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Superovulatory response and embryo recovery in Kankrej cattle

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Abstract
Superovulation is a reproductive technology used in the dairy industry to enhance the reproductive rate of superior female animals. The experiment was conducted to investigate the superovulatory response and embryo recovery in twelve donor Kankrej cattle which were divided in to two groups (A and B) and synchronized with single dose (625 µg, im) of cloprostenol. Superovulation was induced with pFSH (25 mg) in tapering dose started on 8th day of estrus for 4 days. Group-A cows (n=6) were given 10 µg GnRH While, Group-B cows (n=6) did not receive GnRH at the time of breeding. Estrus following superovulation (53±2.15) took less time in hours than induced estrus after synchronization (61.2±5.01). Of the 12 cows, 8 (66.67%) were responding to the superovulation treatment (>2 CL), while 4 (33.33%) were not responding (≤ 2 CL). Using rectal palpation and ultrasonography, total mean CLs were found in Group A to be 4.33±1.27 and 5.67±1.62, and in Group B to be 3.16±.94 and 4.17±.74 respectively. Furthermore, compared to the number of CLs in Group B, there was a substantial (p<0.01) decrease in embryo recovery. Superovulatory response and embryo recovery were non significantly higher using 25mg pFSH in Group-A compared to Group-B.

Keywords: Superovulation, synchronization, pFSH, GnRH

Introduction
Superovulation (SOV) followed by artificial insemination (AI) can be utilised to harvest many embryos from prominent donors. These strategies, which are connected to the embryo transfer (ET) to recipients, are effective tools for sharing top-notch genetics. Embryo transfer is one step in the process of removing one or more embryos from the reproductive tract of a donor female and transferring them to one or more recipient females.
However, the use of embryo transfer still has some limitations, i.e. high cost of embryo production, poor freezability, abnormal fetuses and calves with altered sex ratios. By collecting embryos from genetically elite females and transferring the harvested embryos into females of lesser genetic merit, it is possible to produce more calves from genetically superior females and fewer calves from genetically less valuable females (Youngs, 2007) [31]. Therefore, to achieve this goal, the cattle female ovulate multiple, matured, and viable oocytes, which are capable of being fertilized in-vivo, and which can then continue to develop into embryos. Although early embryo transfer techniques utilized surgical approaches for embryo collection, now all commercial embryo collections are non-surgical procedures requiring trans-cervical catheterization of uterine horns.
In spite of the foregoing limitations in practicing MOET technology in India, many scientists (Patel *et al*., 2004; Babu Rao *et al*., 2005; Mathur *et al*., 2006) [17, 4, 13] have conducted research work on indigenous and crossbred cattle to investigate the response of multiple ovulation treatment and embryo recovery in terms of their quality and quantity. So, under Indian conditions, embryo transfer technology should be adopted to preserve pure Indian breeds which are becoming extinct due to indiscriminate cross-breeding. This is the first study as per our knowledge to study the superovulatory response and embryo recovery in kankrej cattle.

Materials and Methods
This research experiment was carried out at the Department of Veterinary Gynecology and

Obstetrics, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar with the Collaboration of Livestock Research Station, Sardarakrushinagar Dantiwada Agricultural University, Saradarkrushinagar

Animal selection and management

A total of 12 Kankrej cows selected from the herd were kept in the same nutritional, sanitary, and managemental conditions at Livestock Research Station, Sardarkrushinagar. The animals were subdivided into two equal groups, each of 6 cows, as Group A and Group B were managed as under to evaluate the Superovulatory response and embryo recovery.

Estrus synchronization and superovulation

Table 1: Details of synchronization and superovulatory protocol used in the study

