

## Clinical and Ultrasonic Study for Detection of Pregnancy in Iraqi Buffaloes

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### ABSTRACT

The present study was conducted on 10 Iraqi pregnant buffaloes aged between 3-6 years at Al-Qasim district-Babylon province, Iraq from December 2018 to December 2019. Those animals diagnosed by rectal palpation and ultrasonography technique to estimate the reproduction status. The results of this study reported wandering of ovum in 10% of animals (1/10). While ultrasonography characterized by increasing placentomes measurement gradually with development of gestation, and the placentomes recorded  $8.32 \pm 2.46$  mm,  $13.27 \pm 3.25$  mm,  $18.37 \pm 5.34$  mm,  $23.25 \pm 8.63$  mm and  $28.2 \pm 6.87$  mm in the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> month, respectively. Also the total size was recorded by the crown rump length (CRL), which was  $0.77 \pm 0.05$  cm,  $5.81 \pm 2.54$  cm and  $14.09 \pm 0.73$  cm in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> month, respectively. The heart beat and embryonic vesicle during the 1<sup>st</sup> and 2<sup>nd</sup> month were detectable by ultrasonography. In conclusions in the present study, rectal palpation and ultrasonography technique represented one of the most important methods to estimate the gestation period in different stages.

**Keywords: Buffaloes, Rectal palpation, Ultrasonography, Pregnancy detection**

### Introduction

The buffaloes are considered as a major portion of the dairy and meat industries of several south and southeastern Asian countries (1).

Pregnancy diagnosis plays an important role in the reproduction management of ruminants since embryonic mortality has a substantial impact on the fertility of a herd (2).

Determining pregnancy in cattle by rectal palpation is not particularly difficult, but it requires experience, practice, and knowledge of the reproductive system (3). Rectal palpation is a simple procedure that requires little time or equipment to determine pregnancy, which can be performed accurately 35 days from the time of breeding.

However, palpation cannot tell us if the embryo is viable or if the cow is beginning to abort the embryo (4). Trans-rectal ultrasonography diagnosis in buffaloes can be adopted successfully from day 28-30 after service (5).

On day 30 it is possible to observe the fetal heartbeat (6). The sensitivity reaching 100% from day 31 after mating onward (6,7). The main advantage of the ultrasound scanning is that it can give an accurate diagnosis earlier than rectal palpation (8).

Both rectal palpation and ultrasonography are reliable and relatively quick methods of pregnancy determination (9). Because of little studies about pregnancy diagnosis in Iraqi buffaloes we conducted this study.

### Materials and Methods

This study was represented by 10 healthy adult local Iraqi buffaloes, aged between 3-6 years at Al-Qasim district- Babylon province, Iraq and performed from December 2018 to December 2019. Pregnancy diagnosis was carried out by rectal palpation and ultrasonography for recognizing the most characteristic features, including the stages of pregnancy, and the diagnosis included ovaries,

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cervix, uterine horns, amniotic vesicle, fetal size, placentomes size and thrilling of middle uterine artery. The animals were divided according to their reproductive status by using rectal palpation and ultrasonography through ultrasound machine containing trans-rectal probe 7.5 MHZ into four stages and assigned as stage 1 non-pregnant (control), stage 2: first trimesters (1-3 months of pregnancy), stage 3: second trimesters (4 - 6 months of pregnancy), and stage 4: third trimesters (7-10 months of pregnancy). Analysis was arranged in different stages according to mean, Chi-square, standard error and F-test (10).

