SUPEROVULATORY RESPONSE, EMBRYO COLLECTION AND TRANSFER IN CROSSBRED COWS

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"SUPEROVULATORY RESPONSE, EMBRYO COLLECTION AND TRANSFER IN CROSSBRED COWS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis, entitled **"SUPEROVULATORY RESPONSE, EMBRYO COLLECTION AND TRANSFER IN CROSSBRED COWS"** is a record of research work done independently by **Sri. M.P. Unnikrishnan**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Dedicated to my late father

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

INTRODUCTION

Role of reproduction as a factor influencing the efficiency of animal production requires no emphasis. Artificial insemination has already made major impact in dairy cattle industry in many countries around the world but, improvements in the efficiency of animal production through direct manipulation of female reproductive process have been minimal. Cattle in common with all mammals, produce thousands of gametes which are never fully utilized in their normal reproductive life. Though artificial insemination has made it possible the widespread dissemination of genes carried by valuable males, the genetic potential of cattle could not be fully exploited due to our inability to utilise larger number of these gametes from genetically superior females. However, in the past few decades, a greater variety of exceedingly viable techniques have been developed to collect these oocytes, which offered ways of increasing commercial animal production.

Because of the low reproductive rate and long generation interval in cow, the embryo transfer technology is specially useful to yield the same rate of genetic progress in 10 years as that could have been achieved in 30 years by using the conventional systems of breeding now in practice (Arthur *et al.*, 1989).

The term embryo transfer in a broader sense has been accepted to cover a range of allied techniques starting from superovulation of donor to the deposition of a viable embryo into the uterus of a recipient. It took more than 80 years since the reporting of first embryo transfer in rabbit, for the technology to be commercially used in cattle breeding from 1970's. Notable developments occurred in this decade with respect to non-surgical recovery, transfer and cryopreservation of embryos. In the last two decades, the technology has developed further, leading to *in vitro* fertilization, sexing, transgenic animal production, embryo splitting, cloning, nuclear transplantation etc. With these developments and more realistic economic motivations, embryo transfer now plays a useful role in cattle industry in many countries.

Although India has a cattle population of nearly 1/6th of the world's population, most of the animals are low producers owing to the poor genetic make up and inadequate feeding. Even after the introduction of exotic germplasm by artificial insemination using semen from proven bulls, the increase in milk production is not adequate to meet the nutritional requirements of people.

In Kerala, milk production has shown substantial increase with cross breeding using exotic bulls and their

crosses. It is envisaged to achieve a target of 2500 kg milk per cow per lactation and 30 lakh tonnes of milk per day from the state by 2000 A.D. Embryo transfer is a bio-technique available to scientists and technicians to strive for a breakthrough in cattle reproduction and thus contribute to achieve the target in animal production. Embryo transfer technique is also recommended for conservation of native germ plasm so that, they can be used for future genetic research or for possible re-introduction into the population.

Considering the above, it was decided to conduct a study using comparatively cheaper and locally available gonadotrophin, to assess the superovulatory response in crossbred cattle.

Review of Literature

REVIEW OF LITERATURE

The earliest embryo transfer was performed by Heape (1891) at Cambridge in rabbits. It was only during 1930's further developments in this field occurred. Warwick and Berry (1949) first reported the birth of a lamb by embryo transfer technique. Umbaugh (1949) recorded the first embryo transfer in bovine, while birth of the first calf through egg transfer was reported by Willet *et al.* (1951). Application of embryo transfer technology on a commercial scale for speedy development of livestock industry was envisaged only in early 1970's. Non-surgical embryo collection and cryopreservation of embryos were also developed during this period. Successful freezing of mouse embryos was reported by Whittingham *et al.* (1972). Various methods were described to sex embryos in vitro (Keeler *et al.*, 1983; King, 1984).

2.1 Superovulation

Casida *et al.* (1943) were the first to carry out superovulation trials in cattle. Foote and Onuma (1970) also tried extensive superovulation trials with gonadotrophins. Promising results were reported by many workers (Marshall and Struther, 1978; Schneider *et al.*, 1980; Seidel, 1981). Hahn (1991) found that superovulation was unsuccessful in about one-third of the cows tried and 40 per cent of oocytes

recovered were unfertilized or degenerated. Gorin and Budevich (1992) recorded an average yield of 5.5 transferable embryos. Pokorney (1993) stated that average number of viable embryos per cow was 6.5 in a study at Switzerland.

2.2 Pregnant mare serum gonadotrophin (PMSG/eCG)

Schams *et al.* (1978) noted that PMSG has two molecular components which affect the biological half life of the hormone, a short half life and a longer half life components. Saumande (1980) opined that PMSG was less effective in controlling superovulation. Monnieaux *et al.* (1984) observed that higher concentration of LH in PMSG induced premature ovulations in preantral and tiny follicles, due to stimulation of mitosis. Murphey *et al.* (1984) and Donaldson and Ward (1985) opined that preparations of gonadotrophins containing lower LH fraction induced better ovarian response. Moore *et al.* (1985) found abnormal protein synthesis when PMSG was administered. Seidel and Seidel (1988) stated that prolonged half life of PMSG in cattle (5 days) resulted in continued recruitment of follicles after superovulatory heat, very high progesterone levels and probably abnormalities in ovum transport. Arthur *et al.* (1989) also reported that longer half life of PMSG was a disadvantage in superovulation since its effects persisted even after the induced oestrum, resulting in poor embryo recovery rate.

Boland *et al.* (1991) observed a high proportion of unovulated follicles when PMSG was used. Zeitain *et al.* (1991) found that the percentage of cows exhibiting estrus, ovulating and percentage of transferrable embryos decreased with an increasing dose of PMSG from 1500 to 6000 units but ovarian and total corporalutea weight increased linearly with increasing PMSG dose. Contrary to this, Santamaria and Escamilla (1991) found that the dose of PMSG had no significant effect on number of embryos recovered or number suitable for transfer. They also found that use of anti-PMSG caused an increase in ovulation rate and a decrease in the number of large follicles. But there was no change in the number of total and transferable embryos recovered. Zeitain *et al.* (1991) also made similar observation. Domeki (1991) was of the opinion that PMSG antibodies did not increase the number of embryos recovered but increased the number of good quality embryos. Callesen *et al.* (1992) conducted extensive study in this area, but could not demonstrate any significant effect on ovulation rate, number of follicles at collection, total yield of ova, fertilization rate, number of transferable embryos, pregnancy rate after transfer or period required by donor cows for resumption of normal reproduction.

Becker and Pinheiro (1986) opined that single PMSG injection was less stressful to the animal than repeated FSH injections. An econometric study by Slenning and

Wheeler (1989) showed that PMSG induced superovulation was more cost effective than FSH induced superovulation. In a comparative study with FSH, Alrarez *et al*. (1989) found no significant difference in the number of follicles, corporalutea or embryos; but cows treated with FSH had a significantly higher percentage of transferable embryos than those treated with PMSG. Goulding *et al*. (1991) found that the number of corporalutea and number of embryos were significantly high in FSH treated group but the number of transferrable embryos did not differ significantly. Slimane and Ouali (1991) observed that ovulation rate was significantly high with PMSG than FSH but no significant difference in the number of embryos recovered. Rommer (1992) reported a higher ova recovery for PMSG treated group than FSH treated group (8.2 vs. 6.3). Availability of PMSG in large quantity at a low cost and easiness of administration was noted as an advantage of PMSG (Alfuraji *et al*, 1993).

2.3 Time of administration of gonadotrophin

Sreenan and Gosling (1977) obtained better superovulatory response in heifers when gonadotrophin was given in the midluteal stage than in early stages of estrous cycle. Moore *et al*. (1984) recorded good response with FSH when administered between day zero to five or day 9 to 13. Donaldson (1984b) noticed no difference in the total embry

or transferable embryo count when the treatment was started on any day between 9 to 13 of the estrous cycle of donor. Seidel and Seidel (1988) found that mean response was lower if the treatment started later or earlier in the cycle especially prior to day five of estrous cycle. Goulding *et al.* (1990) reported better embryo recovery when gonadotrophin treatment was initiated on day 10 of oestrous cycle than on day two. Calder and Rajamahendran (1992) also made similar observations. Nair (1992) suggested day 11 as ideal for starting superovulation.Nasser *et al.* (1993) was of the opinion that higher superovulatory response could be obtained if treatment started on the day of or the day before follicular wave emergence, compared with later treatment.

2.4 Prostaglandin

2.4.1 Action of PGF_2 alpha on superovulation

Behrman (1975) observed that PGF_2 alpha inhibits progesterone production by direct antagonism with LH and later by causing reduction of the number of LH receptor sites in the corporalutea. Seidel and Seidel (1988) described the role of PGF_2 alpha in superovulation as to induce luteolysis and initiate superovulatory heat. They stated that use of PGF_2 alpha increased the embryo recovery and superovulatory heat became more predictable. Pineda (1989) stated that

PGF_2 alpha caused contraction of utero-ovarian vessels leading to ischemia and starvation of luteal cells by interference with progestrone synthesis. Domeki (1991) studied endocrine profile of superovulated animals and found that plasma progesterone level decreased rapidly after treatment with PGF_2 alpha. A similar observation was made by Vishnevskii and Rozgoni (1991). Gallo *et al.* (1992) observed that cloprostenol injection in heifers induced more number of follicles to ovulate. They opined that PGF_2 alpha or its analogues improved synchrony of ovulation in superovulated heifers. Canseco *et al.* (1992) observed that divided doses of PGF_2 alpha injection resulted in better oestrus response.

