Identifying Early Conception Using Ultrasound in Ovsynch Treated Postpartum Anestrus Gir Cows

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ABSTRACT

The aim of this study was to detect early conception using transrectal ultrasonography in postpartum anestrus Gir cows treated with Ovsynch protocol (Group-I, n=11), keeping the same number of normal cyclic cows as control (Group-II, n=11). Ultrasonography examinations were performed using a real-time B-mode ultrasound scanner equipped with 7.5 MHz convex array transducer on day 11, 22 and 32 post-AI to monitor ovarian structures and uterine changes. Cent percent of anestrus animals responded to the Ovsynch protocol with clear signs of estrus. The conception rates at induced and second estrus in the treatment group were 45.45 and 33.33%, respectively, with an overall conception rate of 2 cycles post-treatment as 63.63%. In the control group, first service, second service and overall conception rates of 2 cycles were 36.36, 14.28 and 45.45%, respectively. The results obtained were better with the Ovsynch protocol. Ultrasound scanning on day 22 and 32 revealed visualization of the fluid and embryonic vesicle, respectively. Early embryonic death occurred in one cow out of six between days 22 and 32 post-AI in ovsynch treated group.

Keywords: Gir cows, Anestrus, Ovsynch, Ultrasonography, Conception rate.

Ind J of Vet Sci and Biotech (2019): 10.21887/ijvsbt.14.4.7

INTRODUCTION

Reproductive performance is one of the important determinants for achieving higher profitability. In order to gain maximum dairy profit as well as one calf per year, early resumption of postpartum ovarian activity, efficient heat detection, and a high first service conception rate are the relevant features. The biological possibility of a new conception by 60–80 days postpartum is based on coordination between hypothalamus, pituitary, ovaries, and uterus, following uterine involution and an early resumption of ovarian activity. The conception rate with Ovsynch protocol does not go beyond 30–40% in Bos taurus (Thatcher *et al.,* 2002), Bos indicus (Fernandes *et al.,* 2001) as well as in crossbreds (Ahuja and Montiel, 2004).

Early conception is possible as early as 25 days post AI by the detection of fluid in the apex of the uterine horn and the presence of corpus luteum using ultrasound (Glatzel *et al.,* 2000). Ribadu *et al.,* (1994) reported an accuracy of 95.7% with ultrasonography, whereas, it was about 85% with rectal palpation in detecting CL. Several reports (Pieterse *et al.,* 1990, Badtram *et al.,* 1991) indicate the presence of an embryonic vesicle as early as day 25 of gestation. Nation *et al.,* (2013) recorded the sensitivity and specificity of early conception in lactating dairy cows based on ultrasonographic detection of uterine fluid as well as embryonic membranes from 28 to 35 days after AI as 96% and 97%, respectively. Ultrasonography can more accurately detect the cases of early embryonic mortality, which are misdiagnosed as early pregnancy by serum progesterone estimation (Abdullah *et al.,* 2014). Hence, this study was planned to evaluate the efficacy of Ovsynch protocol in anestrus Gir cows and accuracy of ultrasonography in early conception and to estimate the early embryonic loss.

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MATERIALS AND METHODS

The study was conducted on 22 Gir cows selected from the herd maintained at the Cattle Breeding Farm, JAU, Junagadh. Of these, 11 cows were true anestrus (Group I), whereas other 11 cyclic cows were kept as normal control (Group II). The cows had moderate body condition and were of 2 to 5 parity with the average milk production of 2500 to 3000 L per lactation. All animals were maintained in similar feeding and managerial farm practices as well as kept under a loose housing system of management. The cows were screened gynaeco-clinically for their reproductive status. Anestrus cows were confirmed by palpating smooth inactive ovaries per rectum twice 10 days apart and were subjected to Ovsynch protocol (n = 11) with fixed time artificial insemination.

Treatment protocol consisted intramuscular administration of injection GnRH analog, Buserelin acetate, 20 μg (Receptal, 5 mL, MSD, Intervet) on day 0, followed by an

Table 1: Estrus induction response and fertility in anestrus Gir cows treated with Ovsynch and untreated control group

Reproductive status	Treatment group	No. of cows	Estrus induction response (%)	Conception rate (%)		
				First service	Second service	Overall
Anestrus Gir cows	Ovsynch	11	100 (11/11)	5/11 (45.45)	2/6 (33.33)	7/11 (63.63)
Normal cyclic cows	Untreated control	11	–	4/11 (36.36)	1/7 (14.28)	5/11 (45.45)
Chi-square value		–	–	0.188	0.660	0.733
p value		–	–	0.6646	0.4164	0.3918

injection 500 µg PGF2α (Pragma, 2 mL, Intas) on day 7, and a second GnRH injection of 20 µg on day 9. Fixed time AI was performed 22 hourrs after the second GnRH injection. The second group of 11 normal cyclic cows that expressed spontaneous estrus within 90 days postpartum and inseminated served as normal cyclic control group. Cows in spontaneous or induced estrus were inseminated using good quality frozen-thawed semen by a single technician. Transrectal ultrasonography was carried out at days 11, 22 and 32 post-insemination using a real-time B-mode ultrasound scanner (DB355M, IMAGOS, ECM, France) equipped with a 7.5 MHz rectal probe. The scanning of ovaries and uterine horns was done for the presence of corpus luteum and/or any other palpable structures and for fluid and/or embryonic vesicle, respectively. Early conception was based on the detection of an anechoic elongated area of varying size representing the fluid filled allantoic cavity, the embryonic vesicle (Pieterse *et al.,* 1990). The result of ultrasound scanning on different days was correlated with the actual pregnancy by palpation per rectum on day 60 post-AI to judge its accuracy. Animals detected in estrus subsequent to FTAI were re-inseminated up to one more cycle and pregnancy diagnosis was followed. The variation between groups in estrus response and conception rate was compared by Chi-square test.

