SUPEROVULATION AND EMBRYO RECOVERY IN RABBITS

By
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THESIS

Submitted in Partial fulfilment of the
requirement for the degree

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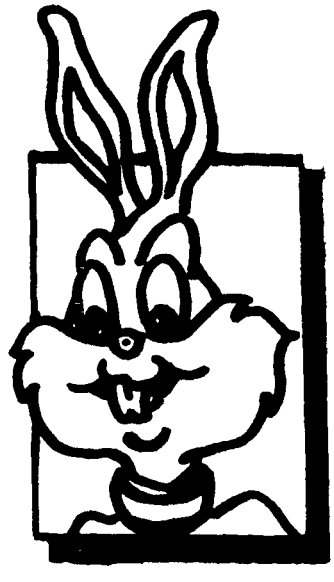
Department of Animal Reproduction
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY. THRISSUR

1997

Dedicated to my
beloved parents



DECLARATION

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

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CERTIFICATE

Certified that the thesis, entitled "**SUPEROVULATION AND EMBRYO RECOVERY IN RABBITS**" is a record of research work done independently by **Dr. Sathesh Kumar, S.**, 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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Introduction

INTRODUCTION

Most of the revolutionary improvements in animal agriculture in the last three decades could be attributed to the development and application of assisted reproductive technology. While it has been possible to exploit the genetic potential of outstanding sires through artificial insemination, the embryo transfer technology offers further avenue to exploit the superior genetic material from both sire and dam. When used in combination with multiple ovulation technique, embryo transfer exponentially increases the availability of valuable genetic material. If embryo transfer studies were to take a patron saint, it would have to be necessarily - Walter Heape. This is, just, not because he performed the first embryo transfer at the end of the last century but because of his immense influence on reproductive sciences that are fundamental to the research work of today (Betteridge, 1981). Although, the transfer study of Heape (1891) in rabbits was aimed to answer the scientific question about the influence of uterine environment on embryo's phenotype, his contribution to applied reproductive science laid a foundation to the animal breeding industry with emphasis on its economic importance.

Laboratory animal models are indispensible for comprehensive training and continuing education in the wide

range of disciplines embraced by reproductive biology. The basic research with rabbits and other lab animals has contributed much for the potential application of biotechnology in the farm animals. The major factor hindering the basic research to be conducted in domestic species is the non-availability of such costly animals for experimental purpose. The hi-tech knowledge for the recovery, evaluation, manipulation, storage and transfer of rabbit and mouse embryos is an excellent training for handling the valuable embryos of domestic animals also.

Rabbits are used all over the world as models for biomedical research because they are particularly useful species for the study of embryos. They are one of the few species in which ovulation is induced by mating or by hormonal stimulation, thus allowing an exact timing for reproductive events. Furthermore migration of embryos between uterine horns is very limited and thus correlations of ovulation rate in one ovary with embryo survival in the ipsi-lateral and contra-lateral uterine horns can be estimated separately (Blasco *et al.*, 1993). Therefore, the rabbit has many technical advantages over other polytoccus species.

As an agricultural species, rabbits are also used for meat and pelt production. The present livestock population in the third World countries are not adequate to solve the

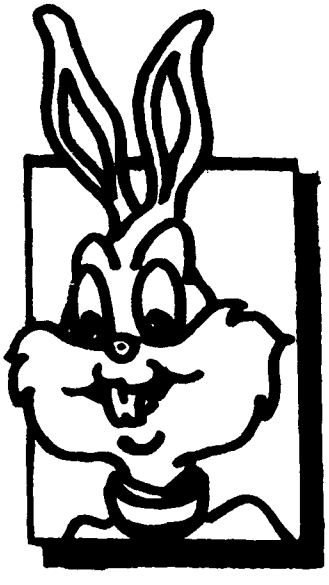
problem of increasing protein shortage. Needless to say that with the disappearance of grazing lands and fragmentation of land holdings, sheep and goat rearing has become rather a costly affair for marginal farmers thereby pushing up the mutton prices. To meet the increased demand for animal protein, rearing of non-traditional meat animals suitable for small scale farming such as broiler rabbits need to be introduced and encouraged. Rabbit is an ideal choice in this regard with its desirable characters like rapid growth, short generation interval, high prolificacy, lower meat cholesterol level and lesser cost involved in rearing. According to Lifton (1980) one doe can produce 14 times her own bodyweight as meat in one year.

In India, the importance of rabbit as meat animal has been realised only recently and so rabbit farming has gained momentum only in the recent past. Kerala is one of the most ideal state for development of rabbitry as a major industry, as it has an overwhelming majority of meat consumers with no social taboo or sentiment against rabbit meat consumption. According to 1987 census, the rabbit population in Kerala was estimated to be 77, 198 (Raja and Mukundan, 1995). If appropriate attention and encouragement is given rabbitry will emerge as a prosperous meat industry.

For rabbit industry to be profitable it is necessary that the doe produces as many kids as possible per unit time. This can be manipulated by proper reproductive management of rabbits. Various biotechniques are routinely used to secure and increase production of embryos. Embryo transfer technology has been used as a major breeding tool for rapid multiplication of genetically superior animal, so as to improve the embryo-potential. A critical aspect of embryo transfer technology is the use of gonadotrophins to induce multiple ovulations from the ovaries thereby increasing the embryo yield from a single animal. Superovulation in polytoccus species especially rabbits produces a homogenous group of ova by drawing on a large pool of follicles that have attained some degree of maturity. With current procedures, superovulation increases the yield of normal embryos, about five fold in the rabbit (Hafez, 1993).

Thus considerable work on techniques like superovulation of donors, embryo recovery, storage of embryos and transfer to recipients, has made it possible to increase the reproductive rate of the rabbit doe to a significant degree. Implementation of these techniques in the breeding and production of rabbits can make rabbit farming a subsidiary occupation for additional income to the rural populace.

The present work, in rabbits, is aimed at studying the superovulatory response and embryo recovery in two different breeds of rabbits, Newzealand White and Soviet Chinchilla. The ultimate objective is to employ the superovulation and embryo transfer technology as a tool for the active promotion of broiler rabbit industry in our state.



Review of Literature

REVIEW OF LITERATURE

2.1 Superovulation treatment

Superovulation treatments are used in embryo transfer programmes to increase the yield of embryos. In rabbits variety of gonadotrophins are used for this. Usually, subcutaneous or intramuscular injections of PMSG or FSH are given to stimulate additional follicular growth which is then followed by intravenous administration of HCG or LH a few days later to induce ovulation. Gonadotrophin preparations vary considerably in potency and hence a wide range of dosage has been employed by many workers.

2.1.1 PMSG with HCG

Hafez (1961 and 1970) and Maurer *et al.* (1968) obtained good superovulatory response in rabbits administered with 50 IU of PMSG subcutaneously for three days followed by intravenous injection of 50 IU HCG on the fourth day of PMSG treatment.

Ishijima *et al.* (1967 and 1969a) induced superovulation in rabbits by administering 150 IU PMSG for three days followed after 24 hours by 20 rabbit units HCG.

Hirano (1971) superovulated rabbits by subcutaneous administration of 40 IU PMSG for 5 days followed by 50 IU HCG on the sixth day.

Adult rabbits were superovulated by Fujimoto *et al.* (1973, 1974a and 1974b) with intramuscular injection of 100 IU PMSG for 4 days followed by 100 IU HCG on the fifth day.

Rutkis and Bekhtina (1975) successfully superovulated does by administering 100 IU PMSG followed 72 hours later by 250 IU HCG.

Rottmann and Lampeter (1981) and Rottmann and Bieniek (1982) stated that rabbits could be superovulated with 60 IU PMSG followed 72 hours later by 60 IU HCG. Yuqi and Min (1981) and Mkrtchyan (1985) also obtained better superovulatory response with a similar schedule.

Akhtar *et al.* (1982) induced superovulation by administering 75 IU HCG after being primed with 50 IU PMSG. At the same time Sakuma *et al.* (1983) suggested that intravenous administration of 100 IU PMSG followed 72 hours later by 75 IU HCG could produce good response.

Illera *et al.* (1988) reported that rabbits could be superovulated with 120 IU PMSG followed after few days by 60

IU HCG. A similar regimen was successfully tried by Kosec and Petac (1989).

Wischark *et al.* (1989) and Tandle *et al.* (1993b) superovulated rabbits by administering 150 IU PMSG followed by 100 IU HCG on the third day after PMSG treatment.

Successful superovulation response was obtained by Taneja *et al.* (1990b) with intramuscular administration of 75 IU PMSG followed by 100 IU HCG by intravenous injection. Bavin *et al.* (1990) tried a similar superovulation treatment with a slightly higher dose (100 IU) of PMSG.

Tandle *et al.* (1993a and 1993b) superovulated rabbits with 150 IU PMSG. They allowed the animals to mate 60-72 hours after PMSG treatment and administered 150 IU HCG post coitum.

Gravance (1994) induced superovulation in rabbits by a single combined intramuscular injection of 72 IU PMSG and 36 IU HCG.

According to Pabuccuoglu and Ileri (1994) rabbits can be superovulated with a single dose of 225 IU PMSG followed by 150 IU HCG.

For superovulation in Japanese White adult rabbits Ishijima *et al.* (1968 and 1969b) administered 0.1 mg

oestradiol benzoate followed by 40 IU PMSG for 5 days. Further, HCG was administered 24 hours after the end of PMSG treatment.

2.1.2 FSH with LH

Kennelly and Foote (1965) reported that rabbits can be superovulated by administering 0.3-0.5 mg FSH subcutaneously twice daily for 3 days followed by intravenous injection of 2.5 to 3 mg LH on the 4th day. The same regimen was successfully tried by Varian *et al.* (1967), Maurer *et al.* (1968), Maurer and Foote (1971), Michelmann and Paufler (1974), Tsunoda *et al.* (1978) and Kane (1983). A modified treatment schedule was adopted by Gabler (1970), who administered FSH in a single dose of 3 mg instead of divided doses.

2.1.3 FSH with HCG

Overstreet (1973) induced the does to superovulate with 0.5 IU porcine FSH for three days followed by 100 IU HCG on the fourth day. Similarly Sofikitis *et al.* (1996) in their superovulation study administered 15 IU FSH for three days. They induced ovulation by administering 100 IU HCG intravenously along with the last priming dose of FSH.

Superovulation in rabbits was also successfully achieved by some workers using GnRH (Heird *et al.*, 1987 and Gosalvez *et al.*, 1994) and human menopausal gonadotrophin (Kanayama *et al.*, 1992 and Usta and Ileri, 1994).

2.2 Oestrous response after PMSG treatment

Hafez (1970) observed that the rabbits exhibited oestrus symptoms 66-72 hours after PMSG treatment. Identical results were reported by Rottmann and Lampeter (1981), Sakuma *et al.* (1983), Illera *et al.* (1988), Kosec and Petac (1989), Besenfelder *et al.* (1993), Bonanno *et al.* (1993), Tandle *et al.* (1993a and 1993b), Alabiso *et al.* (1994), Armero *et al.* (1994) and Boiti *et al.* (1995).

While Mkrtchyan (1985) in his study recorded that rabbits were sexually receptive 96 hrs after PMSG treatment, Ximenez and Vincent (1990) and Theau-Clement and Lebas (1994) reported sexual receptivity of the treated animals within 24 h and 48 hrs respectively.

2.3 Superovulation response

According to Maurer *et al.* (1968) the number of ovulations averaged 13.6 in rabbits superovulated by administering PMSG with HCG. Similarly an average of 14.8, 13 and 17.6 ovulation were observed by Yuqiand Min (1981),

Taneja *et al.* (1990b) and Gravance (1994) respectively. Fujimoto *et al.* (1973) recorded a higher rate of ovulations (23.8) per animal after suprovulation. Similar findings were also reported by Park *et al.* (1980), Illera *et al.* (1988) and Tandle *et al.* (1993a).

