Original Research

Ultrasonographic Studies on Early Embryonic Development and Ageing in Murrah Buffaloes

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Abstract

The present research was instigated to diagnosis of early pregnancy and embryonic ageing through trans-rectal ultrasonography in buffaloes under farm conditions. Total 830 Murrah buffaloes were scanned by standard ultrasonographic procedure after 26 days of Artificial insemination. Among 830 buffaloes, 327 were confirmed pregnant on the basis of presence of embryonic vesicles, embryo proper, heart beats, eye orbits, limb buds whereas 503 buffaloes were diagnosed as non-pregnant based on hypoechoic images on during 26 to 41 days of AI. In pregnant buffaloes, embryonic crown rump length (CRL), body diameter (BD) and bi-parietal diameter (BPD) were measured by in built electronic caliper of the Ultrasonography machine. Mean CRL of the embryo increased from 6.03±0.07 mm on day 26 to 22.12±0.35 mm on day 41 post-insemination. The BD and BPD of embryos were possible to measure from day 29 to 41. The average BD of the embryo varied from 4.33±0.12 mm on day 29 to 11.10±0.10 mm on day41 whereas, the average BPD was recorded 3.22±0.06 on day 29 to 8.98±0.16 mm on day 41. Other embryonic parameters such as the heart beats, amnion, optic lens, limb buds and umbilical cord were detectable first time on day 27.8, 29.8, 33.4, 35.3, 39.9 and 40.5 post-insemination, respectively. Ultrasonography has 100 percent predictive value for diagnosis of pregnancy as well as non-pregnancy on day 28 onwards in Murrah buffaloes. Embryonic measurements viz. CRL, BD and BPD can be utilized for determination of embryonic age as well as gestational days in Murrah buffaloes having unknown breeding history.

Key words: Early Pregnancy Diagnosis, Embryonic Ageing, Murrah Buffaloes, Ultrasonography

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Introduction

In India, buffaloes contribute significantly to the livestock industry producing milk, meat, hides and draft power (Nanda and Nakao, 2003). In most of states, buffalo is the principal dairy animal and plays an important role in the socioeconomic development of rural India. Optimum reproductive efficiency is a

prerequisite to realization of high life-time production from buffaloes. Buffaloes experience problems related to reproduction, especially long calving interval, delayed puberty and high incidence of anestrus. An early and accurate pregnancy diagnosis is an important criterion for better reproductive management in buffaloes. Early pregnancy diagnosis is crucial to shorten the calving interval through enabling the farmer to identify open animals, so as to treat and/or rebreed at an earliest. The clinical examination by trans-rectal palpation of uterus is the most widely used technique for pregnancy diagnosis which is commonly employed after two months of breeding in buffaloes. Ultrasonography is a minimally invasive, accurate, and efficient technique for early pregnancy diagnosis and has made diagnosis possible as early as day 28 post insemination in buffalo and even earlier in mare. Transrectal ultrasonography has the added advantage of providing additional information on ovarian structures, identification of twins and determination of fetal viability, age and sex. Transrectal ultrasonography has made the thorough examination of reproductive health of animal possible and therefore, it has now become an established research tool to study bovine reproductive biology in buffaloes. The early embryonic period in cattle has been described to be lasting for approximately 42 days post insemination, encompassing a series of events starting with fertilization and culminating in implantation. There is paucity of research on the recoding of embryonic development in Murrah buffaloes. The series of embryonic development studies during different days can standardize the embryo aging criteria in Murrah buffaloes. Considering the idea, research was undertaken for diagnosis of early pregnancy and embryonic ageing through trans-rectal ultrasonography in buffaloes under farm conditions.

Materials and Methods

The present work was carried out for two years at organized buffalo farm located near Mumbai having capacity of more than 500 Murrah buffaloes with ideal management, optimum nutrition and breeding through artificial insemination (AI). Buffaloes with known breeding history of insemination were selected from day 26 to 41 post AI. These buffaloes were subjected to transrectal ultrasonography (Sonoscape A5 machine, China made) using B-mode, linear array transducer having 6 – 8 MHz frequency. All buffaloes were examined in chute for the safety of machine and operator. The buffaloes were scanned on the respective day of gestation by ultrasonography using standard operating procedure for early pregnancy diagnosis and cross verified later on by per-rectal palpation after 60 days of breeding. Buffaloes confirmed as pregnant were examined further for measurement of embryonic crown rump length (CRL), body diameter (BD) and bi-parietal diameter (BPD) after freezing the ultrasonographic images and those saved in internal disc of USG machine. Others parameters such as embryonic heart beats, amniotic membrane, eye orbits, limb buds and umbilical cord were also examined during scanning. The sensitivity, specificity and predictive values were measured as follows: correct positive diagnosis (a), incorrect positive

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diagnosis (b), correct negative diagnosis (c), incorrect negative diagnosis (d). From these data the sensitivity [100 x a / (a + d)], the specificity [100 x c / (c + b)], the positive predictive value [100 x a / (a + b)], the negative predictive value [100 x c / (c + d) and the overall accuracy [100 x (a+ c) / (a + b + c + d)] were calculated (Pieterse *et al.*, 1990) in bovines.