Days	Group-A (N=6)		Group-B (N=6)	
-2(d)	Cloprostenol (@ 625 µg, IM)		Cloprostenol (@ 625 µg, IM)	
0(d)	(ESTRUS)		(ESTRUS)	
6th (d)	GnRH (@ 10 µg, IM)		GnRH (@ 10 µg, IM)	
8th (d)	**Morning**	**Evening**	**Morning**	**Evening**
	pFSH (@ 50 µg; IM)	pFSH (@ 50 µg; IM)	pFSH (@ 50 µg; IM)	pFSH (@ 50 µg; IM)
9th (d)	pFSH (@ 35 µg; IM)	pFSH (@ 35 µg; IM)	pFSH (@ 35 µg; IM)	pFSH (@ 35 µg; IM)
10th (d)	pFSH (@ 25 µg; IM) + Cloprostenol (@ 625 µg, IM)	pFSH(@ 25 µg; IM)	pFSH (@ 25 µg; IM) + Cloprostenol (@ 625 µg, IM)	pFSH(@ 25 µg; IM)
11th (d)	pFSH (@ 15 µg; IM)	pFSH (@ 15 µg; IM)	pFSH (@ 15 µg; IM)	pFSH (@ 15 µg; IM)
12th (d)	Breeding (1st) + GnRH (@ 10 µg,IM)	Breeding (2nd)	Breeding (1st)	Breeding (2nd)
13th (d)	Breeding (3rd)		Breeding (3rd)	
20th (d)	Flushing (Embryo recovery)		Flushing (Embryo recovery)	

Embryo recovery and transfer

The donors were examined per rectally on day 7 after the first insemination to identify the various ovulatory responses post pFSH treatment. Donors were considered to have reacted to pFSH treatment if they had two or more corpora lutea (CLs) on one or both ovaries, as opposed to those who had fewer than two CLs. Both responding as well as non-responding were subjected to uterine flushing for embryo recovery by non-surgical technique with Embryo flushing media (emXcell, IMV, India) and a 18 gauze embryo flushing catheter (Minitub, Germany). Foley catheter was passed through the cervix and the tip was placed in the uterine body passage, caudal to the external bifurcation of the uterus. The balloon was inflated with passing sufficient air and about 500 ml of flushing medium was used in each uterine horn by using foley catheters, 'Y' junction tubing and flushing bottles.

Donors were allowed to rest in a holding pen and the remaining DPBS was recovered by manipulation of the uterus. The inlet and outlet valve was opened alternatively so the Flushing media went inside the uterus and came out after the milking procedure of horns. Outcoming media was passed through a pre-sterilized embryo filter (Milex®-GS, Tullagreen) (0.22 µm pore size). Embryo flushing was immediately followed by intrauterine flushing with 20ml Gentamicin (Gentamor-vet, Morvel Laboratories, India) and 2 ml Cloprostenol (Pragma @ 500 µg) was also injected intramuscularly for the lysis of multiple corpora lutea. The recovered fluid in the embryo filter after flushing of the uterus was immediately transferred to the laboratory and searched the embryo with the help of stereo zoom microscope.

Ultrasonography

The transrectal ultrasonography was performed using a real-time B-mode ultrasound scanner (Titan, Sonosite Ltd; Hitchin, UK) equipped with a 5-10 MHz linear array transducer designed for intra-rectal placement before the starting of FSH protocol to know the ovarian status and before flushing to know the superovulatory response in donor animals. Superovulation response was determined based on total CL present on ovary and no. of embryo collected.

Statistical analysis

The estrus characteristics parameters were recorded as Mean ± S.E. in an excel sheet and ovarian response between respondent and non-respondent, No. of CL count, Embryo recovery in Group A and B, were analyzed using an independent t-test.

Results

The estrus synchronization response of 83.33 percent in Kankrej cows observed in the present study (Table 1).

Table 2: Characteristics of the estrus in Kankrej donor cows after synchronization

S. No.	Parameters	Characteristics of the estrus
1.	Estrus response (percent cows)	83.33%
2.	Interval between treatment and onset of estrus (hrs)	61.2±5.01 (36 to 96 hrs)
3.	Duration of estrus (hrs)	17.1±1.29 (12 to 24 hrs)
4.	Intensity of estrus (percent cows)	
A	Intense %	33.33
B	Intermediate %	41.67
C	Weak %	8.33
D	No response	16.67

Estrus expression was observed to occur within the range of 36 to 96hrs after synchronization. The overall mean estrus induction interval in cows synchronized with single PGF2ά was 61.2±5.01 hrs.

Estrus duration was observed to occur within the range of 12 to 24 hrs after synchronization. The mean estrus duration (hrs) in cows synchronized with single PGF2ά was 17.1±1.29. (Table.2) Cows exhibiting the estrus showed weak, intermediate and intense estrus responses in 8.33, 41.67 and 33.33 percent respectively, after synchronization of donors with single PGF2ά (Table 2). The estrus response of 100 percent in Kankrej cows (n=12) was observed after

the superovulation. The mean estrus induction (hrs) and estrus duration (hrs) were 53±2.15 and 20.67±1.07, respectively. The intensity of estrus after superovulation comprised weak, intermediate, and intense estrus as 25, 41.67 and 33.33 percent, respectively.

