SUPEROVULATION, SYNCHRONIZATION OF OESTRUS AND EMBRYO TRANSFER IN CROSSBRED COWS

By

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THESIS

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Dedicated to my beloved father

DECLARATION

I hereby declare that this thesis entitled **SUPEROVULATION, SYNCHRONIZATION OF OESTRUS AND EMBRYO 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

Mannuthy
29 4 1992

29/4/92

Dr S P SURESAN NAIR

CERTIFICATE

Certified that this thesis entitled **SUPEROVULATION, SYNCHRONIZATION OF OESTRUS AND EMBRYO TRANSFER IN CROSSBRED COWS** is a record of research work done independently by Sri S P Suresan Nair 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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(S P Suresan Nair)

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Introduction

INTRODUCTION

The rate of genetic improvement within a breed depends on variable traits like genetic variation selection of dams selection intensity and generation interval Breeding value of a cow depends upon its ability to transmit the desirable traits such as high milk and meat production to its offsprings Embryo transfer is a modern biomedical innovative technology which can influence all the above variables and considerably improve the rate of progress The term embryo transfer though refers to the collection of embryo from a dam and its placement in the reproductive tract of a recipient the term on a broader sense has been accepted as a wide range of allied techniques starting from the induction of superovulation of donor to the deposition of embryos into recipient after various manipulative procedures Embryo transfer technology can be used to cut the generation interval and to yield the same rate of genetic progress in ten years as that can be achieved with thirty years by using the conventional systems now in vogue (Arthur et al 1989)

The first successful embryo transfer was reported by Heape (1891) in rabbit Further studies in rabbits by Pincus (1949) and Dowling (1949) at Cambridge showed that 80 per cent of the eggs transferred to suitable recipients developed normally Warwik and Berry (1949) were the first to do successful embryo transfer in sheep Willet et al (1951) produced the first calf

by embryo transfer Rowson et al (1969) however advanced the technique sufficiently for practical use in the cattle breeding field by claiming 91 per cent success rate in embryo transfer in cattle which created much enthusiasm among many workers in the field Subsequent studies on mammalian embryos in the last two decades have considerably widened the knowledge of the physiological changes during fertilization and early stages of embryo By adopting multiple ovulation embryo transfer technology a cow can yield a minimum of 10 calves per year and a maximum of many hundreds in her reproductive life span

Over the years embryo transfer has been and continues to be a valuable research tool It has been used extensively in studies of uterine capacity uterine environment maternal recognition of pregnancy embryo uterine relationship and endocrinology of pregnancy It has also been used in disease transmission studies and to investigate on the genetics influenced reproductive traits like litter size gestation length birth weight and post natal performances The rapid development of new techniques are now expanding the scope of Embryo transfer for further research in animal reproduction (Fraser 1986)

Commercial exploitation of embryo transfer was made possible with the advancement of efficient non-surgical

techniques for recovery of embryos and effective methods for preserving embryos by freezing Further advancement in the micro manipulation of embryos helped in the sex determination before transferring them into recipients which has considerable economic advantage also Leonard et al (1987) developed a Y chromosome specified D N A probe for sexing embryos successfully Land and Wilmut (1987) reported that from the embryos genes can be isolated and can be multiplied and modified in the laboratory and transferred into another These modern developments in the technology have resulted in dramatic increase in demand for embryo transfer service all over the world

In recent years marked progress has also been made in the formulation of media that support the development of embryo Increased depth of understanding of the secretory activity of the reproductive tract in the female and development of techniques with very high sensitivity to demonstrate with marked precision the appearance and disappearance of various hormones in the biological fluids and tissues have further advanced the technolgy Similarly refinement of various techiques for the safe deposition of embryo into the genital tract of synchronized recipient and establishment of a suitable environment in the uterus of a nonbred recipient animal for continued growth of the fertilized ovum is a breakthrough

This was made possible by controlling or altering certain main events of reproduction by scientific techniques.

In India although cows are numerically preponderant among milch animals they account only 43 per cent of the total milk output while buffaloes which account only 37 per cent contribute 57 per cent of the total milk output (Nair 1985) This paradoxical situation of low productivity of our cows is primarily due to inferior genetic make up and low level of feeding In our country livestock industry is in the threshold of changes and milk and milk products are the second largest contributors of the gross agricultural produce next to rice So there is necessity for speedy genetic improvement of our cattle though vigorous selection of superior germ plasm and by its propagation on a wider scale

In Kerala during the past decade the total production of milk and the productivity of animals have shown considerable increase mainly due to the genetic improvement resulted by the introduction of artificial insemination and the incorporation of exotic germ plasm by cross breeding At present 50 per cent of the total breedable animals are cross breds But this progress in milk production has to be doubled especially because of the low land holding per farmer resulting in the limitations in providing proper feed qualitatively and

quantitatively This necessitates the need for additional technologies for speedy genetic improvement but at the same time limiting the birth of unwanted calves

Embryo transfer technology appears to be an effective tool for quick genetic progress and for the development of livestock industry The physiological response of cross bred cows in a particular agroclimatical condition to superovulation technique with exogenous hormones has to be studied exhaustively before embryo transfer work can be carried out in them According to Bindon et al (1986) unpredictable variations of response could occur and the reason was rather biological than technical Luteolytic drugs and gonadotropic hormones are expensive and so their judicious use can further reduce the cost of embryo transfer programme considerably

Standardisation of optimal doses of drugs for complete luteolysis and induction of multiple ovulation in crossbred cows is therefore a primary requisite for successful embryo transfer programme in our state Similarly it is also necessary to study the various factors which affect the successful superovulation of donor and fix certain standards before selection of the cows It is also necessary to study the optimum time for synchronisation of oestrus of recipients and factors influencing the successful synchronisation of oestrus

Hence it was considered worthwhile to undertake a detailed investigation of superovulation and embryo transfer with the ultimate objective of standardising the non surgical embryo transfer technology in cattle The work therefore has the following objectives

1 To standardise the dose of superovulation treatments in crossbred cows

2 To ascertain the appropriate time for harvesting the pre implantation embryo from the donor

3 To ascertain the percentage of transferable and non transferable embryos

4 To standardise the appropriate time for synchronisation of oestrus of donor

5 To study the hormonal profile of superovulated cows

Review of Literature

REVIEW OF LITERATURE

1 Embryo transfer technique since its origin in 1891 has developed tremendously that the technology has now attained commercial status in many livestock development programmes The earliest successful embryo transfer was reported by Heape (1891) in rabbit and later Warwick and Berry (1949) reported the birth of first lamb by surgical transfer of embryo In cattle the first transfer of bovine embryo was reported by Umbaugh (1949) and the the first calf through egg transfer by Willet et al (1951) Although the technology was commercially used as early as 1970 for multiplying exotic breeds of cattle in United Kingdom and North America low pregnancy rate was reported (Graham 1974) However using improved technology about 30 000 pregnancies were recorded by embryo transfer in the year 1979 in Canada and United States (Seidel 1981)

1 1 Though research on embryo transfer in sheep was started as early as 1930 it was Warwick and Barry (1949) and Lopyrin et al (1950 1951) who reported successful embryo transfer with limited pregnancy rate Commercial exploitation of embryo transfer was found possible in sheep to boost the population of certain breeds when Averill (1958) claimed 80 per cent pregnancy rate by embryo transfer using improved technology

1 2 Hammond (1950) and Rowson (1971) also made several reports on embryo transfer in sheep Rowson et al (1972) recorded better pregnancy rates which gave encouragement for subsequent studies in other animals also In swine Curnock et al (1976) recommended embryo transfer as an alternative to hysterectomy for getting disease free piglets for developing specific pathogen free herd Polge (1980) reported commercial use of the technology for inducing new genetic material into closed herds for disease control One of the restrictions in the genetic progress in cattle has been the inability of cows to produce more than one offspring per year Though artificial insemination gained momentum as an easy and cheap means for bringing about genetic improvement in cattle it was later realised that embryos containing the complete genome or the full quota of chromosomes for the particular individuals can be transferred to a foster mother of known or unknown genetic background without the risk of any genetic change (Hafez 1987)

1 3 The ability to increase reproductive rate of the dairy cow with embryo transfer has made wide possibilities to raise selection response at short generation intervals (Rice et al 1970 and Ruane 1988) The technology further achieved new dimensions following the inventions of the low temperature preservation of mammalian ova

1 4 Dowling (1949) Umbaugh (1949) Rowson and Dowling (1949) and Willet et al (1951) were the pioneers to report intense research on embryo transfer in cattle However it was not until much later that the technique advanced sufficiently to be of practical use in cattle development programmes

2 Superovulation

Casida et al (1943) were the first to conduct superovulation trials in cattle Foote and Onuma (1970) reported extensive superovulation studies with gonadotrophins Graham (1974) obtained less than 4 fertilized eggs per donor in early superovulation studies On the contrary Betteridge (1977) Marshall and Struther (1978) Schneider et al (1980) and Seidel (1981) obtained much better results It was seen that gonadotropins increased the yield of normal embryos about five fold or more in cows when given in multiple doses at specific intervals of reproductive cycle and it was possible to stimulate the release of 100 or more oocytes from an individual cow at a time but fertilization and embryo recovery rate were not satisfactory when this number exceeded twenty (Betteridge 1980)

3 1 Selection of Gonadotrophins

The two important gonadotrophins used for superovulation in cattle are pregnant mare serum gonadotrophin (PMSG) and follicle stimulating hormone (FSH) Schams et al (1978) reported 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 On the other hand FSH has very short biological half life and so repeated injections are to be given in divided doses Murphy et al (1984) and Donaldson and Ward (1985) reported that preparations of gonadotrophins with lower LH fraction induced better ovarian responses They also reported that superovulation response was reduced when PLH was added to FSH P Chupin et al 1985) also observed similar results They also observed that wide variations were found among different breeds of cattle

3 2 Donaldson (1984 a) observed no change in the proportion of good quality embryos when the ovaries of donors are over stimulated with FSH P Monnieaux (1984) observed premature ovulations in preantral and tiny follicles due to stimulation of mitosis by higher concentration of LH in PMSG Moore et al (1985) found abnormal protein synthesis when PMSG was administered In a comparative study with PMSG and FSH Callesen et al (1986) observed premature ovulation at a rate

around 14 and 9 per cent respectively Arthur et al (1989) reported that the longer half life of PMSG was a disadvantageous factor in superovulation since its effects persisted even after the induced oestrum resulting in poor embryo recovery rates

4 1 Selection of donors

Embryo transfer amplifies dramatically the reproductive efficiency of cows and according to Seidel (1975) healthy donor herd is one of the foremost concerns of a reputable embryo transfer programme and the best cows in the lot has to be selected as donor He also stressed that a donor cow should be a high milk yielder with all desirable traits and having a normal oestrous cycle as the cycle has to be characterised accurately since superovulation treatment is timed in relation to the next anticipated oestrus Newcomb et al (1979) and Newcomb (1980) reported that breed of the animal and even strains within a breed can differ markedly in sensitivity to superovulation treatment He also reported that Friesian cows responded well to certain particular dose of PMSG

4 2 Erickson (1966) observed a sharp decrease in the number of vesicular follicles from fourth year of age to virtual absence at 15 to 20 years in cows Moore (1975) noticed

greater ovulatory response in heifers than in cows It was also noticed that ovarian function becomes erratic well before all follicles disappear (Erickson et al 1976) But Hasler et al (1981) noticed no significant difference in ovulation rate with advance in age Donaldson (1984 d) observed at 10 years of age the percentage of transferable embryos collected declined while the total number of embryos per collection remained the same for all ages

4 3 Hill et al (1970) noticed that poor plane of nutrition affected the development of follicles in the midluteal period Lamond (1972) observed that fasting the donor cows during superovulation treatment can reduce ovulation response Mourrasse et al (1980) also opined that negative energy balance affected follicular population in cows Zanwar and Deshpande (1988) opined that the top 10 to 20 per cent of elite cows in the herd has to be selected as donor cows

5 Time of administration

The effect of hormones on superovulation at various stages of reproductive cycle was studied in detail by Phillipo and Rowson (1975) and reported that better response was noticed when FSH was given in the midluteal stage than in early stages Similar response was also noticed by Sreenan and Gosling

(1977) But Moore et al (1984) reported good response with FSH when administered between day 0 to 5 or day 9 to 13 Donaldson (1984 b) noticed no difference in the total embryo or transferable embryo count when FSH treatment was started on any day between 9 to 13 of donors oestrus However Lindsell et al (1986) observed better response on day 9 of the cycle than on day 3 Karihaloo (1987) and Jain et al (1989) also concurred with the above Goto et al (1987) concluded that the functional status of corpus luteum on the first day of treatment with FSH was an important factor for reliable superovulation in cattle

6 Dose

Many workers have tried different doses of FSH for superovulation Bellows et al (1969) noticed a linear relationship between gonadotrophic dose increase and ovarian characteristics For maintaining effective blood levels Seidel (1975) administered FSH as 10 injections of half day intervals from day 15 to 20 of the cycle Elsden et al (1976) also successfully superovulated cows with FSH administered at divided doses morning and evening each in descending level of 5 mg 4 mg 3 mg 2 mg and 2 mg respectively commencing from day 10 of the oestrous cycle They reported that higher doses of FSH induced oestrus earlier Looney et al (1981) noticed no

change in the onset of oestrus when higher dose of gonadotrophin was administered in divided doses But Barnes et al (1982) noticed oestrus after 42 0±5 0 hr with higher doses of FSH and 52 8±1 2 hrs with lower doses Donaldson (1984 c) found significant effect of dose of FSH on embryo production in superovulated cows He harvested 5 9 and 2 7 transferable embryos with 28 mg and 60 mg of FSH repectively and recommended 28 mg as ideal dose for superovulation He also observed that over stimulations of the ovaries had no effect on the quality of embryos However in ewes Moore et al (1985) reported premature ovulation when FSH was used in higher doses Pawlyshyn et al (1986) found that when dose of gonadotrophins was increased beyond an optimal level the number of fertilized ova and transferable embryos decreased in cows They also recommended an optimum dose of 30 mg of FSH for satisfactory superovulation Becker and Pinheiro (1986) noticed heavy increase in the size of ovary resulting in reduced embryo uptake by the infundibulam with higher doses of FSH Bodhipaksha (1988) administered 32 mg of FSH in 4 decreasing levels and 50 mg thrice daily for 5 days in different trials with limited success But Zanwar and Deshpande (1988) administered 28 mg of FSH in descending doses twice daily and 40 mg in constant dose of 5 mg each from day 10 to 13 of oestrus cycle with successful results adu et al (1989) also

reported higher incidence of unfertilized ova with higher dosage of FSH in crossbred cows They surmised that higher doses of FSH resulted in continuous follicular stimulation even after ovulation leading to persistant large follicles accompanied by higher oestrogen level In buffaloes Subramaniam et al (1989) reported oestrus on the 6th day with larger doses of gonadotrophin and on the 4th day with smaller doses respectively They reported that the number of corpora lutea ranged from 5 to 9 and 1 to 5 with higher and lower doses respectively and the response was similar to that in cows Totey et al (1991) also reported that the total number and transferable embryos were higher with 28 mg of FSH and concluded the same as the optimal dose for superovulation

