COMPARATIVE EFFICACY OF PROGESTERONE ASSAY AND ULTRASONOGRAPHY FOR EARLY PREGNANCY DIAGNOSIS IN BUFFALOES

H.C. NAKHASHI[1], B.N.SUTHAR, V.K.SHARMA[3], G.M.SIDDIQUEE[4], J.B. PATEL[5] and F.S.KAVANI[6]

*Department of Gynaecology and Obstetrics,
Veterinary College, Sardarkrushinagar Dantiwada Agricultural University
Sardarkrushinagar - 385 506 (Gujarat.)*

ABSTRACT

The comparative efficacy of ultrasound scanning and plasma P_4 assay was studied to diagnose early pregnancy in Mehsana buffaloes. The sensitivity and specificity for early pregnancy diagnosis through ultrasound scanning on days 20, 25 and 30 of gestation were 75.0,66.7 and 100; 66.7,100 and 100, respectively. Both negative and positive predictive values improved as the pregnancy advanced whereas, diagnostic accuracy was 100 per cent using ultrasonography against 86.2 per cent by P_4 assay on 30th day post breeding. It was possible to visualize the embryonic vesicle and/or embryo clearly in every animal that was found pregnant from day 30 onwards from breeding. The technique of ultrasound scanning facilitated the diagnosis of all pregnant animals as early as on day 25 post-service.

Key words: Early pregnancy, Ultrasonography, P4 assay, Mehsana buffaloes

INTRODUCTION

The Real-time, B-mode ultrasonography detects pregnancy in cattle as early as by day 9 and 12 of gestation (Boyd *et al.*, 1988,Pierson *et al.*, 1984) with its improved accuracy as the stage of pregnancy advances - day 18 , 20 and 22 (Kastelic *et al.*, 1989). On the other hand, Glatzel *et al.* (2000) reported serum progesterone levels of 2 to 3 ng / ml in water buffaloes to diagnose pregnancy between days 25 and 35 of pregnancy where as, Rao *et al.* (1983) documented 0.4 to 4.4 ng / ml of progesterone in Mehasana buffaloes after 21-25 days of insemination. However, meager work has been carried out on comparative accuracy of these techniques. Therefore, the present study was undertaken on Mehsana buffaloes to compare the reliability of ultrasonography and progesterone assay as diagnostic tool for early pregnancy.

1. Corresponding author, 3. Professor, Teaching Veterinary Clinical Complex, Deesa; 4. Professor, Dept of Clinics; 5..Research Scientist, LRS, SDAU, Sardarkrushinagar 6.Professor & Head (Rtd), Deptt. of ARGO, CVSc., AAU, Anand

MATERIALS AND METHODS

The pleuriperous Mehsana buffaloes bred to natural service were included in the present study of early pregnancy diagnosis by ultrasound (n=16) and progesterone assay(n=29). The ultrasound examinations were performed on days 20,25,30,35,38, and 42 post breeding using a Real-time B-Mode Ultrasound Scanner (Sigma-110 Master-Vetson, Kontron Medical, S.A.S., France) equipped with a 6.5 MHz convex linear array transducer designed for intra-rectal placement. Positive diagnosis of pregnancy was made by visualization of embryonic vesicle and other characteristics as documented by Pieterse *et al.* (1990). Desired images of pregnancy associated structures were frozen and printed on thermal printer (Sony, UP-895 MD, Sony Corporation, Japan). The diagnostic accuracy, Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) of ultrasound technique were determined (Badtram *et al.,* 1991). On the other hand, blood samples were collected on day 20th and 30th post-breeding and plasma was obtained for Progesterone estimation by RIA using standard method (Kubasic *et al.*, 1984). The

progesterone level 1 ng per ml was considered as a positive indicator of pregnancy. The findings of two techniques were confirmed on day 60 by per rectal observations of pregnancy. The results were compared to assess the accuracy of early pregnancy diagnosis.

