



MONITORING OF EARLY PREGNANCY FETOMETRY IN EGYPTIAN BUFFALOES USING HIGH FREQUENCY TRANSRECTAL B-MODE AND COLOR DOPPLER ULTRASONOGRAPHY

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ABSTRACT

The current study aimed to screen the growth and viability of embryo from Day 21 to Day 56 post-breeding in Egyptian buffaloes. Twenty buffaloes were synchronized and examined serially by means of real-time B-mode ultrasound equipped with 12 MHz endorectal transducer. The developing embryonic sac diameter (ESD), crown rump length (CRL), head diameter (HD), trunk diameter (TD), and eye ball diameter (EBD) were measured. The heart rate and cardiac blood flow were sequentially examined to verify the fetal viability using color Doppler mode. Data showed the feasibility of first detection and measurement of ESD, CRL, HD, TD and EBD from Day 24.33 ± 0.67, 27.00 ± 1.73, 34.67 ± 1.76, 34.67 ± 0.88 and 33.67 ± 0.88, respectively. The fetal heart rate was 232.00 ± 12.35 beat/min at the day of first examination (Day 24.50 ± 1.30) after breeding. The cardiac blood flow was examined from Day 27 onwards. A highly significant ($P < 0.0001$) correlations were recorded between the ESD ($r^2=0.55$), CRL ($r^2=0.85$), HD ($r^2=0.85$), TD ($r^2=0.93$) and EBD ($r^2=0.85$) and fetal age in buffaloes. It could be concluded that the high-frequency ultrasonography is a valuable diagnostic tool for the detection of early pregnancy from Day 24-26 post-breeding in Egyptian buffaloes. Verification of fetal heart beating (starting from Day 24-26) and cardiac blood flow (starting from Day 26-27) provide a reliable non-invasive promising technique for the fetal viability evaluation. The high correlation between TD and fetal age signified its clinical value, over other estimated fetometric parameters, in determining gestation period in Egyptian buffaloes during early pregnancy.

Keywords: Buffalo, Cardia blood flow, Color Doppler, Fetometry, High, Pregnancy.

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INTRODUCTION

Ever since its introduction into human obstetrics in the late 1950s and thereafter in veterinary field in 1980s, ultrasound has essentially participated in the characterization of the normal embryo and fetal development, and the detection of intrauterine growth retardation (Kähn, 2004). Improvements in the image quality and scanning ability allow the speculation of greater anatomical details, which in turn guide to more sophisticated analysis of pregnancies' progression in bovines (White *et al.*, 1985; Kähn, 1989; Ali, 2004; Rosiles *et al.*, 2005).

Antenatal ultrasonography is capable of providing sequential measurements of the same

conceptus over different developmental stages in bovines. However, according to the best of our knowledge, few ultrasonographic studies examined normal embryo/fetal development in buffaloes (Awasthi *et al.*, 2011; Ferreira *et al.*, 2012; Naikoo *et al.*, 2013), specifically the Egyptian native breed (Karen *et al.*, 2007; Ali and Fahmy, 2008). The period between 28-60 days of gestation is extremely important in buffaloes due to the high incidence of embryonic mortality (Vecchio *et al.*, 2007). Therefore, the current study aimed to monitor the fetal viability and development during early pregnancy (from 21-56 days post-breeding) in Egyptian buffaloes, and establish a biometric threshold of the embryonic vesicle and different

embryo/fetal parts using high-frequency transrectal ultrasonography.

MATERIALS AND METHODS

Experimental animals

This study was carried out at Buffaloes' farm, in Mostorod district, Cairo, Egypt belonging to Department of Animal Production, Faculty of Agriculture, Al-Azhar University during the period from early February to May 2016. Twenty buffaloes, 4-7 years of age, weighing 470–540 kg, reared in an open yard barn (30 × 70 m²) under natural conditions of temperature and the light-dark ratio, were used in this study. Animals were daily fed a mixed ration consisting of 50–55% forage and 45–50% concentrate, and 16% crude protein / dry matter. Tap water and green fodder were provided *ad libitum* and straw as a bulky material.