2.4.2 Time of administration of PGF_2 alpha

Sreenan (1975) obtained successful superovulation when PGF_2 alpha was administered at midluteal phase of oestrous cycle. Betteridge (1977) and Seidel *et al.* (1978) also obtained identical results. Dieleman *et al.* (1983) found that newly formed CL did not respond to PGF_2 alpha. Rodrigues and Grigory (1986) noticed no significant difference in the quality of embryos when PGF_2 alpha was administered at different intervals after the beginning of superovulation treatment. Manicken *et al.* (1990) injected 2000 IU of PMSG and 25 mg PGF_2 alpha on day 10 and 13 respectively of the cycle and inseminated the cow on day 15 and 16. Thomas *et al.* (1991)

administered prostaglandin 48 h after PMSG injection. Similar treatment schedule was followed by Agarwal *et al.* (1992a). Nair (1992) administered FSH-P from day 11 to day 15, while PGF_2 alpha treatment was done on day 14.

2.4.3 Dose of PGF_2 alpha

Sreenan (1975) suggested 30 mg of PGF_2 alpha as the ideal dose for superovulation treatment. Marshall and Struther (1978) and Seidel *et al.* (1978) also recommended the same dose. On the contrary, Subramanyam *et al.* (1989) recommended lower doses like 12.5 mg and 5 mg PGF_2 alpha by IM and intravaginal route respectively in buffaloes and the response was similar to that in the cows. Many workers suggested double spaced injection of PGF_2 alpha or its analogue at 48 and 60 h after PMSG injection (Santamariya and Escamilla, 1991; Goulding *et al.*, 1991; Pleshkerich *et al.*, 1992; Canseco *et al.*, 1992). Gallo *et al.* (1992) observed that cloprostenol had a positive linear effect on ovulation rate. Staigmiller *et al.* (1992) obtained better result with 25 mg PGF_2 alpha. Calder and Rajamahendran (1992) used 35 mg PGF_2 alpha for superovulation treatment. Datta *et al.* (1992) conducted superovulation studies by administering 625 ug (micrograms) PGF_2 alpha analogue in Hariana cows. Holy *et al.* (1992c) used 500 ug cloprostenol for superovulation. Nair (1992) observed that 25 mg

PGF_2 alpha induced satisfactory superovulation but 15 mg PGF_2 alpha resulted in better embryo quality.

2.5 Methods of assessment of superovulation response

2.5.1 Rectal palpation

Dawson (1975) accurately assessed superovulation in 67 per cent of treated animals, by rectal examination. Elsden *et al.* (1976) successfully estimated the number of follicles and ovarian dimensions clinically. Many workers employed rectal palpation of CL as reliable method for assessment of superovulation response (Monnieaux *et al.*, 1983; Sharifuddin and Jainudeen, 1983; Donaldson, 1985). Seidel and Seidel (1988) opined that it was very difficult to assess the response accurately by rectal palpation, if there was overstimulation of ovary. But the same technique was followed by many other workers (Dabas and Sud, 1989; Manickam *et al.*, 1990; Subramanyam *et al.*, 1990; Thomas *et al.*, 1991; Agarwal *et al.*, 1992c).

2.5.2 Hormonal profile

Some scientists studied the hormonal profile of superovulated animals and could not observe any relation between the number of corporalutea and plasma progesterone level (Lamond and Gaddy, 1972; Rajamahendran *et al.*, 1976).

Many other scientists suggested progesterone assay of milk and blood as a measure to assess superovulation response (Bulman and Laming, 1978; Pope and Swinburne, 1980). Nair (1992) observed a gradual increase in progesterone level, following gonadotrophin treatment. He also noted wide variation in the level of progesterone in different animals and the level did not influence the respone to superovulation.

2.5.3 Ultrasonography

Pierson and Ginther (1988) suggested that ultrasonography provided more accurate information about superovulation responses than rectal palpation. Leidel and Wolff (1992) and Bartmann (1993) also used this technique for assessing superovulation response. Botz (1993) stated that rectal palpation could detect only 60 per cent of the CL detected by ultrasonics. Robertson *et al.* (1993) found that ultrasound scanning consistently underestimated the luteal structures.

2.6 Superovulation response

Vorontsova *et al.* (1988) reported that 61 per cent of animals responded to 2500-3000 IU of PMSG. Identical results were obtained by Subramaniyam *et al.* (1990).

Manickam *et al.* (1990) superovulated 11 cows with 2000 IU of PMSG and none of the cows had more than four corporalutea. Sergeev *et al.* (1991) recorded a response of 84 per cent. Thomas *et al.* (1991) attained 90 per cent response to PMSG. Khanna *et al.* (1994) stated that only 66.66 per cent of animals responded to PMSG.

Pawshe *et al.* (1992) and Kharche *et al.* (1995) observed that all animals responsed to PMSG.

2.6.1 Intensity of superovulatory heat

Nair (1992) observed that only about one-third of animals exhibited intense heat signs whereas Pawshe *et al.* (1992) reported intense heat signs in all superovulated animals. Kharche *et al.* (1995) watched intense heat signs among those donors which came into heat within 48 hours of prostaglandin administration.

2.6.2 Onset of superovulatory heat

Eddy (1977) opined that wide variation seen in the onset of oestrus after PGF_2 alpha administration in superovulated cows was due to variability in the duration of pro-estrus period. Lindsell *et al.* (1986) and Yadav *et al.* (1986) observed the onset of estrus in superovulated cows 42 h after administration of PGF_2 alpha. Schallenberger *et al.* (1988)

reported estrus between 22 to 48 h of PGF_2 alpha administration. Manickam *et al.* (1990) reported estrus after 48 h of PGF_2 alpha administration. Mohmood *et al.* (1991) stated that 70 per cent of animals came into estrus within 72 h of PGF_2 alpha treatment. Totey *et al.* (1991b) observed all animals in standing heat 36 to 48 h after PGF_2 alpha administration. Holy *et al.* (1992c) reported the onset of heat 48-56 h after PGF_2 alpha administration. Nair (1992) observed wide variation in the onset of oestrus, but all the animals came into heat within 36 h to 60 h after administration of 20 mg FSH and 25 mg PGF_2 alpha.

An early onset of oestrus was reported by many workers in cows superovulated with PMSG (Datta *et al.*, 1992; Robertson *et al.*, 1993; Kharche *et al.*, 1995). The onset oestrus reported by these workers ranged from 34.8 to 36.6 h.

2.6.3 Duration of superovulatory heat

A shorter duration of 24 to 36 h was reported by many workers (Angle, 1979; Shea *et al.*, 1983; Looney, 1986). Pawshe *et al.* (1992) recorded a duration of 36 ± 2.16 h.

Maxwell *et al.* (1978) found that 90 per cent of ovulations occurred within 48 h. Nair (1992) reported that 43.75 per cent of animals exhibited heat for a duration of 48 h.

2.6.4 Number of corporalutea

Brand *et al.* (1977) detected more than three corporalutea in 74 per cent of cows superovulated. Greve and Lehn-Jensen (1977) also obtained similar results. Betteridge (1980) and Gordon (1983) opined that anything less than three ovulations could not be considered as superovulation. Donaldson (1985) classified superovulation response in cattle as good, moderate and poor when the number of CL were 12, 6 to 12 and 1 to 5 respectively. Kadu *et al.* (1989) recorded a mean value of 12 ± 4 CL in superovulated crossbred cows. Dabas and Sud (1989) observed a range of 3 to 10 with a mean of seven corporalutea. Thomas *et al.* (1991) and Slimane and Ouali (1991) obtained a superovulatory response of above 8 corporalutea using PMSG in cows. Goulding *et al.* (1991) recorded a value of 13.9 ± 0.84 among the beef heifers treated with 2000 IU of PMSG. Pawshe *et al.* (1992) reported an average response of 10.42 ± 2.37 corporalutea in crossbred cows treated with PMSG. Kadu *et al.* (1993) reported a very high value of 17.5 corporalutea in crossbred cows treated with PMSG.

Manickam *et al.* (1990) superovulated 11 cows with PMSG and none of them had more than four corporalutea. Datta *et al.* (1992) obtained a lower response of 2.8 corporalutea on an average in Hariana cows treated with PMSG.

Sarvayia *et al.* (1992); Chauhan *et al.* (1994) and Arora *et al.* (1996) recorded a corporalutea count ranging from 6.6 to 6.69.

Many workers reported that right ovary responded better than left (Becker and Pinheiro, 1986; Dabas and Sud, 1989; Nair, 1992; Datta *et al.*, 1992). Contrary to this Totey *et al.* (1991b) found that both ovaries responded equally. Kharche *et al.* (1995) found that though right ovary response was better than left, the difference was not statistically significant.

2.6.5 Number of unovulated follicles

Kesner *et al.* (1982) opined that very high progesterone level on the day of superovulatory heat interfered with normal LH surge and thereby leaving many follicles unovulated. Monnieaux *et al.* (1984) opined that longer half life of PMSG resulted in more unovulated follicles. Boland *et al.* (1991) also observed a high proportion of unovulated follicles when PMSG was used. Datta *et al.* (1992) recorded 5 ± 1 unovulated follicles in Hariana cows treated with PMSG. Identical values were recorded by Pawshe *et al.* (1992) (4 ± 0.3) and Kharche *et al.* (1995) (5.6 ± 0.5) in crossbred animals treated with PMSG.

Kadu *et al.* (1993) noted a very high value of 13.5 unovulated follicles on an average.