### Results and Discussion

The results of rectal palpation during the 1<sup>st</sup> to 6<sup>th</sup> months of pregnancy are showed in Table 1. In the first 3 months of pregnancy, the corpus luteum can be palpated, the presence of CL in right ovary was reported in 80% (8/10) in comparison with 20% (2/10) on the left ovary in pregnant buffaloes during the 1<sup>st</sup> month of pregnancy, while it was 70% (7/10) in the right ovary compared with 30% (3/10) in the left ovary during the 2<sup>nd</sup> and 3<sup>rd</sup> months of pregnancy. The site of pregnancy documented 80% (8/10) on the right horn with 20% (2/10) being in the left horn at the 1<sup>st</sup> months of pregnancy, then it was 70% (7/10) on the right horn compared with 30% (3/10) on the left horn from the 2<sup>nd</sup> month of pregnancy on word in the pregnant Iraqi buffaloes (Table 1). In the 2<sup>nd</sup> month of pregnancy, the embryonic vesicle was palpable (size of a mouse), then it was about the size of a rat in the 3<sup>rd</sup> month reaching a size of a rabbit in the 4<sup>th</sup> month of pregnancy; however, in the 5<sup>th</sup> and 6<sup>th</sup> month the fetus cannot be palpated (sinking stage). Placentomes (caruncle and cotyledon) were palpable from the 3<sup>rd</sup> month of gestation and their size was about the size of (peas, bean, broad beans and pigeon egg) at the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> months, respectively. One of the pregnancy diagnosis parameters studied was the thrilling of the middle uterine artery which started from the 4<sup>th</sup> month of gestation and was 70% (7/10) on the right side compared with 30% (3/10) on the left side (Table 1). Many changes occurred in the reproductive system including ovaries, uterus and embryo/fetus during pregnancy which could be recognized by rectal palpation in pregnant buffaloes. During the 1<sup>st</sup> month of pregnancy the CL was palpated and this is in agreement with (11, 12) who found that CL can be palpated easily on the buffaloes ovaries, but dis-

agreed with (13) who found that CL on buffaloes ovaries was mostly embedded deeper in ovarian stroma and project less on the ovarian surface which made it diagnosis per rectum to be difficult. The presence of CL was (8/10) in the buffaloes right ovary in a percentage of 80%, while it was (2/10) 20% present on the left ovary in the 1<sup>st</sup> month of pregnancy, and this result agreed with the results of many researchers on large number of cows indicating that the right ovary was more active than left (14, 15). It has been found that 60-70% of the ovulation occur on the right ovary (15), but this finding dis-agreed with (14) who showed that the follicular waves and ovulation had no significant difference between the right and left ovary in buffaloes. The presence of CL was 7/10 (70%) on the right ovary compared with 3/10 (30%) on the left ovary during the 2<sup>nd</sup> and 3<sup>rd</sup> month of pregnancy in our study. The presence of CL was in the right ovary during the 1<sup>st</sup> month, then it was located in the left ovary in the 2<sup>nd</sup> and 3<sup>rd</sup> month due to the presence of a new follicular wave in the left ovary during pregnancy that lead to formation of CL (with or without ovulation) accompanied by regression of the CL on the right ovary. The site of pregnancy was 8/10 (80%) on the right uterine horn and 2/10 (20%) on the left and this agreed with the findings of the presence CL. Nevertheless, during the 2<sup>nd</sup> and 3<sup>rd</sup> month the site of pregnancy was 7/10 (70%) on the right horn, while it was 3/10 (30%) on the left horn, and this may be due to the wandering of ovum (trans uterine migration) and this result agreement with (16).

The parameters determined by trans-rectal ultrasonography (7.5 MHZ) during the 1<sup>st</sup> six months of pregnancy in Iraqi buffaloes are shown in Table (2). The range of placentomes measurements were 6-10 mm, 11-15 mm, 16-20 mm, 21-25 mm and 26-30 mm at the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> month, respectively. While the means of measurements were  $8.32 \pm 2.46$ ,  $13.27 \pm 3.25$ ,  $18.37 \pm 5.34$ ,  $23.25 \pm 8.63$  and  $28.2 \pm 6.87$  with a significant difference of  $P < 0.01$  related to the 6<sup>th</sup> month of pregnancy compared with other months as well as presence of a significant difference of  $P < 0.01$  between the 5<sup>th</sup> and 4<sup>th</sup> month, 4<sup>th</sup> and 3<sup>rd</sup> month, 3<sup>rd</sup> and 2<sup>nd</sup> month. Fetal size (crown rump length) was 0.5-0.9 cm, 2.7-8.6 cm and 12-17 cm in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> month of pregnancy in Iraqi buffaloes (Figure 1), while the means of the length were  $0.77 \pm 0.05$ ,  $5.81 \pm 2.54$  and  $14.09 \pm 5.73$  with superior significant differences ( $P < 0.01$ ) for the 3<sup>rd</sup> month of gestation compared with the 1<sup>st</sup> and 2<sup>nd</sup> stage as well as between the 2<sup>nd</sup>