7 Route of Administration

Dees et al (1984) reported the use of FSH entrapped in stable liposomes as multilamellar vesicles for embryo transfer in cows Wubishet (1986) observed better response when FSH was administered intramuscularly than subcutaneous infusions Schallenberger et al (1988) found that continuous subcutaneous administration of FSH through an osmotic pump gave better ovarian response However Manickam et al (1990) opined that it was difficult to surmise relation of the dose or route of administration with superovulation unless other variables

pertaining to nutrition genetic and management were controlled strictly

8 Prostaglandin $F_2\alpha$

Role of $PGF_2\alpha$ in superovulation by causing effective luteolysis has been well reviewed Behrman (1975) observed that $PGF_2\alpha$ inhibit progesterone production by direct antagonism with LH and later by causing reduction of the number of LH receptor sites in the corpora lutea He further reported that progesterone depression before the onset of functional luteolysis is caused without interfering with LH binding to its receptar sites Pineda (1989) surmised that $PGF_2\alpha$ causes contraction of the uteroovarian vessels leading to ischemia and starvation of luteal cells by interferance with progesterone synthesis

9 Time of administration

Sreenan (1975) reported successful superovulation with $PGF_2\alpha$ when administered at mid luteal phase of the cycle Betteridge (1977) observed better results when $PGF_2\alpha$ was given around 10 to 15 days of cycle Marshall and Struther (1978) and Seidel et al (1978) also reported similarly Dieleman et al (1983) observed that the newly formed corpora lutea from

premature ovulations did not respond for a few days to $PGF_2\alpha$ Tanabe and Hann (1984) observed that the time of $PGF_2\alpha$ administration influenced the degree of oestrum synchrony and time of onset of oestrus and reported day 11 and 15 of cycle as the best time for $PGF_2\alpha$ administration On the other hand Rodrigues and Gregory (1986) reported no significant difference in the quality of embryos when $PGF_2\alpha$ was administrated at different intervals after the beginning of superovulation treatment Arthur et al (1989) reported that the newly developed corpora lutea were refractory to $PGF_2\alpha$ for the first three to five days and responded promptly from day 13 of oestrous cycle in cows They further observed that injection of $PGF_2\alpha$ at an interval of 11 days caused functional luteolysis and oestrus Manickam et al (1990) administered $PGF_2\alpha$ on day 13 of oestrous cycle and induced oestrum successfully

10 Dose

Dose of $PGF_2\alpha$ was also reported to influence super ovulation Douglas and Ginther (1975) reported that when the dose of $PGF_2\alpha$ was increased the onset of ovulations and inter ovulatory interval decreased in mares Sreenan (1975) observed 30 mg of $PGF_2\alpha$ as ideal dose for superovulation treatment Betteridge (1977) and Marshall and Struther (1978) reported

successful superovulation with the same dose of $PGF_2\alpha$ Seidel et al (1978) also reported identical results Subramanian et al (1989) recommended lower doses of 12 5 mg and 5 mg of $PGF_2\alpha$ by intramuscular and intravaginal route in buffaloes and reported the response to be similar to that in cows Rosenberg et al (1990) observed 25 mg of $PGF_2\alpha$ for inducing oestrus earlier in aged donor cows Manickam et al (1990) also reported satisfactory results with 25 mg of $PGF_2\alpha$ in cows

11 Onset of oestrum

Nair and Madhavan (1984) reported that 98 5 per cent of the cows treated with $PGF_2\alpha$ exhibited oestrus at an average period of 53 20 ±1 03 hr Kariavanov (1986) observed oestrus 42 0 to 45 0 hr after $PGF_2\alpha$ therapy in buffaloes Yadav et al (1986) and Lindsell et al (1986) also observed onset of oestrum in cows after 42 hr of administrations of $PGF_2\alpha$ According to Eddy (1977) the wide variations seen in the onset of oestrum after $PGF_2\alpha$ administration in superovulated cows were due to variability in the duration of pro oestrus period Schallenberger et al (1988) reported oestrus between 22 to 48 hr and Manickam et al (1990) after 48 hr of $PGF_2\alpha$ treatment Mohmood et al (1991) reported 70 per cent of animals in oestrus within 72 hr of treatment with $PGF_2\alpha$

12 Time of ovulation

Douglas and Ginther (1975) noticed that onset of ovulation was increasingly proportional with quantity of $PGF_2\alpha$ administered In superovulated cows Maxwell et al (1978) noticed ovulations within 18 hrs of the onset of oestrus with 45 and 90 per cent of ovulations completed in 24 hr and 48 hrs respectively Angle (1979) noticed completion of ovulations in 24 hours Shea et al (1983) recorded ovulations at 12 hrs from the onset of oestrus and 70 per cent of ovulations completed within 24 hrs Yadav et al (1988) noticed ovulations at 24 hrs after the onset of oestrus with duration of 12 hrs Loone (1986) observed ovulation spreading over a period of 18 hrs Similar observations were made by Madan (1988) also Mohmood et al (1991) reported ovulations with 72 hours of treatment with $PGF_2\alpha$

13 Assessment of superovulation

Several methods have been used to assess the superovulation response in cattle Dawson et al (1975) assessed superovulation accurately in 67 per cent of the animals superovulated by rectal examination Elsden et al (1976) also successfully estimated the number of follicles and ovarian dimensions clinically Sharifuddin and Jainudeen

(1983) and Monniaux et al (1983) also reported rectal palpation of corpora lutea as a reliable method like any other method in vogue Gordon (1983) opined that anything less than three ovulations could not be considered as superovulation

13 2 Highly sensitive radioimmunoassay of progesterone is reported as an additional means of checking ovulating response in animals (Bulman and Lamming 1978) While Lamond and Gaddy (1972) and Rajamahendren et al (1976) observed no relation between the number of corpora lutea and plasma progesterone level Bulman and Laming (1978) confirmed ovarian activity by progesterone assay in serum Pope and Swinburne (1980) also opined progesterone assay of milk and blood as a measure of superovulation response Moore et al (1984) noticed in animals responding well to superovulation oestradial 17 as dominating for 15 to 17 hrs after the LH surge and thereafter progesterone They also observed that premature LH surge resulted in luteinization of follicles leading to abnormal progesterone level during oestrus which affected the quality and quantity of embryo recovery adversely

14 Progesterone profile

Stabenfeldt et al (1969) reported 0 1 to 0 4 ng/ml of serum progesterone on day one of oestrous cycle Similar values

were reported by Robertson (1972) Wattermann (1974) and Dobrowalski (1974) also Increase in the level of progesterone immediately with gonadotrophins treatment was reported by Henricks et al (1973) Rajamahendran et al (1976) Sreenan and Cosling (1977) Saumande (1980) Maurer and Echternkamp (1982) Jensen et al (1982) Waltan and Stubbings (1986) and Goto et al (1987) Booth et al (1975) observed that the increased level of progesterone declined 4 days before oestrus and reached the lowest level on day 2 Fournier et al (1976) noticed lowering of progesterone level from 7 01± 0 45 ng/ml to 0 94 ± 0 17 ng/ml after 3 days of $PGF_2\alpha$ therapy But Saumande (1980) observed lower level from 10 to 32 hrs itself Jensen et al (1982) obseved progesterone level of > 1 ng/ml 2 hr after PGF_2 treatment • Similar observations were made b Greve et al (1984) Callesan et al (1986) and Springman e al (1986) However Lindsell et al (1986) reported an initial increase in the serum progesterone level when gonadotrophin was started on any day of the oestrus cycle

14 2 Lamond and Gaddy (1972) reported that on day 15 in animals with single corpus luteum progesterone level was 4 6 to 9 9 ng/ml and with 3 and 9 corpora lutea 120 ng/ml and 3 2 ng/ml respectively But Lindsell et al (1986) observed no relation between level of progesterone and number of corpora

lutea However Goto et al (1987) noticed more number of corpora lutea when the pretreatment progesterone levels were >3 ng/ml rather than when it was <3 ng/ml

14 3 Wide variations with abnormal levels of progesterone were encountered by many workers When Fournier et al (1976) reported a mean progesterone value of 6 89 ± 0 77 ng/ml on day 12 of $PGF_2\alpha$ treatment Saumande (1980) observed 70 to 100 ng/ml on day 7 and 200 to 300 ng/ml on day 10 Animals treated with PMSG had higher levels (14 62 ng/ml) than that with FSH (3 52 ng/ml) on day 10 (Yadav et al 1986) On the contrary Lindsell et al (1986) and Goto et al (1987) observed higher values in animals treated with FSH

15 Corpora lutea

Brand et al (1977) recorded more than 3 corpora lutea in 74 per cent of the cows superovulated Greve and Lehn (1977) also recorded similar values Donaldson (1985) observed superovulation response in cattle as good moderate and poor when the number of corpora lutea were 12 6 to 12 1 to 5 respectively Zanwar and Deshpande (1988) recorded mean value of corpora lutea as 8 0 16 13 and 13 5 in different trials Kadu et al (1989) obtained a mean value of 12 ± 4 corpora lutea in superovulated cows Bhattacharya et al (1989)

observed ovulation response of over 12 and 6 to 12 corpora lutea in different groups following super ovulation treatment While Dabas and Sud (1989) recorded a range of 3 to 10 with a mean of 7 corpora lutea

15 2 Corpora lutea formed on superovulations were reported to be 6 0 ± 0 9 with varying doses of FSH (Barnes et al 1982) Saumande (1980) Betteridge (1977) Kosugiyama et al (1979) and Manickam et al (1990) also reported variability in the response to individual animals to gonadotrophins They reported 54 per cent response in right ovary 9 per cent in eft ovary and 27 per cent in both ovaries with no response in 9 per cent cases Donaldson and Perry (1983) reported a decline in ovarian response when superovulated repeatedly

15 3 Though Crister et al (1980) did not observe any effect by season on superovulation many workers like Betteridge (1977) Haupat (1979) and Shea et al (1984) reported wide variations in the superovulatory response in individual animals and during months in each year and in successive years On the other hand,Massey and Oden (1984) found no seasonal effect on superovulations response But in Indian cattle Randel (1984) reported seasonal influence on the reproductive functions and opined that endocrinology of these

animals differed from that of Bos taurus Jordt and Lorenzini (1988) also found no influence of season on superovulation

16 Embryo harvest

The earliest uterine egg harvested was a day 5 embryo with 16 cells collected by Winters (1942) He also collected embryos with 32 cells on day 6 embryo with developed blastocoel on day 7 and without pellucida on day 9 which showed considerable amount of yellow pigments making recognitions very difficult Elsden et al (1976) collected more eggs on day 6 and 7 than on day 5 and 8 According to Ramakrishna and Vasanth (1990) on day 6 embryo reached 32 cell morulla stage and by day 7 it developed to a compact morulla It was also reported that by day 8 transformation of morulla to blastula takes place which hatch by day 10

17 Day of harvest

Sreenan (1978) observed 41 per cent embryo recovery rate on day 7 and 8 and 29 per cent on day 5 and 6 respectively Seidel et al (1978) also harvested embryos on day 3 and 6 post oestrous and reported that recovery tended to be marginally higher on day 3 Under different regimen of gonadotropnin and

PGF_2 they collected 5 5 4 9 and 5 7 transferable embryo per donor Greve et al (1977) observed high embryo recovery rate on day 7 and 8 than on day 6 and 10 Lampeter (1978) obtained 5 2 embryos per donor with 58 per cent transferable embryos Newcomb (1980) reported that embryo recovery rate was lower in younger cows than in older ones Donaldson (1986) observed increase in the 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

18 Quality

Linares and Pleon (1981) collected embryo on day 6 with 17 per cent transferable quality Donaldson (1983) observed an increase from 3 8 to 5 4 embryos per animal when 50 mg of $PGF_2\alpha$ was given as three divided dose rather than as two dose Donaldson (1984 d) observed that in animals over 10 years of age percentage of transferable embryos declined Massey and Oden (1984) observed more transferable embryos per donor in European breeds than in Brahman cows in which number of ova per collection was higher Hensel (1985) reported 6 fertilised ova per superovulation Donaldson (1986) encountered embryos of different stages on the same day of collection Under farm conditions Anon (1987) obtained 38 8

per cent recovery of embryos with a mean of 3 5 from Holstein Friesien cows Bodhipaksha (1988) collected 0 to 3 number of embryos Zanwar (1988) on an average collected 6 morphologically normal embryos from donors Zanwar and Deshpande (1988) collected 6 22 and 12 88 embryos on an average from 50 per cent Holstein cows using different doses of gonadotrophins Ambrose et al (1989) collected 11 embryos out of 15 attempts from 10 cows of which 6 embryos were of transferable quality Jain et al (1989) collected 26 embryos from 26 attempts in superovulated buffaloes of which 84 5 per cent were found fertile Bhattacharya et al (1989 a) collected 77 (33 9 per cent) ova from 227 ovulations with an average yield of 2 1 eggs and found only 12 9 per cent as transferable But Singla et al (1989) harvested 69 6 per cent eggs of which 45 8 per cent were excellent quality and 22 9 per cent degenerated while rest were unfertilized Kadu et al (1989) recorded a mean embryo collection of 8 33 with 0 66 transferable quality and 7 66 were unfertilised Madan et al (1989) collected three transferable embryos on an average from superovulated cows Manickam et al (1990) noticed an embryo corpora lutea ratio of 1 4 2 5 3 6 and 3 8 in different animals with a mean embryo collection of 0 8 within a range of 0 to 3 Subramaniam et al (1991) obtained in average embryo recovery of 2 45 with 57 4 per cent transferable quality

Subramanıam and Devaragan (1991) collected 22 embryos from fıve flushıngs Thomas et al (1991) reported an embryo corpora lutea ratıo of 1 2 8 5 wıth 33 3 per cent transferable embryos ın crossbred cows

19 Recovery of flushıng fluıd

Lıterature on the percentage of fluıd recovery after flushıng appear to be scanty Brand et al (1977) reported 90 per cent whıle Greve et al (1977) reported 96 per cent fluıd recovery durıng flushıng Brand et al (1978) reported 55 per cent success ın embryo flushıng They observed dıfficulty ın passıng the catheter ın 12 per cent of the anımals Newcomb et al (1978) encountered sımılar problems but obtaıned 76 9 per cent of total embryos ın the fırst 100 ml of fluıd recovered Greve et al (1977) reported bleedıng ın few anımals durıng flushıng Accordıng to Manıckam et al (1990) dıfficulty of passıng the catheter was due to poor alıgnment of cervıcal folds resultıng ın low flushıng effıcıency