RESULTS AND DISCUSSION

All the anestrus (n = 11) Gir cows (100%) came in the heat with obvious signs after Ovsynch treatment. The conception rates obtained at induced and second estrus in cows under treatment group were 45.45% and 33.33%, respectively, with an overall conception rate of two cycles as 63.63%. In untreated normal cyclic group (n = 11), the conception rates at the first service, second service and overall of two cycles were 36.36%, 14.28 and 45.45%, respectively. The overall conception rate was higher in Group-I as compared to Group II, but the difference was statistically non-significant (*p* >0.05).

The findings with Ovsynch protocol are in line with Dhami *et al.,* (2015) as 50.00% and 40.00% first and second service conception rate (overall 3 cycles 80%) in anestrus crossbred cows. However, the lower first service conception rate of 30.00% with Ovsynch protocol was found by Patel *et al.,* (2013). Nak *et al.,* (2011) reported an overall conception

rate of 42.18% in non-cycling cows and 44.07% for heifers. Further, the conception rate of 90.00% was also noted in cows by Muneer *et al.,* (2009). In Sahiwal cows, Ovsynch and prostaglandin protocols yielded comparable but low ovulation rates and pregnancies per AI (Hassan *et al.,* 2017). The possible reasons for variation could be the reproductive status or stage of estrus cycle at the beginning of the protocol, nutritional, managerial, lactation, drug source, age and breed and variations in geographical locations. The success of ovsynch protocol is influenced by the number and the length of follicular waves (Pursley *et al.,* 1997) as well as the stage of estrus cycle when the initial dose of GnRH is administered (Wiltbank and Pursley, 2014). Thus, the first dose of GnRH, when administered at a random stage of the estrus cycle, induces ovulation only in cows with a functional dominant follicle. Perry *et al.,* (2005) concluded that GnRH-induced ovulation of follicles ≥ 11 mm resulted in decreased pregnancy rates and increased late embryonic/fetal mortality, but follicle size had no effect on pregnancy rate when ovulation occurred spontaneously.

USG facilitates diagnosis of non-pregnant animals at an early date and is, therefore, more advantageous than pregnancy diagnosis by per rectal examination at a later date. The animals those conceived were confirmed by the presence of CL and an anechoic area having a varying size of amniotic vesicle on 32 days post-AI (Figs 1 and 2). The presence of CL at days 11, 22 and 32 post-AI was observed to differ significantly (*p* <0.01). CL was observed in all treated cows (n = 11) on day 11 post-AI. However, out of 11 inseminated cows, 6 (54.54%) had CL of mean 22.62 ± 0.63 mm size on day 22, while only 5 cows had CL on day 32 (45.45%). This indicated that one cow lost embryo (1/6 = 18.66%) from 22 to 32 days post-AI. Abdullah *et al.,* (2014) recorded 7.69% early embryonic mortality rate between 30-45 days post-service in Karan fries and Sahiwal cows, while Whitlock and Maxwell (2008) noted it as 5-10% between days 28 to 42 in lactating dairy cows. Fricke *et al.,* (1998) reported conception loss in dairy cattle between 28 and 56 days after AI as 13.5%. Specific physiological mechanisms responsible for the early embryonic loss in dairy cattle may include lactational stress associated with

Fig. 1: Ultrasound image of 32 days of pregnancy in Gir cows

Fig. 2: Ultrasound image of 32 days pregnancy CL

increased milk production, negative energy balance, toxic effects of urea and nitrogen (Butler *et al.,* 1995) or reduced ability to respond to increased environmental temperature (Hansen *et al.,* 1992).

The results of the present study showed that trans-rectal ultrasound scanning for early conception in cows on day 22 post-service was less accurate than day 32 post-service. Awasthi *et al.,* (2011) observed that embryonic vesicle was detected from day 23 onwards in uterine horn ipsilateral to ovary bearing CL in all animals, which were later, confirmed pregnant by palpation per rectum. In 148 dairy cows, pregnancy diagnosis from day 21 to day 25 was 65% accurate, whereas diagnosis of pregnancy from day 26 to day 33 was 93% accurate (Pieterse *et al.,* 1990).

It can be thus concluded that anestrus in Gir cows could be resolved by Ovsynch protocol; hence, it can be practiced by field veterinarians in anestrus cows to improve their reproductive efficiency and thereby the farmers economy. The early pregnancy diagnosis using ultrasonography can be made on or after 32 days post-service in Gir cows as it was less accurate on day 22 post-service.

Acknowledgments

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