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CIDR + ovsynch protocol for synchronisation of recipient cattle for IVF embryo transfer

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Abstract

The present study was conducted to know the efficiency of CIDR + Ovsynch protocol for synchronisation of recipient cows for IVF (in vitro fertilization) embryo transfer. IVEP (in vitro embryo production) in combination with synchronisation and ET can play an important role in faster multiplication of superior germplasm. 60 animals were selected randomly and assigned into 2 groups. Group 1- Natural heat (n=20) cows exhibiting natural estrus were selected. Group 2- CIDR + Ovsynch (n=40) on day 0 CIDR was inserted + inj. GnRH- 10 µg, on day 7 CIDR removal + inj. Vetmate- 500 µg, on day 9 inj. GnRH- 10 µg. Embryo transfer was done in the recipient animals having CL by using a non-surgical method after 7-8 days of estrus by depositing the embryos in the uterine horn ipsilateral to the ovary having CL. In the CIDR+ Ovsynch group 72.5% of animals exhibited estrus among these eligible recipients for transfer 86.2% and conception rate in embryo transferred were 13.23%. In natural estrus group, eligible recipients for transfer (83.3%) for transfer and its conception rate is 0%. There was a positive correlation ($p < 0.05$) between the CL diameter and conception rate of recipient cows, the mean CL diameter of pregnant and non-pregnant animals was 14.18 ± 0.84 mm and 8.97 ± 0.52 mm. In summary, higher synchronisation, eligible animals for transfer and conception rates were observed with timed ET in CIDR + Ovsynch protocol than in natural estrus animals.

Keywords: Estrus synchronisation, CIDR + Ovsynch, embryo transfer, *in vitro* embryo production

Introduction

Embryo transfer technology (ETT) has emerged as an important tool to improve livestock at a faster rate, as in this technique, genetic contribution of both the male and female are utilized simultaneously (Purohit, 2018) [13]. Although many factors influence the outcome of embryo transfer, estrus synchronisation of recipient females is a vital component of a successful programme, as recipients which had better express estrus have greater pregnancy rates in embryo transfer. To achieve estrus synchrony, various hormonal protocols were used to increase the proportion of suitable recipients to receive embryos and improve the efficacy of IVEP programs (Marinho *et al.*, 2012) [11]. Ovsynch protocol has been used to synchronise ovulation in recipients for *in vivo* ET (Ambrose *et al.*, 1999) [2]. CIDR-based protocols prevent premature follicular maturation and ovulation in beef cows, once CIDR is withdrawn, serum progesterone levels will be reduced and negative feedback on the hypothalamus is withdrawn resulting in a gonadotropin surge which causes maturation of follicle, synchrony of estrus and ovulation. To increase the better estrus synchronisation by reducing the interval of ovulation among the synchronised animals, the combination of CIDR + Ovsynch is used. CIDR + Ovsynch had higher synchronisation and the conception rates following timed ET in postpartum cattle (Kawate *et al.* 2007) [9]. The present study was taken to evaluate the efficacy of CIDR + Ovsynch protocol for synchronisation of recipient cows for IVF embryo transfer.

Material and Methods

A total of 60 animals were selected and randomly assigned into two groups irrespective of their stage of estrus cycle. Group 1-Natural Heat: In this group, a total of 20 cows were selected which were in natural estrus. Group 2 - CIDR + OVSYNCH: In this group a total of 40 cows were selected for estrus synchronization by using CIDR + Ovsynch protocol. Day 0 CIDR insertion per vaginum + Inj. GnRH – 10 µg I/M, day 7 CIDR removal + Inj. Cloprostenol – 500 µg I/M, day 9 Inj. GnRH – 10 µg I/M. Embryo transfer was done in the recipient animals having CL by using a non-surgical method after 7-8 days of estrus by depositing the embryos in the uterine horn ipsilateral to the ovary having CL. The recipients were

examined by using trans rectal ultrasonography one day before embryo transfer or on the day of embryo transfer. At that time, the presence of a CL was confirmed and measured by taking the average of the CL width and length in mm. Measurements were done with inbuilt electronic caliper of ultrasound scanner in freeze mode.



Fig 1: Insertion of CIDR in recipient animal for synchronisation

Statistical Analysis

Statistical analysis was done using SPSS software (version 20.0). Estrus response rate and conception rates in different groups were compared by Chi-square test. The effect of CL diameter on conception rate was analysed by using t-test.

Results and Discussion

Estrus Synchronisation Rate

The estrus synchronisation rate in CIDR + Ovsynch group (72.5%). Among these animals eligible recipients for transfer (having CL) in CIDR + Ovsynch group (86.2%) was greater ($p>0.05$) than in natural estrus group (83.3%). On contrary to this Kawate *et al.*, (2007) [9] have registered higher estrus synchronisation (100%) and eligible recipients for transfer (100%) with CIDR + Ovsynch protocol.