20 Synchronızatıon

Wıde varıatıons ın the effect or synchronızatıon were reported among recıpıent anımals Tanabe and Hann (1984) notıced that the stage of cycle when PGF α was admınıstered

influenced both the degree of oestrus synchrony and time of onset Cavestany and Foote (1985) successfully synchronized within 4 days of $PGF_2\alpha$ administration two third of the cows treated Munar and Nigro (1986) reported 53 2 per cent synchronization success and opined that twice as many animals would be required as the number of recipients required Davis et al (1987) administered two injections of $PGF_2\alpha$ eleven days apart and found all animals in heat within 80 hr after the second injection Rosenberg et al (1990) noticed that older animals showed oestrus earlier than younger animals with the same dose of $PGF_2\alpha$ Pant and Singh (1991) observed oestrus at 69 3 hr with 25 mg of $PGF_2\alpha$

21 Transfer of embryos

1 Review of literature revealed varying reports on the success of surgical and non surgical transfers Foote and Onuma (1970) reported limited success rate with trans cervical embryo transfer Low pregnancy was also reported by Rowson et al (1972) Sreenan and Beehan (1974) and Sreenan (1975) Newcomb et al (1978) reported low pregnancy due to failures in trans cervical transfer in the recipients Christie et al (1980) also reported poor pregnancy due to early embryonic mortality in the synchronized cows Anon (1987) reported 22 2 per cent pregnancy in Holstein Friesian cows under farm conditions Poor pregnancy was also noticed by Shea et al

(1984) when transfer was made during winter months Bodhipaksha (1988) failed to establish any pregnancy in swamp buffaloes Zanwar (1988) reported five per cent pregnancy while Subramaniam et al (1990) obtained 20 per cent calving in cows Lampeter (1978) reported 32 per cent conception rate Brand et al (1977) also concurred with the above They also noticed abortions between day 60 and 90 after transfer Subramaniam et al (1991) and Subramaniam and Devaragan (1991) reported 38 7 and 30 per cent pregnancy rate respectively under field conditions Totey et al (1991) also reported similar values

22 1 Pregnancy

High rate of pregnancy has been reported by several authors While Seidel et al (1978) and Heyman et al (1987) reported 64 and 57 7 per cent pregnancies Greve et al (1977) obtained 54 per cent pregnancy rate through egg transfer Jain et al (1989) reported 50 per cent pregnancy rate even with poor ovarian response and embryo recovery This increase in pregnancy rate was attributed (Seidel 1981) to improvement in the technology

22 2 Poor pregnancy rates in embryo transfer were attributed to the introduction of pathogens and consequent sensitisation of uterus resulting in damage or expulsions of embryos (Rowson et al 1972) But Seidel et al (1978) did not concur with

this view Newcomb et al (1978) attributed the low pregnancy to wrong site of transfer and difficulty in passing the A I gun with embryo transcervicaly Christie (1980) opined that the low pregnancy rate was due to the position of egg within the uterine lumen after transfer resulting in death of embryo within day 17 of transfer King (1985) noticed more than half the embryos from superovulated cattle undergoing abnormal development by day 8 He also observed that even when morphologically normal embryos were transferred substantial losses were incurred subsequently due to chromosome abnormalities and mutant genes He attributed that the impact of even minor changes in DNA like a single mutant gene could lead to timed embryonic death Heyman (1985) estimated 30 per cent embryonic loss in cattle after direct blastocyst transfer Wilmut et al (1985) observed variation in hormone profile and embryo stage which resulted in embryonic loss leading to an asynchronous relationship They also suggested supplementing hormones to recipients while transferring embryos at particu ar stage of development in precise interval after initiation of treatment Ginther (1985) found that the pathogenesis of embryonic loss between day 11 and 15 might involve divergent process relating to uterine health as well as luteal adequacy He suggested that embryonic death in a healthy uterus could be associated with normal blockage of uterine induced luteolysis blocked cervix trapping of the embryos by debris and

maintenance of the corpus luteum He also observed that embryonic death might be associated with luteal regression prematurely and expulsion of embryonic vesicle through the resulting patent cervix Moore et al (1985) observed the uterus secreting certain proteins which established an environment hostile to embryos but the progesterone secreted prior to oestrus prevented this Suboptimal sperm transport and ovulation being spread over to longer duration of time were also attributed for low pregnancy by Kadu et al (1989)

23 Preservation of embryos

1 Long term preservation of embryos were studied by many workers Hafez and Sugie (1963) made early attempts to store cattle embryos in the reproductive tract of rabbits with little success But later Lawson et al (1972) and Gordan (1983) reported that cattle embryos could develop in the rabbit uterus and observed that high percentage of these were capable of continuing as normal embryos after retransfer to recipient cows

23 2 Wilmut and Rowson (1973) stored embryos at 196 C in frozen state with good survival rate after thawing Since then many workers reported different methods for long term preservation of embryos of farm animals (Masip and Mulnad 1980 and Jensen et al 1981)

Material and Methods

MATERIAL AND METHODS

Healthy cross bred cows kept under identical conditions of feeding and management maintained at University Livestock Farm Mannuthy formed the material for the present study

(a) Selection of donor

Genetically superior normally cycling cows within the parity of two to five as evidenced by records and gynaecological examination were selected as donors A total of 32 cows were selected as donors and they were closely watched for length of oestrous cycle and clinicaly examined for the time of ovulation and corpus lutem formation Normaly ovulating cows with twenty one days oestrous cycle with well developed genetalia and free from reproductive diseases were selected for superovulation treatment

(b) Superovulation

Follicle Stimulating Hormone Pituitary (FSH P)* and Dinofertin ($PGF_2\alpha$)** were used for superovulation treatment Superovulatory responses for three doses of F S H P and t o doses of Dinofertin were studied in these animals FSH P was administered subcutaneously in two divided doses at morning and

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* distributed by M/S Schering Corporation U S A

** by M/S Alved India

evening at an interval of 8 hours $PGF_2\alpha$ was given as single intramuscular injection in the gluteal region

All the experimental animals were divided into 6 groups based on the dose of FSH and $PGF_2\alpha$ as shown below

Group 1

Consisted of 6 cows and received 34 mg of FSH and 25 mg of $PGF_2\alpha$ administered in the following manner

Day of treatment from 0 day	Dose of FSH & $PGF_2\alpha$ M <- 8 hr -> E	
Day 11	5 mg	5 mg
Day 12	4 mg	4 mg
Day 13	4 mg	4 mg
Day 14	4 mg	$PGF_2\alpha$ 25 mg
Day 15	2 mg	2 mg

Group 2

Consisted of 6 animals and treated with 34 mg of FSH and 15 mg of $PGF_2\alpha$ as shown below

Day of treatment from 0 day	Dose of FSH & $PGF_2\alpha$ M <- 8 hr -> E
Day 11	5 mg 5 mg
Day 12	4 mg 4 mg
Day 13	4 mg 4 mg
Day 14	4 mg $PGF_2\alpha$ 15 mg
Day 15	2 mg 2 mg

Group 3

Five animals were treated in this group with 20 mg of FSH and 25 mg of $PGF_2\alpha$ in the following manner

Day of treatment from 0 day	Dose of FSH & $PGF_2\alpha$ M <- 8 hr -> E
Day 11	4 mg 4 mg
Day 12	2 mg 2 mg
Day 13	2 mg 2 mg
Day 14	2 mg $PGF_2\alpha$ 25 mg
Day 15	1 mg 1 mg

Group 4

Five animals in this group received 20 mg of FSH along with 15 mg of $PGF_2\alpha$ from day 11 as seen below

Day of treatment from 0 day	Dose of FSH & $PGF_2\alpha$ M < 8 hr > E
Day 11	4 mg 4 mg
Day 12	2 mg 2 mg
Day 13	2 mg 2 mg
Day 14	2 mg $PGF_2\alpha$ 15 mg
Day 15	1 mg 1 mg

Group 5

In this group 5 animals were treated with 13 mg of FSH while 25 mg of $PGF_2\alpha$ was administered

Day of treatment from 0 day	Dose of FSH & $PGF_2\alpha$ M < 8 hr > E
Day 11	3 mg 3 mg
Day 12	1 mg 1 mg
Day 13	1 mg 1 mg
Day 14	1 mg $PGF_2\alpha$ 25 mg
Day 15	1 mg 1 mg

Group 6

In the last group also 5 animals were treated with the same dose of FSH as above while $PGF_2\alpha$ was reduced to 15 mg

Day of treatment from 0 day	Dose of FSH & $PGF_2\alpha$ M <- 8 hr -> E
Day 11	3 mg 3 mg
Day 12	1 mg 1 mg
Day 13	1 mg 1 mg
Day 14	1 mg $PGF_2\alpha$ 15 mg
Day 15	1 mg 1 mg

Each donor cow was watched for heat symptoms before the start of the treatment and standing heat was taken as day O On day eleven of the cycle FSH-P treatment was started and continued until day fifteen The follicular development and ovarian dimensions were evaluated by clinical examination at two day intervals until the day of flushing $PGF_2\alpha$ was given on day 14 evening skipping the second FSH dose on the day On observing heat symptoms subsequent to the $PGF_2\alpha$ treatment donor cows were inseminated three times at an interval of 8 hr with 3 ml of good quality diluted semen

c Selection and synchronization of recipient cows

Recipient cows were selected 6 to 8 weeks prior to the start of superovulation treatment Healthy cows with normal and active reproductive tract though inferior in production were selected Oestrum of these cows were synchronized with that of the donor cows by giving a double spaced injection of 25 mg of $PGF_2\alpha$ (Dinofertin) each The first dose was given to the recipient on day O and the second dose on day 13 of the oestrous cycle of the donor cow Second dose was thus given one day ahead of the $PGF_2\alpha$ dose given to the donor (day 14) Recipient and donor cows were watched for heat symptoms and clinicaly examined for ovulation and corpus luteum formation Recipient cows were not inseminated while in oestrum

d Flushing of donor cows

Three and more than three corpora lutea were considered as effective superovulation and these animals were subjected to nonsurgical flushing (Elsden et al 1976 and Monnieaux et al 1983) Clinical examination was done to ascertain the number of corpora lutea in the ovaries and to ascertain the size of the uterus and cervix for choosing the appropriate sized Foley cathter Flushing of the donor cow was done between day 6 and 8 of first insemination The media used was

Dulbeccos Modified Eagle* to which 1 per cent heat treate foetal calf serum 100 000 IU of penicillin G-sodium and -50mg of streptomycin sulphate and 1 0gm dextrose per liter were added (Fig 1 to 4) (Elsden et al 1976)

Foetal calf serum was collected by bleeding new born calves before feeding colostrum Serum was separated from the blood and was Millipore - filtered and heat inactivated by keeping at 56°C for 3 mts (Hafez 1987)

e Preparation of flushing medium

About 500ml of sterile double distilled water was taken in a 1000 ml volumetric flask along with a medium sized clean sterile magnet One vial of Dulbeccos Modified Eagle Medium was added into the flask The vial was rinsed with sterile double distilled water to deliver all the reagents in it The flask was kept in a magnetic stirer till all the salt was dissolved Add 100 000 IU of Penicillin G Sodium and 50 mg of streptomycin sulphate and 1 0 gm dextrose were added and mixed by stirring until dissolved Ten ml of heat treated foetal calf serum was gently added and allowed to settle and dissolve No stirring was done as it might cause bubble formation The solution was

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* marketed by Himedia

diluted to 1000 ml by adding double distilled water with gentle stirring The media was then collected in two sterile clean 500 ml drip bottles with tight caps The mouth and neck region of the bottles were covered with aluminium foil and they were kept in the refrigerater until use They were used within 10 days of preparation

f Preparation of donor cows for flushing

Donor cows were deprived of food and water for 24 hr prior to flushing The hind quarters were washed well and prepared by scrubbing the tail head with spirit The animal was controlled in a ramp with the anterior portion raised about one foot above the level of posterior end Epidural anaesthesia with 4 to 8 ml of 4% Procaine hydrochloride depending on the size of the animal was given between the sacro coccygial area in the extradural space When the tail became flacid it was held on to one side and secured on to the neck rope by using twine to give way for easy manipulation

Rectum was emptied off and the corpora lutea on either ovaries were counted and recorded The vulva and neighbouring areas were again scrubbed clean An ordinary glass pipette used for artifical insemination was passed through the vulva without contaminating it at vulval end and also guided by hand in rectum for dilating the cervical lumen The pipette was

manipulated gently through the cervix until the tip was felt over the body of the uterus Once the lumen was dilated well the pipette was removed and replaced by a two way Foley Catheter The catheter was made stiff by passing the inner piece of a French straw gun through it as a stilette before introducing The stilette was secured with clamp at the base inorder to prevent it from slipping out through the inner holes of the catheter thus causing injury on the uterine lumen The catheter was introduced into the expanded lumen of the cervix gently and manipulated deep beyond the palpable bifurcation of the horns The baloon was slowly inflated with sufficient air so as to secure the catheter well in position in the horn thus preventing the backflow of the flushing fluid beyond the bulb

The stilette was gently removed and the catheter was fitted to a T connecter attached with a 6 mm inside diameter tygon drainage tube of 1 6 meter in length coming from the drip bottle containing the flushing media The drip bottle was suspended about one meter above the body level of the animal The other end of the T connector was fitted to another piece of tygon tube of about one meter in length and leading into a separating funnel for drawing the embryos along with the fluid from the uterus The flow of the fluid from the drip bottle and to the separating funnel was regulated by metal clamps fitted close to the T connector

About 25 ml of the medium was first allowed to flow through the system by releasing the clamp and the fluid was collected back into the funnel to check the patency of the fluid circuit When the drainage to and from the uterine horn was established the horn was filled with the medium while the metal clamp in the drainage tube leading into the seperating funnel was held in the closed position The uterine horn was gently tapped or massaged per rectum to dislodge the embryos into the media When the uterine horn was sufficiently inflated with media the flow was stopped by locking the clamp The clamp on the outflow tube was released and as the fluid from the distended horn flowed out the horn it was gently massaged to flush out the entire fluid along with the dislodged embryos As the horn was emptied again media was filled into the uterus from the drip bottle with the drainage tube in locked position This was repeated several times until about 500 ml of the media was passed through each horn

Once the flushing was completed in one horn the T connector was detached and the Foley catheter was gently taken out after deflating the bulb The catheter was washed with media into a clean searching dish to collect embryos if any sticking inside The stilette was replaced into the catheter and was passed into the other horn for flushing in the same manner After completing the flushing the fluid was

placed in an incubator at 37°C All the donor cows after flushing were treated with intrauterine infusion of 10^6 units of penicillin G Sodium and 1 0 gm of Streptomycin Sulphate to prevent any uterine infection This was followed by 25 mg of $PGF_2\alpha$ as intramuscular injection for promoting luteolysis (Karihaloo 1987)

The fluid was drained slowly into petridishes after giving time for the embryos to sediment down in the funnels The dishes were marked with columns on the bottom area for locating the embryos easily Each column was examined under a zoom microscope As the embryos were located they were drawn into pasteur pipettes and transferred into fresh media in depression slides for further morphological studies These embryos were kept in B O D incubator at 37°C in fresh media until further use

For transferring into the synchronised recipients the embryos were collected in 0 5 ml semen straws using micropipettes A small quantity of air was drawn as a margin first into the straw followed by a column of media Small column of air was again drawn followed by a larger column of fluid along with the embryo and before sealing the end of the straw another column of air and media were again drawn in The tip was sealed by using a hot artery forceps