2.7 Embryo collection

2.7.1 Day of collection

Elsden *et al.* (1976) collected more eggs on day six and seven than on day five and eight. Greve *et al.* (1977) and Sreenan (1978a) obtained more embryos on day seven and eight. Donaldson (1986) observed increase in total number of embryos from 8.5 to 15.3 and mean transferable embryos from 3.1 to 6.5 when day of collection was increased from 6 to 7.5. Seidel and Seidel (1988) found that although embryos could be collected non surgically as early as four days after oestrus, prior to day six, recovery rates were lower. They also found that embryos could be collected from day 9 to 14 after oestrus, but since they hatch from zona pullucide it was difficult to identify. Day six to eight embryos were found to be more resistant to damage while handling (Seidel and Seidel, 1988). Ramakrishna and Ramachandraiah (1989) selected day five and six for collection. Subramanyam and Devarajan (1991) collected 22 embryos from five cows on sixth day after first insemination. Wichmann (1990) obtained 1.5 more embryos than average, from cows flushed on 8th day after insemination and 1.8 less embryos than average for cows

flushed on day six. Nair (1992) collected embryos on day six, seven and eight and suggested day six as ideal, though analysis showed no significant difference in the quality of embryos collected at different days.

2.7.2 Embryo recovery rate

Manickam *et al.* (1990) reported an embryo recovery ranging from one to four. An embryo recovery ranging from 4 to 4.83 was reported by Subramaniyam and Devarajan (1991), Thomas *et al.* (1991), and Arora *et al.* (1996) when PMSG was used.

Mishra *et al.* (1992) recorded a embryo recovery of 2 and 0.8 among crossbred Holstein Friesian and crossbred Jersey cows respectively when treated with PMSG. Similar lower values ranging from 0.22 to 2.60 were recorded by Subramanyam *et al.* (1990); Datta *et al.* (1992); Sarvaiya *et al.* (1992), Chauhan *et al.* (1994) and Kharche *et al.* (1995).

A higher embryo recovery rate of above eight was reported by Goulding *et al.* (1991), Rommer (1992) and Saner (1995).

Kadu *et al.* (1993) reported a recovery percentage of 62.7. Agarwal *et al.* (1995) obtained recovery percentage of 26.5, 28.3 and 50.7 embroys respectively for donors with

three to six, 7 to 10 and more than 10 corporalutea. Arora *et al.* (1996) achieved a higher embryo recovery percentage of 72.5.

Harper and Chang (1971) stated that non-recovery of embryos in superovulated cattle was probably due to premature ovulations, as evidenced by early accelerated rise of estrogen (E-2). They also stated that post-ovulatory rise in estrogen level accelerate egg transport through oviduct.

Booth *et al.* (1975) opined that high post ovulatory estrogen level might result in expulsion of embryos into vagina, leading to non-recovery of embryos. Becker and Pinheiro (1986) opined that lack of egg uptake by infundibulum due to intense ovarian reaction might lead to non-recovery of embryos during flushing. Agarwal *et al.* (1995) observed that 37.1 per cent of donors did not yield any embryo.

2.7.3 Embryo quality

Massey and Oden (1984) obtained more transferable embryos per donor in European breeds than in Brahman. Donaldson (1986) encountered embryos of different stages on the same day of collection. Seidel and Seidel (1988) opined that an average of six transferrable embryos could be obtained from a donor cow. Alfuragi *et al.* (1993) obtained

6.3 transferrable embryos on an average from heifers treated with 2000 IU of PMSG. Saner (1995) reported an average transferrable embryo recovery of 5.1 from European countries.

Kadu *et al.* (1989) recorded a mean collection of 8.33 ova of which 0.66 were transferable and 7.66 were unfertilized. Madan *et al.* (1989) collected three transferable embryos on an average, from superovulated cows. Brown *et al.* (1990) reported a transferrable embryo recovery of 1.3 in Welsh Black cattle. Agarwal *et al.* (1992d) recorded a transferrable embryo recovery of 1 ± 0.408. Sarvaiya *et al.* (1992) obtained a transferrable embryo recovery of 1.2 ± 0.49 in crossbred cows treated with PMSG while Arora *et al.* (1996) reported a value of 2 ± 0.85 in crossbred cows treated with PMSG.

Agarwal *et al.* (1992b) reported 20 per cent transferrable embryo recovery when FSH was used. Kadu *et al.* (1993) and Khanna *et al.* (1994) recorded above 33 per cent transferrable embryo recovery rate in cows.

Subramaniyam *et al.* (1991) and Agarwall *et al.* (1992a) obtained a higher percentage of transferrable embryo recovery of above 55. Arora *et al.* (1996) also reported a higher value of 75.77 per cent.

Foote and Ellington (1988) reported that 15 to 20 per cent of donors flushed non-surgically did not yield any transferrable embryos. Hahn (1991) and Boland and Roche (1992) noted that 40 per cent of oocytes recovered were unfertilized or degenerated. Alfuraji *et al.* (1993) opined that even at optimum doses of gonadotrophins, loss of embryo quality could occur owing to ovarian stimulation after ovulation as a consequence of prolonged action of PMSG. Greve *et al.* (1995) opined that this was because the conditions for oocytes maturation, fertilization and early embryo development were disturbed due to alteration of normal reproductive physiology of donor animal.

2.7.4 Factors affecting embryo recovery

2.7.4.1 Age of donor cow

Moore (1975) reported greater ovulation response in heifers. Decreased reproductive performance with advancing age in cattle as well as in other mammalian species had been reported by Talbert (1978). Donaldson (1984a) observed that in animals over 10 years of age, percentage of tranferable embryos declined. Katska and Smorag (1984) opined that decrease in ovulatory response in older cows was because of reduction in the number of follicles in the ovary with increasing age. Walton and Stabbing (1986) found that

younger animals produced more embryos. Breuel *et al.* (1991) reported that the number of transferable embryos was affected by the age of donor. Agarwal *et al.* (1992d) also stated that the percentage of transferable embryos was less in cows aged more than 10 years. Nair (1992) could not find any influence of age on embryo recovery.

2.7.4.2 Parity of donor cow

Kadeka and Makovkova (1992) found that donors with a minimum of three lactations yielded more embryos. Marsalek *et al.* (1992) reported that embryo quality was not affected by age but decreased with increasing lactation number. Pawshe *et al.* (1992) opined that crossbred cows responded better than heifers.

2.8 Recovery of flushing fluid

A fluid recovery of 90 per cent was reported by Brand *et al.* (1977) while Greve *et al.* (1977) recorded 96 per cent fluid recovery. The former authors felt difficulty in passing the catheter in 12 per cent of animals and the latter authors reported bleeding in a few animals during flushing. Newcomb *et al.* (1978b) encountered similar problems but obtained 76.9 per cent of total embryos in the first 100 ml

of fluid recovered. Sreenan (1978b) and Newcomb (1978a) obtained better fluid recovery with longer catheters. Seidel and Seidel (1988) stated that the single most common error in non-surgical recovery was overinflation of balloon, leading to rupture of endometrium and poor fluid recovery. They also found that if uterus was over distended, loss of fluid through oviduct could occur leading to poor fluid recovery. Ramakrishna and Ramachandraiah (1989) failed to flush two cows out of ten superovulated, due to rupture of endometrium. Kadu *et al.* (1989) obtained a fluid recovery rate of 94.53 ± 0.22 per cent. Manickam *et al.* (1990) faced difficulty in passing the catheter resulting in low flushing efficiency. Nair (1992) obtained an overall flushing efficiencies of 68.2 per cent. He opined that a dose of 20 mg FSH and 25 mg PGF_2 alpha was found to be optimal for cervical dilatation and maximum fluid recovery.

2.9 Selection of recipient animals

2.9.1 Based on parity and age of recipients

King *et al.* (1985) preferred cows as recipients than heifers because of less difficulty at the time of calving. Seidel and Seidel (1988) pointed out that heifers were easier to manage since they were not lactating. They also opined that heifers generally had higher fertility than cows, but

non-surgical transfer would be difficult in heifers than in cows. Huhn *et al.* (1991) reported better fertility rates in heifers than cows. Petrikovic and Svtlansk (1991) reported that most successful transfers were with heifers of 18 to 22 months of age.

2.9.2 Based on quality of corpus luteum

Geim (1990) claimed that recipient selection based on quality of CL was reliable. Saito (1991) and Karykin (1992) had similar opinion. The latter author also stated that recipient selection based on quality of CL assessed by rectal examination was 75 per cent accurate. On the contrary, Budzevich (1993) stated that selection based on CL evaluation per rectum was inaccurate.

2.10 Preparation of recipient

Tanabe and Hann (1984) noticed that the stage of cycle when PGF_2 alpha was administered influenced both the degree of estrus synchrony and time of onset. Cavestany and Foote (1985) observed that two-third of animals came into heat within 4 days of PGF_2 alpha treatment. Munar and Nigro (1986) obtained 53.2 per cent synchronization success. Davis *et al.* (1987) administered two injections of PGF_2 alpha eleven days apart and found all animals in heat 80 h after

the second injection. Rosenberg *et al.* (1990) noticed that with the same dose of PGF_2 alpha older animals showed oestrus earlier than younger animals. Geisert *et al.* (1991) observed that administration of progesterone early in the oestrous cycle of recipient could advance uterine reciptivity for transfer of older asynchronous embryos. Parez and Florin (1992) recorded a synchronization success of 89.9 per cent by double spaced injection of prostaglandin in heifers at 11 days apart and animals showed estrus 50.4 h (Av) after the second injection. Nair (1992) synchronized the recipients by double spaced injection of 25 mg PGF_2 alpha. The use of a combination of progestrone releasing intravaginal device (PRID) and PGF_2 alpha for estrus synchronization was described by Broadbent *et al.* (1993).