Still higher ovulation rate of 33 to 38 were noted by Sakuma *et al.* (1983) Wischark *et al.* (1989) and Kosec and Petac (1989) in superovulated animal.

2.3.1 Superovulation response in right and left ovaries

Sakuma and Ishijima (1964) in their superovulation study observed that there was no significant difference in ovulation rate between right and left ovaries. Ishibashi (1967), Kabayashi *et al.* (1981) and Gosalvez *et al.* (1994) also confirmed the same. On the contrary, Ibrahim (1988) reported that, in rats right ovaries showed significantly higher ovulation rate than the left.

2.4 Embryo collection

2.4.1 Time of collection

According to Tsutsumi and Hafez (1974), after 78 hours post coitum most of the blastocysts occupied the proximal half of the uterine horn. Similarly Hodgson and Pauerstein (1976)

and Agarwal and Bhattacharya (1983) reported that the embryos in the early blastocyst stage reached the uterus between 72-96 hours after mating.

Babinet and Bordenave (1980), Torres *et al.* (1987), and Taneja *et al.* (1990a and 1990b) successfully recovered blastocysts from uterus 96 hours after mating.

2.4.2 **Flushing medium**

Fox (1968), Longley and Black (1968), Fox and Krinsky (1968), Adams (1973), Fujimoto *et al.* (1974a), Fischer and Adams (1981) and Nowak and Bahr (1983) used sterile physiological saline as the flushing medium.

Sterile Modified Dulbecco's Phosphate buffered saline with bovine serum albumin was used as flushing medium by Taneja *et al.* (1990a and 1990b) and Drakakis *et al.* (1995). Similarly Dulbeccos PBS with rabbit serum and Dulbeccos PBS with foetal calf serum was used by Tandle *et al.* (1993b) and Besenfelder *et al.* (1993) respectively for flushing.

2.4.3 **Flushing technique**

Hafez (1961 and 1970) sacrificed the donor rabbits by bleeding or dislocating the neck. He exteriorized the reproductive tract and flushed the embryos *in vitro*. Likewise, Kennelly and Foote (1965), Fox (1968), Pratt (1987)

and Fischer and Odenkirchen (1988) sacrified the rabbit with an overdose of barbiturate and recovered the embryos *in vitro*. Fox and Krinsky (1968) also followed a similar technique but repeated flushing until maximum number of embryos were recovered.

Longley and Black (1968) described a segmentation technique, in which they exteriorized the oviducts and cut them into ten equal segments. They flushed each segment separately from both the ends.

Bivin and Timmons (1974) suggested that laparotomy could be performed under anaesthesia for *in vivo* flushing of embryos, so that rabbits needed for the future studies could be retained alive. Accordingly Maurer (1978), Agrawal *et al.* (1979), Nowak and Bahr (1983), Kuzan *et al.* (1984) and Tandle *et al.* (1993a) flushed the uterine cornuae and oviducts in anaesthetized rabbits by performing mid ventral laparotomy.

Taneja *et al.* (1990a) in their modified surgical technique, milked out the flushing medium from the uterus and collected it into a sterile siliconised glass petri dish direct through an incision on the dorsal vaginal wall. They repeated this procedure for complete recovery of the flushing media.

2.4.4 Recovery rate

Sakuma *et al.* (1964) reported an embryo recovery rate of 85 per cent from superovulated rabbits. Similarly Adams (1970), Maurer and Foote (1971), Fujimoto *et al.* (1974a), Sakuma *et al.* (1983), Nowak and Bahr (1983), Kim *et al.* (1988) and Taneja *et al.* (1990a), recovered more than 80 per cent of the embryos.

But, Adams (1973) harvested only 27 to 62 per cent of embryos from the superovulated rabbits. Similarly, low recovery rate of 22 to 37 per cent were recorded by Akhtar *et al.* (1982), Taneja *et al.* (1990b) and Tandle *et al.* (1993a). A moderate recovery rate of 45-68 per cent was observed by Agrawal *et al.* (1979). Kosec and Petac (1989) and Lee *et al.* (1991) also reported a moderate recovery rate of 59 per cent and 68 per cent respectively.

2.5 Embryos

2.5.1 Fertilization rate

Kennelly and Foote (1965) reported that only 49.0 per cent of the ova recovered from superovulated rabbits were fertilized. But Kilicoglu and Tekeli (1981) observed a fertilization rate of 96.3 per cent. Tandle *et al.* (1993a)

also recorded 92 per cent fertilization rate in superovulated does.

2.5.2 Quality

Tesh (1966) suggested that ova produced by superovulation were in someway deficient when compared with the ova from control animals with regards to fertilization rate.

Shaver (1970) found that two out of the 84 blastocysts recovered, showed chromosomal abnormalities. Likewise Hofsaess and Meacham (1971) identified three degenerated and nine chromosomally abnormal eggs in a collection of 75 embryos. Bubbles in the Zona pellucida of fertilized embryo was the only kind of abnormality observed by El-Din and Fulka (1974).

Braun (1979) reported 98.2 per cent of embryos in early morula stages to be free of abnormalities both in control and superovulated Newzealand White rabbits. However, he observed a sharp fall in the percentage of normal embryos (62.9%) at late morula stage in treated group only. Similarly, a higher incidence of morphologically abnormal embryos was recorded in Newzealand White rabbits (Braun and Liedl, 1980). Agarwal and Bhattacharya (1983) noted 94.2 per cent of the recovered embryos to be normal, while the remaining 5.8 per cent of embryos showed abnormalities on size, shape and segmentation.

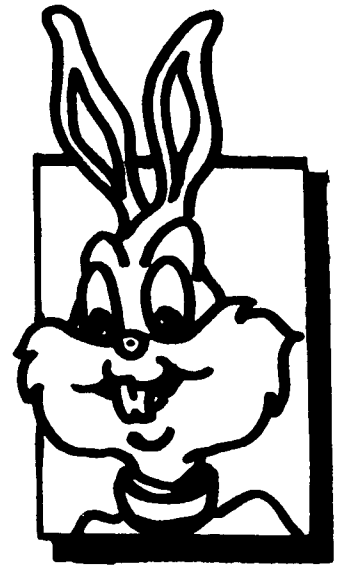
Similarly mean percentage of viable embryo of 91.67 was recorded by Taneja *et al.* (1990a). But Peinado *et al.* (1995) recorded only 33.3 per cent transferrable embryos in superovulated rabbits.

2.6 Breed/strain variation

The breed of donors in embryo transfer studies has not been considered as a source of variation by many workers, though Donaldson (1984), Nuti *et al.* (1987) and Driancourt *et al.* (1992) noticed variation on superovulatory response between breeds of cows, goats and gilts respectively.

In case of rabbits, Kodituwakku and Hafez (1969) found that ovulation rate and embryo recovery rate were relatively higher in New Zealand White breed when compared with Dutch-Belted breed. Breed variation in rabbits on these aspects was also confirmed by Hulot and Matheron (1979), Meunier *et al.* (1983), Hulot and Mariana (1985) and Torres *et al.* (1987). Schmidt *et al.* (1984) and Hetherington (1987) stated that different strains of mice responded quite variantly in superovulation treatment.

But, Hulot *et al.* (1988) observed no difference in superovulation response between California and New Zealand breed of rabbits. Similarly Bavin *et al.* (1990) also reported that there was no significant difference in superovulatory response between New zealand White and Giant Chinchilla breeds.



Material and Methods

MATERIALS AND METHODS

Twenty four healthy rabbit does of two breeds, New Zealand White (12) and Soviet Chinchilla (12), in the age group of six to 12 months maintained under ideal and identical managemental conditions formed the material for the study. Six animals randomly selected from each breed were allotted in group A and B for superovulation studies, while the remaining six does from each breed formed the controls groups, A_1 and B_1, respectively.

Healthy bucks of known fertility, from the same breeds, maintained in the University rabbit farm attached to the College of Veterinary and Animal Sciences, Mannuthy, were utilized for breeding.

All the does were isolated in individual cages for atleast 30 days, to preclude the development of pseudopregnancy.

3.1 Superovulation

Animals in the experimental group A and B were superovulated with 150 IU of pregnant mare serum gonadotrophin

(PMSG)* intramuscularly as a single dose (0 day). These animals were closely observed for the heat symptoms and double mating with two bucks of the same breed was allowed at the induced heat following PMSG treatment for effective fertilization. Human chorionic gonadotrophin (HCG)** 150 IU was administered intravenously soon after mating to ensure ovulation.

All the animals in the control groups, A_1 and B_1, were also monitored regularly for heat signs and allowed double mating in the same manner with the bucks of the same breed.

Surgical collection of embryos was done by laparotomy performed 96 hours post coitum. Both the ovaries and the uterus were exteriorized for recovery of embryos. Embryos were collected by flushing the uterine horns in vitro.

3.2 Embryo collection

3.2.1 Preparation of flushing medium

About 500 ml of Millipore water was taken in a 1000 ml volumetric flask and one vial of Dulbecco's Modified Eagle

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* Folligon 1000 IU 10 ml Marketed by Intervet International, Holland

** Chorulon 1500 IU 10 ml Marketed by Intervet International, Holland

Medium* was added into the flask. The vial was rinsed several times with Millipore water to deliver all the reagents in it. Two gram of D-Glucose (Dextrose) was also added to this and the fluid was agitated in a magnetic stirrer till all the contents were dissolved. One lakh IU of penicillin G sodium and 50 mg of streptomycin were added to this. The media was made upto 1000 ml by adding Millipore water. The media was filtered through a sterile 0.22 μm Millipore filter. The flask containing the media was properly sealed with aluminium foil and sterilized in autoclave at 121°C under 15 lb pressure for 15 minutes. After sterilization the flask was kept in refrigerator until use. The media was used within ten days of preparation. Before flushing the pH of the media was adjusted between 7.2-7.6 with 1N sodium hydroxide solution.

3.2.2 Preparation of the animal

Animals were fasted for 24 hours prior to surgery. Ventral abdominal area, between the sternum and last pair of nipples, was chosen as the surgical site. The operation site was prepared and sterilized by standard method.

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* Marketed by Himedia

All the animals were administered with Xylazine*** (5 mg/kg body weight) followed 10-15 minutes later by Ketamine**** (50 mg/kg body weight) intramuscularly for proper sedation.

All the animals were secured on dorsal recumbency with limbs stretched well apart for good exposure of the surgical area.

3.2.3 Surgical procedure

Laparotomy was performed with a longitudinal midline incision (approximately two and a half inch length) along linea alba. The muscles and peritoneum were incised so as to expose the visceral organs of the area.

Both the ovaries were traced out. The number of ovulation points showing evidence of luteinization were counted to arrive at the number of corpora lutea on the ovaries, while those follicles of comparable size to mature

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*** Xylaxin (Inj) 10 ml. Manufactured by Indian Immunologicals, Hyderabad.

Each ml contains 23.32 mg xylazine hydrochloride

**** Ketmin 50 (Inj) 2 ml - Manufactured by Themis Chemicals Ltd., Hyderabad

Each ml contains 50 mg ketamine hydrochloride

follicles were taken as anovulatory follicles. The uterine cornuae and the oviducts were traced upto the fimbriated end. Fimbria of both oviducts were ligated. Two artery forceps were applied on the pedicle cranial to the ovary to prevent bleeding and ligature was applied in front of the cranial forceps. Ovaries and horns were severed from the pedicle. Major blood vessels supplying the horns were also ligated to avoid bleeding. A forceps was applied in the anterior portion of the cervix and a ligature was applied posterior to the forceps in the cervix itself and the uterus was severed in between these points. The tubular genitalia with the ovaries were kept in petri dish containing sterile media until further studies. Laparotomy incision was closed by standard procedure.

Following surgery the animals were kept under antibiotic umbrella and the wound was dressed periodically.

3.2.4 Collection procedure

The excised reproductive tract was cleaned by trimming excess adipose tissue and washed with normal saline. After ligating the cornuae at both the ends, the horns were separated. A ligature was also applied just posterior to the uterotubal junction in both the horns.