and 1<sup>st</sup> month. Both means of placentome weight and length increased significantly throughout the gestation with a relative increase in placentome length as pregnancy advances, this result agreed with (17, 18). Some studies have reported that placentome growth continues throughout the gestation period (19, 20); however, (21-23) reported that it ceased around day 200 of gestation. Fetal size (crown rump length) was significantly increased during pregnancy in Iraqi buffaloes, while the means of the length were significantly different (P<0.01) in favor for the 3<sup>rd</sup> month of gestation compared with 1<sup>st</sup> and 2<sup>nd</sup> stage as well as between

the 2<sup>nd</sup> and 1<sup>st</sup> month. Heart beats were obvious in 1<sup>st</sup> and 2<sup>nd</sup> month and the embryonic vesicle (Figure 2) was also visible in the 1<sup>st</sup> and 2<sup>nd</sup> months of gestation. During the 2<sup>nd</sup> month of pregnancy, fetal buds were visible and the skeletal structure (ribs, skull and long bones) were observed from the 3<sup>rd</sup> month (Figure 3), as well as fetal membranes were noticed from the 2<sup>nd</sup> month of gestation. In conclusion, rectal palpation and ultrasonography technique represented one of the most important methods to estimate the gestation period in different stages.

**Table 1. Characteristics of pregnancy (ovaries, corpus lutein, fetal size, placentome and thrilling) by rectal palpation according to month of pregnancy in local Iraqi buffaloes**

Months	Ovaries with CL		Fetal size	palpable	Placentome size	Thrilling	
	Right %	Left %	Right %			Right %	Left %
1 <sup>st</sup>	(8) (+) 80%	(2) (+) 20%	(8) (+) 80%	-	-	-	-
2 <sup>nd</sup>	(7) (+) 70%	(3) (+) 30%	(7)* (+) 70%	Mouse	-	-	-
3 <sup>rd</sup>	(7) (+) 70%	(3) (+) 30%	(7)* (+) 70%	Rat	+	peas	-
4 <sup>th</sup>	-	-	(7)* (+) 70%	Rabbit	+	bean	(7) (+) 70%
5 <sup>th</sup>	-	-	(7)* (+) 70%	-	+	Broad beans	(7) (+) 70%
6 <sup>th</sup>	-	-	(7)* (+) 70%	-	+	pigeon egg	(7) (+) 70%

\*Wandering of ovum

**Table 2. Parameters determined by trans rectal transducer during 1st to six months of pregnancy in Iraqi buffaloes**

Parameters	1 <sup>st</sup> month Mean±SE	2 <sup>nd</sup> month Mean±SE	3 <sup>rd</sup> month Mean±SE	4 <sup>th</sup> month Mea ±SE	5 <sup>th</sup> month Mean±SE	6 <sup>th</sup> month Mean±SE
Placentome measurement	-	(6-10 mm.) 8.32±2.46 <sup>e</sup>	(11-15 mm) 13.27±3.25 <sup>d</sup>	(16-20 mm) 18.37±5.34 <sup>c</sup>	(21-25 mm) 23.25±8.63 <sup>b</sup>	(26-30 mm) 28.2±6.87 <sup>a</sup>
Fetal size (CRL) cm	(0.5-0.9 cm) 0.77±0.05 <sup>c</sup>	(2.7-8.6 cm) 5.81±2.54 <sup>b</sup>	(12-17.5 cm) 14.09±5.73 <sup>a</sup>			
Heart beats	+	+	-	-	-	-
Embryonic vesicle	+	+	-	-	-	-
Fetal buds	-	+	+ skeletal structure (ribs, skull and long bones)	+	+	+
Fetal membranes	-	+	+	+	+	+