Estrous synchronization and animal breeding

Animals were synchronized according to regimen described by **Kelley et al. (2016)**. Briefly, controlled internal drug release (CIDR) insert (EAZIBREED, inter Age, Hamilton, New Zealand) impregnated with 1.38 g progesterone was placed in the vagina for seven days. Cloprostenol sodium (Estrumate[®], MSD Animal Health New Zealand) was given IM at a dose of 500 µg (equivalent to 2.0 ml) at the 6th day after CIDR insert. Females were naturally bred upon estrus detection at 3-5 days after CIDR withdrawal.

Ultrasonographic examinations

Serial transrectal ultrasonographic examinations were accomplished twice weekly between the third and eighth weeks post-breeding. The examinations were performed using real-time, B-mode, diagnostic ultrasound (SonoAce R3 ; Samsung, Medision, South Korea) equipped with high frequency (12MHz) endorectal transducer. The device was also equipped with color Doppler mode.

Fetometric parameters

According to **Kähn (1989)**, and **Ali and Fahmy (2008)**, the Buffalo developing embryos/fetuses were measured for the embryonic sac diameter (ESD), crown rump length (CRL; measured between the occipital bone and the first vertebrae of the tail), eye ball diameter (EBD; the largest diameter of the orbit), trunk diameter (TD; the widest point of the abdomen at the level of the liver and stomach) and head diameter (HD; the largest distance between the outer surfaces of the cranial bones i.e. brain cavity) .

Color Doppler Ultrasonography

The heart rate and cardiac blood flow of the embryo/fetus were sequentially examined to verify

the fetal viability using color Doppler mode according to **Herzog and Bollwein (2007)**.

Statistical analysis

Pearson correlation coefficients of the embryo/fetal parameters in relation to the time post-breeding (in days) were analyzed using standard linear statistics (**Quinn and Keough, 2002**) using SPSS (ver. 16) software. The data showed the date and estimates at the first examination of embryo/fetal structures were presented as a mean ± standard error of means. The significance level was set at $P < 0.05$.

Statement of Animal Rights

This study was performed in accordance with The Use and Animal care guidelines of National Research Centre, Cairo University, and Al-Azhar University.

RESULTS

In the current study, ultrasound monitoring of buffalo gestation was carried out from the third-week post-breeding with the feasibility of embryonic mass detection from 24.33 ± 0.67 , 27.00 ± 1.73 , 34.67 ± 1.76 , 34.67 ± 0.88 and 33.67 ± 0.88 days for ESD, CRL, HD, TD and EBD, respectively (Fig. 1). At that age, they reached 1.37 ± 0.26 , 0.85 ± 0.04 , 0.35 ± 0.04 , 0.61 ± 0.01 and 0.13 ± 0.01 cm, respectively (Table 1). Concomitantly, the uterine lumen contained a variable quantity of anechoic fluid produced by the conceptus. Special attention during the examination of the anechoic fetal fluid was adopted to explore the presence of the embryo, particularly close to the uterine wall and/or folds.

Fetal heart motion activity, one of the earliest signs affirmed its viability, was detectable at Day 24.50 ± 1.30 post-breeding with heart rate was 232.00 ± 12.35 beat/min. Prior to Day 27 onwards, it was possible by using color Doppler to view the heart blood flow in the hypoechoic mass representing the heart inside the echogenic embryonic mass (Fig. 2).

A highly significant ($p < 0.0001$) correlations were outlined between the ESD ($r^2=0.55$), CRL, HD, EBD ($r^2=0.85$) and TD ($r^2=0.93$), and fetal age in buffalo fetuses (Table 1). The corresponding regression line for these parameters was represented by the equations $Y = 0.082 X - 1.855$, $Y = 0.096 X - 1.979$, $Y = 0.053 X - 1.956$, $Y = 0.045 X - 1.207$ and $Y = 0.012 X - 0.437$, respectively. The high correlation between trunk diameter and fetal age signified its clinical value, over other estimated fetometric parameters, in determining gestation period in Egyptian buffaloes, with special emphasis to the period from 21-56 days post-breeding (Fig. 3).