Results and Discussion

Total 830 Murrah buffaloes were scanned by trans- rectal ultrasonography in between day 26 to 41 after AI. In pregnant buffaloes, crown rump length, bi-parietal diameter and body diameter were measured by electronic caliper of USG machine. Total 327 buffaloes were confirmed pregnant on 26 to 41 day of gestation whereas 503 buffaloes were found non-pregnant.

Table 1: Embryonic parameters from 26 to 41 days post breeding in Murrah buffaloes

S. No.	Day of Pregnancy	No. of Non-Pregnant Buffaloes	No. of Pregnant Buffaloes	Average CRL ± S.E.M. (mm)	Average BD± S. E.M. (mm)	Average BPD ± S. E.M. (mm)
1	26	8	6	6.03±0.07	--	--
2	27	9	7	6.44±0.09	--	--
3	28	14	7	6.91±0.11	--	--
4	29	26	16	8.67±0.14	4.33±0.12	3.22±0.06
5	30	46	31	9.72±0.12	4.45±0.06	3.54±0.05
6	31	47	22	10.25±0.18	4.70±0.06	3.63±0.18
7	32	57	39	10.40±0.10	6.13±0.13	4.35±0.12
8	33	38	23	11.44±0.39	5.70±0.15	4.45±0.08
9	34	32	15	12.63±0.19	5.94±0.09	4.74±0.21
10	35	36	22	14.35±0.31	6.62±0.56	5.24±0.07
11	36	21	18	16.11±0.13	7.23±0.05	5.43±0.09
12	37	40	17	17.68±0.09	7.98±0.51	6.03±0.45
13	38	34	27	18.13±0.15	8.18±0.30	6.50±0.32
14	39	39	38	19.33±0.22	8.46±0.16	7.34±0.15
15	40	28	23	20.11±0.11	9.81±0.28	8.24±0.29
16	41	28	16	22.12±0.35	11.10±0.10	8.98±0.16

In all pregnant buffaloes, the most peculiar characteristic of the image was compartmentalization of embryonic vesicle with typically two to three anechoic sections visualized through the chorionic vesicle. An embryo, which was little more intense than that of the neighbouring endometrium, noticed in one of these compartments in pregnant buffaloes. Bhosrekar and Hangare (2000) in buffaloes and Kahn (1990) in bovines recorded similar findings regarding compartmentalization and visualization of embryo in bovines. Flickering echoes of the heart-beats were visualized in the centre of embryo from average day 27.8 (27 to 29 days), whereas, Sharma *et al.* (2012) detected the same on day 27.1 in pregnant buffaloes. However, in the present study, embryonic heart-beats were detected slightly earlier than mentioned by Pawshe *et al.* (1994) and Glaztel *et al.* (2000) who detected heart-beats as early as day 29.6 ± 1.57 and day 28 in buffaloes,

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respectively, which could be attributed to old generation of machine used by them. The mean CRL of the embryo increased from 6.03±0.07 mm on day 26 to 22.12±0.35 mm on day 41 post-insemination. Almost similar progression of CRL was mentioned by Pawshe *et al.* (1994) and Sharma *et al.* (2012) who started to measure CRL of embryo from day18 and day 22, respectively. The average BD of embryo was recorded as 4.33±0.12 mm on day 29 and 11.10±0.10 on day 41 of gestation. Such finding has not been cited anywhere in literature and hence, present findings regarding the BD of embryo in between day 29 to 41 of gestation could not be compared. The average BPD progression from 3.22±0.06 mm on day 29 to 8.98±0.16 mm on day 41 of gestation was also recorded in the present study. Mali (2006) reported BPD measurements from day 46 to 60 of gestation which is not comparable with the current findings. The embryonic anatomical features such as the amnion, optic lens, limb buds and umbilical cord were first visualized on post-insemination day 29.8, 33.4, 35.3, 39.9 and 40.5, respectively. Similar observations were reported by Pawshe *et al.* (1994), Bhosrekar and Hangare (2000), Rane *et al.* (2002), Mali (2006), Ali and Fahmy (2008), Ingawale *et al.* (2012) and Sharma *et al.*(2012) in buffaloes.