Different small letters mean significant differences (P≤0.01) between stages



Figure 1. Buffalo embryos on the day 30 using trans-rectal ultrasonography



Figure 2. Buffalo embryo on the day 65 shows crown rump length

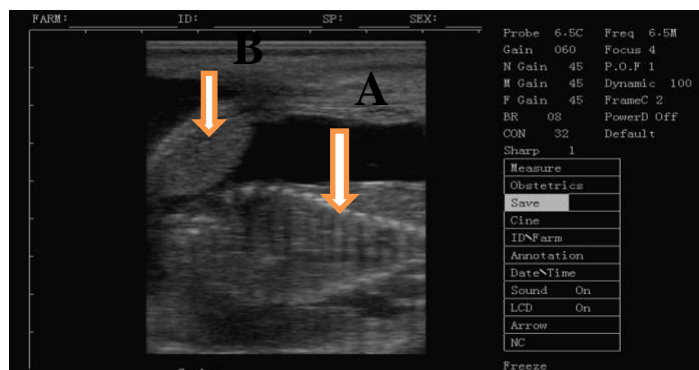


Figure 3. A: Buffalo fetus in the 3rd month shows fetal ribs ,B: Placentomes

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### Conflict of Interest

The authors declare that they have no conflict of interest.

### References

1. El-Wishy AB. The postpartum buffalo: I. Endocrinological changes and uterine involution. *Anim. Reprod. Sci.* 2007; 97(3-4): 201-215.
2. Barbato O, Barile, V L. The pregnancy diagnosis in buffalo species laboratory methods. *J Buffalo Sci.* 2012; 1(2): 157-162.
3. Pawshe CH, Rao KA, Totey SM. Ultrasonographic imaging to monitor early pregnancy and embryonic development in the buffalo (*Bubalus bubalis*). *Theriogenology.* 1994; 41(3): 697-709.
4. Reese ST, Pereira MC, Vasconcelos JL M, Smith MF, Green JA, Geary TW. *et al.* Markers of pregnancy: How early can we detect pregnancies in cattle using pregnancy-associated glycoproteins (PAGs) and microRNAs. (*AR*). 2018; 13(3): 200-208.
5. Ali A, Abdel-Razek AK, Abdel-Ghaffar S, Glatzel PS. Ovarian follicular dynamics in buffalo cows (*Bubalus bubalis*). *Reprod. Domest. Anim.* 2003; 38(3): 214-218.
6. Karen A M, Darwish S, Ramoun A, Tawfeek K, Van Hanh N, de Sousa NM, Beckers JF. Accuracy of transrectal palpation for early pregnancy diagnosis in Egyptian buffaloes. *Trop Anim Health Prod.* 2011; 43(1): 5-7.
7. Pawshe CH, Purohit GN. Approaches for diagnosis of pregnancy in female buffaloes. *Bubaline Theriogenology.* IVIS, Ithaca NY A. 2013; 5708:0613 [updated: 2013 Jun26]. available from: <http://www.ivis.org>.
8. Jerome A, Singh SK, Agarwal SK. Interaction analysis of buffalo pregnancy associated glycoprotein-1 in silico. *Indian J Anim Sci.* 2013; 83(11): 1149-1154.
9. Medan MS, El-Aty AA. Advances in ultrasonography and its applications in domestic ruminants and other farm animals reproduction. *J. Adv. Res.* 2010; 1(2): 123-128.
10. Steel RG, Torrie JH. Principles and procedures of statistics: a biometrical approach. 4<sup>th</sup> Ed. New York: Mcgraw-Hill; 1982. 633p.
11. Bekele N, Addis M, Abdela N, Ahmed W M. Pregnancy diagnosis in cattle for fertility