RESULTS AND DISCUSSION

The diagnostic predictives of pregnancy by ultrasound and progesterone assay are presented in Table 1. The results revealed that sensitivity, specificity PPV and NPV of ultrasound on day 20 of pregnancy were lower (75.0, 66.7, 75.0 and 66.7 per cent) than that of plasma progesterone (81.8, 85.7, 94.74 and 60 percent). The over all accuracy of these predictives for ultrasound technique increased as the pregnancy advanced and was observed to be 100 per cent from day 30 onwards while it remained low (82.80 per cent) for progesterone assay. The findings of ultrasound scanning are in agreement to earlier reports in buffaloes (Glatzel et al., 2000) and cows also (Pieterse et al., 1990). However, Pawshe et al. (1994) reported pregnancy detection in 12 out of 26 animals as early as day 19 post breeding. Whereas the accuracy of progesterone assay on day 20 and 30 post breeding was low, the average values of hormone did not show any demarkable variation at this stage (Table -2). It averaged to 2.56±0.32 ng/ml and 2.30 ± 0.35 ng/ml in buffaloes diagnosed pregnant correctly on day 20 and

30, respectively. Almost similar plasma P_4 in pregnant buffaloes on day 20 has been reported (Jain and Pandey, 1991 and Glatzel et al., 2000). Rao et al. (1983) also reported lower accuracy of progesterone assay to detect pregnancy in buffaloes. In comparative progesterone profiles of pregnant buffaloes, one buffalo which was diagnosed pregnant incorrect had exceptionally higher progesterone level 9 5 ng/ml on day 20 post breeding in the present study which might be an indicator of embryonic loss. Awasthi et al. (2004) also diagnosed two buffaloes nonpregnant with higher plasma progesterone.

The sonographic images of pregnancy associated structures are depicted in Fig. The uterus was visualized well within the pelvic cavity in all buffaloes where as, the embryo was observed as a faint streak at 20th day in only one buffalo but it appeared distinctly on days 30, 35, 38 and 42 as the pregnancy progressed, which resulted to diagnosis of pregnancy on visualization of embryo proper in 12 out of 18 observations in 12 buffaloes while rest of the buffaloes were diagnosed pregnant at 20th and 25th day on the basis of presence of amniotic fluid. This finding corroborated the earlier reports of Awasthi et al. (2004) and Nation et al. (2003) that identification of embryonic fluid is more efficient rather than finding an embryo which is hardly of 6mm size at this stage in convoluted uterine horn.

Table 1. Diagnostic predictive of early pregnancy by ultrasound scanning and plasma progesterone assay in Mehsani buffaloes

Diagnosis results / Predictive values	Day of ultrasound scanning						Plasma P_4 assay (Days)	
	20	25	30	35	38	42	20th	30th
	N=7	N=5	N=3	N=3	N=5	N=4	N=29	N=29
Pregnant correct (A)	3	2	2	2	3	3	18	19
Pregnant incorrect (B)	1	0	0	0	0	0	1	1
Non-pregnant correct (C)	2	2	1	1	2	1	6	6
Non-pregnant incorrect (D)	1	1	0	0	0	0	4	3
Sensitivity 100 x A/ (A+D)	75	66.7	100	100	100	100	81.8	86.4
Specificity 100 x C/ (C+B)	66.7	100	100	100	100	100	85.7	85.7
Positive Predictive Value 100 x A/ (A+B)	75	100	100	100	100	100	94.74	95.0
Negative Predictive Value 100 x C/ (C+D)	66.7	66.7	100	100	100	100	60	66.7
Diagnostic Accuracy 100 x (A+C)/(A+B+C+D)	71.4	80	100	100	100	100	82.8	86.2

Table 2. Plasma progesterone (ng/ml) during different status of pregnancy in Mehsani buffaloes

Diagnostic result Group of animals	Pregnancy status			
	Number of Buffaloes	Day - 20	Number of Buffaloes	Day - 30
Pregnant correct (A)	18	2.56±0.32 (1.10-6.00)	19	2.30±0.35 (1.01-7.00)
Pregnant incorrect (B)	1	5.00	1	2.66
Non-pregnant correct (C)	6	0.40±0.09 (0.18-0.73)	6	Returned to estrus
Non-pregnant incorrect (D)	4	0.20±0.01 (0.01-0.36)	3	0.73± 0.10 (0.54-0.84)

Figures in the parenthesis denote the range values

REFERENCES

Awasthi, M.K. (2004). "Follicular dynamics, early pregnancy diagnosis and fertility management in postpartum Mehsana buffaloes through ultrasonography" A Thesis submitted to AAU, Anand, Gujarat

Badtram, G.A., Gaines, J.D., Thomas, C.B. and 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**: 1153-1167.

