in the timing in initiation of luteolysis among animals that are not pregnant. The duration of the estrous cycle in buffalo has been reported to vary ranging from 17 to 26 days (Jainudeen and Hafez, 1993). These variations in the length of the estrous cycle (abnormally short and long estrous cycles) may be attributed to various factors including nutrition, adverse environmental conditions, and the parity effect (Kaur and Arora, 1982; Nanda et al., 2003; Perera, 2011).

The second explanation may be the occurrence of early embryonic mortality, which would likely result in a lengthening of the period from insemination to expression of estrus subsequent to AI if there was early embryonic mortality (Campanile et al., 2005; Samir et al., 2016). Embryonic mortality in buffalo can occur before Day 21, and has been termed as early embryonic mortality, and from Day 21 to 45 which has been termed late embryonic mortality (Campanile and Neglia, 2007; Campanile et al., 2016). Previous studies in domestic buffalo indicate that the incidence of late embryo loss is between 30% and 45% (Campanile et al., 2005; Diskin and Morris, 2008).

The P4 secretion in buffalo is closely related to blood flow to the CL (Campanile et al., 2010). In the current study, the marked association between the P4 concentrations and scores of LBF especially at Day 17 post-TAI and subsequent to this time post TAI may explain the functional capacity of CL during early pregnancy in buffalo, and in turn, be a predictor of the non-pregnant status. Similar findings were reported in cows (Guimarães et al., 2015).

Early optimum growth of the CL is associated with an increased blood flow, and greater secretion of P4, and in turn, is associated with a greater likelihood of continued development of an embryo and the establishment of pregnancy (Papa et al., 2007; Neglia et al., 2015; Kanazawa et al., 2016). In the current study, buffalo with a score of IV (greatest LBF) for LBF at Day 6 and that were considered to be pregnant and in most animals there was confirmation of the pregnant status at Day 35, as determined by B-mode ultrasonography. In addition, the animals with a score of IV for LBF had functional secretory CL as indicated by relatively greater P4 concentrations. Buffalo with a score of I (relatively lesser LBF) for LBF at Day 14 and subsequently post-TAI, and were, therefore, considered to be non-pregnant and were in most animals there was subsequent confirmation of the non-pregnant status at Day 35 post-TAI. The results in the present study utilizing LBF as an indicator of pregnancy status were similar to those reported for sheep (Arashiro et al., 2018). Furthermore, buffalo that had relatively lesser concentrations of P4 in sera, especially at Day 17 and subsequently following TAI, had CL vascularization scores of I or II (correct negative classification of pregnancy status). These findings are consistent with those reported by Balestrieri et al. (2013).

Importantly, buffalo that were wrongly diagnosed as pregnant at Day 21 based on subjective scale scores of LBF (incorrect positive), supposedly as a result of late embryonic development did not have relatively lesser concentrations of P4 in the sera. These findings may support, in part, the involvement of other factors as predisposing causes of embryonic mortality (Campanile et al., 2005; Scully et al., 2015; Samir et al., 2016). The variations in the length of the estrous cycle, however, should be taken into consideration when assessing results in studies that focus on diagnosis of pregnancy in the early stages subsequent to the time of AI.

5. Conclusion

Subjective evaluation of LBF by color Doppler ultrasonography is considered a practical method to provide accurate and real-time detection of pregnancy in Egyptian buffalo. The reliable accuracy of identification of pregnancy status, especially in animals that were not pregnant was accomplished at Day 17 with an accuracy of 80.4% and there was a maximum accuracy (96.4%) at Day 21 post-TAI. In addition, P4 concentrations tend to be positively associated with the scores of LBF especially at Day 17 and 21 post-TAI.

Declaration of Competing Interest

No conflict of interest.

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