2.11 Embryo transfer and conception

First successful non-surgical embryo transfer in bovine was reported by Mutter *et al.* (1964). Rowson *et al.* (1972) obtained a poor pregnancy rate for non-surgical transfer and reason was attributed to the introduction of pathogens during transfer. Newcomb *et al.* (1978b) attributed the low preganancy to wrong site of transfer and difficulty in passing the transfer gun through cervix. Christie *et al.* (1980) opined that low pregnancy rate was due to position of

egg within the uterine lumen after transfer resulting in death of embryo within day 17 of transfer. King (1985) noticed that more than half of the embryos from superovulated cattle undergoing abnormal development by day eight. He also observed that even when morphologically normal embryos were transferred, substantial losses occurred subsequently due to chromosome abnormalities. Ginther (1985) attributed premature luteal regression and expulsion of embryonic vesicle through the resulting patent cervix, as the cause of poor pregnancy rate. Seidel and Seidel (1988) concluded that rate of pregnancy would be very low in the beginning of any embryo transfer programme till the technician became proficient with the technique. Suboptimal sperm transport and ovulation being spread over a long duration of time were also attributed to low pregnancy rates (Kadu *et al.*, 1989). Basile *et al.* (1994) observed pregnancy rate among recipients that received embryo from FSH treated donors was significantly higher (56.1 per cent) than that received embryos from PMSG treated donors (37.8 per cent).

2.11.1 Factors affecting conception rate

Many workers attributed the asynchrony of hormone profile of the recipient with the stage of embryo, to the low conception rate after transfer (Wilmut *et al.*, 1985; Breuel *et al.*, 1991; Geisert *et al.*, 1991). Ashworth (1992) also

stressed on the potential contribution of asynchrony to low pregnancy rates.

Many workers opined that relying on natural synchrony of recipients tended to depress the conception rate in non-surgical transfer (Elsden, 1980; Subramanyam *et al.*, 1991; Javed and Ullah, 1995).

Breuel *et al.* (1991) attributed low pregnancy rate to poor quality of embryos. Totey *et al.* (1991b) reported that pregnancy rate was higher for excellent quality blastocyst than for fair quality blastocyst (80% Vs 54.5%). Subramanyam (1992) obtained a very low pregnancy rate after transferring embryo with damaged Zona (12.5%) when compared with transfer of embryos with intact Zona (35.3%).

2.11.2 Conception rate

After transfer of embryos to recipients varying results were reported by many workers. Jordt and Lorenzini (1988) reported 20 per cent conception in the recipient animals. But higher rate of above 30 per cent pregnancy was reported by many workers (Subramaniyam *et al.*, 1991; Subramaniyam and Devarajan, 1991; Totey *et al.*, 1991; Nair, 1992; Lee *et al.* (1993).

A still higher rate of pregnancy ranging from 60 to 83.3 per cent was recorded by Yamashina (1989), Hahn (1991); Holey *et al.* (1992a) and Kruger *et al.* (1992).

None of the animals was reported pregnant after transferring good quality embryos by Manickam *et al.* (1990). Similarly Javed and Ullah (1995) also reported zero pregnancy rate when embryos were transferred in the farmers' animals at their premises.

Incidence of abortion was reported by some workers (Brand *et al.*, 1977; Subramanyam *et al.*, 1990; Nair, 1992; Kadu *et al.*, 1993).

2.12 Effect of superovulation on subsequent breeding of donor cows

Holy *et al.* (1992b) reported that 98 per cent of donor cows, treated with luteolytic agents after flushing were inseminated at an average interval of 25.1 days after flushing. The number of inseminations required for conception post flushing was 1.78 and the overall conception rate was 88 per cent in the above trial. Hackett and McAllister (1992) reported that 52 out of total 64 animals superovulated and flushed came into regular oestrous cycle subsequently and 41 animals (79%) became pregnant within a range of one to four insemination. Reshetnikova *et al.* (1992)

noticed a prolongation of service period by 15 days in donor cows than in control animals. Nair (1992) observed estrus in 62.5 per cent of animals within three months of flushing and 21.9 per cent within three to six months. It was also recorded that 50 per cent of flushed animals conceived again. Knickel (1993) found that 71.4 per cent of flushed cows conceived again after a post flushing gap of 44.5 days. Miksik *et al*. (1993) observed no reduction in milk yield subsequent to superovulation.

Materials and Methods

MATERIALS AND METHODS

The study was conducted in animals belonging to University Livestock Farm, Mannuthy and those of farmers. For superovulation studies, animals of the farm and for transfer of embryos farmers' animals were used.

3.1 Selection of donor cows

Eleven normally cycling healthy cows of the University Livestock Farm, maintained under identical conditions formed the material for superovulation trials. These cows were observed for two consecutive cycles to check the regularity of oestrum and ovulation. Normally cycling cows with oestrous cycle length of 19-21 days and apparently free from reproductive disorders were subjected to superovulation trials.

3.2 Superovulation

*Pregnant mare serum gonadotrophin (PMSG) along with **prostaglandin F_2 alpha (PGF_2 alpha) were used to induce

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* Trophovet: Each vial contains 1000 IU of PMSG. From Indian Immunologicals, Hyderabad.

** Lutalyse: 10 ml vial contains 5 mg Dinoprost per ml. From Up John Co. Marketed by Unichem Laboratories, Bombay.

Fig.1 Hormones and drugs used for superovulation

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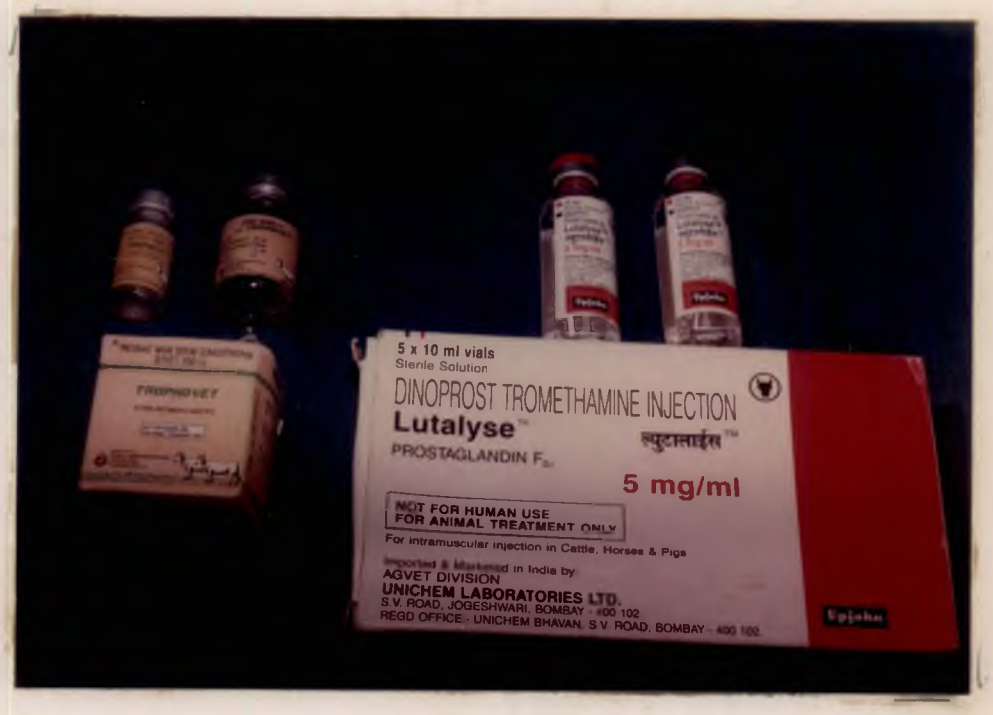
5 x 10 ml vials
Sterile Solution
DINOPROST TROMETHAMINE INJECTION
Lutalyse™
लुटालाईस™
PROSTAGLANDIN F2α
5 mg/ml
NOT FOR HUMAN USE
FOR ANIMAL TREATMENT ONLY
For intramuscular injection in Cattle, Horses & Pigs
Imported & Marketed in India by
AGVET DIVISION
UNICHEM LABORATORIES LTD.
S.V. ROAD, JOGESHWARI, BOMBAY - 400 102
REGD OFFICE - UNICHEM BHAVAN, S.V. ROAD, BOMBAY - 400 102

oestrum and superovulation in donor cows (Fig.1). Each cow was watched for signs of heat before the start of treatment. Standing heat was taken as day '0'. On day 11, PMSG 2000 I.U was administered, followed by 25 mg of PGF_2 alpha after 48 h, intramuscularly. The treated animals were watched for heat symptoms and inseminated with good quality chilled semen from known fertile bulls towards the latter half of oestrum. Inseminations were repeated at 8th and 16th h of first insemination with semen from the same bull.

3.3 Selection of recipient cows

Recipient cows were selected from animals brought to Artificial Insemination Centre, attached to the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy. Recipients were selected from cows reported in oestrum on the same day as donors. Those animals which had regular oestrous cycle with normal breeding history were only considered for transfer of embryos. Ovulation was confirmed by palpation of corpora lutea on 5th day of heat and again on the day of transfer of embryo.

3.4 Preparation of flushing media

One unit (9.98 g) of *Dulbecco's Modified phosphate buffer medium was dissolved in autoclaved demineralised

double distilled water taken in a volumetric flask and the volume was made upto 1000 ml. Antibiotics were added at the rate of 100 I.U of penicillin G sodium and 50 microgram of streptomycin sulphate per ml. The prepared media was rendered sterile by positive pressure filtration through a membrane filter of 0.2 micron pore size. Foetal calf serum was added at 0.5 per cent and 10 per cent levels respectively in the flushing and holding media. Holding media was filtered again through membrane filter of the same pore size and stored in refrigerator at 5°C until use.