Boyd, J.S., Omran, S.N. and Ayliffe, T.R. (1988). Use of a high frequency transducer with real-time, B-mode ultrasound scanning to identify early pregnancy cows. *Vet. Rec.,* **123** : 8-11.

Glatzel, P.S., Ali, A., Gilles, M. and Fidelak, C. (2000). Diagnosis of early pregnancy in 30 water buffalo heifers by transrectal palpation with and without ultrasonography. *Tierarztliche Umschau.,* **55** (6): 329-332 (c.f. *Anim. Breed. Abstr.* **68**: 7459).

Jain, G.C. and Pandey, R.S. (1991). Circulatory levels of prostaglandin F_2 alpha and progesterone following breeding in buffalo heifers. *Buffalo Bull.,* **10** (2): 38-44.

Kastelic, J.P., Curran, S.and Ginther, O.J. (1989). Accuracy of ultrasonography for pregnancy diagnosis on days 10 to 22 in heifers. *Theriogenology,* **31**: 813-820 .

Kubasic, N.P., Hallauer, G.D. and Brodows, R.G. (1984). Evaluation of direct solid-phase RIA for progesterone, useful for monitoring luteal function. *Clinical Chemistry,* **30** (2) : 284-286.

Nation, D.P., Malmo, J., Davis, G.M. and Macmillan, K.M. (2003). Accuracy of bovine pregnancy detection using ultrasonography at 28 to 35 days after insemination. *Aust.Vet. J.,* **81** (1&2): 63-65.

Pawshe, C.H., Appa Rao, K.B.C. and Totey, S.M. (1994). Ultrasonographic imaging to monitor early pregnancy and embryonic development in the buffalo heifer. *Theriogenology,* **41** (3): 697-709.

Pierson, R.A. and Ginther, O.J. (1984). Ultrasonography of bovine ovary. *Theriogenology,* **21**: 495-504.

Pieterse, M.C., Szenci, O., Willemse, A.H., Bajcsy, C.S.A, Dieleman, S.J. and Taverne, M.A.M. (1990). Early pregnancy diagnosis in cattle by means of linear array real-time ultrasound scanning of uterus and a qualitative and quantitative milk progesterone test. *Theriogenology,* **33**: 697-707.

Rao, A.V.N., Sreemannarayana, O. and Rao, V.N. (1983). Early pregnancy diagnosis in the buffalo by serum progesterone radioimmunoassay. *Livestock Advisor,* **8**:11-13.
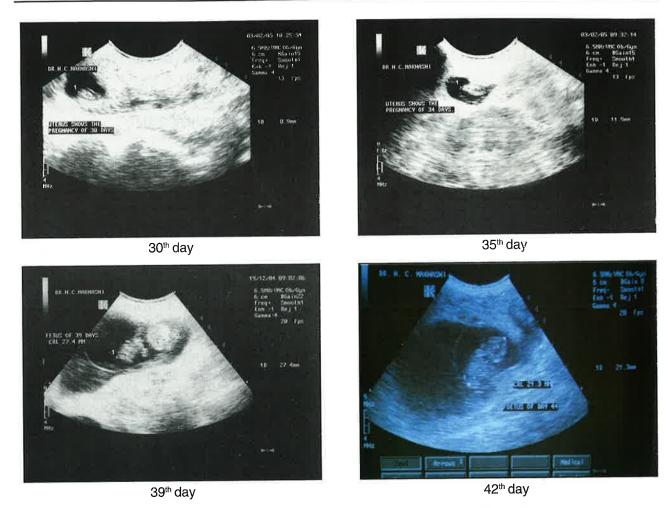
30th day   35th day

39th day   42th day

Fig. Sonographic visualization of pregnancy associated structures at different days of pregnancy

ISSAR AWARD

R.D. SHARMA AWARD

☞ The award is for the best presentation in Obstetrics by a young life member of ISSAR below 35 years of age.

☞ The application along with five copies of the article with certificate of proof of age may be submitted to the General Secretary, ISSAR.

☞ He should also send the abstract and full text of the article to the Organizing Secretary of the convention.

☞ Application form may be obtained from General Secretary, ISSAR.