Table 1: Relationship between fetometric parameters and gestation age during early pregnancy in buffaloes

Fetal Structure	Age at the first examination (Days)	Measures at the first examination (cm)	Regression equation	r ²	Sig.
Embryonic sac diameter	24.33 ± 0.67	1.37 ± 0.26	Y= 0.082 X - 1.855	0.55	< 0.0001
Crown rump length	27.00 ± 1.73	0.85 ± 0.04	Y= 0.096 X - 1.979	0.85	< 0.0001
Head diameter	34.67 ± 1.76	0.35 ± 0.04	Y= 0.053 X - 1.956	0.85	< 0.0001
Trunk diameter	34.67 ± 0.88	0.61 ± 0.01	Y= 0.045 X - 1.207	0.93	< 0.0001
Eye ball diameter	33.67 ± 0.88	0.13 ± 0.01	Y =0.012 X - 0.437	0.85	< 0.0001

Data presented as mean ± SE. r² denoted Pearson correlation coefficient. Y is representing the parameter in mm where X is representing the age of pregnancy in days.

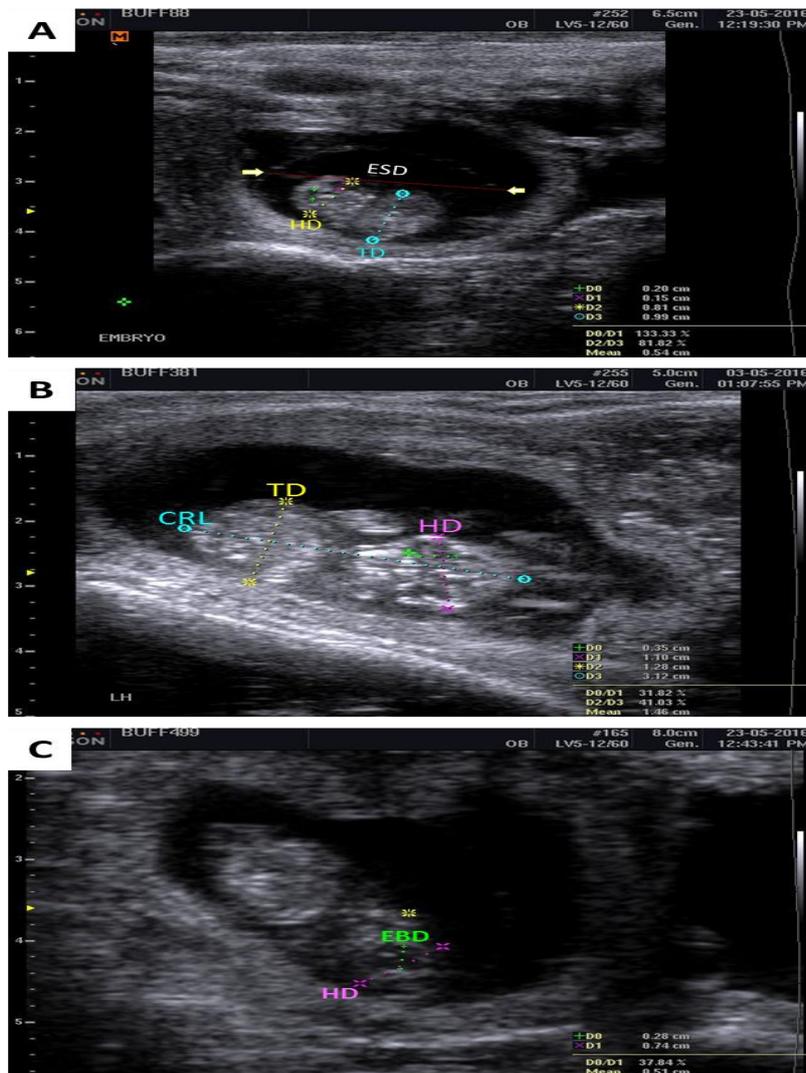


Fig. 1: Showed ultrasound biometry of buffalo fetus during early gestation period. ESD: Embryonic sac diameter. CRL: Crown Rump Length. HD: Head diameter. TD: Trunk diameter. EBD: Eye ball diameter. A, B and C ultrasound images of fetus at Day 28, 42 and 49 of pregnancy, respectively.