3.5 Flushing of donor cow

Non-surgical flushing, as described by Nair (1992) was followed for harvesting the embryos. Donor cow was restrained in the flushing chute and epidural anaesthesia was given with 4 ml of 2 per cent lignocaine hydrochloride. Number of corpora lutea and unovulated follicles in both the ovaries were assessed by clinical examination and recorded. Cervix was dilated gently by using an insemination pipette. A two way Foley's catheter (Fig.2) of appropriate size fitted with a stillete was then introduced through the dilated cervix into the right uterine horn, close to the tubal junction. The catheter was secured in position, by inflating the baloon

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* Dulbecco's Modified Eagle Medium marketed by Himedia Lab Pvt. Ltd., Bombay.

with required volume of air by using a 20 ml syringe. The stillete was then gently removed and the outlet of the catheter was fitted to a T-connector attached with two siliconised rubber tubings, one leading to the bottle containing flushing media and the other to a glass cylinder for collecting the fluid from uterine horn. The flow of fluid from the drip bottle and to the cylinder was regulated by metal clamps fitted near the T-connector. About 25 ml of the media was first allowed to flow through the system by releasing the clamp and fluid was collected back into the glass cylinder, to check the patency of fluid circuit. When the drainage to and from the uterine horn was established, the horn was filled with media with the metal clamp in the drainage tube leading to the glass cylinder in closed position. Uterine horn was gently tapped to dislodge the embryos into the media. When the uterine horn was sufficiently filled with media, the flow was stopped by locking the clamp. Then the clamp on the outflow tube was released allowing the fluid from the distended horn with the dislodged embryos to flow into the glass cylinder. This was repeated several times until 500 ml of media had passed through the horn.

Once the flushing was completed in right horn, the T-connector was detached and the Foley's Catheter was gently taken out after deflating the baloon. The catheter was washed

Fig.2 Equipments used for embryo recovery

1. Foley's catheter with stillete and inflated baloon
2. Foley's catheter
3. Embryo concentrator

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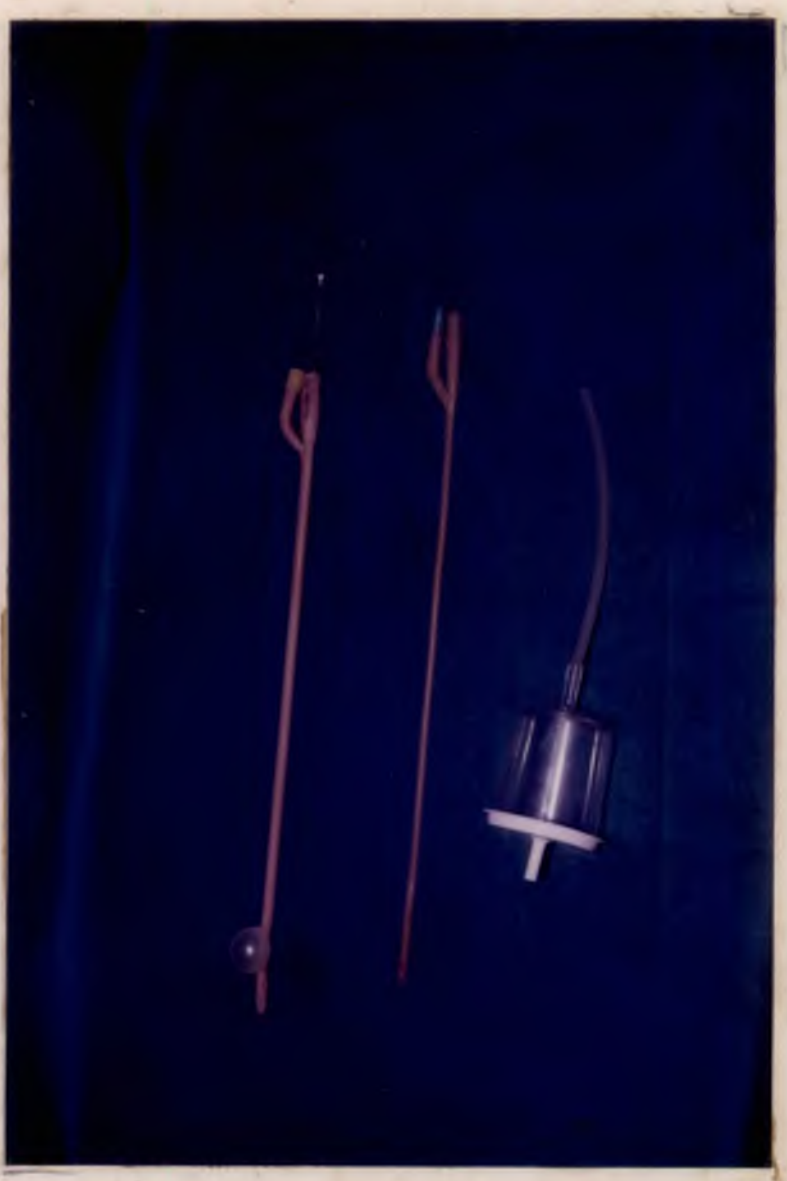

with media, into a clean searching dish to collect embryos if any, sticking on the sides. The stillete was replaced into the catheter which was passed into the left horn for flushing in the same manner. After completing flushing of both the horns, the media was incubated in a BOD incubator at 37°C. The donor cows were administered with 25 mg PGF_2 alpha as intramuscular injection to promote luteolysis. They were also given a course of antibiotic to prevent uterine infection.

3.6 Transfer of embryos

The flushed fluid was filtered through an *Embryo concentrator (Fig.2). The fluid in small volumes was then searched for embryos in petridishes with marked columns, under zoom microscope. The transferrable embryos based on their morphology were washed in fresh media and then transferred into cavity slides having holding media, with the help of micropipettes. The embryos were then loaded into straws and transferred to naturally synchronised recipients. (Nair, 1992).

The recipients were watched for oestrus signs and those which did not return to cycle were checked for pregnancy at 60 days.

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* Embryo Concentrator : Marketed by EmCon, Vet Concepts Inc., USA.

Results

RESULTS

The results of the study on superovulation of 11 crossbred cows with 2000 IU of PMSG on day 11 followed by 25 mg of PGF_2 alpha on day 13, with respect to superovulatory response, embryo recovery, embryo quality, success rate on transfer and subsequent reproduction performance of donors are presented in Tables 1 to 10 and Fig.3 to 6.

4.1 Superovulation

4.1.1 Oestrus response

Out of the 11 cows subjected to superovulation, nine (81.8%) responded by coming into oestrum and ovulating. All these cows evinced oestrum at an interval of 31 to 52 h with a mean of 39.44 ± 2.44 h after PGF_2 alpha injection. Intensity of oestrum was graded as intense, moderate and weak based on the degree of behavioural signs, external signs of heat and the nature of mucus flow. Out of the nine cows observed in heat, six (66.7%) exhibited intense heat while the remaining three (33.3%) showed moderate heat. The duration of oestrum was found to range from 36 to 48 h with a mean of 40.00 ± 2.00 h (Table 1).

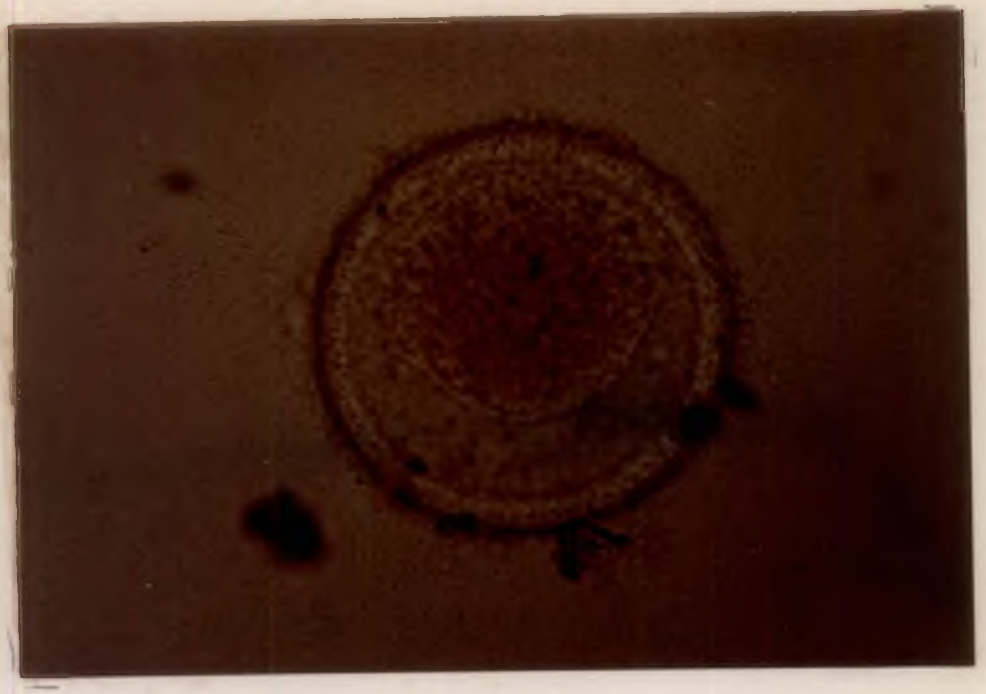
4.1.2 Number of corpora lutea and unovulated follicles