The mean time required for induced estrus (hrs.) was higher after synchronization (61.2±5.01) as compared to estrus after superovulation (53±2.15). The mean estrus duration (hrs) in cows after synchronization was lower (17.1±1.29) as compared to estrus duration after superovulation (20.67±1.07).

Table 3: Characteristics of the estrus in Kankrej donor cows after superovulation

S. No.	Parameters	Estrus after superovulation
1.	Estrus response (%)	100%
2.	Interval between treatment and onset of estrus (hrs)	53±2.15 (36 to 60 hrs.)
3.	Duration of estrus(hrs)	20.67±1.07 (15 to 24 hrs.)
4.	Intensity of estrus	
A	Intense %	33.33
B	Intermediate %	41.67
C	Weak %	25

The superovulatory response was recorded based on the number of corpora lutea palpated at the time of embryo recovery in the cows of different groups which was then validated by transrectal ultrasonography. The cows were considered as the respondent when the ovulatory response was of more than two corpora lutea, while the cows with ≤ 2 corpora lutea were marked as non-respondents. Out of all the 12 cows, 8(66.67%) responded to the superovulation treatment whereas, 4 cows (33.33%) did not show the superovulatory response. Ultrasonographically, an equal superovulatory response was observed as 4 (66.67%) respondents in each group with nonsignificant differences between the groups of donors.

Table 4: Ovarian response to superovulation in Kankrej donors of different groups

Group	No. of cows		Total	X2 (Pvalue)
	Respondents	Non-respondents		
A	4 (66.67%)	2 (33.3%)	6	1.000 (df 1)
B	4 (66.67%)	2 (33.3%)	6	
Total	8	4	12	

Total numbers of corpora lutea were higher on the right ovary as compared to the left ovary in both groups of cows. A similar finding was observed by Stevenson (2019) [28] in dairy cattle. The variation in the total number of CL in the results might be due to the fact that the right side ovary is more functional as compared to the left side ovary in the cattle.

Table 5: Numbers of corpora lutea in the donor cows at various stages using ultrasonography

Periods No. of CL	Group A (n=6)		Group B (n=6)	
	Right ovary	Left ovary	Right ovary	Left ovary
8th day (At the initiation of FSH)	4	1	4	1
Flushing day	20	14	15	10

The total mean number of corpora lutea found through rectal palpation and ultrasonographically were 4.33±1.27 and 5.67±1.62 in Group A and 3.16±94 and 4.17±.74 in Group B, respectively. The statistical difference was found to be non-significant between and within the groups.

Table 6: Number of CLs (Mean±SE) on the day of flushing in different groups of Kankrej cows

Method No. of CLs	Group A (n=6)	Group B (n=6)	P value
RCL	4.33±1.27	3.16±.94	0.475
UCL	5.67±1.62	4.17±.74	0.430
P value	0.531	0.426	

*****RCL = CL count through rectal palpation
*UCL= CL count through ultrasonography

All the treated cows of both groups were subjected to flushing for embryo collection irrespective of the number of corpora lutea present. The overall total means number of corpus lutea and mean recovery of total embryos were 5.67±1.62 and 2.50±1.12 in Group A and the corresponding values were found to be 4.17±.75 and 1.17±.48 in Group B, respectively. Whereas, the non-respondent donor cows with less than two CLs in both the groups yield no embryo upon flushing. Embryo recovery was found to be significantly (p<0.01) lesser when compared with the number of CLs within Group B. However, it was found to be non significantly higher in Group A as compared to Group B. These findings are in accordance with Mishra *et al*. (2002) [33] who reported a mean total embryo as 2.5±0.68 in Sahiwal cows.