For transferring embryos into recipient cows the straw was loaded in an ordinary 0 5 ml French A I gun and deposited deep into the horn ipsilateral to the ovary where the Corpus luteum was noticed

Study on Superovulation response

Optimum level of F S H and P G $F_2\alpha$ for inducing successful superovulation was studied with three doses of FSH and two doses of $PGF_2\alpha$ Animals with three FSH dose in groups 1 to 6 were clinicaly examined before flushing and the number of the corporalutea were counted and recorded Effect of 25 mg and 15 mg of $PGF_2\alpha$ on superovulation in each of these groups were also studied

One experimental animal each from group 1 to 4 was slaughtered after flushing to confirm the clinical findings on superovulation The genetalia was collected and the number of corpora lutea was estimated on each ovary

Study on the appropriate time for embryo harvest

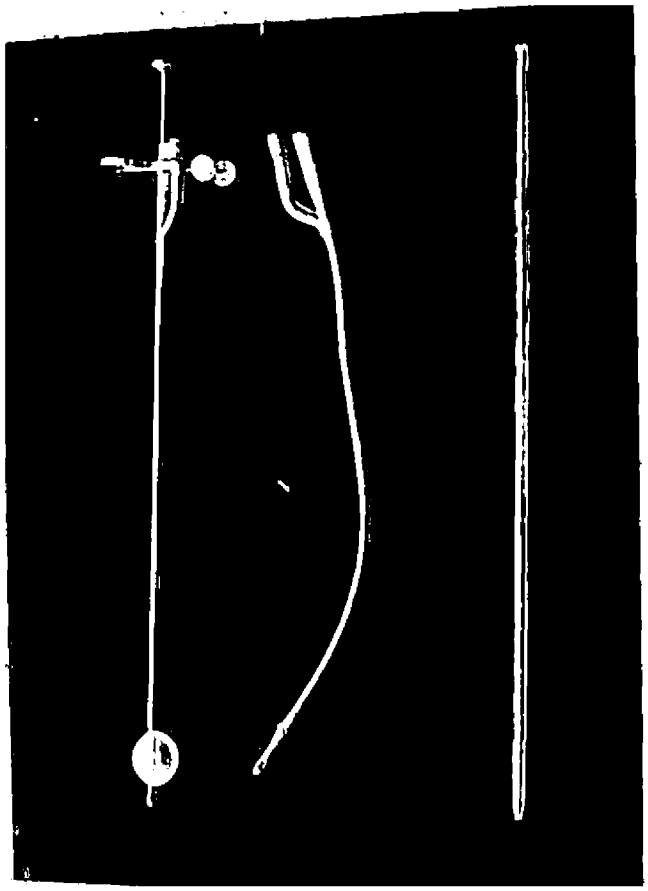
Superovulated cows were flushed for embryo collection on day 6 7 and 8 after the first insemination for ascertaining the appropriate time of harvesting embryos Percentage of fertilized and defective embryos and unfertilized ovum were noted

Hormonal Profile

Level of progesterone in the superovulated cows were studied and for this blood from six randomly selected superovulated cows were collected on day 11 12 13 14 15 16 of oestrum till coming into standing heat About 20 ml of blood was collected in the morning and in the evening serum was separated and drained into P V C vials and stored in liquid nitrogen until analysis The level of hormones was estimated by Radio Immuno Assay technique The serum samples were run in a single assay system and radioactivity was counted in Rack-beta Scintillation system counter programmed to calculate sample concentrations using smoothing spline plot of binding per cent against concentration of standards

Transfer of embryo

Oestrum of twelve recipent cows were synchronised with that of donor cows by giving a double spaced injection of 25 mg of PGF_2 on day 0 and 13 of oestrous cycle of donor cows Embryos collected from donors on day 6 to 8 were transferred into these cows and they were closely watched for oestrum following transfer Pregnancy was confirmed after 60 days of transfer by clinical examinations



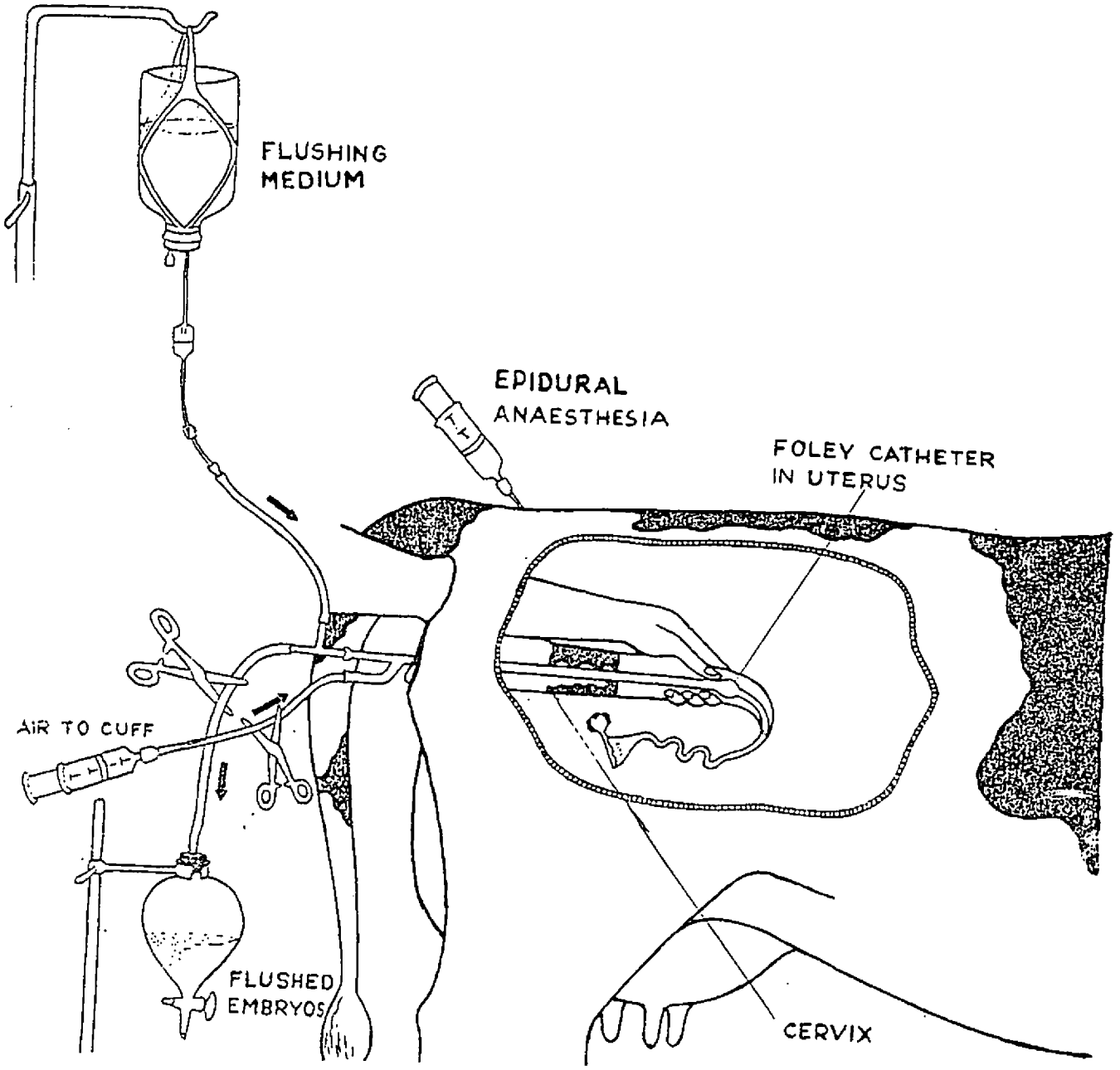


Fig. 2. Procedure of Embryo collection - Diagramatic representation

Fig.3. Procedure of Flushing

Fig. 4. Embryo collection in progress

Results

RESULTS

With the aim of fixing optimal dose of FSH and PGF_2 for inducing satisfactory superovulation yielding maximum number of normal viable embryos thirty two crossbred cows were selected from the University Livestock Farm and randomly allotted into six groups based on three doses of FSH and two doses of $PGF_2\alpha$ with 6 animals each in first two groups and 5 animals each in the other groups Results obtained during the period of study from April 1989 to December 1991 on (1) superovulation assessed by the number of corpora lutea revoked (2) number of transferable and nontransferable embryos collected by flushing of donor cows (3) suitable day for harvesting the embryos from the donor cows (4) results of embryos transferred into recipient cows and (5) hormone profile of superovulated cows are presented in Tables 1 to 9 and Fig 5 to 13

Influence of parity age and season on superovulation observations on onset intensity and duration of oestrum in superovulated cows uterine infection due to embryo collection if any problems in cervical dilatation affecting fluid recovery post flushing oestrum and subsequent reproductive status of the donor cows are presented in Tables 10 to 21

Superovulation response

The data on the effect of 34 mg of FSH and 25 mg of $PGF_2\alpha$ on superovulation are presented in Table 1 and Fig 9 & 5 Perusal of data revealed that the number of corpora lutea developed in the right ovary ranged from 5 to 9 with a mean of 6 7±0 615 In the left ovary the values were 3 to 6 with an average of 4 3±0 422 It could also be seen that the total number of corpora lutea produced in both the ovaries averaged 11 00± 0 966 with a range of 8 to 15

It could also be seen from the Table that number of embryos collected ranged from 2 to 6 with an average of 3 7 The total number of embryos collected from all the animals in this group was 22 (33 1 ± 4 0.6 per cent) of which 16 (69 4±11 915 per cent) were transferable (mean 2 7) and 6 non transferable (30 6±11 915)

The table also revealed that the recovery of fluid after flushing ranged from 65 to 90 per cent with a mean of 77 5±3 354 per cent

The data on the effect of superovulation with 34 mg of FSH and 15 mg $PGF_2\alpha$ on superovulation are presented in Table 2 and Fig 10 While the number of corpora lutea developed in

the right ovary ranged from 0 to 7 with an average of 4 25±0 946 the values for left ovary ranged from 0 to 4 with a mean of 2 75±0 479 It may also be seen that the total number of corpora lutea formed in both the ovaries averaged 7 0±1 414 and ranged between 0 to 11

The number of embryos harvested ranged from 0 to 4 with an average of 3 25 Though a total of 13 (52 5±11 327 per cent) embryos were collected the transferable embryos collected in the group ranged from 0 to 3 with a mean of 2 2 It could also be seen that a total of 68 7±2 075 per cent embryos were transferable T o animals did not respond to the superovulation treatment The percentage of recovery of fluid after flushing ranged from 50 to 90 per cent with a mean volume of 77 5±9 242 per cent

Results of treatment with 20 mg of FSH and 25 mg of PGF_2 are presented in Table 3 The number of corpora lutea in the right ovary was within a range of 4 to 9 with a mean of 6 0±0 837 while in the left ovary the number of corpora utea it varied from 4 to 5 with a mean of 4 6±0 245 The total number of corpora lutea in both the ovaries was 10 6±1 030 with a range of 8 to 14

Embryos harvested under this regimen from the experimental animals varied from 1 to 3 with a mean of 2 4 It could also be seen that the total number of embryos collected was 12 (24 6±5 462 per cent) with 63 3±11 061 per cent of transferable embryos

Recovery of flushing media from the donor cows varied from 60 to 95 per cent with an average of 81 0±6 00 per cent

Superovulation response of the donor cows with 20 mg of FSH and 15 mg of $PGF_2\alpha$ presented in Table 4 revealed that the right ovary responded within a range of 2 to 5 corpora lutea with a mean value of 3 6±0 510 Similarly the number of c pora lutea in the left ovary varied from 1 to 5 with a mean or 3 4±0 678 The total number of corpora lutea ranged from 3 to 8 with a mean of 7 0±1 00

The total number of embryos collected ranged from 0 to 3 from the experimental animals with a mean of 1 4 It could also be seen that all the embryos (100 per cent) harvested were of transferable quality

The rate of recovery of fluid from the donor cows ranged from 50 to 85 per cent with a mean of 68 0±5 612 per cent No embryo could however be collected from one animal although it responded to the treatment with 3 corpora lutea Fluid recovery from this animal was 85 per cent

Superovulation responses with 13 mg of FSH and 25 mg of $PGF_2\alpha$ are furnished in Table 5 Data indicate that the response of right ovary in terms of corpora lutea was within a range of 0 to 2 with a mean of 0 6 while in the left ovary only one responded with a single corpus luteum The total number of corpora lutea in both the ovaries together ranged from 0 to 3 with an overall mean of 0 8 corpora lutea

It could also be observed that no embryo could be collected from any animal in this group Only one animal responded to the treatment and the number of corpora lutea in the right and left ovary were 2 and 1 respectively

None of the animals in this experimental group yielded any embryo though one animal responded to the treatment with 3 corpora lutea Fluid recovery from this animal after flushing was 90 per cent

Superovulation responses with 13 mg of FSH and 15 mg of PGF_2 are furnished in Table 6 Data show that response of right ovary in terms of corpora lutea was within a range of 0 to 2 with a mean of 0 8 while the same for left ovary was seen ranging from 0 to 1 with a mean of 0 2 It could also be seen that the total number of corpora lutea developed in both the ovaries together ranged from 0 to 3 with an overall mean of 1 0

The overall mean response of the experimental animals in all the groups are presented in Table 7 The mean number of corpora lutea ranged from 0 6 to 6 7 in the right ovary and 0 2 to 4 6 in the left ovary The mean number of embryos ranged from 0 8 to 11 It could be also seen that none of the animals in group 5 and 6 responded to superovulation

Statistical analysis revealed significant difference in the response of right ovary between groups ($P < 0.05$) Significant difference was observed between groups 1 and 2 1 and 4 and 3 and 4 However the difference was not significant between groups 1 and 3 2 and 3 and 2 and 4 Analysis also revealed that the difference in the response of left ovary of animals in different groups are not significant

Analysis of data revealed significant difference between groups ($P < 0.05$) in total number of corpora lutea formed in both ovaries Group 1 showed significant difference from group 2 and 4 and group 2 from group 4 ($P < 0.05$) Similarly group 1 and group 2 differed significantly from group 4 also with regard to the number of corpora lutea developed However difference noticed in the number of total corpora lutea formed in animals treated under group 1 and 3 was not significant

Embryo harvest

Total number of embryos harvested from anımals ın dıfferent groups was found to be sıgnıfıcantly dıfferent at (P <0 05) It ıs seen from the anova that group 2 was sıgnıfıcantly dıfferent from group 3 and 4 It was also seen that group 2 sıgnıfıcantly dıfferent (P < 0 05) from group 3 whıle no dıfference ın the response was notıced between group 1 and 2 group 1 and 3 group 1 and 4 and group 3 and 4