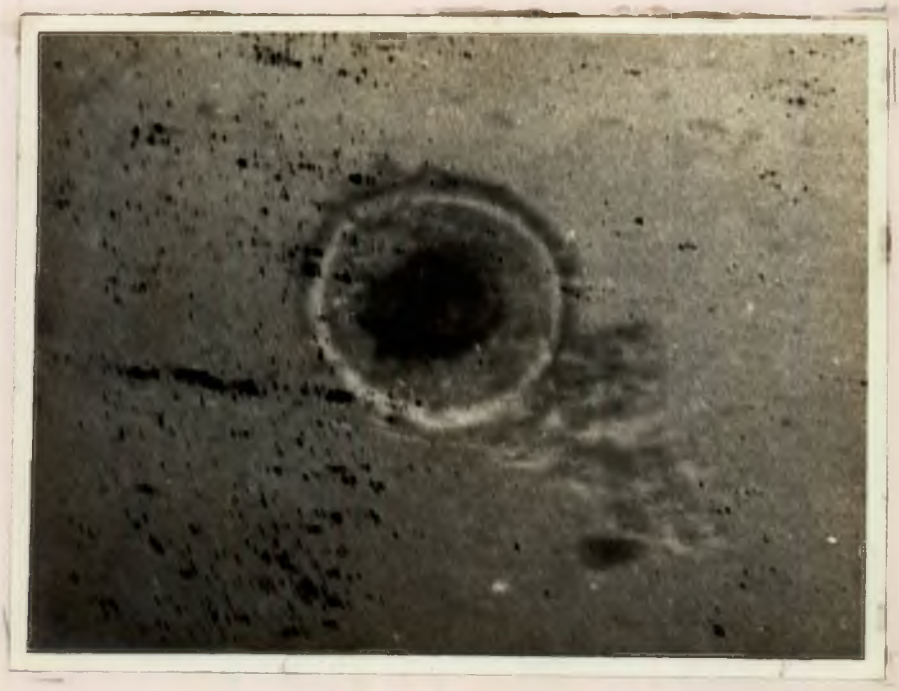
Superovulation response was assessed by counting the number of corpora lutea (CL) in both the ovaries by per rectal examination. The number of corpora lutea in the left ovary ranged from 2 to 4 with a mean of 3.11 ± 0.26 as against 2 to 6 with a mean of 3.56 ± 0.44 in the right ovary. The total number of corpora lutea was found to range from 4 to 9 with a mean of 6.67 ± 0.57. There was no significant difference in the ovarian response to superovulation treatment between the left and the right ovaries (Table 2).

It could also be noted that the number of unovulated follicles in the left ovary varied from 2 to 5 with a mean of 2.78 ± 0.46 whereas the number in the right ovary ranged from 2 to 5 with a mean of 3.44 ± 0.29. The total number of unovulated follicles in both the ovaries put together ranged from 3 to 9 with a mean of 6.22 ± 0.57. The superovulatory response in terms of the total number of corpora lutea and unovulated follicles is also presented in Table 2. The average number of corpora lutea and unovulated follicles in both the ovaries put together was 12.89 ± 0.53 with a mean of 5.89 ± 0.42 and 7.00 ± 0.50 respectively for the left and the right ovaries. Statistical analysis revealed no significant difference in the response between left and the right ovaries

Fig.3 Embryo recovered on flushing (Morula)

Fig.4 Embryo recovered on flushing (Degenerated)





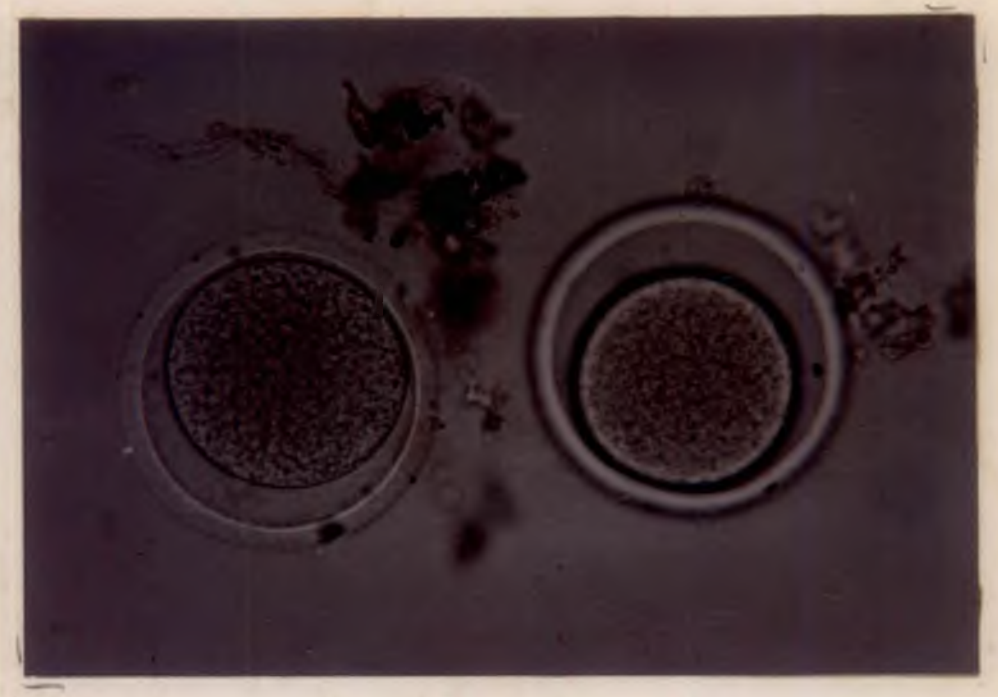
with respect to the total number of corpora lutea and unovulated follicles.

4.2 Embryo recovery

The data on fluid and embryo recovery are presented in Tables 3 and 4. Although nine animals responded to superovulation treatment, flushing could be done successfully for embryo recovery in only eight animals. In one cow, flushing was unsuccessful due to difficulty in passing the catheter due to kinking of the cervix. The mean percentage of flushing medium recovered from the left horn was 75.38 ± 10.89 as against 83.63 ± 6.66 from the right horn with mean recovery percentage of 79.50 ± 9.24 (Table 3). The average number of embryos collected from both the uterine horns put together was 3.38 ± 0.70 with a mean of 1.75 ± 0.49 from the left horn and 1.63 ± 0.49 from the right horn. It may be noted that out of the total 27 embryos collected, 14 were from the left horn (54.16 ± 15.02%) and the remaining 13 were from the right horn (47.93 ± 11.97%). Eventhough there were a total of 52 corpora lutea in both the ovaries of the eight flushed animals, only 27 embryos could be recovered on flushing giving an embryo recovery percentage of 53.11 ± 10.83 (Table 4).

Fig.5 Ova recovered on flushing (Unfertilized)

Fig.6 Ova recovered on flushing (Unfertilized)



4.3 Embryo quality

The quality of the recovered embryo in terms of its fitness for transfer is presented in Table 5. Out of the total 27 embryos recovered from the experimental animals, only 12 (33.34 ± 10.14%) were found fit for transfer based on its morphology. The remaining 15 embryos (54.16 ± 11.83%) were non-transferrable being unfertilized or morphologically abnormal. The mean number of transferrable embryos from both the horns put together was 1.5 ± 0.53 with the left horn yielding 66.67% and the right 33.39% transferrable embryos.

The data on the influence of parity and age of the donors on superovulation response are presented in Tables 6 and 7. The average number of corpora lutea in donors whose parity below three was 5.33 while that of the cows of parity over three was 7.33. Similarly the mean number of embryos recovered from the corresponding groups were 3.00 and 3.60 respectively. The percentage of transferrable embryos in the above two groups were 33.33 and 50.00 per cent respectively.

The average number of corpora lutea and the embryos harvested in the group of donors aged 6 to 9 years were respectively 6.40 and 2.75. The corresponding figures for the

donors aged 10 years and above 10 years were 7.00 and 4.00 respectively. The percentage of transferrable embryos in the younger age group was 45.45 per cent as against 43.75 per cent in the older age group.

4.4 Success rate on embryo transfer

Out of the ten naturally synchronised recipient animals in which embryos were transferred, seven (70%) returned to oestrum within the normal oestrous cycle length indicating failure of conception. The remaining three cows (30%) did not return to cycle within the next 45 days and were confirmed to be pregnant at 60 days. However, two of these cows were reported to have early abortion on 75th and 90th day of transfer respectively. Only one recipient completed gestation and gave birth to a male calf (Table 8). Influence of parity of recipient on the success rate of transfer is given in Table 9. Out of the four heifers receiving the embryo, two conceived giving a success rate of 50 per cent. However one of the heifers aborted while the other successfully completed the gestation and gave birth to a male calf. In contrast, only one out of the six recipient cows (16.25%) conceived but subsequently aborted.

The effect of superovulation treatment on subsequent oestrous cycle of the donor cows is presented in Table 10.

The percentage of cows returning to cycle within two months, between two and three months and between 3 and 4 months of superovulation treatment were respectively 36.36 per cent, 27.27 per cent and 36.36 per cent.