It was possible to clearly visualize the embryonic vesicle in all 327 predicted pregnant buffaloes, whereas embryo proper was seen in all predicted pregnant buffaloes from day 28 onwards. Two buffaloes on day 26 and one buffalo on day 27were diagnosed as false positive in which there was no visualization of embryos. The sensitivity of early pregnancy diagnosis by ultrasonography on day 26, 27 and 28 onwards was found 66.67, 85.71 and 100 per cent; whereas, specificity was recorded as 75, 88.89 and 100 per cent, respectively. The positive predictive value in the present study was 70, 87.89 and 100 per cent on day 26, 27 and 28 onwards, respectively. Similar observations were recorded by Pawshe *et al.* (1994), Glatzel *et al.*(2000), Bhosrekar and Hangare (2000), Rane *et al.* (2002), Awasthi (2004) and Karen *et al.*(2007)in buffaloes, who carried out ultrasonographic scanning for confirmed pregnancy or non-pregnancy on day 28 onwards.

Ultrasonographic Images of Embryonic Vesicle, Embryo, Amniotic Membrane, CRL, BD and BPD of Embryo from Day 26 to 41 of Gestation (USG Image 1 to 16)

USG Image 1: Day 26

USG Image 2: Day 27

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USG Image 3: Day 28

USG Image 4: Day 29

USG Image 5: Day 30

Image 6: Day 31

USG Image 7: Day 32

USG Image 8: Day 33

USG Image 9: Day 34

USG Image 10: Day 35

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USG Image 11: Day 36

USG Image 12: Day 37

USG Image 13: Day 38

USG Image 14: Day 39

USG Image 15: Day 40

USG Image 16: Day 41

Conclusion

Ultrasonography has cent per cent predictive value for diagnosis of pregnancy as well as non-pregnancy on day 28 onwards post breeding in buffaloes. There is great feasibility and value of ultrasonographic embryometry in buffaloes for evaluation of embryonic development and ageing from day 30 of gestation. The present results can be utilized for determination of embryonic age as well as gestational days in Murrah buffaloes having unknown history of breeding.

References

1. Ali, A. and S. Fahmy (2008).Ultrasonographic fetometry and determination of fetal sex in buffaloes *(Bubalus bubalis). Animal Reproduction Science* 106: 90–99.

DOI 10.5455/ijlr.20180720025719

2. Awasthi, M.K. (2004). Follicular dynamics, early pregnancy diagnosis and fertility management in postpartum Mehsana buffaloes through ultrasonography. Ph.D. Thesis, Anand Agri. University, Anand, India
3. Bhosrekar, M. R. and I. M. Hangare (2000). Ultrasonography for early pregnancy diagnosis in buffaloes.*Indian J. Anim. Reprod*., 21 (2): 143 – 144.
4. Glatzel, P. S., A. Ali., M. Gilles and C. Fidelak (2000).Diagnosis of early pregnancy in 30 water buffalo heifers by transrectal palpation withand without ultrasonography.*Tierarztliche Umschau*, 55 (6): 329–332 (c.f. Anim. Breed. Abstr.48: 537.)
5. Ingawale, M.V., S. A. Bakshi, H. S. Birade, S. R. Chinchkar and S. U. Gulavane (2012).Early detection of pregnancy using ultrasonography in buffaloes. *Buffalo Bulletin*.31 (4).202-208.
6. Kahn, W. (1990).Sonographic imaging of the bovine fetus.*Theriogenology*.33 (2): 385-396.
7. Nanda, A.S. and T. Nakao (2003). Role of buffalo in the socioeconomic development of rural Asia: Current status and future prospectus. *Anim. Sci. J.,* 74: 443-455.
8. Pawshe, C. H.; R. B. C. AppaRao and S. M. Totey (1994).Ultrasonographic imaging to monitor early pregnancy and embryonic development in the buffalo.*Theriogenology*.41: 697-709.
9. Pierson, R. A. and O. J. Ginther (1984).Ultrasonography for detection of pregnancy and study of embryonic development in heifers.*Theriogenology*.22: 225-273.
10. Pieterse, M. C.; O. Szenci; A.H. Willemse; C. S. A. Bajesy; S. J. Dieleman and M. A. M. Tayerne (1990). Early pregnancy diagnosis in cattle by means of linear array real time. Ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. Theriogenology. 33 (3): 697-707.
11. Rane, R. S.; R. H. Jadhav; R. A. Thorat and S. S. Swami (2002).Early pregnancy diagnosis by ultrasound techniques in buffaloes. XVII Annual Convention and National Symposium on Reproduction Tech. for augmentation of fertility in Livestock, Izzatnagar: 153.
12. R. K. Sharma, J. K. Singh, S. Khanna, S. K. Phulia, S. K. Sarkar and I. Singh (2012).Fetal age determination in Murrah buffaloes from days 22 through 60 with ultrasonography. *Indian Journal of Animal Sciences* 82 (4): 374–376.
13. Tiwari, R. P.; M. K. Awasthi and R. P. Gautam (2002).Principles, methods and clinical uses of ultrasonography in bovine reproduction. *Intas Polivet*. 3 (1): 78-85.

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