From the analysıs ıt was seen that the number of transferable embryos collected ın group 4 vas sıgnıfıcantly dıfferent from group 1 2 and 3 (P <0 05) But ıt was also notıced that anımals ın group 1 2 and 3 responded sımılarly wıth varyıng treatments and showed no sıgnıfıcant dıfference between groups However no sıgnıfıcant dıfference was notıced ın the number of non transferable embryos harvested from anımals ın the dıfferent groups Analysıs also revealed no sıgnıfıcant dıfference between groups wıth regard to the fluıd recovery

The effect of dıfferent days of collectıon on the qualıty of embryos are presented ın Table 8 As may be seen from the table embryos collected on day 6 7 and 8 numbered 15 15 and 24 respectıvely The number of transferable embryos beıng 14 (93 3 per cent) 10 (66 7 per cent) 15 (62 5 per cent)

respectively The corresponding numbers of non transferable embryos were 1 5 and 9 (6 7 per cent 33 3 per cent and 37 5 per cent) respectively Out of 14 transferable embryos collected on day 6 4 embryos were transferred to recipient cows resulting in 75 per cent pregnancy rate Similarly out of 10 transferrable emrbyos collected on day 7 one cow became pregnant out of 3 embryos transferred None of the animals to which 6 embryos transferred out of 15 transferable eggs collected on day 8 conceived Out of 16 non transferable embryos collected 9 were unfertilized and 6 damaged which constituted of 2 zona broken and 4 degenerated embryos (Table 9 Fig 11 to 13) Analysis showed no significant difference on the quality of embryos collected at different days

The effect of parity on superovulation of different animals are presented in Table 10 The average number of corpora lutea formed in animals with parity of two and below was 5 3 Twenty four embryos were harvested from this group out of which 17 were transferable (70 8 per cent) In cows with parity of more than two presented an average number of 6 6 corpora lutea Out of 30 embryos harvested 22 were transferable and 8 non transferable The percentage of transferable emrbyos was 73 3

Statistical analysis revealed that the difference on superovulation response and embryo harvested between parity of animals was not significant

Effect of age of donors on superovulation is presented in Table 11 While cows below 6 years of age yielded an average of 4 9 corpora lutea the corresponding number for cows above 6 years was 6 9 The number of embryos harvested was 21 and 33 with 15 and 24 transferable embryos respectively in the two groups

Statistical analysis revealed the difference between the two age groups on superovulation response and embryos harvested was not significant

The data on the effect of season on superovulation is presented in Table 12 The average number of corpora lutea observed in summer rainy and winter season was 7 7 5 3 and 3 7 respectively The number of embryos collected during the 3 seasons was 24 23 and 7 respectively and transferable embryos were 70 8 82 6 and 42 8 per cent respectively

Difference in the response between seasons was not significant on statistical analysis

It may be noted that the percentage of anımals evınced oestrus wıthın 36 hrs (Table 13) of treatment ın sıx dıfferent groups were 50 0 20 0 20 and 0 per cent respectıvely wıth an overall percentage of 15 7 whıle those showed oestrus wıthın 36 to 48 hours ın the respectıve groups were 33 3 16 7 40 100 40 and 60 per cent wıth an overall percentage of 46 8 Correspondıng values for those showed oestrus wıthın 48 to 60 hrs were 16 7 83 3 40 0 40 and 40 per cent wıth overall percentage of 37 5

Data presented ın Table 14 revealed that weak oestrus symptoms were observed ın 50 16 7 0 40 80 and 80 per cent of anımals subjected to superovulatıon ın the sıx experımental groups respectıvely wıth an overall percentage of 43 8 Percentage of anımals observed wıth ıntermedıary symptoms were 0 50 60 20 20 and 20 respectıvely wıth 28 1 overall percentage Intense oestrus symptoms vere noted ın 50 33 3 40 40 0 and 0 per cent of anımals ın grous 1 to 6 respectıvely wıth an overall 28 1 percentage

The duratıon of oestrum ın the varıous experımental anımals are presented ın Table 15 Duratıon of oestrum of 24 hours was observed ın 50 50 75 25 75 and 60 pert cent of anımals respectvely wıth an overall mean of 56 2 per cent Length of oestrum of 48 hours vas observed ın 50 50 25 75

25 and 40 per cent of cows with an overall mean of 43 75 per cent in the respective treatment groups

The data on the onset of oestrum in the recipient cows after the second dose of $PGF_2\alpha$ is consolidated in Table 16 It could be seen that 30 8 per cent of animals showed onset of oestrum within 36 hours and equal number of animals between 36 to 48 hours while 38 4 per cent animals were in oestrum between 48 to 60 hrs after the administration of $PGF_2\alpha$

The details of flushing efficiency are presented in Table 17 Flushing was efficient in 66 7 and 50 per cent animals in group 1 and 2 80 and 60 per cent in groups 3 and 4 and 20 per cent each in group 5 and 6 respectively The overall efficiency averaged 68 2 per cent Flushing was difficult in 33 3 50 20 and 40 per cent of animals in group 1 to 4 respectively while in group 5 and 6 one animal each was flushed and both were easy It could also be seen that irrespective of groups in 31 8 per cent of animals flushing was difficult

The reproductive status subsequent to superovulation and flushing of donor cows are presented in Table 18 It is seen from the data that normal reproductive status was noticed in 66 7 and 50 per cent respectively in group 1 and 2 and 80 per

cent each in group 3 to 6 It may also be noticed that 71 9 per cent of animals showed normal reproductive cycle It is also seen that genital disorders were noticed in 33 3 and 50 per cent of animals in group 1 and 2 and 20 per cent each in group 3 to 6 respectively Table also revealed that a total of 28 1 per cent of animals developed genital disorders subsequent to superovulation

Effect of superovulation on subsequent oestrum of the donor cows after the induced oestrum in different treatment groups is consolidated in Table 19 Oestrum was noticed within 3 months in 50 16 7 and 60 per cent of animals in group 1 to 3 respectively and 80 per cent of animals in group 4 to 5 All the animals (100 per cent) in group 6 evinced oestrum within 3 months The overall percentage of animals showing oestrum within 3 months was 62 5 Oestrum was noticed within 3 to 6 months in 16 7 and 33 3 per cent of animals in group 1 and 2 and 20 per cent each in group 3 and 4 None of the animals were in oestrum in group 5 and 6 It was also noticed that 15 66 per cent showed oestrum within 3 to 6 months irrespective of groups Similarly 33 3 and 50 per cent of animals in group 1 and 2 20 per cent each in group 3 and 5 showed oestrum after 6 months The overall percentage of animals showing oestrus within 3 to 6 months was 21 9

The details of conception rate of donor animals on subsequent breeding are presented in Table 20 The percentage of animals conceived was found to be 66 7 per cent in group and 2 and 40 per cent each in group 3 to 6 The overall percentage of conception was 50

Level of progesterone in the serum of six superovulated cows from day of treatment till day of heat is presented in Table 21

It could be seen from the table that the level o progesterone was within the range of <0 008 ng/ml to 0 428 ng/ml in different animals during day 11 to 17 of the cycle

It is also seen that the mean value in the morning on day one was 0 01 and in the evening 0 08 On day 2 the level showed a sharp rise to 0 06 in the morning but again dropped to 0 01 in the evening Gradual increase in the level was noticed on day 3 to reach the maximum level of 0 06 and 0 14 in morning and evening respectively on day 4 Gradual lowering of progesterone level was noticed from day 5 to reach the lower minimum (0 008) level on day of oestrum (Fig 14)

Fig.5. Superovulated ovary. Group I
Multiple corpora lutea and unovulatory follicles
6 days after superovulation

Fig. 6. Superovulated ovary. Group 3.
Multiple corpora lutea - 6 days after superovulation

Fig.7. Superovulated ovary - Group 2. No response and with single corpus luteum. 7 days after superovulation

Fig.8. Superovulated ovary - Group 4. No response. No ovulation.

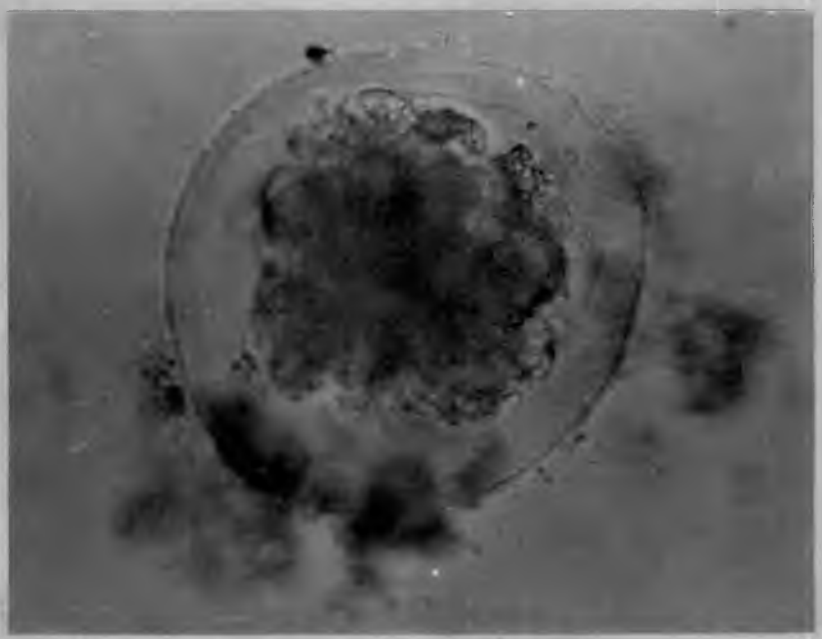


Fig.9. Day 6 embryo (Morula) (x1000).

Fig.10. Day 7 embryo
(x 750)

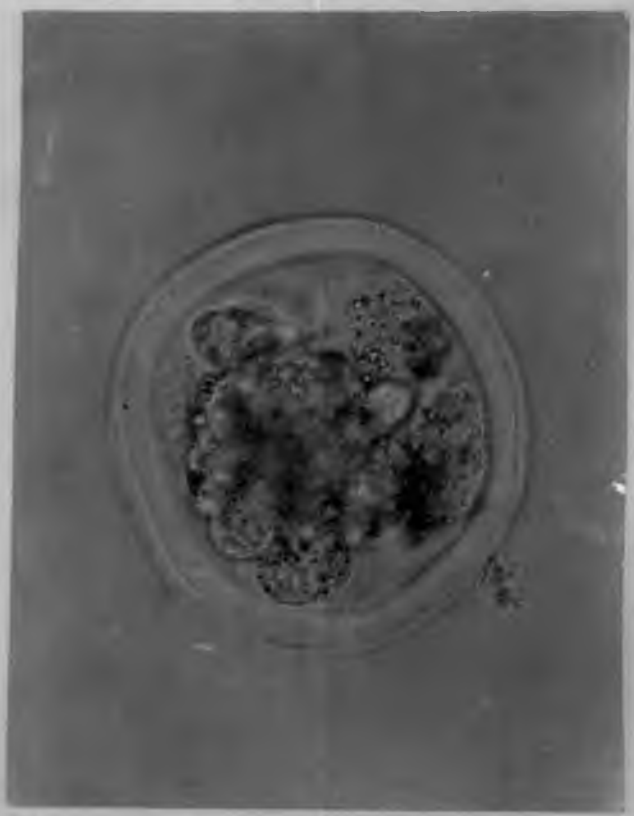


Fig.11. Day 8 embryo
(blastocyst) (X 1000)

Fig. 12. Unfertilized ovum- day 7 (X 1000).

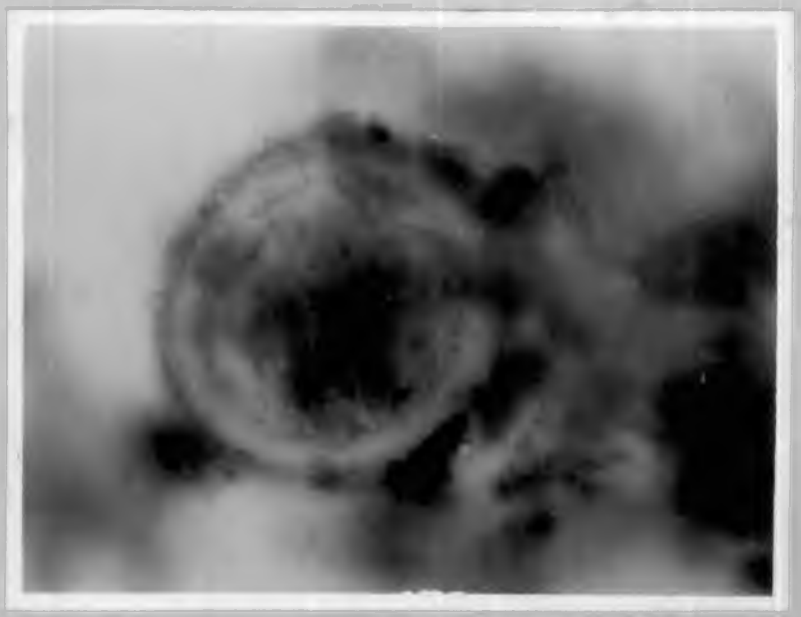


Fig. 13. Degenerating embryo - day 6 (X 750).