Table 1. Oestrus response in superovulated cows

Sl.No.	Interval after PGF_2 alpha administration (h)	Duration of estrus (h)	Intensity
1.	31	48	Intense
2.	34	36	Moderate
3.	Not responded		
4.	Not responded		
5.	52	36	Moderate
6.	34	48	Intense
7.	36	36	Intense
8.	36	48	Intense
9.	40	36	Intense
10.	42	36	Intense
11.	50	36	Moderate
Mean±SE	39.44±2.44	40.00±2.00	

Table 2. Ovarian response to PMSG

Sl.No.	No. of CL* formed in the ovary			No. of UOF** in the ovary			No. of CL + UOF in the ovary		
	Left	Right	Total	Left	Right	Total	Left	Right	Total
1.	4	3	7	3	3	6	7	6	13
2.	2	4	6	2	3	5	4	7	11
3.	3	5	8	3	4	7	6	9	15
4.	3	4	7	4	2	6	7	6	13
5.	3	6	9	2	4	6	5	10	15
6.	3	2	5	3	5	8	6	7	13
7.	2	2	4	5	4	9	7	6	13
8.	4	3	7	0	3	3	4	6	10
9.	4	3	7	3	3	6	7	6	13
Mean± SE	3.11± 0.26	3.56± 0.44	6.67± 0.50	2.78± 0.46	3.44± 0.29	6.22± 0.57	5.89± 0.42	7.00± 0.50	12.89± 0.53

* Corpora lutea

** Unovulated follicles

Table 3. Fluid recovery percentage

Sl.No.	Percentage of fluid recovery		
	Left	Right	Average
1	30	50	40
2	30	50	40
3	Could not be flushed		
4	60	80	70
5	92	98	95
6	96	96	96
7	96	100	98
8	99	99	99
9	100	96	98
Mean ± SE	75.38± 10.89	83.63± 7.66	79.50± 9.24

Table 4. Percentage of embryo recovery

Sl.No.	No. of CL in the ovaries			No. of embryos collected from the horns			% of embryo recovery		
	Left	Right	Total	Left	Right	Total	Left	Right	Total
1.	4	3	7	2	0	2	50	0	28.6
2.	2	4	6	0	0	0	0	0	0
3.	3	4	7	0	2	2	0	50	28.6
4.	3	6	9	1	4	5	33.3	66.7	55.6
5.	3	2	5	3	1	4	100	50	80.0
6.	2	2	4	2	1	3	100	50	75.0
7.	4	3	7	4	2	6	100	66.7	85.7
8.	4	3	7	2	3	5	50	100	71.4
Mean±	3.13±	3.38±	6.50±	1.75±	1.63±	3.38±	54.16±	47.93±	53.11±
SE	0.29	0.46	0.53	0.49	0.49	0.70	15.02	11.97	10.88

Table 5. Quality of embryos collected

Sl.No.	No. of total embryos			Transferable embryos			% of transferable	Non-transferable embryos			% of non-transferable
	Left	Right	Total	Left	Right	Total		Left	Right	Total	
1.	2	0	2	1	0	1	50	1	0	1	50
2.	0	0	0	0	0	0	0	0	0	0	0
3.	0	2	2	0	0	0	0	0	2	2	100
4.	1	4	5	1	1	2	40	0	3	3	60
5.	3	1	4	2	0	2	50	1	1	2	50
6.	2	1	3	0	0	0	0	2	1	3	100
7.	4	2	6	2	2	4	66.7	2	0	2	33.3
8.	2	3	5	2	1	3	60	0	2	2	40
Mean±	1.75±	1.63±	3.38±	1.00±	0.50±	1.50±	33.34±	0.75±	1.13±	1.88±	54.16±
SE	0.49	0.49	0.706	0.32	0.26	0.53	10.14	0.31	0.39	0.35	11.83

Table 6. Effect of parity of donor on superovulation and embryo quality

Parity	No.of CL	No.of embryos harvested	Transferable		Non-transferable	
			No.	%	No.	%
Below three	16 (3) Av.5.33	9 (3) Av.3.00	3	33.33	6	66.67
Three and above	44 (6) Av.7.33	18 (5) Av.3.60	9	50.00	9	50.00

Number of animals is given in parenthesis

Table 7. Effect of age of donor on superovulation and embryo quality

Age	No.of CL	No.of embryos harvested	Transferable		Non-transferrable	
			No.	%	No.	%
6-9 years	32 (5) Av.6.40	11 (4) Av.2.75	5	45.45	6	54.55
10 and above	28 (4) Av.7.00	16 (4) Av.4.00	7	43.75	9	56.25

Number of animals is given in parenthesis

Table 8. Result of transfer of embryos

		Recipients returned to regular cycle	Recipients conceived		
			Aborted	Calved	Total
Number of embryos transferred	10	7	2	1	3
	Percentage	70	20	10	30

Table 9. Effect of parity of recipient on conception

Parity of recipient	No. of transfers conducted	No. of conception	No. of calving
Cow	6	1	Nil
Heifers	4	2	1
Total	10	3	1

Table 10. Effect of superovulation on subsequent reproductive cycle of donor cows

	Returned to cycle within 2 months	Between 2 and 3 months	Between 3 and 4 months	Non-cycling
No. of animals	4	3	4	Nil
Percentage	36.36	27.27	36.36	Nil

Discussion

DISCUSSION

The objective of the study was to evaluate the superovulatory response, embryo recovery and embryo viability in 11 crossbred cows treated with 2000 IU of PMSG on day 11 of the cycle followed by PGF_2 alpha 25 mg on day 13. Those of the cows which came to oestrum were inseminated towards the latter half of heat and thereafter twice at 8 and 16 h of first insemination. The superovulatory response was assessed by counting the number of corpora lutea and unovulated follicles in both the ovaries. The embryos were flushed out on day 7th of insemination using Dulbecco's modified phosphate buffer medium. The embryo quality was assessed microscopically based on morphology and the transferrable embryos were non-surgically deposited in the uterus of naturally synchronised recipients. The recovery rate and success rate on transfer of embryos was evaluated.

5.1 Superovulation

5.1.1 Oestrus response

Out of eleven animals treated with PMSG, only nine responded (81.8%). Vorontsova *et al.* (1988), Subramanyam *et al.* (1990), Sergeev *et al.* (1991), Thomas *et al.* (1991) and Khanna *et al.* (1994) also reported a failure of superovulatory response to PMSG to the extend of 10-40 per cent. Contrary to

this, Pawshe *et al.* (1992) and Kharche *et al.* (1995) obtained 100 per cent response. Out of the cows responding to treatment, only 66.7 per cent showed intense heat as against 33.3 per cent exhibiting moderate heat signs. This observation is in contrast to the earlier report by Pawshe *et al.* (1992) who recorded intense heat in all animals subjected to superovulation treatment with PMSG. But Nair (1992) reported that only 1/3 of cows superovulated with FSH-P showed intense heat signs.

The onset of superovulatory heat varied within the range of 31-52 h with a mean of 39.44 ± 2.44 h which is in agreement with the earlier studies (Datta *et al.*, 1992; Robertson *et al.*, 1993; Kharche *et al.*, 1995). Kharche *et al.* (1995) had remarked that early onset of oestrum might be due to early accelerated production of estradiol after gonodotrophin treatment.

The mean duration of oestrum was 40.00 ± 2.00 h which is comparatively longer than in natural cycles. Pawshe *et al.* (1992) also reported a duration of 36.00 ± 2.16 h when crossbred cows were superovulated with PMSG. In contrast a shorter duration of oestrum in cows superovulated with FSH-P has been reported by many workers (Looney *et al.*, 1986; Yadav *et al.* , 1986; Nair, 1992). The probable reason for longer

duration of oestrum in cows superovulated with PMSG could be attributed to longer half life of the hormone.

5.1.2 Number of corpora lutea and unovulated follicles

Average number of corpora lutea in both the ovaries put together was 6.67 ± 0.50. Similar values were reported by Sarvaiya *et al.* (1992); Chauhan *et al.* (1994) and Arora *et al.* (1996). It was found that there was no statistically significant difference between response of right and left ovaries. Kharche *et al.* (1995) also obtained similar result. Many other workers on the other hand, observed significantly better response in the right ovary than in the left (Dabas and Sud, 1989; Manickam *et al.*, 1990; Nair, 1992). Average number of unovulated follicles in both the ovaries put together was 6.22 ± 0.57 which was comparable with the earlier reports (Datta *et al.*, 1992; Kharche *et al.*, 1995). A comparatively smaller number of unovulated follicles have been reported from cows superovulated with FSH-P (Seidel and Seidel, 1988; Boland *et al.*, 1991; Agarwal *et al.*, 1992a; Datta *et al.*, 1992, Kharche *et al.*, 1995). Monnieaux *et al.* (1984) and Seidel and Seidel (1988) opined that PMSG induces continued recruitment of follicles even after superovulatory heat leading to the production of a larger number of unovulated follicles, which could be attributed to longer half life of PMSG (5 days) in cattle.

Total ovarian response (number of corpora lutea and unovulated follicles considered together) was found more in right ovary (7.00 ± 0.50) than in left ovary (5.89 ± 0.42), eventhough not statistically significant. Becker and Pinheiro (1986) and Datta *et al.* (1992) also obtained more total ovarian response in right ovary.

5.2 Embryo recovery

It is noted that one animal could not be flushed due to difficulty in passing the catheter through cervix. The same problem was encountered by Brand *et al.* (1977) and Manickam *et al.* (1990). The mean percentage of fluid recovery was 79.50. Similar value was reported by Nair (1992). However, higher recovery rates were reported by Brand *et al.* (1977) and Greve *et al.* (1977). Lower fluid recovery rate in the present study may be due to shorter length of Foley's catheter, which warrant further improvement in the technique of flushing as well as refinement of flushing equipments.

The average embryo recovery in the present study was 3.38 ± 0.70. A lower embryo recovery than the present value (ranging from 0.2 to 2.6) was reported by Subramanyam *et al.* (1990); Datta *et al.* (1992), Chauhan *et al.* (1994) and Kharche *et al.* (1995). A higher recovery rate ranging from 4 to 4.83 were reported by Subramanyam and Devarajan (1991); Thomas

et al. (1991) and Arora *et al.* (1996). However, these values are much lower than those reported from developed countries (Gouding *et al.*, 1991; Rommer, 1992; Saner, 1995). The percentage of embryo recovery was 53.11 ± 10.88, which is lower than the values reported by Kadu *et al.* (1993) and Arora *et al.* (1996). This lower embryo recovery rate is on account of very low fluid recovery (40%) obtained from two donor cows yielding only unusually small number of embryos. Poor embryo recovery may also be attributed to poor "egg pick up" mechanism by infundibulum, due to intense ovarian reaction (Becker and Pinheiro, 1986), premature or delayed ovulation, delayed or accelerated embryo descend into uterus and also to hormonal imbalance (Harper and Chang, 1971 and Becker and Pinheiro, 1986).

