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Superovulatory Responses and Embryo Recoveries in Rathi (*Bos indicus*) Cattle with Different Doses of Folltropin-V

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Abstract

The superovulatory responses and embryo recoveries in Rathi cows superovulated with different doses of Folltropin-V were tested. Post-partum cows were superovulated 60 days post-partum using 400, 300, 200 or 240 mg of Folltropin-V administered in 8 descending doses 12 h IM starting from day 8-10 of a CIDR-B. Cows were induced to estrus by IM administration of a prostaglandin (Inj Cyclix) and withdrawal of CIDR-B at the time of last injection of FSH. Cows were inseminated thrice at 12 h interval using frozen semen. Embryos were recovered by non-surgical means on day seven of insemination. Transrectal ultrasonography was performed on the day of embryo recovery to record the number of unovulated follicles and the number of corpora lutea. Estrus expression was poor in superovulated donors which posed a difficulty in precise timing of insemination. The total ovulatory responses and the total embryo recoveries were non-significantly different between all the groups. However, the mean number of transferable grade embryos was significantly higher in the 300 mg group compared to all other groups. A large number of unovulated follicles were visible ultrasonographically on the day of estrus in the 400 mg group. A total of 2, 5, 3 and 2 animals yielded no embryos in 400, 300, 200 and 240 mg groups respectively and in a total of 5 animals the flushing catheter could not be traversed from the cervix. Transfer of embryos to 29 recipients did not result in pregnancy. It was concluded that superovulation and embryo recoveries are difficult in Rathi (*Bos indicus*) cows due to anatomic differences and that 300 mg of Folltropin-V appears to be the optimum dose for induction of superovulation.

Keywords*:* Bos indicus, embryo, Folltropin-V, Rathi, superovulation

Introduction

Superovulatory responses and embryo recoveries continue to be highly variable (Hasler, 1992; Misra *et al*., 2005) and no single protocol has been successful in reducing the variability in the superovulatroy responses (Hasler, 2003; Bo *et al*., 2009). *Bos indicus* cattle are known to have lower ovulatory response compared to *Bos taurus* cows (Baruselli

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et al. 2006) because of inherently lower follicular populations on the ovary (Gaur and Purohit, 2007) reduced duration of estrus (Plasse *et al*., 1970; Rea *et al*., 1999), a shorter period from onset of estrus to the LH surge as well as from the LH surge to ovulation (Randel, 1984) and different levels of circulating hormones especially estradiol and insulin like growth factor (Purohit *et al*., 2000a; Sartori *et al*., 2010). Attempts to superovulate Rathi (*Bos indicus*) cows have yielded highly variable responses depending upon the type of hormone used (Purohit *et al*., 2000b) and repeated superovulation (Chandra *et al*., 2002). Some other limiting factors are the cervical hypertrophy that poses difficulty in embryo recovery (Purohit *et al*., 2000b). In general, porcine FSH tends to result in better embryo recoveries compared to eCG (Purohit *et al*., 2006). However, the dosage rates of porcine FSH suggested for *Bos taurus* cows result in high ovulatory response but poor transferable grade embryo recoveries (Purohit *et al*., 2006). The present study evaluated the effect of different doses of Folltropin-V on superovulatroy responses and embryo recoveries in Rathi cows and the pregnancy rates on transfer of recovered embryos to synchronized recipients.

Materials and Methods

Superovulation and breeding

Post partum Rathi cows were superovulated 60 days post-partum using 400, 300, 200 or 240 mg of Folltropin-V administered in 8 descending doses 12 h IM starting from day 8-10 of a CIDR-B insertion. Cows were induced to estrus by IM administration of a prostaglandin (PG) (Inj Cyclix) and withdrawal of CIDR-B at the time of last injection of FSH. Cows were inseminated thrice at 12 h intervals using frozen semen from proven bulls. Recipients were synchronized by injecting a dose of PG one day before the injection to donors.

Embryo recovery and transfer

Embryos were recovered by non-surgical means on day seven post insemination as per procedures described previously (Purohit *et al*., 2006). Briefly, the uterus of cows was flushed using 1 to 1.5 L of DPBS (Sigma, USA) infused into the uterus employing foley catheters. The recovered fluid was passed through embryo filters (IMV, France) and searched under a stereo-zoom microscope (Nikon, Japan) to locate the embryos and evaluate their quality as described previously (Lindner and Wright, 1983). Transferrable grade embryos (morula and blastocysts) were transferred to synchronized recipients in the uterine horn ipsilateral to the CL bearing ovary the same day.

Transrectal ultrasonography

Transrectal ultrasonography (TRUS) was performed on superovulated cows using a portable ultrasound device (Agroscan, France) with a dual (5.0-7.5 MHz) frequency linear array probe to

record the ovulatory response (number of corpora lutea) and unovulated follicles on the day of embryo recovery. TRUS was also performed on recipient cows at day 40 post transfer of embryos for evaluation of pregnancy.

Results

The total ovulatory responses (corpora lutea on ovaries Fig 1) and the total embryo recoveries were non-significantly different between all the groups (Table 1). However, the mean number of transferable grade embryos were significantly (P<0.05) higher in the 300 mg group compared to all other groups. A large number of unovulated follicles were visible sonographically on the day of embryo recovery in the 400 mg group (Fig 2). Estrus expression was poor in superovulated donors which posed a difficulty in precise timing of insemination.