Fig.2: Doppler ultrasonographic imaging of the fetal cardiac blood flow (arrow) in the echogenic embryonic mass (F) at different periods after breeding.

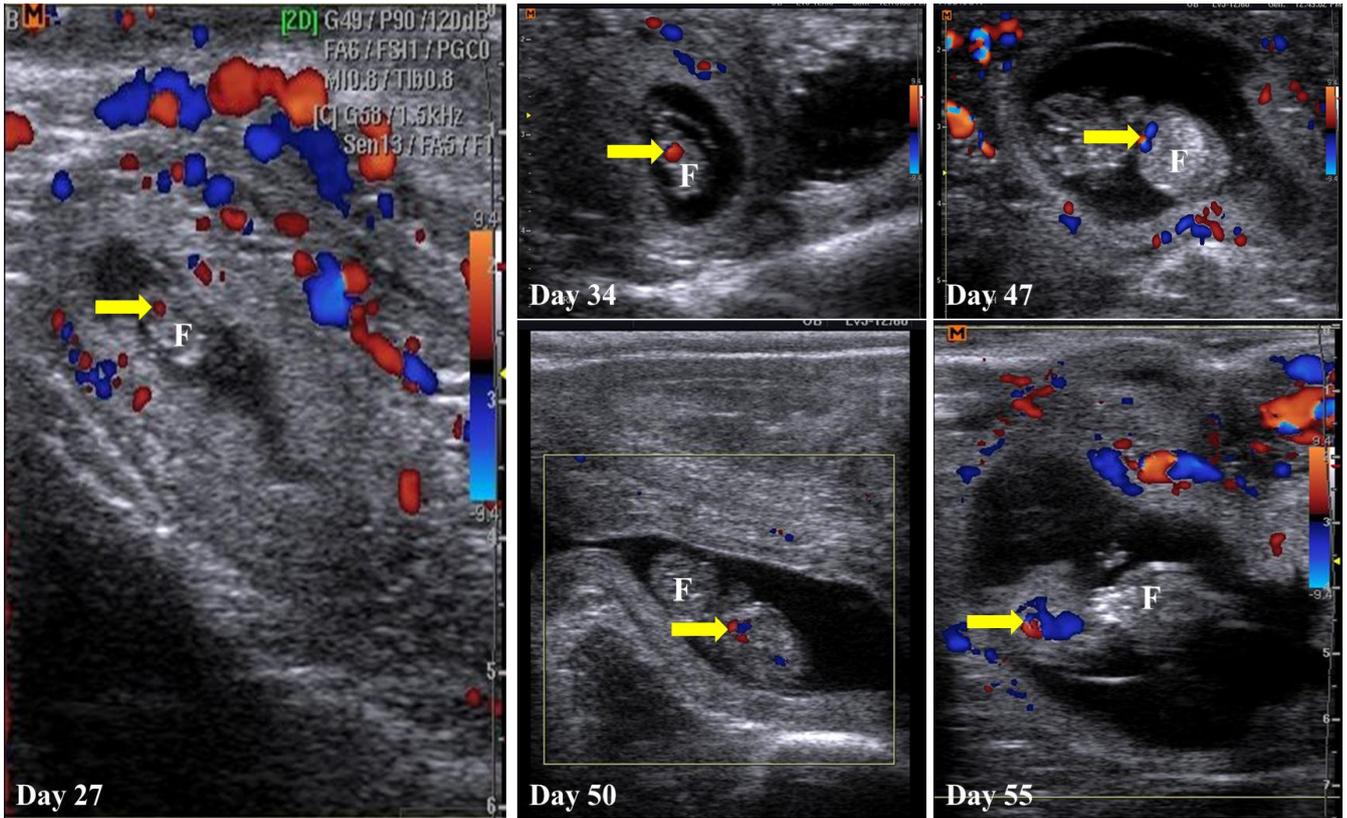
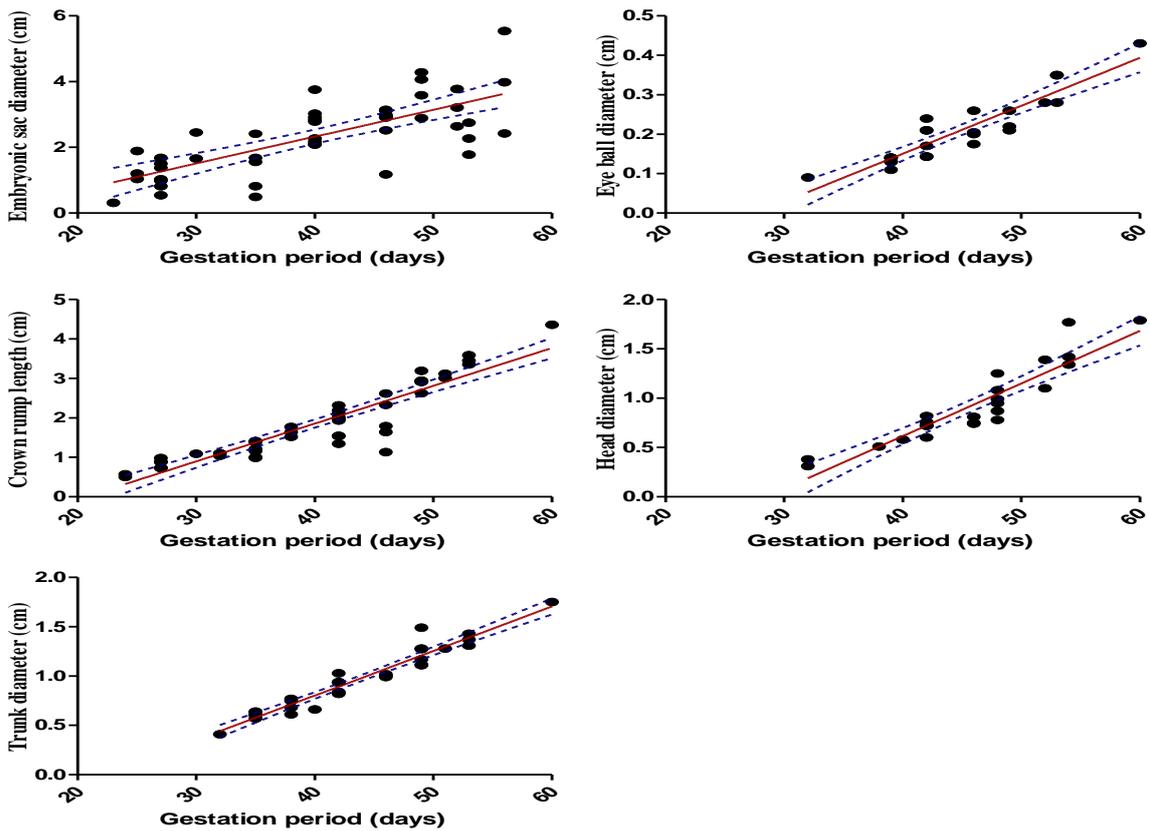


Fig. 3: Growth curves of gestation sac diameter, crown rump length, head diameter, trunk diameter and eye ball diameter during early pregnancy in buffaloes



DISCUSSION

Early pregnancy estimates become a very important participant in the modern farm animals' management protocols. Special considerations are directed to early pregnancy period in buffaloes with special emphasis to the period between 28 and 60 days' post-service due to the high incidence of embryonic mortality (**Vecchio *et al.*, 2007**).