5.3 Embryo quality

The percentage of transferrable embryos was 33.34 ± 10.14. Identical results were reported by Kadu *et al.* (1993) and Khanna *et al.* (1994). Higher percentage was reported by Subramanyam *et al.* (1991) and Arora *et al.* (1996). The percentage of non-transferrable embryos in the study was 54.16 ± 11.83 which were either unfertilized ovum or defective embryos. Hahan (1991) and Boland and Roche (1992) also obtained a sizable proportion of non-transferrable embryos. Alfuraji *et al.* (1993) noticed that even at optimum doses,

there might be loss of embryo quality owing to ovarian stimulation after ovulation, as a consequence of prolonged action of PMSG. The observation of larger number of unfertilized ovum tends to support the above theory. Average number of transferrable embryos recovered was 1.50 ± 0.53. This is in agreement with the findings of Brown *et al.* (1990) and Sarvaiya *et al.* (1992). It may be seen from Table 5 that three animals did not produce any transferrable embryos which was the reason for a lower transferrable embryo count. Foote and Ellington (1988) also found that 15 to 20 per cent of donors did not yield any transferrable embryos. The reason for this might be due to the fact that the conditions for oocyte maturation, fertilization and early embryo development were disturbed due to alterations of normal reproductive physiology of donor (Greve *et al.*, 1995).

Ovarian response, embryo recovery and percentage of transferrable embryos was better in donor of parity over three than in those below three. This is in agreement with the findings of Kadeka and Makovkova (1992) and contradictory to that of Marsalek *et al.* (1992).

Although ovarian response and embryo recovery was more in animals of older age group, percentage of transferrable embryos was higher in younger age group animals. Agarwal *et al.* (1992d) also opined that there was a reduction in fertilization

rate and deterioration in embryo quality with advancement in age, particularly beyond 10 years. Donaldson (1984a) and Breuel *et al.* (1991) also made similar conclusions. Contrary to this Marsalek *et al.* (1992) reported that age of donor had no adverse effect on embryo quality. Nair (1992) could not find any influence of age of donor on superovulation response or embryo recovery.

5.4 Success rate on embryo transfer

The conception rate (30%) achieved in the study is comparable to that reported by Totey *et al.* (1991b), Subramanyam *et al.* (1991) and Nair (1992). Manickam *et al.* (1990) and Javed and Ullah (1995) did not get any pregnancy after transfer studies. However, conception rate in this study is lower than the values reported by many workers (Yamashina, 1989; Hahn, 1991; Holy *et al.*, 1992; Kruger *et al.*, 1992). A low conception rate in the present study may be because of the fact that naturally synchronised animals were used as recipients. Relying on natural synchrony of recipient tends to depress the success rate of non-surgical transfer (Elsden, 1980; Subramanyam *et al.*, 1991; Javed and Ullah, 1995). Basile *et al.* (1994) opined that conception rate would be low if embryos were obtained from donors superovulated with PMSG. King (1985) noted that chromosomal abnormalities of embryo occurring due to the effect of gonadotrophin might add to low

conception rate. Accidental introduction of pathogens into uterus at the time of transfer might also contribute to low conception (Rowson *et al.*, 1972). Seidel and Seidel (1988) opined that pregnancy rate would be very low in the beginning of any embryo transfer programme, till the technician becomes proficient with the technique. The rate of success of pregnancy could not be assessed properly in the present study as the number of transfers was not sufficient. Heifer recipients had registered an apparently better conception in this study. This is in agreement with the findings of Seidel and Seidel (1988), Hahn (1991) and Patrikovic and Svetlansk (1991). Contrary to this, King *et al.* (1985) obtained higher fertility in cows after transfer of embryos. Out of the three animals conceived, two animals aborted. Higher incidence of abortion in recipients was reported earlier also (Brand *et al.*, 1977; Subramanyam *et al.*, 1990; Nair, 1992; Kadu *et al.*, 1993).

The percentage of donor cows returning to cycle within two months, between two and three months and between three and four months of superovulation treatment was respectively 36.36, 27.27 and 36.36. Nair (1992) also found that 62.50 per cent of animals exhibited oestrum within three months. It may be noted that all superovulated animals started cycling within four months indicating that superovulation and flushing did not seriously affect the subsequent oestrous cycle of donor cows.

It may be concluded that more than 80 per cent of animals responded to superovulation with an average copora lutea count of 6.67 ± 0.50 which is comparable to many other previous studies conducted in crossbred animals using PMSG. Relatively early onset of oestrum was noticed in superovulated animals. Comparatively lower fluid recovery and embryo recovery warrant use of improved technique and equipments. The presence of more unovulated follicles and higher percentage of non transferrable embryos, especially unfertilized ova was attributed prolonged action of PMSG, casting doubt on the usefulness of PMSG in superovulation of crossbred cows. Transfer studies indicate that heifers are better recipients than cows. Superovulation and flushing did not seriously affect the subsequent oestrous cycle of donor cows. Since the study was conducted only in limited number of animals, a detailed study in a large group, including heifers and cows and at different dose levels of PMSG is warranted before making any final conclusion.

Summary

SUMMARY

The present study was conducted with the objective of evaluating superovulatory response, embryo recovery and embryo viability, in crossbred cows superovulated with 2000 IU of PMSG and 25 mg PGF_2 alpha. The success rate after transfer of embryos to naturally synchronised recipients was also studied.

The percentage of animals responded to superovulation was 81.80, with majority of them (66.7%) exhibiting intense heat symptoms. An early onset (mean 39.44 ± 2.44 h) and longer duration of oestrum (mean 40.00 ± 2.00 h) was observed in animals which responded to superovulation. The early onset might be attributed to early accelerated production of estradiol after PMSG treatment. The longer duration of oestrum might be attributed to longer half life of the hormone.

Average number of corpora lutea in both ovaries put together was 6.67 ± 0.50. There was no statistically significant difference between the response of right and left ovaries. Average number of unovulated follicles in both ovaries put together was 6.22 ± 0.57 which might be attributed to the longer half life of PMSG, causing continued recruitment of follicles. Total ovarian response was more in right ovary than in left ovary, eventhough not statistically significant.

Average fluid recovery obtained on flushing was 79.50 per cent. Improvement in flushing technique and use of longer catheters was suggested to improve flushing efficiency. The average embryo recovery and percentage of embryo recovery in the present study was 3.38 ± 0.70 and 53.11 ± 10.88 respectively. The lower rate of embryo recovery was mainly attributed to poor fluid recovery in the first two donors flushed. The number and percentage of transferrable embryos recovered averaged 1.50 ± 0.53 and 33.34 ± 10.14 respectively. The lower percentage of transferrable embryo was attributed to alterations of normal reproductive physiology of donor due to prolonged action of PMSG which in turn leads to disturbance in oocyte maturation, ovulation, fertilisation and early embryo development.

Donors of parity over three exhibited better ovarian response, embryo yield and transferrable embryo recovery than those below three. Although cows of older age group showed better ovarian response and embryo recovery, percentage of transferrable embryos was higher in younger animals. This might be attributed to the reduction in fertilisation rate and embryo quality with advancement of age, particularly beyond 10 years.

Conception rate achieved in the study was 30 per cent. Lower conception rate might be attributed to the use of

naturally synchronised animals as recipients in which proper synchronisation of the cycle of the recipient with the donor does not occur. Accidental introduction of pathogens at the time of transfer, chromosomal abnormalities of embryo and non-perfection of the techniques might have added to low conception rate. Heifers appeared to be better recipients than cows, in the present study. Out of three animals conceived, two aborted and one animal successfully completed gestation.

All superovulated donors started cycling within four months indicating that their reproductive cycle was not seriously affected by the treatment.

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SUPEROVULATORY RESPONSE, EMBRYO COLLECTION AND TRANSFER IN CROSSBRED COWS

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ABSTRACT OF A THESIS

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ABSTRACT

Out of eleven crossbred cows superovulated with 2000 IU of PMSG on day eleven and 25 mg PGF_2 alpha 48 h later, nine animals (81.8%) exhibited oestrum after an average interval of 39.44 ± 2.44 h. Average duration of oestrum was 40.00 ± 2.00 h with 66.7 per cent of them exhibiting intense heat signs and 33.3 per cent exhibiting only moderate heat signs. Average number of corpora lutea and unovulated follicles in both the ovaries put together was 6.67 ± 0.50 and 6.22 ± 0.57 respectively. Ovarian response was more in right ovary than in left ovary, though not statistically significant.

Average fluid recovery on flushing was 79.5 per cent which was comparatively low. The average embryo recovery and percentage of embryo recovery were 3.38 ± 0.70 and 53.11 ± 10.18 respectively, which was comparatively lower. The reason for poor recovery of embryo was attributed to poor fluid recovery on flushing. The average number and percentage of transferrable embryo recovered were 1.50 ± 0.53 and 33.34 ± 10.14. Reason for these lower rates were attributed to loss of embryo quality, due to prolonged action of PMSG. Donors of parity over three performed better on superovulation and flushing, than those below three. Animals of age group six to nine years produced more transferrable embryos than cows of age group ten and above.

A conception rate of 30 per cent was achieved after transfer of embryos to naturally synchronised recipients. Heifers appeared to be better recipients than cows. Although a high incidence of abortion was encountered, birth of an embryo transfer male calf was also recorded in the study. All the donor cows came into regular oestrous cycle within four months of superovulation treatment.