For embryo collection from oviduct, a 22 G hypodermic needle attached to a syringe containing the media was inserted through the uterotubal junction. After removing the ligature a polyethylene tubing of 2 mm diameter was inserted through the ostium of fimbria, to a depth of about one centimetre and held firmly in position between the fingers. The oviduct was flushed with 3 to 6 ml of media and the fluid was collected in a sterile petri dish.

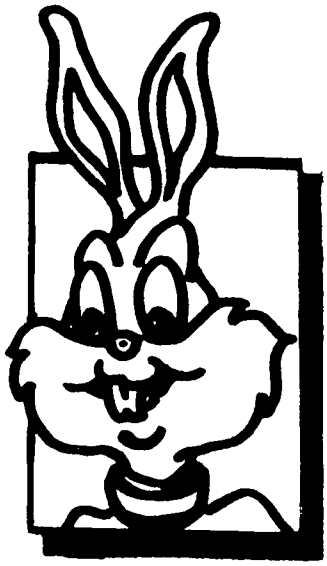
For collection from uterine horn, a 22 G hypodermic needle with syringe containing the media was inserted into the anterior end of the horn closer to the utero tubal junction. Another blunt smooth edged 16 G hypodermic needle was inserted at the posterior end of the horn and held at position as described by Agrawal *et al.* (1979). About 10 ml of flushing medium was injected into the lumen and collected into a sterile petri dish through the needle at the base of the horn. The uterine horn was flushed in both directions for harvesting the embryos.

Same procedure was repeated in the other side of the genitalia also.

Stereoscopic binocular zoom microscope was used for the identification and collection of embryos in the medium.

Embryos were located and transferred into fresh medium in depression slides for further morphological studies.

The data were tabulated and statistically analysed, by standard procedures (Snedecor and Cochran, 1967).



Results

RESULTS

With the aim of studying the superovulatory response and embryo recovery rate in Newzealand White and Soviet Chinchilla breeds of rabbits, 12 healthy animals from each breed were selected from University rabbit farm. Six animals from each breed assigned as Group A and B were subjected to superovulation treatment by administering 150 IU PMSG as a single dose followed by natural mating with two fertile bucks of same breed at induced heat. Soon after mating 150 IU HCG was administered. Remaining six animals from each of the corresponding breeds were maintained as control Groups A_1 and B_1. Embryos were recovered 96 h post coitum from all the animals by *in vitro* flushing of excised genitalia.

Onset of oestrum on superovulation treatment and intensity of oestrum based on changes in vulval mucosa, restlessness and lordosis in both the breeds are presented in Table 1 and 2. Results on superovulatory response of two treatment groups and the number of transferrable and non-transferrable embryos collected from animals of all four groups are presented in Table 3 and 12. Effect of treatment within breeds and between breeds on oestrus induction, superovulatory response, embryo recovery rate, fertilization rate and percentage of transferrable embryos are illustrated in Figures 1 to 5.

4.1 Superovulation

4.1.1 Oestrus response after PMSG treatment

The effect of administration of PMSG on oestrus induction in Newzealand White and Soviet Chinchilla rabbits are shown in Table 1 and 2, respectively.

All the animals in both the treatment groups evinced oestrus at an interval of 48-72 h (mean 56.0 ± 5.1 h) after the PMSG administration. Among the six Newzealand White rabbits, four (66.7%) exhibited intense heat and remaining two (33.3%) evinced moderate heat symptoms. While five (83.3%) out of six Soviet Chinchilla rabbits exhibited intense heat, only one animal (16.7%) evinced moderate heat.

4.1.2 Superovulatory response

Data on ovulation response in Newzealand White rabbits belonging to both control and treatment groups is presented in Table 3.

The table reveals that in control group, the number of corpora lutea indicating ovulation, in the right and left ovaries averaged 2.5 ± 0.86 and 2.2 ± 0.5 respectively with a total of 4.7 ± 1.2 ovulations from both the ovaries. The number of anovulatory follicels on the right and left ovaries

averaged 1.0 ± 0.45 and 1.3 ± 0.6. The total number of ovulations from both the ovaries averaged 2.3 ± 0.94. In one animal there was no ovulation.

In case of superovulated animals the mean number of corpora lutea in the right ovary was 11.2 ± 0.82, while those in left ovary were 10.8 ± 1.2. The total number of ovulation points indicative of developing corpora lutea in both the ovaries averaged 22.0 ± 1.35. It could be, further noted that the number of anovulatory follicles on the right and left ovaries averaged 2.5 ± 0.86 and 2.5 ± 0.9 respectively with a overall mean of 5.0 ± 1.6 in both the ovaries.

Statistical analysis revealed that there was significantly more number of corpora lutea ($P<0.01$) in the superovulated group when compared to control group in Newzealand White rabbits. In contrast, no significant difference in number of anovulatory follicles was noticed between the two groups. There was also no significant difference in the number of corpora lutea and anovulatory follicles between the right and left ovaries in both the control and treatment groups.

Data presented in Table 4 shows the ovulation response in Soviet Chinchilla rabbits belonging to both control and treatment groups.

In control group, the total number of corpora lutea from both the ovaries averaged 6.7 ± 0.65 with a mean of 3.8 ± 0.16 from right ovary and 2.8 ± 0.65 from the left ovary. The average number of anovulatory follicles from both the ovaries were 1.5 ± 0.2 with a value of 0.8 ± 0.16 and 0.7 ± 0.20 for right and left ovaries respectively.

The table also revealed that in superovulated group, the number of corpora lutea on the right ovary averaged 11.7 ± 2.0, while in the left ovary it averaged 8.3 ± 1.35, with a overall mean of 20.0 ± 3.2 ovulations in both the ovaries. It was also observed that the number of anovulatory follicles on the right ovary averaged 3.5 ± 0.5, while in left ovary it averaged 4.2 ± 0.82, with an overall mean of 7.7 ± 1.27 on both the ovaries. In one animal bilateral ovaro-bursal adhesion was noticed and hence only a few ovulation points could be detected.

There was significant difference ($P<0.01$) in the number of corpora lutea and anovulatory follicles between the superovulated and control groups of Soviet Chinchilla. However, there is no significant difference in the superovulation response from the right and the left ovaries both in the treatment and control groups.

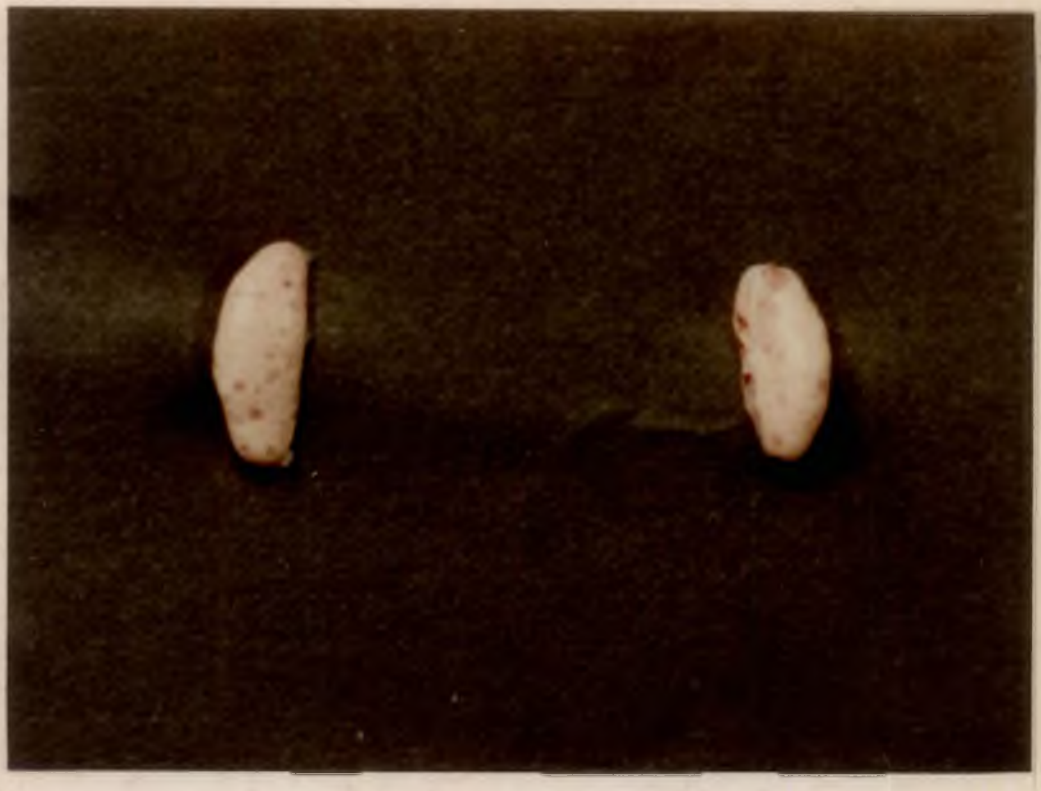
Plate 1. a. Ovulation response in treatment gorup (A) - Newzealand White

b. Ovulation response in control gorup (A_1) - Newzealand White

Plate 2. a. Ovulation response in treatment gorup (B) - Soviet Chinchilla

b. Ovulation response in control gorup (B_1) - Soviet Chinchilla

Plate 3. Ovulation failure control group of Newzealand White



Breed-wise comparison did not also reveal any significant difference in superovulatory response between treatment groups and also between control groups.

4.2 Embryo collection

Table 5 and 6 revealed the results of embryo collection in Newzealand White rabbits belonging to control and treatment groups.

In control group, the number of embryos recovered from right and left horns averaged 1.00 ± 0.37 and 1.50 ± 0.5 respectively with a overall mean of 2.50 ± 0.7 embryos from both the horns. The embryo recovery rate was 47.22 per cent.

It could be seen that in superovulated group, the total number of embryos recovered from both the horns averaged 6.8 ± 3.14, with a mean of 4.00 ± 1.92 from the right horn and 2.80 ± 1.27 from the left horn. The percentage of embryos recovered was 31.67.

Statistical analysis revealed that there was no significant difference in embryo recovery rate between the treatment and control groups of Newzealand White rabbits.

Data presented in Table 7 and 8 showed the results of embryo collection in Soviet Chinchilla rabbits belonging to control and treatment groups.

In control group, the number of embryos harvested from right and left horns averaged 2.00 ± 0.70 and 1.50 ± 0.40. The total number of embryos flushed from both the horns averaged 3.5 ± 0.80. The recovery rate was found to be 53.33 per cent.

In case of superovulated animals the mean number of embryos collected from right horn was 4.70 ± 2.24, while those from left horn were 3.70 ± 1.18. The total number of embryos recovered from both the horns averaged 8.30 ± 3.35. The percentage of embryos recovered was 36.27.

There was no significant difference statistically in the embryo recovery rate between the control and superovulated animals of Soviet Chinchilla breed.

Eventhough, the embryo recovery rate was higher in control groups when compared with treatment groups, statistical analysis revealed that there was no significant difference between the breeds and within the breeds.

4.3 Quality of embryos

Data on the quality of embryos collected from control and treatment groups of Newzealand White rabbits are presented in Table 9 and 10 respectively.

The table revealed that in control group, among the 15 embryos recovered in total from all the animals, 13 (86.70%) were fertilized (mean 2.2 ± 0.70) and 2 (13.3%) were unfertilized (mean 0.3 ± 0.2). Out of the 13 fertilized embryos, 12 (92.3%) were transferrable (mean 2.0 ± 0.57) and 1 (7.7%) was non-transferrable (mean 0.2 ± 0.16).

All the 41 embryos collected from superovulated animals were fertilized (fertilization rate 100%) of which 36 (87.80%) were transferrable (mean 6.00 ± 2.90) and 5 (12.20%) were non-transferrable (mean 0.80 ± 0.41).

Statistical analysis revealed no significant difference in fertilization rate and percentage of transferrable embryos between the control and superovulated groups of Newzealand White rabbits.

