EARLY PREGNANCY DIAGNOSIS THROUGH TRANSRECTAL ULTRASONOGRAPHY AND PLASMA PROGESTERONE ASSAY IN KANKREJ COWS*

H.L. BHORANIYA[1], A.J. DHAMI[2], M. NAIKOO[3] AND B.S. DIVEKAR[4]

Department of Animal Reproduction, Gynaecology and Obstetrics
College of Veterinary Science and Animal Husbandry
Anand Agricultural University, Anand - 388 001, India

The ultrasound examinations were performed on 26 experimentally induced estrus Kankrej cows at day 26 and 40 post-insemination using a real-time B-mode scanner equipped with a 5 MHz linear array transducer. The results were correlated with the findings of plasma progesterone on day 20 and palpation per rectum on day 60 post-insemination with respect to sensitivity, specificity, reliability and accuracy of ultrasound scanning. Ultrasound scanning of uterus on day 26 post-AI revealed the embryo proper in 11 of 12 predicted pregnant cows, though embryonic vesicle was seen in all 12. Moreover, both embryonic vesicle and embryo proper were detected in all 15 predicted pregnant animals on day 40 post-insemination. The sensitivity of ultrasound scanning for early pregnancy on days 26 and 40 was 85.71 and 93.75 %, and the specificity 75.00 and 100.00 %, respectively. The positive predictive values of early pregnancy were 80.00 and 100.00 % on days 26 and 40, while the negative predictive values were 81.82 and 90.91 %. The overall diagnostic accuracy of USG was found to be 80.77 and 96.15 % on days 26 and 40 post-AI, respectively, and that for plasma progesterone assay on day 20 post-AI was 86.66 %. The plasma progesterone concentrations on day 20 post-AI were significantly higher in conceived cows as compared to non-conceived cows in all the experimental animals. Thus transrectal USG is a fairly reliable, instant and accurate means of detecting early pregnancy in zebu cows.

Key Words: Kankrejcows, Early pregnancy diagnosis, Plasma progesterone, Ultrasonography,

INTRODUCTION

The use of ultrasonography has been increasing as an imaging modality in bovine reproduction. Its use can provide solution to a number of unanswered questions in dealing with the bovine reproductive cycle and its concurrent disorders, including early pregnancy diagnosis and detection of early embryonic mortality. Boyd *et al.* (1988) tentatively observed the conceptus at day 13 within the vesicle. The vesicle suddenly enlarged at day 19 and the heart beat was detected in the embryo at day 22. Viability of embryo is essential to increase the profitability from the animal (Tiwari*et al.*, 2002) and ultrasound pregnancy diagnosis is a reliable method of determining the presence of conceptus by day 25 to 28 after breeding in cows(Kahn, 1990). The present study was conducted to detect early pregnancy and embryonic mortality, if any, throughtrans rectal ultrasonography on days 26 and 40 post-AI in relation to plasma progesterone assay on day 20 and per rectal palpation on day 60 post-AI in estrus induced post-partum anestrus Kankrej cows.

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[1]Scientist-1, Amul Research and Development Association, Amul, Anand – Gujarat – 388 001
[2]#Corresponding author: Dr. A.J. Dhami, Professor and Head, E-mail: dhami_1659@yahoo.com.
[3]Ph.D.Scholar, [4]Asstt. Prof.,Livestock Research Station, AAU, Anand.

MATERIALS AND METHODS

The transrectal ultrasound examinations were performed on 26 out of 30 experimental Kankrej cows under different estrus induction/synchronization protocols using a real-time B-mode ultrasound scanner (ALOKA SSD-500, SN-M10408, Japan) equipped with a 5 MHz linear array transducer designed for transrectal placement. The cows were inseminated at fixed time following use of various estrus induction protocols and/or natural estrus. Jugular blood samples were taken in heparinized vials just after AI and again on day 20 post-AI. The plasma separated out by centrifugation of samples was stored in deep freeze at -20°C till analysis.

For USG examinations, the rectum was evacuated by lubricated gloved left hand and both the uterine horns were located. After liberal application of transmission gel the transducer was placed and directed in the rectum through the right hand and carried to the grasped uterine horn. The scanning of uterine horns was carried out on their dorsal and lateral surfaces. The images displayed were thoroughly examined, frozen on the screen and sonograms were taken using videographic thermal printer (Sony, UP-895 MDW, Japan). The pregnancy was then confirmed by per rectal palpation on day 60 post-AI.Plasma progesterone concentrations were determined by standard RIA technique of Kubasic*et al.* (1984). Labeled antigen (I^{125}), antibody coated tubes and standards were procured from Immunotech, France. The sensitivity of assay was 0.1 ng/ml. The intra-and inter-assay coefficients of variation were 5.4 and 9.1 %, respectively. Cross reactivity of the antibody with progesterone, 17α-dihydroprogesterone and 20α-hydroxyprogesterone was 100, 0.13 and 0.96 %, respectively.