FIG - 14
SERUM PROGESTERONE LEVEL (ng/ml)

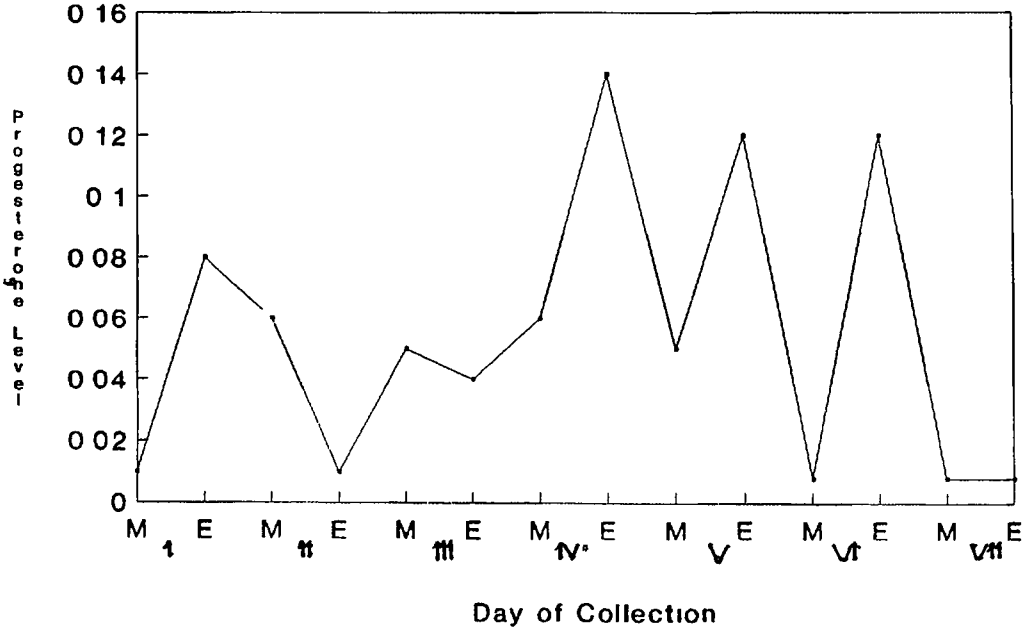


TABLE 1

Group 1

Effect of Superovulation with 34 mg FSH and 25 mg $PGF_2\alpha$

No of Cows	No of corpora Lutea			Embryo collected		Transferable embryos		Non transferable embryos		Fluid recovered (%)
	Right	left	Total	No	percentage	No	percentage	No	percentage	
1	5	3	8	2	25 0	1	50 0	1	50 0	75
2	6	4	10	3	30 0	3	100 0	0	00 0	80
3	6	4	10	4	40 0	3	75 0	1	25 0	75
4	6	5	11	3	27 27	2	66 7	1	33 3	80
5	9	6	15	4	26 66	1	25 0	3	75 0	65
6	8	4	12	6	50 0	6	100 0	0	00 0	90
Mean	6 7±	4 3±	11±	3 7	33 1±	2 7	69 4±	1 0	30 6±	77 5
S E	0 615	0 422	0 966		4 016		11 915		11 915	3 354

TABLE 2

Group 2

Effect of Superovulation with 34 mg FSH and 15 mg $PGF_2\alpha$

No of Cows	No of corpora Lutea			Embryos collected		Transferable embryos		Non transferable embryos		Fluid recovered (%)
	Right	left	Total	No	percentage	No	percentage	No	percentage	
1	3	2	5	3	60 0	2	66 7	1	33 3	85
2	3	2	5	4	80 0	3	75 0	1	25 0	50
3	0	0	0	0	00 0	0		0	00 0	
4	7	4	11	3	27 3	2	66 7	1	33 3	90
5	4	3	7	3	42 9	2	66 7	1	33 3	85
6	0	0	0	0	00 0	0		0	00 0	
Mean	4 25±	2 75±	7 0±	3 25	52 5±	2 2	68 7±	1 0	31 3±	77 5±
S E	0 946	0 479	1 414		11 327		2 075		2 075	9 242

TABLE 3

Group 3

Effect of Superovulation with 20 mg FSH and 25 mg $PGF_2\alpha$

No of Cows	No of corpora Lutea			Embryos collected		Transferable embryos		Non transferable embryos		Fluid recovered (%)
	Right	left	Total	No	percentage	No	percentage	No	percentage	
1	9	5	14	1	7 1	1	100 0	0	00 0	90
2	6	5	11	3	74 3	1	34 3	2	66 7	60
3	4	4	8	3	37 5	2	66 7	1	33 3	95
4	6	5	11	2	18 2	1	50 0	1	50 0	80
5	5	4	9	3	33 3	2	66 7	1	33 3	80
Mean	6+	4 6+	10 6+	2 4	24 6+	1 4	63 34+	1 0	36 7+	81+
S I	0 837	0 245	1 030		5 462		11 061		7 995	6 00

TABLE 4

Group 4

Effect of Superovulation with 20 mg FSH and 15 mg $PGF_2\alpha$

No of Cows	No of corpora Lutea			Embryos collected		Transferable embryos		Non transferable embryos		Fluid recovered (%)
	Right	left	Total	No	percentage	No	percentage	No	percentage	
1	3	5	8	3	37 5	3	100 0	0	00 0	70
2	2	1	3	0	00 0	0	0 0	0	00 0	85
3	5	3	8	1	12 5	1	100 0	0	00 0	50
4	4	4	8	1	12 5	1	100 0	0	00 0	70
5	4	4	8	2	25 0	2	100 0	0	00 0	65
Mean	3 6±	3 4±	7 0±	1 4	21 8±	1 4	100 0			68±
S E	0 510	0 678	1 00		5 984					5 612

TABLE 5

Group 5

Effect of Superovulation with 13 mg FSH and 25 mg $PGF_2\alpha$

No of Cows	No of corpora Lutea			Embryos collected		Fluid recovered (%)
	Right	left	Total	Number	percentage	
1	0	0	0			
2	1	0	1			
3	0	0	0	NIL		
4	0	0	0			
5	2	1	3			90
Mean	0 6	0 2	0 8			90

TABLE 6

Group 6

Effect of Superovulation with 13 mg FSH and 15 mg $PGF_2\alpha$

Sl No	No of Cows	No of corpora Lutea			Embryos collected		Fluid recovered (%)
		Right	left	Total	Number	percentage	
	1	0	0	0			
	2	1	0	0			
	3	1	0	1			
	4	0	0	0			
	5	2	1	3			75
	Mean	0 8	0 2	1 0			75

NIL

Table 7

Effect of superovulation with 3 doses of FSH and 2 doses of $PGF_2\alpha$ (Mean)

No of groups	No of CL (Avg)			No of Embryos (Avg)	Transferable Embryos (Avg)	Non-transferable Embryos (Avg)	Fluid recovery (%)
	Rt	Lt	Total				
Group 1	6 7± 0 615	4 3± 0 422	11± 5 600	3 7	2 7	1 0	77 5± 3 354
Group 2	4 2± 0 946	2 7± 0 479	7 0± 1 414	3 2	2 2	1 0	77 5± 9 242
Group 3	6 0± 0 837	4 6± 0 245	10 6± 1 030	2 4	1 4	1 0	81 0± 6 00
Group 4	3 6± 0 510	3 4± 0 678	7 0± 1 0	1 4	1 4	0	68 0± 5 612
Group 5	0 6	0 2	0 8	0	0	0	90 0
Group 6	0 8	0 2	1 0	0	0	0	75 0

Response of right ovaries between groups
ANOVA Table

Source	DF	SS	MS	F
Group	3	1 66224	0 5540746	4 649198*
Error	16	1 906822	0 1191764	

(Contd)

* (P < 0 05)

(Table 7 Contd)

Total corpora lutea between groups
Anova Table

Source	DF	SS	MS	F
Group	3	2 197159	0 7323863	4 114409*
Error	16	2 848084	0 1780052	

Total Embryos between groups
Anova Table

Source	DF	SS	MS	F
Group	3	948 7149	316 2383	3 77037 *
Error	15	1258 119	83 87461	

Transferable Embryos between groups
Anova Table

Source	DF	SS	MS	F
Group	3	3292 922	1097 641	3 527294 *
Error	15	4667 774	311 1849	

* (P < 0 05)

Table 8

Effect of day of collection on quality of embryos

Sl No	Day of collection	No of Embryos	Transferable Embryos		Non-transferable Embryos		Embryos Transferred		Cows conceived	
			No	%	No	%	No	%	No	%
1	6	15(7)	14	93 3	1	6 7	4	30 8	3	75
2	7	15(7)	10	66 7	5	33 3	3	23 0	1	33
3	8	24(8)	15	62 5	9	37 5	6	46 2	0	0

(Parenthesis No of animals)

Table 9 Percentage of fertilized degenerated and unfertilized ovum collected

No of groups	Total embryos collected	Transferable embryos		Non transferable embryos			
				Degenerated		Unfertilized	
		No	Percentage	No	%	No	%
1	22	16	72 7+ 11 915	2	9 1+ 3 675	4	18 2+ 7 351
2	13	9	69 2+ 2 075	2	15 4+ 1 037	2	15 4+ 1 037
3	12	7	58 3+ 11 061	2	16 7+ 3 199	3	25 0+ 4 799
4	7	7	100	0	--	0	
Mean			72 2+ 6 016	6	11 1+ 1 201	9	16 7+ 1 701

Table 10
Effect of parity on superovulation

Parity	No of CL (Avg)	No of Embryos harvested	No of transferable Embryos		No of non transferable embryos	
			No	%	No	%
Two & below	5 3 (16)	24 (10)	17	70 8	7	29 2
Above two	6 6 (16)	30 (12)	22	73 3	8	26 7

(Parenthesis No of animals)

Table 11
Effect of age on superovulation

Age	No of CL (Avg)	No of Embryos harvested	No of transferable Embryos		No of non transferable embryos	
			No	%	No	%
Below 6 year	4 9 (15)	21 (10)	15	71 5	6	28 5
Above 6 year	6 9 (17)	33 (12)	24	72 7	9	27 3

(Parenthesis No of animals)

Table 12
Effect of season on superovulation

Season	No of CL (Avg)	No of Embryos harvested	No of transferable Embryos		No of non transferable embryos	
			No	%	No	%
Summer	7 7 (13)	24 (11)	17	70 8	7	29 2
Rainy	5 3 (12)	23 (8)	19	82 6	4	17 4
Winter	3 7 (7)	7 (3)	3	42 8	4	57 2

(Parenthesis No of animals)

Table 13

Effect of superovulation on onset of oestrum

Time for onset of oestrum	Groups 1	2	3	4	5	6	Over all (%)
in 36hr	3(50%)	0	1(20%)	0	1(20%)	0	15 7
36 to 48hr	2(33 3%)	1(16 7%)	2(40%)	5(100%)	2(40%)	3(60%)	46 8
48 to 60hr	1(16 7%)	5(83 3%)	2(40%)	0	2(40%)	2(40%)	37 5

Table 14

Effect of superovulation on intensity of oestram

Time for onset of oestrum (symptoms)	Groups 1	2	3	4	5	6	Treat-ment group mean
Weak	3(50%)	1(16 7%)	0	2(40%)	4(80%)	4(80%)	43 8
inter-mediary	0	3(50%)	3(60%)	1(20%)	1(20%)	1(20%)	28 1
intense	3(50%)	2(33 3%)	2(40%)	2(40%)	0	0	28 1

Table 15

Effect of superovulation on duration of oestrum

Duration	Groups						Overall (%)
	1	2	3	4	5	6	
24hr	3(50%)	3(50%)	4(75%)	1(25%)	4(75%)	3(60%)	56 25
48hr	3(50%)	3(50%)	1(25%)	4(75%)	1(25%)	2(40%)	43 75

Table 16

Onset of oestrum in recipients after $PGF_2\alpha$ treatment

Oestrum exhibited	No of animals	Within 36 hr	Between 36 to 48 hr	Between 48 to 60 hr
No of animals	13	4 (30 8%)	4 (30 8%)	5 (38 4%)

Table 17

Effect of superovulation on flushing efficiency

Flushing	Groups						Overall (%)
	1	2	3	4	5	6	
Efficient	4(67 7%)	2(50%)	4(80%)	3(60%)	1(20%)	1(20%)	68 2
Difficult	2(33 3%)	2(50%)	1(20%)	2(40%)	0	0	31 8

Table 18

Effect of superovulation on subsequent reproductive status

	Groups						Overall (%)
	1	2	3	4	5	6	
Normal	4(66 7%)	3(50%)	4(80%)	4(80%)	4(80%)	4(80%)	71 9
Abnormal	2(33 3%)	3(50%)	1(20%)	1(20%)	1(20%)	1(20%)	28 1

Table 19

Effect of superovulation on onset subsequent oestrum

Oestrum	Groups						Overall (%)
	1	2	3	4	5	6	
Within 3 months	3(50%)	1(16 7%)	3(60%)	4(80%)	4(80%)	5(100%)	62 5
3 to 6 months	1(16 7%)	2(33 3%)	1(20%)	1(20%)	0	0	15 66
Over 6 months	2(33 3%)	3(50%)	1(20%)	0	1(20%)	0	21 9

Table 20

Effect of superovulation on subsequent pregnancy

Pregnancy	Groups						Overall (%)
	1	2	3	4	5	6	
Not pregnant within 1 yr	2(33 3%)	2(33 3%)	3(60%)	3(60%)	3(60%)	3(60%)	50
Pregnant within 1 yr	4(66 7%)	4(66 7%)	2(40%)	2(40%)	2(40%)	2(40%)	50

TABLE 21

Effect of superovulation on serum progresterone level (ng/ml)

No. Amimäl	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
	M	E	M	E	M	E	M	E	M	E	M	E	M	E
1	<0.00836	<0.00836	0.1208	<0.00336	0.1164	<0.00836	0.14756	0.34388	0.06036	0.13328	<0.00836	0.10184	<0.00836	<0.00836
2	<0.00836	0.1996	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	0.22696	<0.00836	<0.00836	<0.00836	0.1008	<0.00836	<0.00836
3	<0.00836	0.2076	0.07768	<0.00836	<0.00836	<0.00836	0.11088	0.17808	0.10616	0.18348	<0.00836	0.12504	<0.00836	<0.00836
4	0.04756	0.0546	<0.00836	<0.00836	0.018	0.10568	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836
5	<0.00836	<0.00836	0.14504	0.0468	0.13788	0.1184	0.08412	0.08992	<0.00836	0.40772	<0.00836	0.42824	<0.00836	<0.00836
6	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836	<0.00836
MEAN	0.01	0.08	0.06	0.01	0.05	0.04	0.06	0.14	0.05	0.12	0.008	0.12	0.008	0.008

Discussion

DISCUSSION

With the objective of fixing optimal dose of FSH and $PGF_2\alpha$ for inducing satisfactory superovulation yielding maximum number of normal viable embryos 32 cross bred cows were selected from the University Livestock farm and randomly allotted into six groups Animals in each group were administered FSH and $PGF_2\alpha$ at the rate of 34 mg and 25 mg 34 mg and 15 mg 20 mg and 25 mg 20 mg and 15 mg 13 mg and 25 mg and 13 and 15 mg respectively (Table 1 to 6) FSH P was administered in all animals because of its superior superovulation effect over PMSG (Murphy et al 1984 Donaldson and Ward 1985 Chupin et al 1985) The animals selected for the study were high milk yielders with all desirable traits and having a normal oestrous cycle which is in accordance with that of Seidel (1975) Newcomb et al (1979) and Critser et al (1980 The results obtained and inferences drawn are summarised below

In the present study FSH was administered to donor cows in the mid luteal phase (day 11) and satisfactory response was noticed in all animals which received effective dose of FSH and $PGF_2\alpha$ Phillipo and Rowson (1975) Sreenan (1975) Sreenan and Gosling (1977) Moore et al (1984) Lindsell et al (1986) and Jain et al (1989) also observed better results when FSH was administered at mid luteal phase Lindsell et al (1986)

reported better response on day 9 of cycle than on day 3 to 5 But Moore et al (1984) also recommended FSH administrations between day 0 to 5 also However Goto et al (1987) observed that the presence of functional corpus luteum in the first day of treatment influenced the superovulation response of ovaries The present study revealed that 20 mg FSH and 25 mg of $PGF_2\alpha$ administered at mid luteal phase were optimal doses for inducing satisfactory superovulation on crossbred cows