Data presented in Table 11 and 12, showed the details on quality of embryos in Soviet Chinchilla rabbits belonging to both control and treatment groups.

In control group, the number of embryos harvested from all the animal was 21, of which 16 (77.80%) were fertilized (mean 2.7 ± 0.60) and 5 (22.20%) were unfertilized (mean 0.8 ± 0.33). Among the fertilized embryos 15 (93.8%) were transferrable (mean 2.5 ± 0.60) and one (6.2%) was damaged (mean 0.20 ± 0.20).

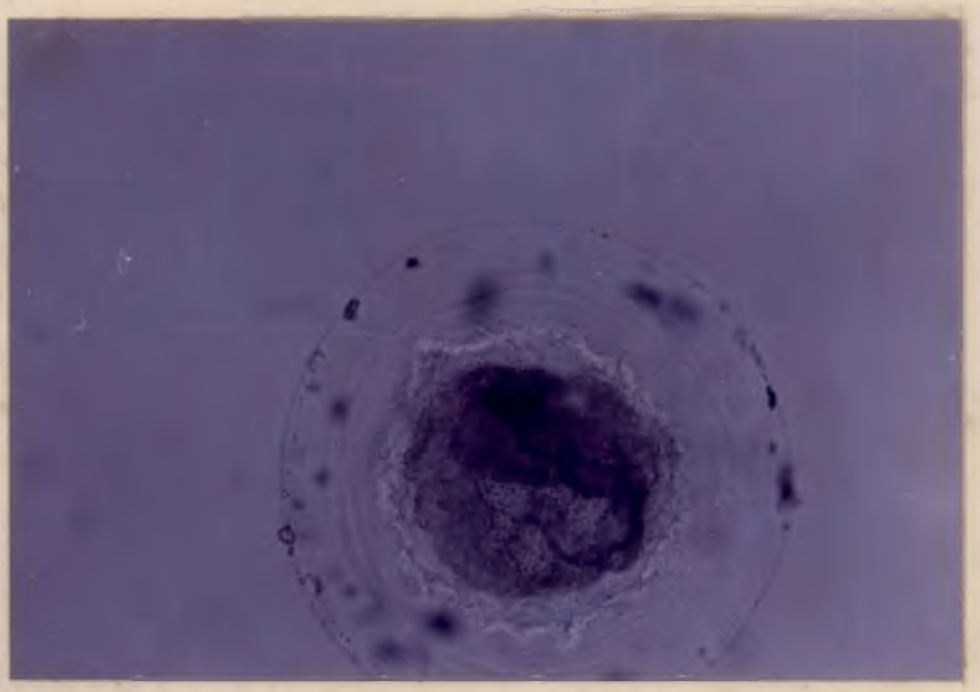
The total number of embryos recovered from all the superovulated animals was 51, of which 29 (69.82%) were fertilized (mean 4.8 ± 1.8) and 22 (31.20%) were unfertilized ova (mean 3.7 ± 1.9). Among the fertilized embryos 27 (93.1%) were transferrable (mean 4.5 ± 1.7) and 2 (6.9%) were non-transferrable (mean 0.30 ± 1.7).

There was no significant difference in the fertilization rate and percentage of transferrable embryos between the control and treatment groups of Soviet Chinchilla rabbits.

Breed-wise comparison did not also reveal any significant difference in percentage of fertilized and transferrable embryos between treatment groups and also between control groups.

Plate 4. a. Day 4 embryo (early blastocyst) - Newzealand White (x160)

b. Unfertilized ovum (day 4) - Newzealand White (x60)



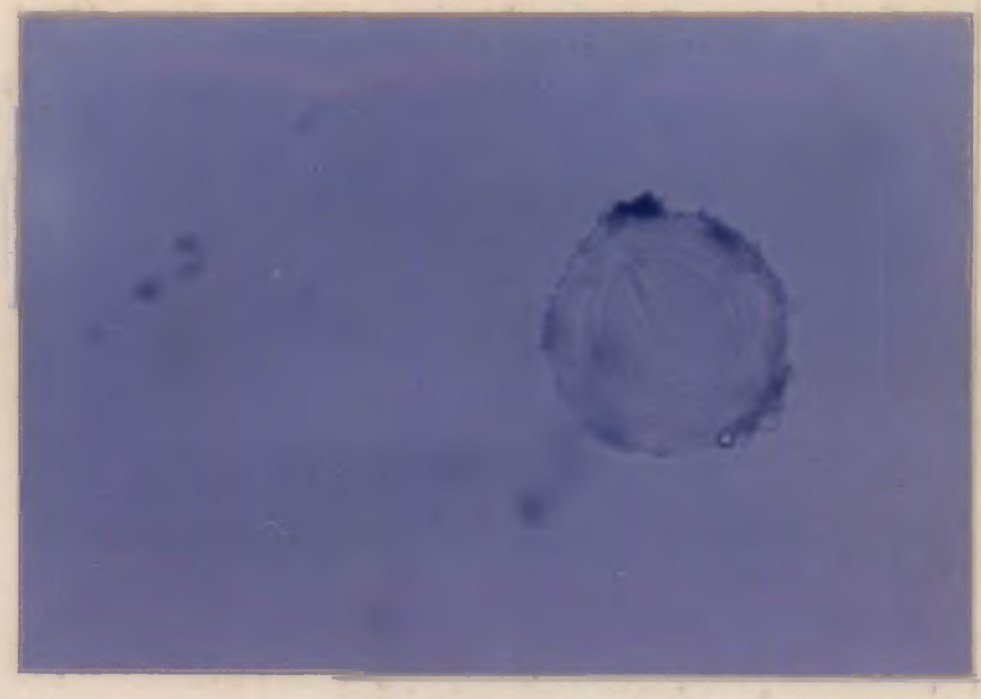
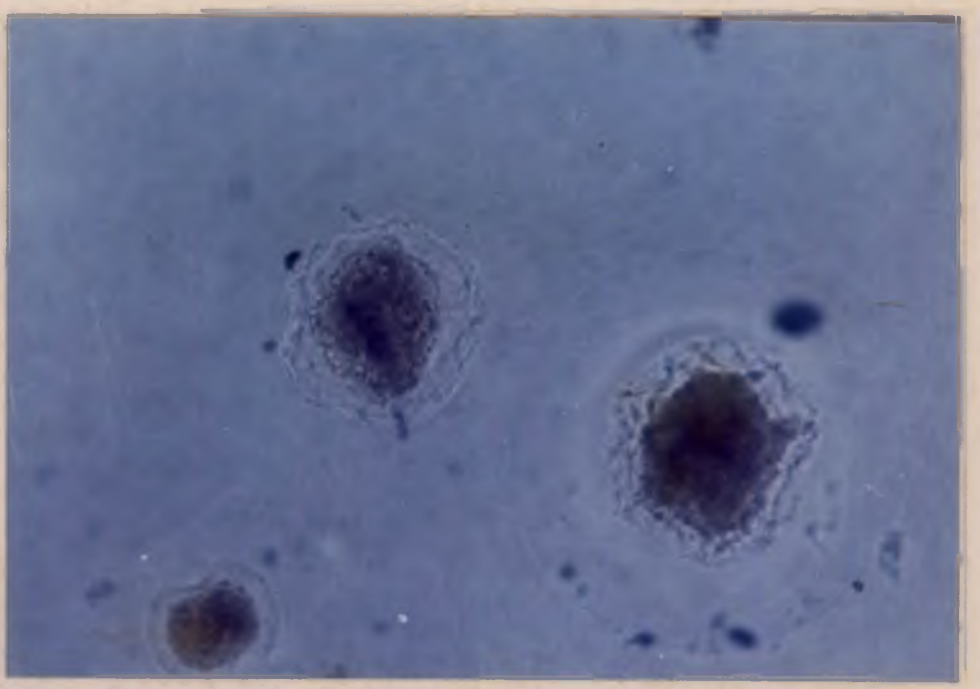


Plate 5. a. **Day 4 embryos (early blastocysts) - Soviet Chinchilla Note an embryo with damaged zonapellucida (x160)**

b. **Unfertilized ovum (day 4) - Soviet Chinchilla (x60)**



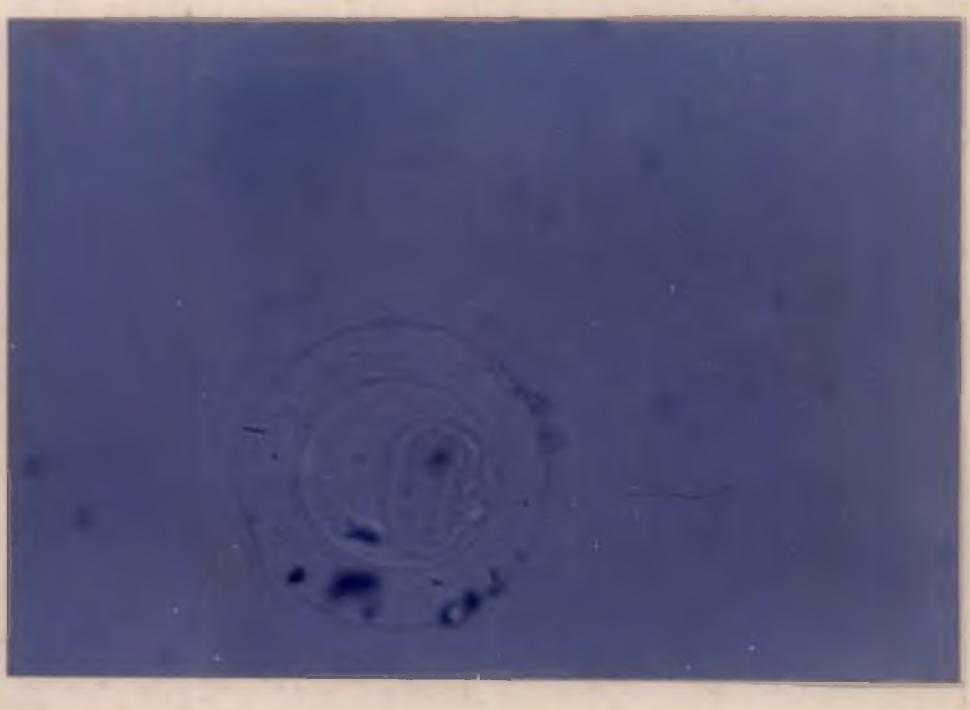


Table 1. Oestrus response and intensity of oestrum in treatment group A - Newzealand White

Sl. No.	Animal number	Time for onset of oestrus after PMSG treatment	Intensity of oestrum
1.	ET(R) 001	48 hrs	+++
2.	ET(R) 002	72 hrs	++
3.	ET(R) 003	48 hrs	+++
4.	ET(R) 004	72 hrs	++
5.	ET(R) 005	48 hrs	+++
6.	ET(R) 006	48 hrs	+++
	Mean ±SE	56.0 ±5.1	

+++ - High
++ - Moderate

Table 2. Oestrus response and intensity of oestrum in treatment group B - Soviet Chinchilla

Sl. No.	Animal number	Time for onset of oestrus after PMSG treatment	Intensity of oestrum
1.	ET(R) 013	72 hrs	+++
2.	ET(R) 014	48 hrs	+++
3.	ET(R) 015	48 hrs	+++
4.	ET(R) 016	72 hrs	+++
5.	ET(R) 017	48 hrs	++
6.	ET(R) 018	48 hrs	+++
	Mean ±SE	56.0 ±5.1	