Ultrasonography is considering a safe, reliable and accurate method for not only detection but also monitoring of pregnancy in ruminants (**Naikoo *et al.*, 2013**; **Karen *et al.*, 2014**; **Samir *et al.*, 2016**). Ultrasonographic fetometry has been shown to provide a precise estimation of gestational age and prediction of calving dates (**Wright *et al.*, 1988**). Growth curves of fetal structures based on ultrasonographic examinations have been reported formerly in cows (**White *et al.*, 1985**; **Kähn, 1989**). However, in buffaloes, transrectal ultrasonography can be embraced successfully starting from Day 28-30 after service (**Glatzel *et al.*, 2000**). In the current study, we observed the heart beats from Day 24-26 that reached 232.00 ± 12.35 beat/min, and the heart blood flow (detected by means of Doppler ultrasound) from Day 26-27 after breeding and onwards in the live fetus. This signified its applicability as an indicator of fetal viability and normal development during pregnancy in buffalo .

Doppler ultrasonography techniques have been found to enhance the understanding of blood flow during important reproductive events, thus permitting the producers to apply specific approaches to improve the reproductive efficacy of farm animals (**Lemley, 2017**). **Pawshe *et al.*, (1994)** detected the heartbeat of the embryo proper on Day 29.6 ± 1.57 that was 203.8 ± 9.0 beats per minute on the first day of detection. However, **Naikoo *et al.*, (2013)** examined Mehsana buffaloes' fetus and found the mean heart rate was 174.60 ± 0.81 and 165.28 ± 1.40 beats per minute on Day 26 and 40 post insemination, respectively. The inconsistency between this study and the aforementioned studies may be attributed to the difference in the transducer frequency used.

Early detection of pregnancy can aid the reproductive performance by minimizing the inter-service interval and assurance of non-pregnancy status with forceful strategies for early animal rebreeding (**Fricke, 2002**). In this study, it was verified the accessibility of ESD, CRL, HD, TD and EBD measurement of the embryonic/fetal mass at 24.33 ± 0.67 , 27.00 ± 1.73 , 34.67 ± 1.76 , 34.67 ± 0.88 and 33.67 ± 0.88 days post-breeding, respectively. These findings suggested that the pregnancy in Egyptian buffaloes could be detected as early as Day 24-26 post-breeding. **Pawshe *et al.*,**

(1994) showed that the embryonic vesicle, embryo proper and optic area were first visible on a mean day (\pm SD) 19.00 ± 2.1 , 19.0 ± 1.69 , 38.2 ± 2.39 post-breeding, respectively. **Awasthi *et al.*, (2011)** observed buffalo embryo on 26.33 ± 0.52 days, and the heartbeats on 29.25 ± 0.36 days' post-service, concluding that the early pregnancy in buffaloes can be verified from Day 28 of pregnancy.

Rosiles *et al.*, (2005) studied pregnancy development in cattle until Day 40 after AI and detected the embryo from Day 24 and the heart contractions from Day 26 with high reliability. The differences might be related to the stage or the day of examination in relation to breeding time, and also the difference in the frequency of the transducer used. Species, breed and strain are additional factors accused the differences between our study findings from others, due to their influence on the fetal size, mostly as a result of the variation in the rate of cell division that is genetically controlled (**Jainudeen and Hafez, 2000**). Moreover, former study declared that the diagnostic accuracy and sensitivity differed between the early (Day 16 to 22) and later stage (Day 23 to 31) after insemination (**Badtram *et al.*, 1991**).

The Growth curves of fetal parts and its correlation with gestation age, based on ultrasonographic examinations, have been reported formerly in cows (**White *et al.*, 1985**; **Kähn, 1989**). In the current study, the growth curve and the correlation coefficient between buffalo fetometry and age (in days) were achieved. The highest correlation was observed with TD ($r^2= 0.93$), followed by CRL, HD and EEB ($r^2= 0.85$) and the lowest was with ESD ($r^2= 0.55$). These data indicated the practicality and value of ultrasonography for the fetal characterization, the evaluation of fetal development, and the estimation of gestational age during the first 8 weeks of pregnancy in buffaloes.