Bellows et al (1969) noticed a linear relationship between dose increase of gonadotrophins and ovarian response Reports on the dose of gonadotrophins varied in different superovulation studies Elsden et al (1976) administered 32 mg FSH in divided doses Looney et al (1981) advised schedules of either once or twice daily FSH injection in small doses for superovulation studies Barnes et al (1982 observed 10 4±1 5 and 6 0±0 9 corpora lutea with 50 mg and 32 mg FSH respectively while Donaldson (1984) harvested 5 9 and 2 7 transferable embryos with 28 mg and 60 mg of FSH respectively and found that 28 mg was ideal Moore et al (1985) reported premature ovulations with larger doses Pawlyshyn et al (1986) recommended 30 mg FSH as optimal dose for superovulation Becker and Pinheiro (1986) observed poor embryo uptake by the infundibulam with higher doses Kadu et al (1989) reported higher incidence of unfertilised ova

with higher dosage of FSH But Subramaniam et al (1989) found the number of corpora lutea ranged from five to nine and one to five with higher and lower doses of $PGF_2\alpha$ also Totey et al (1991) also opined 28 mg FSH as ideal dose for superovulation Zanwar and Deshpande (1988) obtained better results with higher doses of FSH in crossbred Holstein cows The variations in the effect of different doses in these trials could be attributed to the variations in the genetic make up of the animals as observed by Newcomb et al (1979) and Newcomb (1980)

In the present investigation good response was observed when FSH was administered subcutaneously twice daily for five days at 8 hr intervals in tapering doses ranging from 5 mg to 1 mg Seidel (1975) and Elsden et al (1976) also recommended similar regimen of FSH dose for superovulation in cows Mubishet et al (1986) observed better response when FSH was administered intramuscularly while Karihaloo (1987) observed better results with subcutaneous administration Schallenberger et al (1988) found better response with continuous subcutaneous infusion of FSH Bodhipaksha (1988) observed satisfactory results when FSH was administered as 4 injections in descending levels But Zanwar and Deshpande (1988) successfully administered FSH twice daily in descending as well as constant doses in superovulation trials However Manickam et al (1990) attributed these variations in the response to nutritional genetic and managemental factors also

In the present study $PGF_2\alpha$ was administered on day 14th of the cycle to the donor cows in accordance with the findings of Sreenan (1975) Betteridge (1977) Marshall and Struther (1978) However Rodrigues and Gregory (1986) could not observe any significant difference when $PGF_2\alpha$ was administered at different intervals after the beginning of superovulation treatment

Dose of $PGF_2\alpha$ administered in the present study was 25 mg and 15 mg in different groups It could be seen from table 7 that the mean corpora lutea formed in group 1 3 and 5 where 25 mg $PGF_2\alpha$ was administered were 11 0±5 600 10 6±1 030 and 0 8 respectively The corresponding values in group 2 4 and 6 where 15 mg of $PGF_2\alpha$ was administered were 7 0±1 414 7 0±1 0 and 1 00 respectively

Data indicate that animals which received 25 mg of PGF_2 showed better response than the other group This is in ful agreement with the findings of Zanwar and Deshpande (1988) Rosenberg et al (1990) Manickam et al (1990) and Subramanian et al (1991) Though Douglas and Ginther (1975) reported that dose of $PGF_2\alpha$ when increased delayed the onset of ovulations Sreenan (1975) Marshall and Struther (1978) Seidel et al (1978) and Bhattacharya et al (1989) used higher doses of $PGF_2\alpha$ for superovulation treatments with satisfactory results

It could be seen from Table 13 that wide variation existed on onset of oestrum in animals treated with 25 mg and 15 mg $PGF_2\alpha$ In group 1 3 and 5 which received 25 mg of $PGF_2\alpha$ oestrum was exhibited in 36 hr after $PGF_2\alpha$ treatment in 50 20 and 20 per cent of animals and within 36 to 48 hr in 33 3 40 and 40 per cent animals and in 48 to 60 hr in 16 7 40 and 40 per cent of animals respectively These findings are in agreement with Eddy (1977) who observed wide variability in the onset of oestrum in superovulated cows and attributed this to the variability in the duration of pro oestrus period prior to induced oestrum However varying periods were observed in the onset of oestrum by Nair and Madhavan (1984) (42 to 45 hr) Yadav et al (1986) (42 hr) Lindsell (1986) (42 hrs) Schallenberger et al (1988) (42 48 hr) Manickam et al (1990) (48 hr) and Mohmood et al (1991) (72 hr)

Among the superovulated animals 50 per cent in group one showed weak oestrus while rest of the animals showed intense symptoms Similarly 60 and 40 per cent of animals in group 3 showed intermediary and intense symptoms It could be seen that better oestrus symptoms were exhibited by animals in the group 3

Perusal of literature revealed paucity of information on intensity of oestrus in superovulated cows However Douglas

and Ginther (1975) noticed interovulatory period with low intensity when dose of $PGF_2\alpha$ was increased Looney (1986) observed that ovulation was spread over a period of 18 hr when FSH was administered in smaller doses Similar observations were made by Madan (1988) also

In superovulated animals the duration of induced oestrum was 24 hr in 50 per cent of animals in group I and 2 while the same was 75 and 25 per cent in the groups 3 and 4 respectively It was seen when 20 mg of FSH and 25 mg of $PGF_2\alpha$ were administered shorter duration of oestrum was noticed in more number of animals But when the $PGF_2\alpha$ dose alone was reduced to 15 mg the duration of oestrus was higher in majority o animals When 34 mg of FSH was administered with 25 mg as well as 15 mg of $PGF_2\alpha$ no difference in the response was noticed In the present study shorter duration of oestrum was noticed in more number of animals which responded to treatments This agrees with the findings of Angel (1979) Shea et al (1983) Yadev et al (1986) and Looney et al (1986) However Maxwel et al (1978) and Madan et al (1991) reported longer duration for the completion of ovulations in superovulated cows

Superovulation response was assessed by rectal examination of the ovary for the number of corpora lutea developed and the accuracy of this was checked by clinical examination of ovaries

after slaughter of superovulated cows (Fig 5 to 8) The results of the rectal examinations were found to be in agreement as reported by Dawson et al (1975) Elsden et al (1976) Sharifuddin and Jainudeen (1983) and Monnieaux et al (1983)

The level of progesterone in the serum of superovulated cows ranged from <0 008 to 0 42824 ng/ml from day 1 to 7 of induced oestrum The level showed a gradual increase (Table 21 and Fig 14) following gonadotropic treatment in concurrence with the findings of Henrick et al (1973) Rajamahendran et al (1976) Sreenan and Gosling (1977) Saumande (1980) Maurer and Echternkamp (1982) Jensen et al (1982) Waltan and Stubbings (1986) and Goto et al (1987) The level declined 3 days before oestrus and reached to the lowest point on day of oestrum Booth et al (1975) reported the lowering of level from 4 days before oestrus and reached the lowest point on day 2 of oestrus The results of the present study are also in general agreement with the above findings However wide variations in the level of progesterone were observed in different animals Similar variations were encountered by many workers earlier also Fournier et al (1976) Saumande (1980) and Yadev et al (1986) reported contradicting levels of progesterone in the serum while Lindsell et al (1986) and Goto et al (1987) observed uniform

higher values in the superovulated animals Lindsell et a (1986) reported no relation between level of progesterone and superovulation response However Goto et al (1987 noticed more number of corpora lutea when pretreatment progesterone level was higher In the present investigation also it was noticed that the level of progesterone did not influence superouvlation response and varied widely in different animals This is in agreement with the earlier reports

Perusal of data presented in Table 1 revealed that the number of corpora lutea in the right ovary in group 1 ranged from 5 to 9 with a mean of 6 7±0 615 In the left ovary t e values were 3 to 6 with an average of 4 3±0 422 A total o 11 0±0 966 corpora lutea were observed in both the ovaries on an average and ranged from 8 to 15

In group 2 the number of corpora lutea developed in the right ovary ranged from 0 to 7 with an average of 4 25±0 94o while those in left ovary ranged from 0 to 4 with a mean o 2 75±0 479 The total number of corpora lutea developed in bo n the ovaries averaged 7 0±1 414 and ranged between 0 to 11

In group 3 the number of corpora lutea in the right ovar was within a range of 4 to 9 with a mean of 6 0±0 837 while in the left it varied from 4 to 5 with a mean of 4 6±0 245 The

total number of corpora lutea in both ovaries were 10 6±1 030 and ranged between 8 to 14

The number of corpora lutea in group 4 ranged from 2 to 5 with a mean of 3 6±0 510 in right 1 to 5 with a mean of 3 4±0 678 in the left and the total number of corpora lutea ranged from 3 to 8 with a mean of 7 0±1 00

Superovulation response of animals in group 5 indicated that right ovary responded within a range of 0 to 2 with a mean of 0 6 while in one animal only the left ovary responded with a single corpus luteum Total number of corpora lutea in both the ovaries ranged from 0 to 3 with an overall mean of 0 8

In group 6 response of right ovary was within a range of 0 to 2 with a mean of 0 8 while the same for left ovary was ranging from 0 to 1 with a mean of 0 2 Total number of corpora lutea developed in both the ovaries ranged from 0 to 3 with a mean of 1 0

Analysis of data revealed that there was significant influence ($P < 0\ 05$) of different doses on the number of corpora lutea in the right ovary while no such difference was noticed in the left ovary This is in accordance with the findings of Betteridge (1977) Saumande (1980) Murphy *et al* (1984)

Bhattacharya et al (1989) and Manickam et al (1990) who also reported significant influence of different dose of FSH and $PGF_2\alpha$ on superovulatory response of right ovary It was also revealed that there was significant difference between group 1 and 2 group 1 and 4 and group 3 and 4 However no significant difference was noticed between group 1 and 3 and group 2 and 3 The result indicate that 34 mg of FSH and 25 mg of $PGF_2\alpha$ had homogenous effect However from the mean it could be seen that 20 mg of FSH and 25 mg of $PGF_2\alpha$ was superior to 34 mg of FSH and 25 mg of $PGF_2\alpha$ in inducing superovulation

Total number of corpora lutea with varying doses of FSH in both the ovaries ranged from 0 to 15 in the animals under different groups Statistical analysis also revealed identica response in group 1 and 3 It was also noticed that increase in the dose of FSH beyond an optimal level did not improve the superovulation response This is in concurrence with the findings of Bellows et al (1969) Seidel (1975) Donaldson (1984) and Moore et al (1985)

The satisfactory response of superovulation with FSH P in the present study concur with the earlier findings of Murphy et al (1984) Chupin et al (1985) and Callesen et al (1986) which concur the superiority of FSH over PMSC Similarly the time of administ ation of FSH (mid luteal phase)

and selection of donors were also found to influence superovulation response in the study which concur with the findings of Erickson (1966) Hill et al (1970) Newcomb (1979) and Lamond (1972) and also Phillipo and Rowson (1975) Karihaloo (1987) and Jain et al (1988)

From Table 7 it could be seen that the average number of embryos recovered were 3 7 3 2 2 4 and 1 4 in groups 1 to 4 respectively Statistical analysis revealed significant influence of different doses of FSH and $PGF_2\alpha$ on embryo harvest Significant difference ($P < 0.05$) was also observed between groups 2 and 3 and between 2 and 4 It was also seen that group 2 was significantly different ($P < 0.05$) from group 3 and 4 and no difference was noticed between group 1 and 2 and group 1 and 3 and group 3 and 4 Since these groups were found homogenous in action 20 mg and 34 mg of FSH in combination with 25 mg of $PGF_2\alpha$ showed the same influence on embryo harvest Since 25 mg and 15 mg $PGF_2\alpha$ in group 1 2 3 and 4 did not show any difference in the number of embryos it could be assumed that the increase in dose of FSH and $PGF_2\alpha$ beyond an optimal level would not influence embryo yield positively Observations of Donaldson (1983) Anon (1987) Bhattacharya et al (1989) Madan et al (1989) and Subramaniam et al 1991 are in agreement with the present findings However higher rates of embryo yield was reported with higher

dose of FSH and $PGF_2\alpha$ by Elsden et al (1976) Zanwar (1988) Kadu et al (1989) and Manickam et al (1990) also

The total number of transferable embryos harvested from animals subjected to superovulation ranged from 1 to 6 and averaged 2 7 (69 4±11 915 per cent) 2 2 (68 7±2 075 per cent) 1 4 (63 3±11 061 per cent) and 1 4 (100 per cent) in groups to 4 respectively Statistical analysis revealed that animals in group 4 significantly ($P< 0 05$) differ in the number of transferable embryos produced when compared with animals in group 1 2 and 3 But animals in group 1 2 and 3 showed homogenous effect with regard to the percentage of transferable embryos collected

This t appeared that increase in the dose of FSH from 20 mg and $PGF_2\alpha$ from 15 mg did not show ny increase in the eld of transferable embryos The present results concur with the findings of Ambrose et al (1989) Jain et al (1989) Sinha et al 1989) and Madan et al (1989) Donaldson (1985) noticed increase in the percentage of transferable embryos when $PGF_2\alpha$ was given as 3 smaller doses rather than as 2 doses Massey and Oden (1984) reported higher percentage of transferable embryos in exotic breeds However Zanwar and Deshpande (1988) collected more number of transferable embryos with larger doses of gonadotrophins On the contrary lo

percentage of transferable embryos was harvested with lower doses of gonadotrophins also by Anon (1987) Kadu et al (1989) and Bhattacharya et al (1989)

Number of embryos collected on day 6 7 and 8 of oestrum were 15 15 and 24 respectively (Table 8) and analysis of data revealed no significant difference between the day of collection on embryo harvest Though more number of embryos could be collected on day 8 than on day 6 and 7 the percentage of transferable embryos was more on day 6 in the present study Variations have been noticed regarding the success rate of embryos harvested on different days and Elsden et al (1976) collected more transferable eggs on day 6 and 7 than on day 5 or 8 Seidel et al (1978) also reported better harvest on day 6 which agree with the result of present study However Greenan (1978) and Greve et al (1977) observed better rates on day 7 and 8 than on day 5 or 6

Present study revealed that parity had no influence either on the number of corpora lutea formed or on embryos harvested However Moore et al (1975) noticed better ovulatory response in heifers than in older cows

When the data were arranged according to age of animals it as revealed that age had no influence on the number of corpora

lutea produced or embryo harvested (Table 11) Hasler et al (1981) also observed no influence of age on superovulation or embryo survival rate On the contrary Erickson (1966) Erickson et al (1976) Gordon (1983) and Donaldson (1984 d) observed significant influence of age on superovulation

Although season was statistically found to have no influence on superovulation the number of corpora lutea and transferable embryos were more during summer than rainy or winter seasons The seasonal influence on reproductive functions in Indian cattle has been reported by Randel (1984) Variations in superovulation response during the month in each year and in successive years were reported by Shea et al (1984) They noticed poor response during winter months Present study also revealed poor superovulation response during winter season However Crister et al (1980) failed to observe any seasonal effect on superovulation

Recovery of flushing fluid was 77 5 per cent from animals in group 1 and 2 while the same were 81 68 90 and 75 per cent in animals in group 3 to 6 respectively

It may be noticed that the flushing efficiency was more (81 per cent) in group 3 (Table 3 which had maximum cervical dilatation as the animals in the group received 20 mg of FSH

and 25 mg of $PGF_2\alpha$ The results of present investigation appear to indicate that 20 mg of FSH with 25 mg of $PGF_2\alpha$ as optimal dose for better cervical dilatation and maximum fluid recovery There is paucity of information on the relationship between dose of FSH and $PGF_2\alpha$ with regard to flushing efficiency Brand et al (1977) and Greve et al (1977) also reported comparable results However Manickam et al (1990) opined that difficult flushing resulted from unsuccessful penetration of catheter transcervically due to poor alignment of cervical folds