+++ - High
++ - Moderate

Table 3. Ovulation Response - Newzealand White

Sl. No.	Control Group A_1						Treatment Group A					
	No. of corpora lutea			No. of anovulatory follicles			No. of corpora lutea			No. of anovulatory follicles		
	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total
1.	6	3	9	1	0	1	10	16	26	1	2	3
2.	2	3	5	1	1	2	12	9	21	1	1	2
3.	3	3	6	0	1	1	12	11	23	2	2	4
4.	0	0	0	3	4	7	8	8	16	6	7	13
5.	1	2	3	1	1	2	11	12	23	4	1	5
6.	3	2	5	0	1	1	14	9	23	1	2	3
Mean ±SE	2.5 ±0.86	2.2 ±0.5	4.7 ±1.2	1.0 ±0.45	1.3 ±0.6	2.3 ±0.94	11.2 ±0.82	10.8 ±1.2	22.0 ±1.35	2.5 ±0.86	2.5 ±0.9	5.0 ±1.6

Table 4. Ovulation Response - Soviet Chinchilla

Sl. No.	Control Group B_1						Treatment Group B					
	No. of corpora lutea			No. of anovulatory follicles			No. of corpora lutea			No. of anovulatory follicles		
	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total
1.	4	4	8	1	0	1	11	10	21	3	4	7
2.	4	5	9	1	1	2	7	6	13	3	2	5
3.	4	1	5	1	1	2	5	3	8	4	7	11
4.	3	3	6	1	0	1	16	9	25	2	4	6
5.	4	3	7	1	1	2	16	10	26	6	6	12
6.	4	1	5	0	1	1	15	12	27	3	2	5
Mean ±SE	3.8 ±0.16	2.8 ±0.65	6.7 ±0.65	0.8 ±0.16	0.7 ±0.2	1.5 ±0.2	11.7 ±2.0	8.3 ±1.35	20.0 ±3.2	3.5 ±0.57	4.2 ±0.82	7.7 ±1.27

Table 5. Embryo harvest from control Group A_1 - Newzealand White

Sl. No.	Animal number	No. of corpora lutea			Day of flushing (Day '0'- Oestrum)	Embryo harvest			Percentage of embryos recovered (%)
		Right ovary	Left ovary	Total		Right	Left	Total	
1.	ET(R) 007	6	3	9	Day 4	0	3	3	33.33
2.	ET(R) 008	2	3	5	Day 4	1	1	2	40.0
3.	ET(R) 009	3	3	6	Day 4	2	3	5	83.3
4.	ET(R) 010*	0	0	0	Day 4	0	0	0	-
5.	ET(R) 011	1	2	3	Day 4	1	1	2	66.7
6.	ET(R) 012	3	2	5	Day 4	2	1	3	60.0
	Mean ±SE	2.5 ±0.86	2.2 ±0.5	4.7 ±1.2		1.0 ±0.37	1.5 ±0.5	2.5 ±0.7	47.22 ±12.0

* No ovulation

Table 6. Embryo harvest in treatment Group A - Newzealand White

Sl. No.	Animal number	No. of corpora lutea			Day of flushing (Post coitum)	Embryo harvest			Percentage of embryos recovered (%)
		Right ovary	Left ovary	Total		Right	Left	Total	
1.	ET(R) 001*	10	16	26	Day 4	0	0	0	-
2.	ET(R) 002	12	9	21	Day 4	11	8	19	90.5
3.	ET(R) 003*	12	11	23	Day 4	0	0	0	-
4.	ET(R) 004±	8	8	16	Day 4	0	2	2	12.5
5.	ET(R) 005	11	12	23	Day 4	6	2	8	34.8
6.	ET(R) 006	14	9	23	Day 4	7	5	12	52.2
	Mean ±SE	11.2 ±0.82	10.8 ±1.2	22.0 ±1.35		4.0 ±1.92	2.8 ±1.27	6.8 ±3.14	31.67 ±14.4

* Stricture noticed at uterotubal junction

\+ Slight obstruction at uterotubal junction

Table 7. Embryo harvest from control Group B_1 - Soviet Chinchilla

Sl. No.	Animal number	No. of corpora lutea			Day of flushing (Day '0'- Oestrum)	Embryo harvest			Percentage of embryos recovered (%)
		Right ovary	Left ovary	Total		Right	Left	Total	
1.	ET(R) 019	4	4	8	Day 4	0	3	3	37.5
2.	ET(R) 020	4	5	9	Day 4	3	1	4	44.4
3.	ET(R) 021	4	1	5	Day 4	0	0	0	-
4.	ET(R) 022	3	3	6	Day 4	2	2	4	66.7
5.	ET(R) 023	4	3	7	Day 4	3	2	5	71.4
6.	ET(R) 024	4	1	5	Day 4	4	1	5	100.0
	Mean ±SE	3.8 ±0.16	2.8 ±0.65	6.7 ±0.65		2.0 ±0.7	1.5 ±0.4	3.5 ±0.8	53.33 ±14.0

Table 8. Embryo harvest in treatment Group B - Soviet Chinchilla

Sl. No.	Animal number	No. of corpora lutea			Day of flushing (Post coitum)	Embryo harvest			Percentage of embryos recovered (%)
		Right ovary	Left ovary	Total		Right	Left	Total	
1.	ET(R) 001	11	10	21	Day 4	0	3	3	14.3
2.	ET(R) 002*	7	6	13	Day 4	0	0	0	-
3.	ET(R) 003	5	3	8	Day 4	1	1	2	25.0
4.	ET(R) 004	16	9	25	Day 4	14	7	21	84.0
5.	ET(R) 005	16	10	26	Day 4	6	5	11	42.3
6.	ET(R) 006	15	12	27	Day 4	7	7	14	51.9
	Mean ±SE	11.7 ±2.0	8.3 ±1.35	20.0 ±3.2		4.7 ±2.24	3.7 ±1.18	8.3 ±3.35	36.27 ±12.2

* Ovaro bursal adhesion noticed

Table 9. Quality of embryos harvested from control Group A_1 - Newzealand White

Sl. No.	Animal number	Total no. of embryos harvested	Evaluation of Ova				Percentage of fertilized embryos (%)
			Fertilized			Unfertilized	
			Transferrable	Non-transferrable	Total		
1.	ET(R) 007	3	3	0	3	0	100
2.	ET(R) 008	2	2	0	2	0	100
3.	ET(R) 009	5	4	1	5	0	100
4.	ET(R) 010	0	0	0	0	0	-
5.	ET(R) 011	2	1	0	1	1	50
6.	ET(R) 012	3	2	0	2	1	66.7
	Mean ±SE	2.5 ±0.7	2.0 ±0.57	0.2 ±0.16	2.2 ±0.7	0.3 ±0.2	86.7 ±16.4

Table 10. Quality of embryos harvested from treatment Group A - Newzealand White

Sl. No.	Animal number	Total no. of embryos harvested	Evaluation of Ova				Percentage of fertilized embryos (%)
			Fertilized Ova			Unfertilized	
			Transferrable	Non-transferrable	Total		
1.	ET(R) 001	0	0	0	0	0	-
2.	ET(R) 002	19	18	1	19	0	100
3.	ET(R) 003	0	0	0	0	0	-
4.	ET(R) 004	2	2	0	2	0	100
5.	ET(R) 005	8	6	2	8	0	100
6.	ET(R) 006	12	10	2	12	0	100
	Mean ±SE	6.8 ±3.14	6.0 ±2.9	0.8 ±0.41	6.8 ±3.1	0	100

Table 11. Quality of embryos harvested from control Group B_1 - Soviet Chinchilla

Sl. No.	Animal number	Total no. of embryos harvested	Evaluation of Ova				Percentage of fertilized embryos (%)
			Fertilized Ova			Unfertilized	
			Transferrable	Non-transferrable	Total		
1.	ET(R) 019	3	3	0	3	0	100
2.	ET(R) 020	4	2	1	3	1	75
3.	ET(R) 021	0	0	0	0	0	-
4.	ET(R) 022	4	3	0	3	1	75
5.	ET(R) 023	5	3	0	3	2	60
6.	ET(R) 024	5	4	0	4	1	80
	Mean ±SE	3.5 ±0.8	2.5 ±0.6	0.2 ±0.2	2.7 ±0.6	0.8 ±0.33	77.8 ±14.0

Table 12. Quality of embryos harvested from treatment Group B - Soviet Chinchilla

Sl. No.	Animal number	Total no. of embryos harvested	Evaluation of Ova				Percentage of fertilized embryos (%)
			Fertilized Ova			Unfertilized	
			Transferrable	Non-transferrable	Total		
1.	ET(R) 013	3	3	0	3	-	100
2.	ET(R) 014*	0	0	0	0	0	-
3.	ET(R) 015	2	1	0	1	1	50
4.	ET(R) 016	21	9	0	9	12	75
5.	ET(R) 017	11	4	1	5	6	45.5
6.	ET(R) 018	14	10	1	11	3	78.6
	Mean ±SE	8.3 ±3.35	4.5 ±1.7	0.3 ±0.2	4.8 ±1.8	3.7 ±1.9	69.82 ±14.2