Table 7: Total number of corpora lutea and Embryo recovery (Mean ± S.E.) in different groups of Kankrej donors

	Group A (n=6)	Group B (n=6)	P value
Number of CL	5.67±1.62	$4.17±.75^{A}$	0.430
Embryo recovery	2.50±1.12	$1.17±.48^{B}$	0.298
P Value	0.139	0.007	

Means bearing different superscripts within the columns (A-B) differ highly significantly (p<0.01)

Discussion

The estrus synchronization response observed in the present study corroborated well with the findings in Arab zebu cattle (Zeuh *et al*. 2014) [32]; HF X Local crossbred (Mekonnin, 2016) [14], in dairy cattle (Chanylew *et al*., 2018) [6] and crossbred cattle (Ratnaparkhi *et al*., 2020) [19] using single PGF2ά. Whereas, estrus response was higher in the present study than observed in previous studies including Friesian cows (Abdelkhalek *et al*., 2014) [1], non-descript cows using double PGFs in (Sahatpure and Patil 2008) [20], and crossbred cattle using double PGFs in (Ratnaparkhi *et al*., 2020) [19]. Nearly similar intensity of estrus have been reported in crossbred cattle (Ratnaparkhi *et al*. 2020) [19] and in Kangayam donor cows (Mani *et al*., 2021) [12] using double PGF2ά protocol.

The higher proportion of intense and intermediate estrus observed in the present study might be due to the fact that in regular estrus the progesterone concentration obtains a peak level on the 15th day and start to reduce gradually and reaches the basal level on the day of subsequent estrus. A low level of progesterone in the synchronized group during the pro-estrus might be the reason for a higher percent of intense estrus (Chauhan *et al*., 1983) [7]. Higher normal and intense estrus and lower weak estrus in synchronized cows

as compared to control cows observed in the present study is in accordance with the findings of Senthilkumar and Chandrahasan (2015) [22]. The total mean number of corpora lutea found through rectal palpation and ultrasonographically in the two groups were statistically non- significant and these findings corroborate well with that of the in Sahiwal x Jersey crossbred cows (Ullah *et al*., 1998) [30]; in Ongole cows (Babu Rao *et al*., 2005) [4]; in Kamphaeng sean beef cows (Nilchuen *et al*., 2011) [16]; in Gir crossbred cows (Deopurkaretal., 2003) [8]; in crossbred cows (Arosh *et al*., 2000) [3] and in Pesisir cattle using FSH (Afriani *et al*., 2021) [2].

However, a significantly higher average yield of corpus luteum (CL) in Sahiwal cows (Mishra *et al*., 2002) [33]; in *Bos indicus* cattle (Tribulo *et al*., 1991) [29]; in Jersey cows (Sarvaiya *et al*., 2003) [21]; and in North Omani cattle (Hussein *et al*., 2017) [9] have been reported.

A lower superovulatory response as compared to the present findings, 50.00 percent in Sahiwal cattle (Siddiqui *et al*., 2011) [26], and 57.00 percent in crossbred cattle (Ranjan *et al*., 2004) [18] has been reported. Whereas, a much higher 82.60 percent in Ongole cows (Babu Rao *et al*., 2005) [4], 82.00-91.00 percent in Holstein cows (Sharma *et al*., 2002) [23], 100 percent in crossbred cattle (Arosh *et al*., 2000; Kharche *et al*., 2001 and Bhuyan *et al*., 2012) [3, 10, 5] have been reported. These variations observed in the research studies can be ascribed to numerous extrinsic factors, such as dose and type of gonadotrophins, superovulation protocol, seasonal variation and mostly innate individual variation.

In case of embryo recovery the present finding gets support from the those in dairy cattle (Morgan *et al*., 1993) [15]; in Gir cow and its crossbred (Shelar *et al*., 2002) [25] and in Jersey x Red Sindhi (Sharma *et al*., 2006) [24] wherein, GnRH was incorporated at the time of breeding. Changes in endocrine physiology at an older age results in lower superovulatory response as observed in Bos Taurus (Malhi *et al*., 2007) [11] and Bos indicus (Silva *et al*., 2009) [27]. In the present study, It was observed that the donor cows supplemented with GnRH at the time of AI had a positive impact on the number of embryos recovered.

Conclusion

Looking to the overall superovulatory response using 25 mg pFSH in the present study, it is suggested to use a higher dose of Stimufol (pFSH) for further research studies in Kankrej cows. Superovulatory response and embryo recovery were non significantly higher in 25mg pFSH with the GnRH at the time of breeding as compared to without GnRH in Kankrej cattle. Ultrasonography revealed the more number of corpus luteum as compared to the rectal palpation in superovulated Kankrej donors.

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