A total of 2, 5, 3 and 2 animals yielded no embryos in 400, 300, 200 and 240 mg groups respectively and in a total of 5 animals the flushing catheter could not be traversed from the cervix. Thus 32.43% (12/37) cows did not yield any embryos and in 13.51% (5/37) of cows the foley catheter could not be passed up to the uterus for embryo recovery. Transfer of 36 embryos to 29 recipients did not result in pregnancy in any

Table 1. Superovulatory response, total and transferable grade embryo recoveries in Rathi (Bos indicus) cows with different doses of Folltropin-V.

Dose of Folltropin	No of animals	Mean ovulatory response	Total embryo recovery	Transferable grade embryo recovery
400 mg	5	6.0±2.15	2.2±1.02	0.6±0.67
300 mg	13	5.53±0.99	1.86±0.53	1.30±0.45*
200 mg	15	7.13±1.21	3.66±1.38	0.6±0.27
240 mg	4	8.5±0.74	1.75±0.96	0.5±1.15

*Significant (P<0.05)

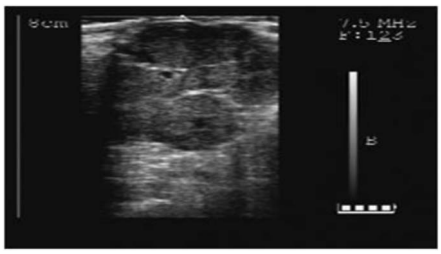

Fig 1. Ultrasonogram of a superovulated cow showing the corpora lutea on the ovary

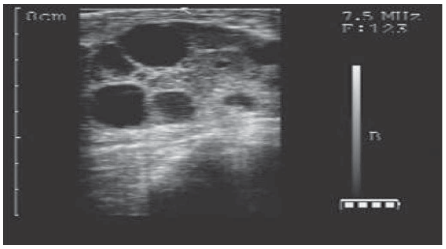

Fig 2. Ultrasonogram of a superovulated cow showing the unovulated follicles on the ovary on the day of embryo recovery. Two CL are also visible.

animal. Thus the limitations to embryo recovery in the Rathi cows were poor ovulatory response of a proportion of cows and the physical inaccessibility of the cervix.

Discussion

The overall mean ovulatory response obtained during the present study is similar to our own previous studies embploying a different porcine FSH for superovulation of Rathi and its crossbred cows (Purohit *et al*., 2000b; Purohit *et al*., 2006) and similar studies in other Bos indicus cows like Sahiwal (Mishra *et al*., 1997), Ongole (Kasiraj *et al*., 2000) and Nelore (Silva *et al*., 2009) recorded nearly similar ovulation rates. However, the total embryo recoveries during the present study are lower compared to our own studies (Datt *et al*., 2000; Purohit *et al*., 2000b; Purohit *et al*., 2006) and studies conducted elsewhere on *Bos indicus* cows (Mishra *et al*., 1997; Kasiraj *et al*., 2000; Silva *et al*., 2009). A valid reason for low total embryo recovery during the present study appears to be the physical problems with the cervix of Rathi cows. In 13.51% of the superovulated cows inspite of a good ovulatory response embryos could not be recovered due to failure of passage of the foley catheter into the uterus while in 32.43% of cows inspite of a proper uterine flushing and a good ovulatory response few or no embryos could be recovered. Such problems have been previously observed with embryo recovery in cows (Hasler, 2003).

The mean number of transferable embryos recovered during the present study are lower compared to those obtained in other *Bos indicus* cows like Sahiwal (Mishra *et al*., 1997) and Nelore (Silva *et al*., 2009) but are similar to those obtained for Ongole (Kasiraj *et al*., 2000), Rathi (Purohit *et al*., 2006) and Sahiwal (Prasad *et al*., 2003) cows.

The 300 mg dose of Folltropin-V was found to be optimum for Rathi cows as this yielded the highest proportion of transferable grade embryos. A few of the previous studies have shown no difference in the number of ovulations, number of unovulated follicles and the yield of transferable embryos in *Bos indicus* cows (Chauhan *et al*., 1994; Silva *et al*., 2009) while other found subtle difference in embryo recoveries with different doses of FSH (Joshi *et al*., 1993; Purohit *et al*., 2000b). The average number of anovulatory follicles increased proportionally to the increasing dose of FSH in *Bos indicus* (De Armas, 2001) and Thai (Sumretprasong *et al*., 2008). During the present study a large number of anovulated follicles were seen sonographically on the day of embryo recovery in cows superovulated using 400 mg of Folltropin-V.

No pregnancy could be established by transfer of 36 embryos to 29 synchronized recipients. Poor synchrony of estrus, viability of the recovered embryos or improper placement of the embryos could be the reasons for failure of pregnancy during

the present study as also mentioned previously (Misra *et al*., 2005). In previous studies in Rathi cows the pregnancy rates were 10.86% (Purohit *et al*., 2000b).

It was concluded that superovulation and embryo recoveries are difficult in Rathi (*Bos indicus*) cows due to physiological differences and that 300 mg of Folltropin-V appears to be the optimum dose for induction of superovulation.

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