* Ovaro bursal adhesion noticed

Fig.1 TIME INTERVAL FOR ONSET OF OESTRUS AFTER PMSG TREATMENT

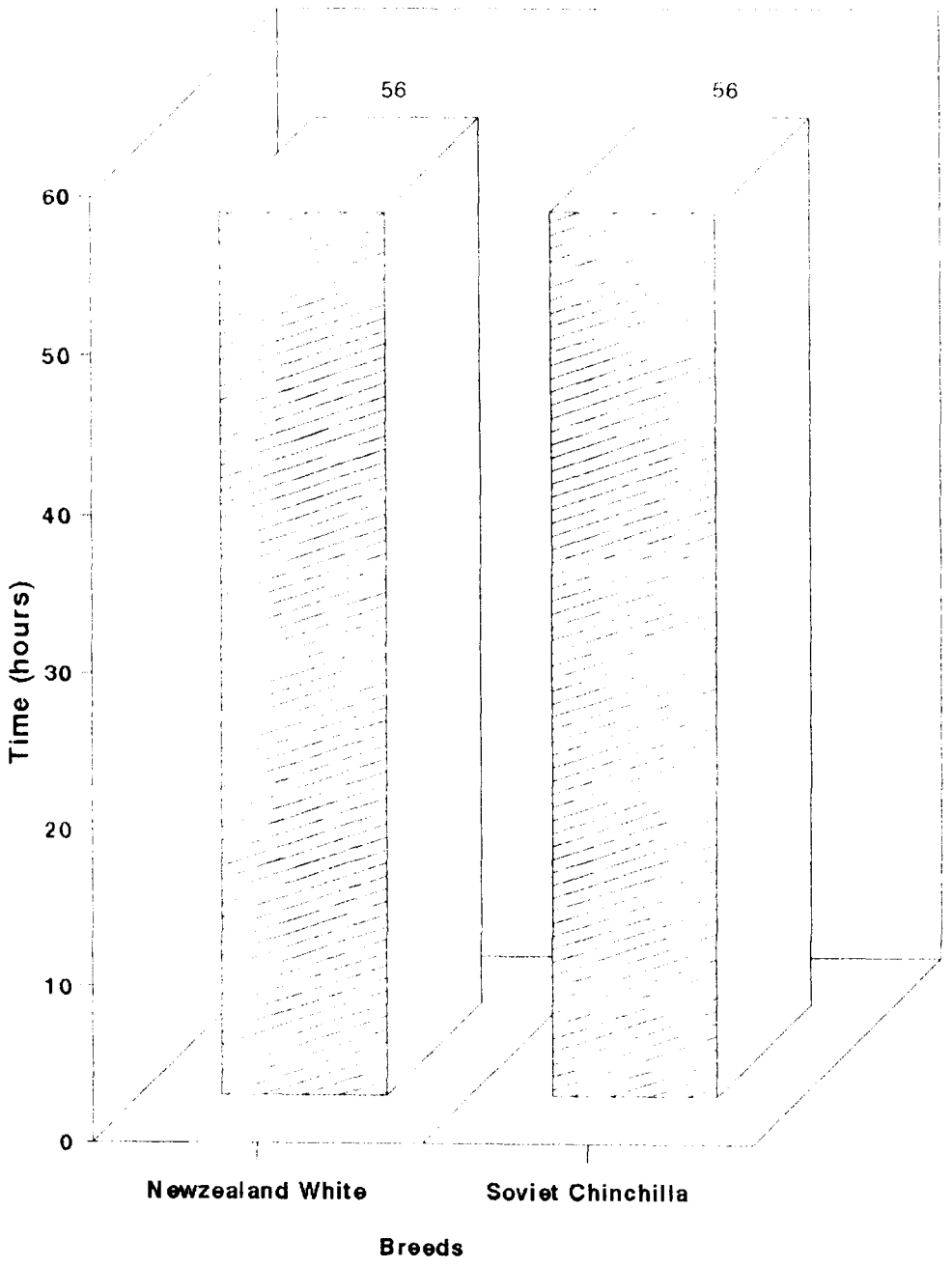


Fig.2 OVULATION RESPONSE

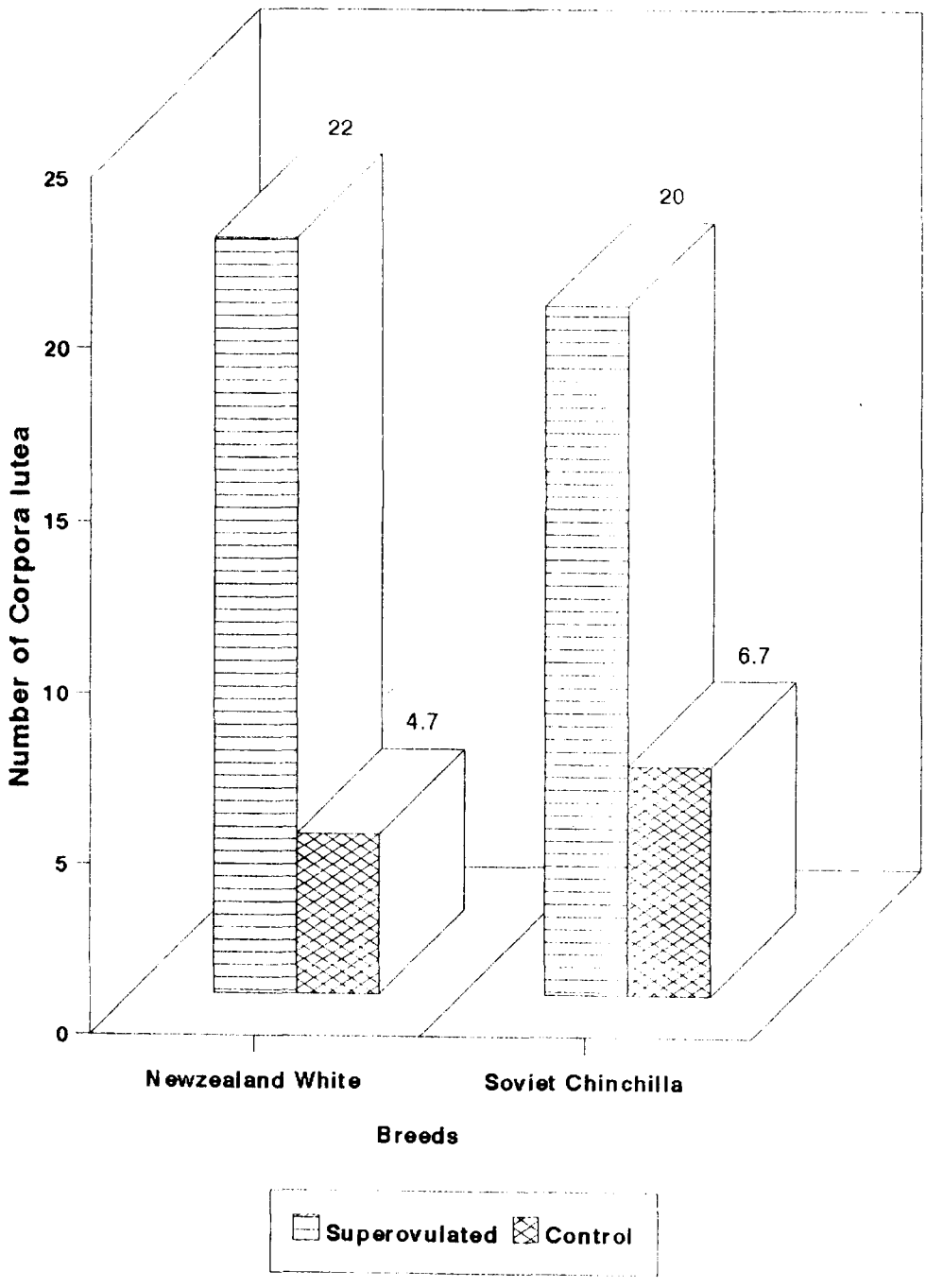


Fig.3 EMBRYO RECOVERY RATE

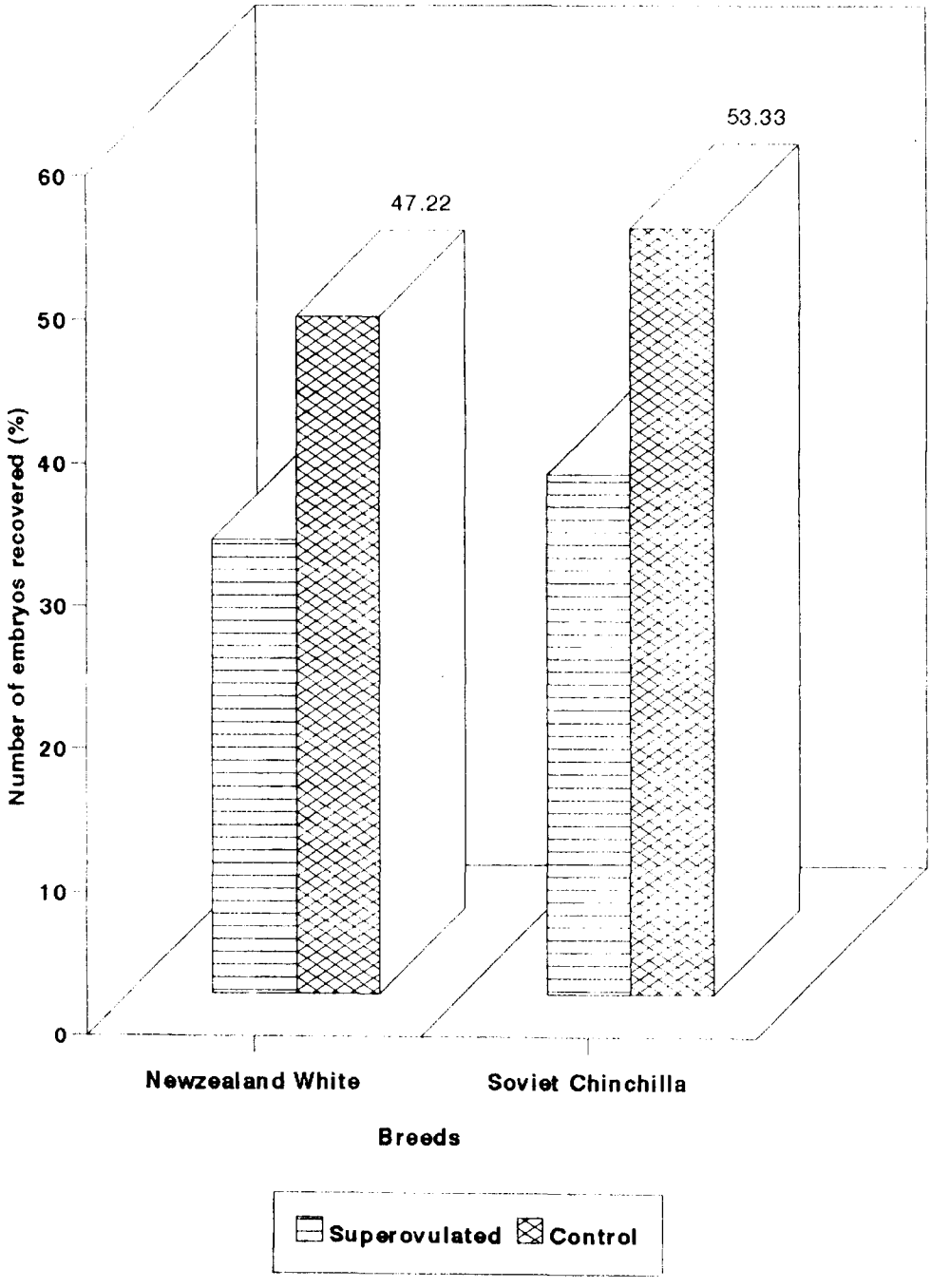


Fig.4 FERTILIZATION RATE

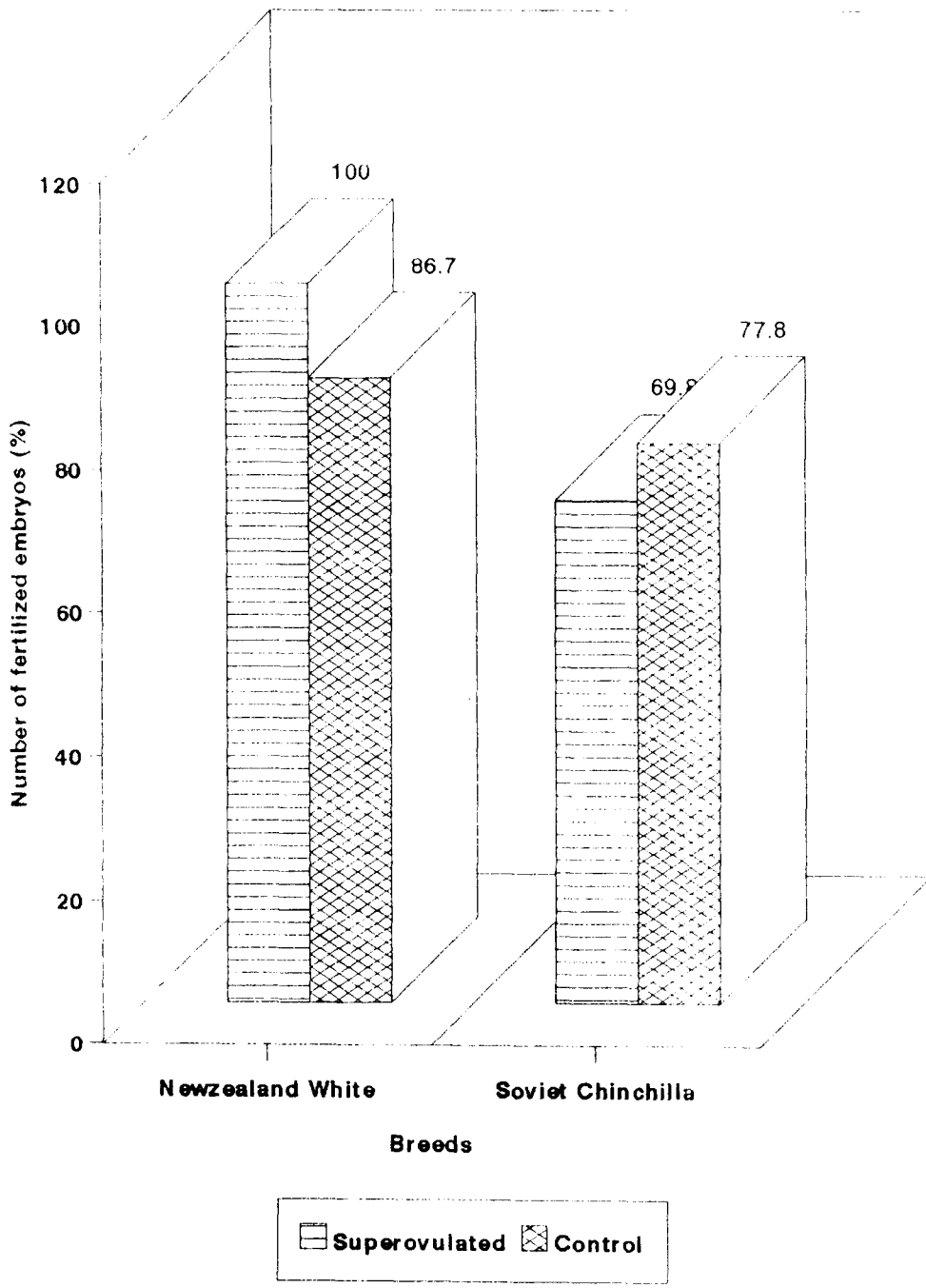
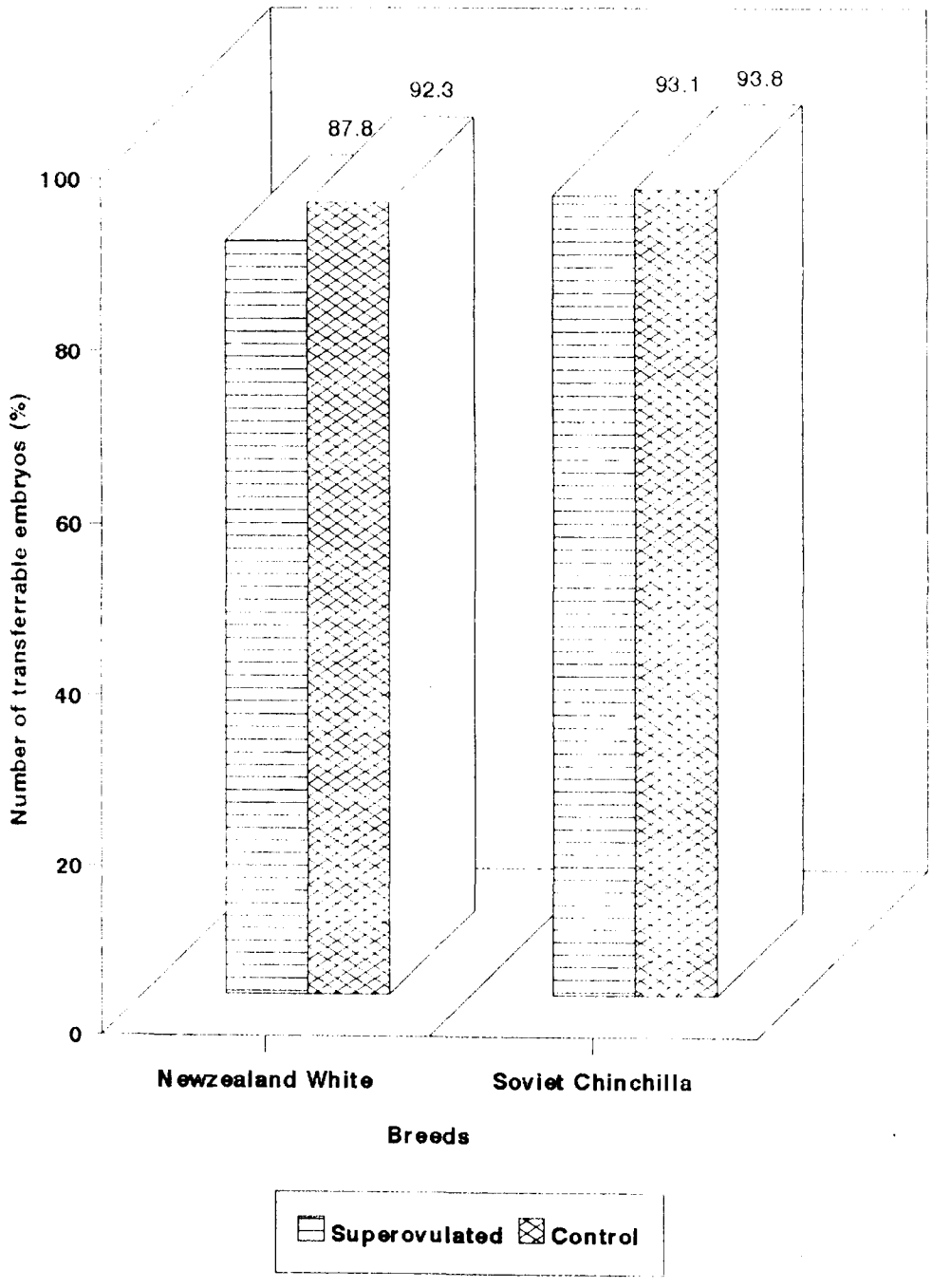
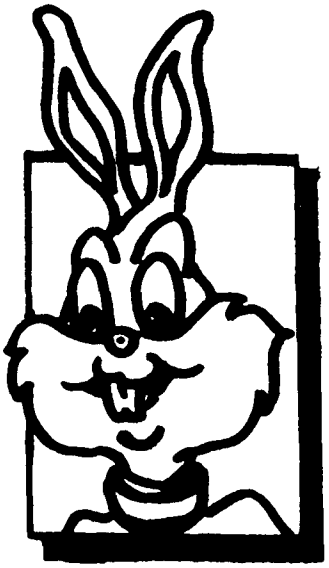


Fig.5 TRANSFERRABLE EMBRYOS





Discussion

DISCUSSION

With a view to study the superovulatory response and embryo recovery rate in Newzealand White and Soviet Chinchilla breeds of rabbit, 12 healthy animals from each breed were selected from University rabbit farm. Six animals randomly selected from each breed were allotted as group A and B, and subjected to superovulation treatment with 150 IU PMSG. Animals were mated twice with two different bucks of the same breed at induced heat and were also administered with 150 IU HCG soon after coitus to ensure better ovulation. Six animals, each of the corresponding breeds were maintained as control groups A_1 and B_1. Animals in these groups were allowed double mating in similar manner when found in oestrum. Embryos were collected 96 h post coitum from all the animals by flushing of the excised genitalia in vitro.

The results obtained and the inferences drawn are summarised below.

5.1 Superovulation

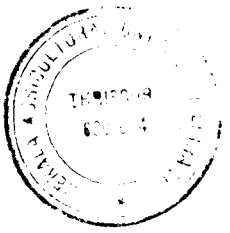
5.1.1 Oestrus response with PMSG treatment

The results of administration of PMSG in Newzealand White and Societ Chinchilla rabbits, as shown in Table 1 and

2 respectively, reveals that the mean interval between the PMSG treatment and onset of oestrus was 56.0 ± 5.1 h for both the treatment groups. This finding is in agreement with Illera *et al.* (1988), who also reported a time interval of 60 h for onset of oestrus after PMSG.

In the present study, it could also be seen that the animals subjected to superovulation with 150 IU PMSG exhibited sexual receptivity within a range of 48-72 h. A similar duration was also observed by Hafez (1970), Rottmann and Lampeter (1981), Besenfelder *et al.* (1993), Bonanno *et al.* (1993), Armero *et al.* (1994), Alabiso *et al.* (1994), Theau-Clement and Lebas (1994) and Boiti *et al.* (1995), after administration of only 20-60 IU PMSG. By administering a dose of 100-150 IU PMSG Sakuma *et al.* (1983), Kosec and Petac (1989) and Tandle *et al.* (1993a and 1993b) reported a time interval of 72 h for oestrus response. But Mkrtchyan (1985) and Ximenez and Vincent (1990) detected the onset of oestrus in 96 h and 24 h respectively after administering PMSG within a similar range of 20-150 IU.

From these results it could be interpreted that there is no relationship with dose of hormone and time interval for onset of oestrum.



5.1.2 Superovulation response

Data presented in Table 3 and 4 reveals that in control group of Newzealand White rabbits, the number of corpora lutea in the right and left ovaries averaged 2.5 ± 0.86 and 2.2 ± 0.5 respectively with a mean of 4.7 ± 1.2 ovulations from both the ovaries put together. It could also be seen that the number of anovulatory follicles from both the ovaries averaged 2.3 ± 0.94 with a value of 1.0 ± 0.45 from right ovary and 1.3 ± 0.6 from left ovary. In control group of Soviet Chinchilla rabbits as shown in Table 4, the overall mean number of ovulations from both the ovaries was 6.7 ± 0.65 with a mean of 3.8 ± 0.16 in right ovary and 2.8 ± 0.65 in the left ovary. The average number of anovulatory follicles in both the ovaries were 1.5 ± 0.2 with a value of 0.8 ± 0.16 and 0.7 ± 0.2 for right and left ovaries respectively. By statistical analysis, no significant difference could be observed in the number of corpora lutea and anovulatory follicles between the two control groups.