The sensitivity, specificity, reliability and accuracy of ultrasound scanning for detection of early pregnancy on different days post-AI and of P_4 assay on day 20 post-AI were calculated as per Pieterse *et al.* (1990),and were interpreted statistically.

RESULTS AND DISCUSSION

It was possible to clearly visualize the embryonic vesicle in all the predicted pregnant animals and embryo proper in 11 out of 12 cows on day 26 post-insemination. Moreover, both embryonic vesicle and embryo proper were detected in all 15 pregnant animals on day 40 post-insemination. Thus, ultrasonography facilitated diagnosis of all non-pregnant animals at an early stage (day 26 post-AI) and is really advantageous than pregnancy diagnosis by rectal palpation, wherein 100 % reliable results cannot be obtained at such an early stage. Kahn (1990), Pieterse *et al.* (1990), Nation *et al.* (2003), Awasthi (2004), Rosiles *et al.* (2005), Sawale *et al.* (2006) and Naikoo *et al.*(2010) reported similar findings in cattle and buffaloes.

Although the sensitivity of early pregnancy diagnosis through ultrasound scanning on days 26 and 40 was 85.71 and 93.75 %, the specificity was 75 and 100 %, respectively. On day 26, three animals were incorrectly diagnosed pregnant, while incorrectly diagnosed non-pregnant animals were 2 and 1 on day 26 and 40, respectively.The results of ultrasound examination on day 26 and 40 post-AI closely correlated, except in one cow, where it was diagnosed as non-pregnant on day 26, but turned out to be pregnant on day 40 by ultrasound scanning and per rectal palpation on day 60.

The positive predictive value (PPV) of the ultrasound scanning was 80and 100 % on days 26 and 40 post-insemination.Similar results were also recorded earlier by some workers (Chauffax *et al.*, 1986; Boyd *et al.,*1988; Awasthi, 2004), while others (Pieterse *et al.*, 1990; Barros and Visintin, 2001) found little lower positive predictive values. The negative predictive values (NPV) of the ultrasound scanning of the present study were 81.82 and 90.91 % on the days 26 and 40 post-insemination. The overall diagnostic accuracy of early pregnancy in the present study was recorded as 80.77 and 96.15 % on days 26 and 40, respectively. Similar diagnostic accuracy was recorded by Kastelic*et al.* (1989). In this study, there was one case of early embryonic mortality between days 40 and 60 post-AI.

Out of 30 animals, 13 cows conceived at the first natural or induced estrus, while 17 cows returned to next estrus at different time intervals. The number of

cows conceived at induced estrus varied from 1 to 4 (16.67 to 66.66 %) in different groups. Mean values of plasma progesterone (ng/ml) on day 20 post-AI were higher (P<0.05) in conceived cows than the non-conceived cows of all treatment protocols and normal cyclic group (Table). On the basis of plasma progesterone estimation at day 20 post-AI, 17 cows were predicted to be pregnant, but later on ultrasound scanning and even palpation per rectum revealed only 13 to be pregnant. Thus, the specificity and accuracy of early pregnancy diagnosis through plasma progesterone assay on day 20 post-AI was 76.47 and 86.66%, respectively, although the sensitivity and negative predictive value were cent per cent. Naikoo

et al.(2010) reported similar results in buffaloes. Muhammad et al. (2000) recorded the plasma P_4 concentrations in 18 normal cyclic HF cows post-insemination to range from 2.30 - 4.00 and 0.10 - 2.20 ng/ml in pregnant and non-pregnant groups, respectively, which is similar to the present finding in Kankrej cows.Raghorteet al. (2009) also documented higher average P_4 levels in pregnant heifers and buffaloes on 21[st]day post-breeding.

Study concluded that transrectal USG is fairly reliable, instant and accurate means of detecting early pregnancy and embryonic death as early as day 26 post-breeding in zebu cows.

TABLE : PLASMA PROGESTERONE CONCENTRATION (NG/ML) ON THE DAY OF AI AND 20 DAYS POST-AI IN KANKREJ COWS UNDER VARIOUS ESTRUS INDUCTION PROTOCOLS

Estrus synchronization protocols	Status at natural/ induced estrus	No. (%)	Plasma P_4 (ng/ml)	
			Day of AI	D-20 post-AI
Ovsynch	Conceived	2 (33.33)	0.23±0.13	3.20*±0.10
	Non-conceived	4	0.70±0.29	2.04±1.07
CIDR	Conceived	4 (66.66)	0.12±0.17	3.45*±0.17
	Non-conceived	2	0.71±0.39	1.60±0.50
Ovsynch + CIDR	Conceived	3 (50.00)	0.66±0.23	3.70*±0.35
	Non-conceived	3	1.18±0.32	2.20±0.89
Heatsynch	Conceived	1 (16.67)	0.17	2.60
	Non-conceived	5	0.57±0.32	1.94±0.81
Normal Cyclic Control	Conceived	3 (50.00)	0.60±0.06	3.53*±0.56
	Non-conceived	3	0.80±0.29	1.03±0.44

* P<0.05 between days within the row.

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