- management. a review. *Glob Vet.* 2016; 16(4): 355-364.
12. Usmani R H, Ahmad M, Inskeep EK, Dailey R A, Lewis P E, Lewis GS. Uterine involution and postpartum ovarian activity in Nili-Ravi buffaloes. *Theriogenology.* 1985; 24(4): 435-448.
  13. Kirkpatrick JF, Bancroft K, Kincy V. Pregnancy and ovulation detection in bison (*Bison bison*) assessed by means of urinary and fecal steroids. *J Wildl Dis.* 1992; 28(4): 590-597.
  14. Gereš D, Ževrnja B, Žubčić D, Zobel R, Vulić B, Staklarević N. *et al.* Asymmetrical functional activities of ovaries and tubular part of reproductive organs of dairy cows. *Vet Arh.* 2011; 81(2): 187-98.
  15. Khaton R, Sarder MJU, Gofur MR. Biometrical Studies of Reproductive Organs of Dairy Cows of Different Genotypes in Bangladesh. *Asian J. Anim. Sci.* 2015; 9(6): 388-395.
  16. Fetrow J, Stewart S, Eicker S, Rapnicki P. Reproductive health programs for dairy herds: analysis of records for assessment of reproductive performance. In *Current therapy in large animal theriogenology* 2007; (473-489). Elsevier Inc..
  17. Karen A, Darwish S, Ramoun A, Tawfeek K, Van Hanh N, De Sousa NM. *et al.* Accuracy of ultrasonography and pregnancy-associated glycoprotein test for pregnancy diagnosis in buffaloes. *Theriogenology.* 2007; 68(8):1150-1155.
  18. Patel MD. Early Pregnancy Diagnosis in Dairy Animals. *Int. J. Agric.* 2016; 8(11): 1134-1136.
  19. Drost M. Bubaline versus bovine reproduction. *Theriogenology.* 2007; 68(3): 447-449.
  20. Schmidt S, Gerber D, Soley JT, Aire T A, Boos A. Histo-morphology of the uterus and early placenta of the African buffalo (*Syncerus caffer*) and comparative placentome morphology of the African buffalo and cattle (*Bos taurus*). *Placenta.* 2006; 27(8): 899-911.
  21. Ali A, Fahmy S. Ultrasonographic fetometry and determination of fetal sex in buffaloes (*Bubalus bubalis*). *Anim. Reprod. Sci.* 2008; 106(1-2): 90-99.
  22. Zaher H, Abdalla H, Labib F, Eidaroos A. Transrectal combined thickness of the uterus and placenta in normal pregnant Egyptian buffalo-cows. *Theriogenology.* 2012; 77(7): 1438-1441.
  23. Estrella CA, Kind KL, Derks A, Xiang R, Faulkner N, Mohrdick M. *et al.* Remodelling of the bovine placenta: Comprehensive morphological and histomorphological characterization at the late embryonic and early accelerated fetal growth stages. *Placenta.* 2017 ;55:37-46.

## دراسة سريرية وباستخدام الموجات فوق الصوتية لتشخيص الحمل في الجاموس العراقي

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### الخلاصة

اجريت الدراسة الحالية على عشرة جواميس عراقية تراوحت اعمارها بين (3-6) سنوات في قضاء القاسم / محافظة بابل للفترة من كانون الأول (2018) الى نيسان (2019). شخّصت هذه الحيوانات بطريقتي الجس عبر المستقيم واستخدام جهاز الموجات فوق الصوتية لغرض تحديد الحالة التناسلية لها، سجلت نتائج التجربة الحالية وجود حالة تجوال البيضة (وجود الجسم الاصفر في جهة والحمل في جهة معاكسة) لواحدة من حيوانات التجربة وبنسبة 10% ويتضح من خلال الفحص بجهاز الموجات فوق الصوتية بأن هناك زيادة تدريجية في قياس الفلقات مع تطور فترات الحمل المختلفة والتي سجلت  $2.46 \pm 8.32$  ملم،  $3.25 \pm 13.27$  ملم،  $5.34 \pm 18.37$  ملم،  $8.63 \pm 23.25$  ملم و  $6.87 \pm 28.2$  ملم. للأشهر الثاني والثالث والرابع والخامس والسادس على التوالي اما ما يخص الطول الناتج للجنين فقد سجلت  $0.05 \pm 0.77$  سم،  $5.81 \pm 2.54$  سم و  $0.73 \pm 14.09$  سم في الشهر الاول والثاني والثالث فقط، كما تم تسجيل وملاحظة دقات القلب والحويلة الجنينية خلال الشهرين الاولين من الحمل، خلاصة الدراسة الحالية طريقة الجس عبر المستقيم وكذلك باستخدام الموجات فوق الصوتية تعتبر واحدة من اهم الطرق لتشخيص الحمل في المراحل المختلفة.

الكلمات المفتاحية: الجاموس، الجس عبر المستقيم، جهاز الموجات فوق الصوتية، تشخيص الحمل