Ali and Fahmy (2008) observed that the highest correlation between the CRL and amniotic vesicle diameter at the early-gestation; the biparietal diameter at the mid-gestation; and the eyeball diameter at the mid-and late-gestation with fetal age. **Ferreira *et al.*, (2012)** found high correlation coefficients between gestational age, and the CRL ($r=0.94$), amniotic vesicle diameter ($r= 0.97$), biparietal diameter ($r=0.89$) and TD ($r=0.86$) during the examination period that extended from Day 15 to 70 after artificial insemination. **Bergamaschi *et al.*, (2004)** showed a high cubic regression between the fetal length ($R^2 = 0.98$) and head diameter ($R^2=0.95$) and gestation period. **Riding *et al.*, (2008)** indicated that the amniotic vesicle fluid volume ($r^2= 0.9275$), length ($r^2=0.9713$) and width ($r^2= 0.9802$) and CRL ($r^2= 0.9899$) confidently correlated with fetal age in beef cattle.

CONCLUSION

It could be concluded that the high-frequency ultrasonography is a valuable tool in detecting pregnancy starting from Day 24-26 post-breeding in Egyptian buffaloes onward. Moreover, studying fetal heart blood flow with Doppler ultrasound provides a reliable non-invasive promising technique for the fetal viability detection from Day 26-27 of pregnancy due to its easiness and high accuracy as compared to the classic ultrasound scanners.