The ovulation rate observed in this study is in accordance with the findings of Agrawal *et al.* (1979). The mean number of ovulations recorded by them was 5.09 ± 0.27.

In the present study, no ovulation was noticed in one animal belonging to control group of Newzealand White breed. This animal exhibited only a low intensity of oestrum when

allowed for mating. A similar finding was observed by Hulot *et al.* (1988) and they suggested that the lack of ovulation following natural mating in non-receptive does might be caused by a lack of LH discharge.

Perusal of data presented in Table 3 reveals that in superovulated Newzealand White rabbits, the mean number of corpora lutea from both the ovaries were 22.0 ± 1.35, with a value of 11.2 ± 0.82 for the right ovary and 10.8 ± 1.2 for the left ovary. It could be further noted that the number of anovulatory follicles on right and left ovaries averaged 2.5 ± 0.86 and 2.5 ± 0.9 respectively with a overall mean of 5.0 ± 1.6 in both the ovaries. In superovulated Soviet Chinchilla rabbits, as shown in Table 4, the number of corpora lutea on the right ovary averaged 11.7 ± 2.0, while in the left ovary ovary it averaged 8.3 ± 1.35, with a mean of 20.0 ± 3.2 ovulations from both the ovaries put together. It was also observed that the number of anovulatory follicles on both the ovaries averaged 7.7 ± 1.27 with a value of 3.5 ± 0.5 for the right ovary and 4.2 ± 0.82 for the left ovary. In one animal bilateral ovaro-bursal adhesion was noticed and hence only a few ovulation points could be detected.

Statistically, there is no significant difference in the superovulation response between the two breeds. It also

reveals that there is significantly more number of corpora lutea ($P<0.01$) in the superovulated group when compared to control group of both breeds.

These results are in agreement with the findings of Tandle *et al.* (1993a) who recorded a mean of 24.66 ± 2.71 ovulations with a protocol of 150 IU PMSG with 150 IU HCG, which was adapted in the present study also. The result is also in accordance with that of earlier works of Fujimoto *et al.* (1973) who observed an average of 23.8 ovulations by administering 100 IU PMSG with 100 IU HCG. Similar results were also reported by Park *et al.* (1980) and Illera *et al.* (1988).

Several other workers have reported a higher ovulation rate following superovulation with PMSG and HCG. An average of 38.5 corpora lutea (range 6-135) was recorded by Hafez (1961) with a split dose of 150 IU PMSG followed by 50 IU HCG. In the above study, 23 per cent of animals did not respond at all. Identical results were recorded by Yuqi and Min (1981) with split dose of PMSG. The reason can be attributed to the variation in some animals when divided dose of PMSG was given. In the present study no such variation was noticed when PMSG was administered as a single dose. Similarly a range of 30 to 38 ovulations were observed by Sakuma *et al.* (1983), Kosec and Petac (1989) and Wischark *et al.* (1989) with 75-150 IU PMSG

and 75-100 IU HCG, when single dose protocol was administered. But Taneja *et al.* (1990b) suggested that the dose rate of hormones was not necessarily a criterion for the ovulation rate.

It has been reported that PMSG will produce antibodies to foreign serum protein and so refractiveness in animals were noticed in subsequent treatments (Muy and Simpson, 1975). But in the present study PMSG was promptly used because there is no necessity for repeated administration of this hormone in the same animals. Also it is relatively cheaper and easily available in the market.

