

Successful Superovulation and Recovery of Embryos in Repeat Breeding Crossbred Cows

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SUMMARY

The present study was undertaken to find out possibilities for superovulation and recovery of freezable/transferable embryo from repeat breeding crossbred cows. For this study a total of 10 Holstein crossbred cows having history of failure of conception even after more than 4~5 inseminations were taken and superovulated using Folltropin-V at 100 to 140% dose schedule. The results indicated that out of 10 donors, 8 responded to superovulatory treatment and yielding a total of 94 embryos, out of which 45 were of transferable/freezable quality. The mean ovulations and mean transferable embryos were 11.5 ± 2.91 and 4.5 ± 1.66 , respectively. These observations suggest that the repeat breeding cows having conception failure after several artificial inseminations can be used as donors for production of embryos and calves through embryo transfer technology.

(Key words : crossbred cows, embryo transfer, superovulation and repeat breeding)

INTRODUCTION

Repeat breeding has been recognized as a serious problem affecting the economy of dairy industry. Higher embryonic mortality has been reported to be a major cause of repeat breeding than fertilization failure (Awasthi et al., 2002). The repeat breeding is one of the challenging problem faced by animal breeder. Approximately, 10~15% cows of a herd show failure of conception after repeated artificial insemination even though the cows appear healthy and free from diseases. This not only results in economic loss to the breeder in terms of milk and calf but also if the cow is high yielder its genetic potential remains unutilized. In view of these circumstances superovulation and

embryo transfer may offer a good hope to utilize the cow for embryo production. There are only two reports on studies on successful pregnancies out superovulation and embryo transfer which was conducted only in one repeat breeder cow (Kharche et al., 1995 and Sharma et al., 1996).

Present study is therefore designed to look into the possibility if more number of repeat breeding cows could be successfully superovulated and recovery of transferable/freezable embryo possible to have offspring of such donors having superior genotype.

MATERIALS AND METHODS

The study was carried out on 10 regular cyclic Holstein crossbred cows having failure of conception

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even after more than 4~5 inseminations. These cows were reared under standard feeding and management conditions at Dairy farm of Raymond Embryo Research Centre, Gopal Nagar-Bilaspur (CG).

The cows were synchronized for oestrus using 25mg of Lutalyse injected intramuscularly(im) 11 days apart. The superovulation was induced as per the following protocol using 100~140% purified FSH preparation Folltropin-V(Vetrepharm Inc. Canada) in descending doses through im route (Table 1).

The donors were injected 25 mg of Lutalyse at 48 and 60 hours after initiation of superovulatory treatment to induce oestrus and were inseminated at 48, 60 and 72 hrs after Lutalyse injection.

The embryos were recovered non-surgically by passing embryo flushing catheter into both uterine horn one after another under epidural anaesthesia. Approximately 500 ml modified Dulbecco's phosphate buffered saline (DPBS-Sigma) containing 0.1 % bovine serum albumin (BSA-Sigma) was used as flushing media and each horn was flushed taking 50 ml media in 50 ml syringe for 10 consecutive times as interrupted flushing method. The flushed media of each horn was filtered through embryo

filter(Emcon filters), approximately 20 ml media containing embryo left in the filter were poured to the petridish in which searching examination, grading etc. were done under zoom stereo microscope as per standard norms of International Embryo Transfer Society(IETS). The embryos were then transferred to another petridish containing holding media(DPBS with 0.4% BSA).

RESULTS AND DISCUSSION

The superovulatory response and embryo recovery has been presented in Table 2. Out of 10 cows, 7 responded to superovulatory treatment with Folltropin-V at 120~140% doses yielding a total of 94 embryos out of which 45 were of transferable/freezable quality. One cow (Cow No. 629) though had a 4 CL but no embryo could be recovered due to poor flushing as the media entered in the myometrium and fluid recovery was only 30%. Remaining two cows (Cow No. ET-110 and 530) did not show any superovulatory response the ovaries were overstimulated and had a palpable size of an apple having few unovulated follicles.

The total number of ovulations/corpus luteum (CL) were 115 with mean ovulations as 11.5 ± 2.91 (Table 3). A total of 94 embryos were recovered out of which 45 were transferable. The number of transferable embryos was 4.5 ± 1.66 which is in agreement as reported in normal crossbred cows (Totey et al., 1989). Among the recovered embryos 74.46% were fertilized and 64.28% were transferable indication that repeat breeding in the cow might be due to developmental abnormalities of embryos rather than failure of fertilization. These observations somewhat differ as reported by Sharma et al. (1996) in which the percentage of fertilized and viable embryos was 89.18% and 81.08%, respectively. Nigam et al. (2001) reported superovulatory response of 12.33 ± 0.49 with Folltropin-V and mean % transferable embryos per donor was

Table 1. Superovulatory protocol

Days	
0	Oestrus
9~12	Superovulation treatment twice daily for 4 days Lutalyse 25mg was administered at 48 and 60 hrs after initiation of superovulation treatment
13 am	Observe Oestrus and AI
pm	AI
14 am	AI
20	Collection of embryos Transfer a fresh/freezing

Table 2. Superovulatory response and embryo recovery in repeat breeding Holstein crossbred cows

S. N.	Cow No.	Folltropin-V dose(mg)	Superovulatory Response			Day 7 Embryo recovery					
			CL	UF	M	EB	B	UF	DEG	TE	TTE
1.	740	480(120%)	19	-	9	2	2	-	4	17	13
2.	ET-17	560(140%)	7	3	2	1	-	-	1	4	3
3.	629	560(140%)	4	-	Very poor flushing. No embryo recovered						
4.	504	560(140%)	8	2	5	-	-	-	1	6	5
5.	B-62	560(140%)	15	3	-	4	2	-	6	12	6
6.	543	560(140%)	25	2	14	-	-	1	7	22	14
7.	B-47	560(140%)	13	3	-	4	-	1	6	11	4
8.	ET-110	560(140%)	-	15	Overstimulation, good flushing-No embryo recovered						
9.	734	480(120%)	24	-	-	-	-	22	-	22	-
10.	530	560(140%)	-	18	Overstimulation excellent flushing-No embryo recovered						
Total			115	46	30	11	4	24	25	94	45

CL-Corpus luteum; UF-Unovulatory follicles; M-morula; EB-Early blastocyst; B-Blastocyst; DEG-Degenerated embryos; TE-Total embryos; TTE-Total transferable embryos.

Table 3. Mean ovulations and transferable embryos in repeat breeding Holstein crossbred cows

Observations	Folltropin-V (Dose 120~140%)
Number of donor	10
Number of donor responded	8
Number of ovulations/CL	115
Number of unovulated follicles	46
Mean CL/cow	11.5±2.91
Number of cows flushed	10
Number of embryos recovered	94
Number of transferable embryos	45
Mean transferable embryos	4.5±1.66

84.6 and mean/donor was 2.4. The lower percentage of fertilized and transferable embryos in our study is due to the fact that one cow yielded all the 22 unfertilized eggs. This failure of fertilization may be due to response of individual cow as the other cows had good response in terms of recovery of fertilized embryos. All the 45 transferable

embryos (Grade- I and Grade- II) were frozen using Ethylene glycol(1.5 M in DPBS + 0.4% BSA) for direct transfer (Voelkel and HU, 1992).

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