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REFERENCES

- ALI, A., FAHMY S. 2008.** Ultrasonographic fetometry and determination of fetal sex in buffaloes (*Bubalus bubalis*). Anim. Reprod. Sci., 106: 90-99.
- AWASTHI, M.K., ABHISHEK, KHARE, KAVANI, F.S., SIDDIQUE, G.M., DHAMI, A.J. 2011.** Early pregnancy diagnosis in water buffaloes using transrectal ultrasonography. Indian Journal of Animal Reproduction, 32 (1): 43-46.
- BADTRAM, G.A., GAINES, J.D., THOMAS, C.B., BOSU, W.T.K. 1991.** Factors influencing the accuracy of early pregnancy detection in cattle by real-time ultrasound scanning of the uterus. Theriogenology, 35 (6): 1153..
- BERGAMASCHI, M.A.C.M., VICENTE, W.R.R., BARBOSA, R.T., MACHADO, R., MARQUES, J.A., FREITAS, A.R. 2004.** Ultrasound assessment of fetal development in Nelore cows. Arch. Zootec., 53: 371-374.
- FERREIRA, J.C.P., IAN MARTIN, IRIKURA, C.R., GIMENES, L.U., FUJIRAH, C.J., JORGE, A.M., OBA, E. 2012.** Ultrasonographic monitoring of early pregnancy development in Murrah buffalo heifers (*Bubalus Bubalis*). Journal of Animal and Plant Sciences, 22 (3 Suppl.): 377-383.
- FRICKE, P.M. 2002.** Scanning the future-ultrasonography as a reproductive management tool for dairy cattle. J Dairy Sci., 85(8): 1918-1926.
- GLATZEL, P.S., ALI, A., GILLES, M., FIDELAK, C. 2000.** Diagnosis of early pregnancy in 30 water buffalo heifers by transrectal palpation with and without ultrasonography. Tierärztliche Umschau, 55: 329-332.
- HERZOG, K., BOLLWEIN, H. 2007.** Application of Doppler Ultrasonography in Cattle Reproduction. Reprod Dom Anim., 42 (Suppl.2): 51-58.
- JAINUDEEN, M.R., HEFEZ, E.S.S. 2000,** Gestation, prenatal physiology, and parturition. In: Reproduction in Farm Animals. Hafez B. and Hafez E.S.E. (Eds.). 7th ed. Lipponcott Williams & Wilkins. USA. Pp. 147.
- KÄHN, W. 1989.** Veterinary Reproductive Ultrasonography. Schlutersche Verlagsgesellschaft mbH and Co, Germany: 83-210.
- KÄHN, W. 2004.** Sonographic fetometry in the bovine. Theriogenology, 31: 1105-1121.
- KAREN, A., DARWISH, S., RAMOUN, A., TAWFEEK, K., VAN HANH, N., DE SOUSA, N.M., SULON, J., SZENCI, O., BECKERS, J.F. 2007.** Accuracy of ultrasonography and pregnancy-associated glycoprotein test for pregnancy diagnosis in buffaloes. Theriogenology, 68 (8): 1150-1155.
- KAREN, A., SAMIR, H., ASHMAWY, T., EL-SAYED, M. 2014.** Accuracy of B-mode ultrasonography for diagnosing pregnancy and determination of fetal numbers in different breeds of goats. Anim Reprod Sci., 147(1-2):25-31.
- KELLEY, D.E., IBARBIA, L., DAETZ, R., BITTAR, J.H., RISCO, C.A., SANTOS, J.E., RIBEIRO, E.S., GALVÃO, K.N. 2016.** Combined use of progesterone inserts, ultrasonography, and GnRH to identify and resynchronize non-pregnant cows and heifers 21 days after timed artificial insemination. Theriogenology, 85(2): 230-237.
- LEMLEY, C.O. 2017.** Investigating reproductive organ blood flow and blood perfusion to ensure healthy offspring. Animal Frontiers, 7: 18-24.
- NAIKOO, M., PATEL, D.M., DERASHRI, H.J. 2013.** Early pregnancy diagnosis by transrectal ultrasonography in Mehsana buffaloes (*Bubalus bubalis*). Buffalo Bulletin, 32: 120-125.
- PAWSHE, C.H., APPA, RAO, K.B.C., TOTEY, S.M. 1994.** Ultrasonographic imaging to monitor early pregnancy and embryonic development in the buffalo (*Bubalus bubalis*). Theriogenology, 41: 697.
- QUINN, G.P., KEOUGH, M.J. 2002.** Experimental design and data analysis for biologist. Cambridge University Press, Cambridge.
- RIDING, G.A., LEHNERTA, S.A., FRENCHB, A.J., HILLC, J.R. 2008.** Conceptus-related measurements during the first trimester of bovine pregnancy. Veterinary Journal, 175: 266-272.
- ROSILES, V.A., GALINA, C.S., MAQUIVAR, M., MOLINA, R., ESTRADA, S. 2005.** Ultrasonographic screening of embryo development in cattle (*Bos indicus*) between days 20 and 40 of pregnancy. Anim. Reprod. Sci., 90: 31-37.
- SAMIR, H., KAREN, A., ASHMAWY, T., ABO-AHMED, M., EL-SAYED, M., WATANABE, G. 2016.** Monitoring of embryonic and fetal losses in different breeds of goats using real-time B-mode ultrasonography. Theriogenology, (2):207.
- VECCHIO, D., DI PALO, R., ZICARELLI, L., GRASSI, C., CAMMARANO, A., D'OCCHIO, M.J., CAMPANILE, G. 2007.** Embryonic mortality in buffalo naturally mated, Italian Journal of Animal Science, 6 (2): 677-679.
- WHITE, I.R., RUSSEL, A.J.F., WRIGHT, I.A., WHYTE, T.K. 1985.** Real-time ultrasonic scanning in the diagnosis of pregnancy and the estimation of gestational age in cattle. Vet. Record, 6: 5-8.
- WRIGHT, I.A., WHITE, I.R., RUSSEL, A.J., WHYTE, T.K., MCBEAN, A.J. 1988.** Prediction of calving date in beef cows by real-time ultrasonic scanning. Vet. Rec., 123: 228-229.