The possible reason for higher eligibility of recipient animals (86.2%) for embryo transfers in CIDR + Ovsynch protocol may be due to action of first GnRH injection which causes

release of enough LH, to ovulate the largest follicle on the ovary and formation of CL and help for the recruitment of small follicles, the inserted CIDR mimics the luteal function by blocking the release of gonadotropins from pituitary. On seventh day CIDR withdrawal and further injection of PGF2 α regress the CL that will cause the decrease of progesterone levels which results in reduction of negative feedback mechanism on gonadotropin leading to LH Surge and ovulation and second injection of GnRH was helpful for maturation of graffian follicle and synchronisation of estrus exhibition and ovulation time.

Conception Rate

The conception rate in CIDR + Ovsynch group (13.23%) was greater ($p>0.05$) than in natural estrus group (0%). Contrary to these results, higher conception rate (57.9%) was registered in recipient animals using CIDR + Ovsynch protocol with embryo transfer by Kawate *et al.* (2007) [9].

CL diameter

The mean CL diameter was found to be higher in pregnant animal (14.18 \pm 0.84 mm), when compared to non-pregnant (8.97 \pm 0.52 mm), statistically difference ($p<0.05$) was observed between CL diameter and conception rates of recipient cows. Ambrose *et al.* (1999) [2], Baruselli *et al.* (2010) [3], Nogueira *et al.* (2012) [12] and Alkan *et al.* (2020) [11] who found CL diameter had an effect on pregnancy after embryo transfer and stated CL diameter increases, better conception rates in embryo transfer. On contrary Ramsen and Rouseel *et al.* (1982) [14], Hasler *et al.* (1987) [8], Coleman *et al.* (1987) [6], Bo *et al.* (2001) [4], Spell *et al.* (2001) [15], Benyei *et al.* (2006) [5], Looney *et al.* (2006) [10] and Erkan *et al.* (2021) [7] has stated there was no correlation between CL diameter and conception. In this study, the higher CL diameter might be responsible for increased secretion of progesterone and it might be helpful for providing better uterine environment for the development of transferred IVF sexed embryo and it resulted better conception rate.

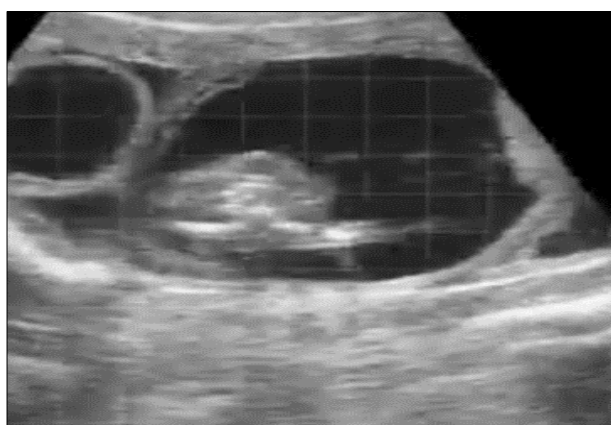


Fig 2: Pregnancy diagnosis in recipient animals after 30 days of embryo transfer and Ultrasonographic image showing the presence of embryo in amniotic vesicle



Fig 3: Checking the recipient animals for presence of CL before embryo transfer and measuring the diameter of CL

Table 1: Showing response of estrus exhibition response, animals eligible for transfer and conception rate in recipient animals of CIDR + Ovsynch and Natural estrus groups

S. No.	Name of the group	Estrus exhibition response	No. of animals eligible as recipients for embryo transfer among estrus exhibited animals	Conception rate
1.	CIDR+ Ovsynch	72.5% (29/40)	86.20 (25/29)	13.63% (3/22)
2.	Control	-	83.33(10/12)	0

Table 2: Showing the comparison of mean CL diameter (mm) of pregnant and non pregnant IVF embryo transferred cows

S. No.	Mean CL (mm) of Non-Pregnant Cows	Mean CL (mm) of Pregnant Cows
1	8.97±0.52	14.18±0.84

Conclusion

In conclusion, there were higher synchronisation and transfer rate in recipient cattle were observed in CIDR + Ovsynch protocol. The conception rate were higher in CIDR + Ovsynch protocol. So, the CIDR + Ovsynch protocol was found to be efficient protocol for ivf embryo transfer over the embryo transferred natural estrus recipients animals. The poor results observed in the study may be due to the primitive stage of this work in india and lesser experience of IVF production of embryos and transfer. And lack of poor scientific feeding conditions of recipient animals.

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