Among the recipient animals synchronised 30 8 per cent each evinced oestrus within 36 hr and 36 to 48 hr after treatment while 38 4 per cent showed oestrum within 48 to 60 hours The results indicate that recipient animals showed oestrus at varying periods after synchronization treatment Munar and Nigro (1986) Davis et al (1987) and Pant and Singh (1991) also observed majority of animals in oestrus within a duration of 80 hr But Cavestany and Foote (1985) observed most of the animals in synchrony earlier after $PGF_2\alpha$ treatment

Results of transfer of embryos to recipient cows showed that out of 4 cows 3 became pregnant (75 per cent) when the day of collection was 6 When the day of collection was 7 the

percentage of conception rate was 33 3 and on day 8 the percentage was 0 The total percentage of conception after embryo transfer was 30 7 It could be inferred from the preset study that the best time for embryo harvest from donor cows is day 6

The calving rate in the present investigation was zero This can be attributed to the early embryonic loss and abortions Abortions were reported by Brand et al (1977) between day 60 and 90 after transfer Christie (1980) also reported embryonic loss due to faulty displacement of embryos in the uterine lumen after embryo transfer However high pregnancy was encountered by many workers also Seidel et al (1978) Heyman et al (1977) Greve et al (1977) and Jain et al (1989) obtained better pregnancy through embryo transfer which can be attributed to improvement in the technology

From the foregoing paragraphs it is evident that administration of 20 mg of FSH P and 25 mg $PGF_2\alpha$ induced satisfactory superovulatory response in crossbred cows as evinced by number of corpora lutea Increase in the level of FSH did not have any beneficial effect on the superovulatory response The response to superovulation was more in the right ovary than in the left ovary Administration of FSH P at mid luteal phase (Day 11) was found to be the ideal time for

induction of superovulation Wide variation in the effect of different doses of hormone in the present investigation could be attributed to the different genetic make up of the animals

Wide variations have been observed in the onset of oestrum after administration of 2 doses of $PGF_2\alpha$ These variations could be attributed to the variability in the duration of prooestrum prior to induced oestrum Shorter duration of oestrum was noticed in more number of animals which responded to treatments

The level of progesterone showed a gradual increase following gonadotropic treatment and declined 3 days before oestrum and reached the lowest level on day of oestrum

Significant influence of different doses of FSH and PGF_2 was observed on embryo harvest However their increase beyond an optimal level had no positive influence on embryo recovery Similarly increase in the dose of FSH from 20 mg and $PGF_2\alpha$ from 15 mg did not show any increase in the yield of transferable embryos Analysis of data also showed no significant differences between day of collection and number of embryos harvested It was also observed that age and parity of the cows did not significantly influence either the number of corpora lutea formed or the number of embryos harvested

Although statistically not significant the number of corpora lutea formed and the number of transferable embryos collected during the summer months were more than those in rainy or winter months Better cervical dilatation and flushing efficiency were observed in animals that received 20 mg FSH and 25 mg $PGF_2\alpha$

Best time for embryo harvest was observed to be day 6 based on conception rates of embryos transferred None of the embryos transferred on day 8 conceived Although the data were not adequate to reach a conclusion the results point to a significant influence of day of collection on conception in recipient cows The calf crop in the present investigtion was zero although the total conception rate was 30 7 This could be attributed to early embryonic loss and early abortions

Since the present investigation cannot be deemed comprehensive further detailed investigation on large number of animals is warranted to ensure higher embryo crop and better calving rate

Summary

SUMMARY

The aim of the present investigation was to fix optimal dose of FSH and $PGF_2\alpha$ for inducing successful superovulation to ascertain the appropriate time for harvesting the pre implantation embryo from the donor cow to estimate the percentage of transferable and non transferable embryos that can be harvested to standardize the proper time for synchronization of oestrum of donor cows and to check the hormonal profile of superovulated cows with the ultimate objective of standardising non surgical embryo transfer technique in crossbred cows The effect of superovulation treatment on onset intensity and duration of induced oestrum influence of parity and age of animal and season of treatment on superovulation were also studied The flushing efficiency and fluid recovery with different doses of FSH and $PGF_2\alpha$ and conception rate after embryo transfer were also studied

The material used for the study consisted of 32 crossbred cows belonging to the University Livestock Farm attached to the College of Veterinary and Animal Sciences Mannuthy They were maintained under identical conditions of feeding and management and were stall fed These animals were randomly allotted into six groups on the basis of 3 doses of FSH and 2 doses of $PGF_2\alpha$ administered in the following manner

Group 1 consisted of 6 animals treated with 34 mg of FSH and 25 mg of $PGF_2\alpha$ group 2 with 6 animals received 34 mg of FSH and 15 mg of $PGF_2\alpha$ group 3 with 5 animals received 20 mg of FSH and 25 mg of $PGF_2\alpha$ group 4 with 5 animals received 20 mg of FSH and 15 mg of $PGF_2\alpha$ Group 5 with 5 animals administered with 13 mg of FSH and 25 mg of $PGF_2\alpha$ while in the last group (6) 5 animals received 13 mg of FSH along with 15 mg of $PGF_2\alpha$

FSH was given subcutaneously as divided tapering doses morning and evening from day 11 to 15 with standing heat taken as day 0 $PGF_2\alpha$ was administered on the evening of day 14 skipping the FSH dose Flushing of donor cow was done from day 6 to 8 of induced oestrum Superovulation response was ascertained by checking the number of corpora lutea developed by clinical examination of ovaries

In animals treated under group 1 the number of corpora lutea in the right and left ovaries was 6 7±0 615 and 4 3±0 22 respectively Overall mean corpora lutea in both the ovaries together was 11 0±0 966 Average embryo harvest in this group was 3 7 and out of 22 embryos collected 16 (72 7±11 91 per cent) were transferable Mean fluid recovery was 77 5±3 35 per cent Animals in group 2 yielded 4 25±0 946 and 2 75±0 479 corpora lutea in right and left ovaries respectively while the

group average was 7 0±1 414 Total number of embryos collected was 12 (mean 3 25) with 68 7 per cent transferable embryos Fluid recovery from the animals in the group was on an average 77 5±9 242 per cent. Corpora lutea in right and left ovaries in animals in group 3 were 6 0±0 837 and 4 6±0 245 respectively Total number of corpora lutea developed in both the ovaries was 10 6±1 030 Out of 12 embryos harvested 7 (6 34±11 06 per cent) were of transferable grade Mean fluid recovery from animals in the group was 81 0±6 00 Animals in group 4 yielded 3 6±0 810 and 3 4±0 678 corpora lutea in right and left ovaries respectively The overall mean value of total corpora lutea in both ovaries was 7 0±1 0 Total number of embryos harvested was 7 and all the embryos (100 per cent) were transferable Mean fluid recovery from the animals in the group was 68 0±5 612 None of the animals in group 5 and 6 showed satisfactory superovulation response and no embryo could be collected from the animals in these two groups

Statistical analysis revealed significant difference in the response of right ovary between groups ($P < 0.05$) Significant difference was observed between group 1 and 2 1 ^ 4 and 3 and 4 The difference noticed between group 1 and 3 2 and 3 and 2 and 4 were not significant Difference in the total number of corpora lutea in both ovaries between groups ($P<0.05$)

was also significant Group 1 showed significant difference from group 2 and 4 Group 2 showed significant difference from group 4 also However homogeneous response was noticed between animals in group 1 and 3 and 2 and 4 It was also seen that response of animals in group 2 was significantly different ($P < 0.05$) from group 3 and 4 with regard to total embryos harvested Similarly group 2 showed significant difference from group 3 ($P < 0.05$) However homogeneous response was noticed in animals in group 1 and 2 1 and 3 1 and 4 and 3 and 4 The number of transferable embryos collected from animals in group 4 was significantly ($P < 0.05$) different from group 1 2 and 3 But response was similar in animals treated in groups 1 2 and 3 Emrbyos collected on day 6 7 and 8 after induced oestrum were 15 15 and 24 respectively with highest number (93 3 per cent) of transferable embryos on day 6 and lowest (62 5 per cent) on day 8 However no significant difference was noticed on the number of transferable embryos collected on different days Similarly it was also observed that parity and age of animals had no influence on superovulation response and embryo harvest The number of corpora lutea was highest in rainy (7 7) and lowest in winter (3 7) seasons Similarly the number of transferable embryos (42 8 per cent) was lowest in winter months However analysis of data revealed no significant difference on number of transferable embryos between seasons Overall percentage of animals evinced oestrus

within 36 hrs 36 to 48 hr and 48 to 60 hr after superovulation treatment were 15 7 46 8 and 37 5 respectively Weak oestrous symptoms were shown by 50 16 7 0 40 80 and 80 per cent of animals (overall 43 8 per cent) in group 1 to 6 respectively while 0 50 60 30 30 and 20 per cent (overall 28 1 per cent) of animals showed intermediary symptoms Intence oestrus was noticed in 50 33 3 40 40 0 and 0 per cent (overall 28 1 per cent) of animals in group 1 to 6 respectively Length of oestrum in superovulated cows was 24 hr in 50 50 25 75 25 and 40 per cent (overall 56 2 per cent) cows 48 hr in 50 50 25 75 25 and 40 per cent (overall 43 75 per cent) of animals in group 1 to 6 respectively In the recipient animals after synchronisation treatment 30 8 per cent of animals each showed oestrum within 36 hr and between 36 to 48 hr while 38 4 per cent showed oestrum between 48 to 60 hr Flushing was efficient in 66 7 and 50 per cent of animals in group 1 and 2 80 and 60 per cent in group 3 and 4 and 20 per cent each in 5 and 6 respectively with an overall mean of 68 2 per cent (Table 16) Flushing was difficult in 33 3 50 20 and 40 per cert of animals in group 1 to 4 respectively while in group 5 and 6 one animal each responded with easy flushing Subsequent to superovulation treatment and flushing (Table 18) normal reproductive status was noticed in 66 7 and 50 per cert in group 1 and 2 and 80 per cent each in groups 3

to 6 The overall mean of animals with normal reproductive tract was 71 9 per cent Genital disorders were seen in 33 3 and 50 per cent of animals in group 1 and 2 and 20 per cent each in groups 3 to 6 On an average 28 1 per cent of total animals showed genital disorders after superovulation treatment Similarly normal oestrum was noticed within 3 months of superovulation in 50 16 77 and 60 per cent of animals in group 1 to 3 respectively and 80 per cent each in group 4 and 5 and all animals in group 6 Overall percentage of animals showing oestrum within 3 months was 62 5 Oestrum was seen within 3 to 6 months in 16 7 and 33 3 per cent of animals in group 1 and 2 20 per cent each in animals in group 3 and 4 and none in groups 5 and 6 Overall percentage of animals in group 1 to 6 which showed oestrum in 3 to 6 months was 15 66 Similarly 33 3 and 50 per cent of animals in group 1 and 2 20 per cent each in groups 3 to 5 and none in group 6 showed oestrum after 6 months (overall percentage 21 9) subsequent to superovulation and flushing Percentage or animals conceived on subsequent breeding after completion of superovulation study was 66 7 in group 1 and 2 and 40 per cent each in group 3 to 6 with an overall percentage of 50 (Table 19)

Serum progesterone level in superovulated cows ranged from <0 008 ng/ml to 0 428 ng/ml in the different animals from day 11 to 17 of the cycle (Table 20) The mean value on day of starting (day 11) superovulation treatment was 0 01 ng/ml in the morning and 0 08 ng/ml in the evening An increase of 0 06 ng/ml and 0 01 ng/ml (morning and evening) was noticed on day 12 The level increased gradualy to 0 6 and 0 14 ng/ml in morning and evening respectively on day 14 Lowering of progesterone level was noticed from day 5 to reach the lowest minimum (0 008 ng/ml) on day of induced oestrum Conception rate after transfer of embryos was 33 3 per cent while calving rate was found to be zero

To sum up it could be stated that significant influence of different doses of FSH and $PGF_2\alpha$ was observed on embryos harvested but 20 mg of FSH and 25 mg of $PGF_2\alpha$ induced satisfactory superovulation response in crossbred cows However increase in the level of gonadotrophin did not show any beneficial effect on superovulation response The response of right ovary was better than the left ovary between groups It was seen that when FSH was administered during the mid luteal stage of cycle (day 11) satisfactory response was shown by most of the animals

Day of collection did not show any significant influence on the number of transferable embryos But percentage of transferable embryos and pregnancy rates were higher when collections were made on day 6 Parity and age were found to have no influence on superovulation and embryo harvest Number of embryos collected and percentage of transferable embryos were lowest during winter months Shorter duration of oestrum was noticed in more number of donor cows which responded to superovulation treatments Recipient animals showed wide variation in the onset of oestrum after synchronization treatment

Better cervical dilatation and flushing efficiency were observed in donor animals that received 20 mg of FSH and 25 mg of $PGF_2\alpha$ The level of progesterone increased following superovulation treatment and declined 3 days before oestrum and reached the lowest level on day of oestrum Pregnancy rate achieved in the study was 33 3 per cent while the calving rate was zero Further detailed investigations on larger number of animals are warranted to ensure higher embryo crop and better cal ving rate

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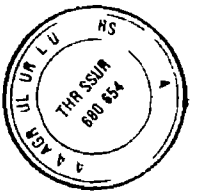

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SUPEROVULATION, SYNCHRONIZATION OF OESTRUS AND EMBRYO TRANSFER IN CROSSBRED COWS

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ABSTRACT OF A THESIS

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ABSTRACT

The object of the present investigation was to ascertain the influence of varying doses of FSH and $PGF_2\alpha$ on various parameters of superovulation and embryo collections with the ultimate aim of fixing the optimum dose of FSH and $PGF_2\alpha$ required for successful superovulation in crossbred cows Detailed investigation was made in a herd of 32 crossbred cows belonging to the University Livestock Farm attached to the College of Veterinary and Animal Sciences Mannuthy during the period from April 1989 to December 1991 The animals were randomly divided into 6 groups based on 3 doses of FSH and 2 doses of $PGF_2\alpha$ administered

It was observed that 20 mg of FSH with 25 mg of PGF_2 induced satisfactory superovulation response and 20 mg of FSH with 15 mg of $PGF_2\alpha$ resulted in better embryo quality Further increase in the dose did not show any improvement in the responses Right ovary showed more response than the left Day 11 was found to be ideal for starting superovulation treatment and day 6 for embryo collection though statistically no significant influence was noticed by day of collection on superovulation response Parity and age of donors had no influence on superovulation Rainy and summer months were found to be better seasons for superovulation

treatment than winter Shorter duration (24 hr) of oestrum was noticed in more number of animals

20 mg of FSH and 25 mg of $PGF_2\alpha$ (group 3) yielded better cervical dilatation and flushing efficiency Level of progesterone increased following superovulation treatment and declined 3 days before oestrum and reached the lowest level on day of oestrum Pregnancy rate achieved was 33 3 per cent but calving percentage was nil