Based on the ovulation rate, the functional ability of right and left ovaries did not show any variation in both the groups. These observations are in agreement with Sakuma and Ishijima (1964), Ishibashi (1967), Kabayashi *et al.* (1981) and Gosalvez *et al.* (1994) who also noticed no significant difference in the number of ovulations between right and left ovaries.

Taneja *et al.* (1990b) recorded an average of three anovulatory follicles in superovulated rabbits, but Tandle *et al.* (1993a) observed a much higher value of 15.66 ± 2.24 anovulatory follicles. The former author also suggested the reason for higher value of anovulatory follicles as overdose

of PMSG. But it could be noticed that when a similar higher dose of PMSG (150 IU) was administered in the present study, the number of anovulated follicles counted were only 5-7 which is comparable with the results observed by Taneja *et al.* (1990b).

5.2 Embryo collection

Data presented in Table 5 reveals that in control group of Newzealand White rabbits, the number of embryos recovered from right and left horns averaged 1.00 ± 0.37 and 1.50 ± 0.5 respectively with a overall mean of 2.50 ± 0.7 embryos from both the horns. The embryo recovery rate was 47.22 per cent. In control group of Soviet Chinchilla rabbits, as shown in Table 7 the number of eggs harvested from both the horns averaged 3.5 ± 0.80 with a mean value of 2.00 ± 0.70 and 1.50 ± 0.40 embryos from right and left horns respectively. The embryo recovery rate was found to be 53.33 per cent. Statistical analysis revealed that there is no significant difference in embryo recovery rate between the control groups of both breeds.

The findings of the earlier workers were found to be higher than the values in the present study. Taneja *et al.* (1990a and 1990b) collected 85.55 ± 4.75 per cent embryos from

the control rabbits. They milked out the flushed medium from the uterus and repeated the technique thrice for complete recovery of flushing media. It may be noted that in the present study such milking out of flushing media was not attempted.

Perusal of data presented in Tables 6 and 8 reveals that the total number of embryos recovered from superovulated group of Newzealand White rabbits was 6.8 ± 3.14 with the values of 4.00 ± 1.92 from right horn and 2.8 ± 1.27 from the left horn. In superovulated group of Soviet Chinchilla rabbits, a mean of 8.3 ± 3.35 embryos were recovered from both the both the horns, with the values of 4.7 ± 2.24 and 3.7 ± 1.18 from the right and left horns respectively. The percentage of embryos recovered from superovulated groups of both breeds were 31.6 and 36.27 respectively.

By analysing the above results, it can be concluded that, eventhough recovery rate was comparatively higher in control groups than treatment groups, statistically no significant difference could be observed between these groups.

These results concur with the findings of many earlier workers. Taneja *et al.* (1990b) recorded an embryo recovery rate of 34 per cent, while Tandle *et al.* (1993a) recovered 37 per cent of embryos from rabbits superovulated with PMSG and

HCG, which were in general agreement with the observations in the present study. An average of 6.83 embryos were collected by Tandle *et al.* (1993b), which is also quite agreeable with the results in the study conducted now. The reason for the identical results may be due to same dosage of hormones administered by them also.

Some other workers have reported a higher rate of embryo collections from superovulated rabbits. Sakuma *et al.* (1964) recovered 85 per cent embryos, while Adams (1970), Fujimoto *et al.* (1974a), Nowak and Bahr (1983) and Kim *et al.* (1988) recorded 75-80 per cent recovery rate. Taneja *et al.* (1990a) also observed a similar higher embryo recovery rate, which he attributed to the complete recovery of flushing medium from the uterine horns.

At the same time a lower recovery rate of 22 to 62 per cent were recorded by Adams (1973), Akhtar *et al.* (1982), Taneja *et al.* (1990b), Lee *et al.* (1991) and Tandle *et al.* (1993a), when cannulation method for embryo recovery was adapted. Agrawal *et al.* (1979) suggested that when cannulation technique of embryo recovery was followed, a hindrance was produced preventing the complete recovery of flushing medium, which led to a low embryo recovery rate. In the present study also, cannulation technique was followed and this may be the reason for comparatively lower recovery rates.

Many reasons for the low recovery rate were suggested by previous workers. Hafez (1961) explained that some ova may be lost into the peritoneal cavity after ovulation. On the other hand, Kennelly and Foote (1965) found that treatment producing overstimulation with excessive follicular development resulted in lower recovery rate and they suggested that it could be due to retension of ova in the ruptured follicles itself. This factor was reported by Fox (1968) also. Maurer and Foote (1971) stated that interference with gamete transport also resulted in a decreased percentage of embryo recovery. Sherwood and McShan (1977) described that PMSG has a longer circulating half life, which is responsible for its tendency of overstimulation. Taneja *et al.* (1990b) in their superovulation study with 75 IU PMSG, suggested that the residual effect of the hormone, due to its over dosage, stimulated the ovaries for a longer period resulting in increased number of anovulatory follicles. They found that the oestrogen secreted by these unruptured follicles interfered with the ovum transport mechanism. Pincus (1965) stated that higher oestrogen concentration during the post-fertilization days in rabbits, caused retension of the ova in the Fallopian tube (tube locking) long past the time they would normally enter the uterus and thereby leading to an eventual degeneration of the cleaning ova. A magnified representation of this view was noticed in three superovulated

Newzealand White rabbits in which a complete or partial obstruction at the uterotubal junction was felt while flushing.

Although, in the present investigation, no hormonal profiles were studied, in the light of above facts, it could be assumed that the interference with ovum transport mechanism, due to an altered oestrogen-progesterone concentration, might be responsible for the reduction in embryo recovery rate.

5.3 Fertilization rate

In the present study, as shown in Tables 9 and 11, out of 15 embryos recovered from Newzealand White rabbits belonging to control group, 13 were fertilized (Fertilization rate 86.7%), while among Soviet Chinchilla rabbits, 16 embryos out of 21 embryos recovered were found to be fertilized (Fertilization rate 77.8%). There was no significant difference in fertilization rate between the control groups of both breeds. The fertilization rate of group A_1 (86.7%) is in general agreement with an observation of 93.8 per cent reported by Agrawal *et al.* (1979) in control animals, while these values in the study conducted now, are lower than the observations of Taneja *et al.* (1990b) who had reported 100 per cent fertilization in untreated animals. It could be seen

from Table 10 and 12 that all the 41 embryos collected from treatment group of Newzealand White rabbits, were found to be fertilized (Fertilization rate 100%), while only 29 embryos were fertilized out of 51 embryos recovered from superovulated Soviet Chinchilla rabbits (Fertilization rate 69.82%). Statistical analysis revealed no significant difference between breeds in fertilization rate.

Kilicoglu and Tekeli (1981) and Tandle *et al.* (1993a) recorded an average fertilization rate of 96.3 per cent and 91.4 per cent respectively in different studies. On the contrary Kennelly and Foote (1965) reported an average of 49 per cent fertilized embryos from rabbits treated with PMSG and progesterone. They explained that the fertilization failure was due to progesterone interference with the sperm transport mechanism and its capacitation. The two above observations were noticed in the present study also, in the animals of both breeds belonging to treatment groups, when in Newzealand White breed 100 per cent fertilization rate was observed, in Soviet Chinchilla rabbits with the same treatment only 69.82 per cent fertilization rate was recorded.

5.4 Quality of embryos

Perusal of data presented in Table 9 reveals that in control group of Newzealand White rabbits, among the 13

fertilized embryos, 12 were (92.3%) transferrable and one was (7.7%) non-transferrable, while in control group of Soviet Chinchilla breed, as presented in Table 11, out of 16 fertilized embryos, 15 were (93.8%) transferrable and one was (6.2%) damaged. No significant difference could be observed in percentage of transferrable embryos between the control groups of both breeds.

These findings are in agreement with Braun (1979) who reported that 98.2 per cent of embryos obtained from untreated animals were free of abnormalities. Similarly Agarwal and Bhattacharya (1983) observed only 5.8 per cent abnormalities in the embryos which is nearly coinciding with the values of the present investigation.

Perusal of data presented in Tables 10 and 12 shows that 87.8 per cent of the fertilized embryos in Newzealand White rabbits belonging to treatment group were transferrable (mean 6.0 ± 2.9), while 12.2 per cent embryos were non-transferrable (mean 0.8 ± 0.4) and in superovulated Soviet Chinchilla rabbits 93.1 per cent embryos were transferrable (mean 4.5 ± 1.7) and 6.9 per cent were non-transferrable. Statistical analysis revealed no significant difference in percentage of transferrable embryos between the treatment groups of both breeds.

These results concur with the findings of El-Din and Fulka (1974). They observed 14 per cent abnormal embryos from superovulated animals. Taneja *et al.* (1990a) reported a mean viable embryo percentage of 91.67, which is also in agreement with the values of present study.

At the same time, Sakuma *et al.* (1983) recorded a maximum of 36.3 per cent degenerated ova in superovulated rabbits, which is much higher than the findings in the study conducted now. Fischer and Odenkirchen (1988) explained the detrimental effects of gonadotrophin treatment on embryonic development. They also reported a higher peripheral progesterone concentrations at the time of mating and during the pre-implantation period in superovulated rabbits, which led to a higher incidence of damaged embryos upto day 4 post coitum. In the present study it is observed that these factors were not influenced by breed of the animal.

5.5 Breed influence

Data presented in Tables 1 to 12 were compared and analysed to study the breed influence on the above parameters.

The inference drawn after statistical analysis of the data is that, superovulated rabbits of both Newzealand White and Soviet Chinchilla breeds responded similarly to the superovulation treatment and there is no significant

difference in the number of embryos recovered, embryo recovery rate, number of fertilized embryos, fertilization rate and in the quality of embryos between these two breeds. Similarly there exists no significant difference statistically, on the above parameters between the control groups of both the breeds. These results concur with the findings of Hulot *et al.* (1988) and Bavin *et al.* (1990). They stated that breed differences in the number of ovulations and recovery rate were not significant in superovulated groups.

Meunier *et al.* (1983) and Torres *et al.* (1987) reported higher ovulation in Newzealand strain rabbits when compared with Californian strain rabbits. Hulot and Mariana (1985) suggested that increased number of ovulations in Californian strain than the Newzealand strain, was due to a significantly higher number of healthy follicles in Californian strain.

From the foregoing paragraphs it is evident that administration of 150 IU PMSG with 150 IU HCG induced satisfactory superovulatory response in rabbits of both the breeds under study. A five fold increase in the ovulation rate is noticed in superovulated does than the control animals with the present protocol. Based on the ovulation rate, the functional ability of right and left ovaries does not show any variation between the groups.

A moderate recovery rate is achieved with the flushing technique followed in this study. Although statistically not significant, the embryo recovery rate is higher in control animals when compared to the treatment groups. Similarly the percentage of fertilized embryos and transferrable embryos does not differ significantly, although fertilization rate in superovulated Soviet Chinchilla breed rabbits was lower when compared to other groups.

Both the Newzealand White and Soviet Chinchilla breeds of rabbits responded similarly to the superovulation treatment and there is no significant difference in ovulation rate, recovery rate, fertilization rate and percentage of transferrable embryos between these two breeds. Thus breed influence on the above parameters could not be appreciated in the present study.

SUMMARY

The effect of superovulation treatment with PMSG and HCG on onset and intensity of induced oestrus, ovulation response, embryo recovery rate and quality of embryos in Newzealand White and Soviet Chinchilla breeds of rabbits were studied.

Six animals randomly selected from each breed assigned to group A and B were superovulated and another six animals from each of the corresponding breeds were maintained as control groups, A_1 and B_1. Animals in the treatment group were administered with 150 IU PMSG intramuscularly. At the induced heat they were mated twice with two different bucks of the same breed and to ensure better ovulation each animal was treated with 150IU HCG intravenously post coitum. Animals in the control groups were allowed in the same manner for double mating when found in oestrum. Embryos were collected from all the animals 96 h post coitum by in vitro flushing of excised genitalia.

All the animals in the treatment groups of both breeds exhibited oestrous symptoms at an interval of 48-72 h (mean 56.0 ± 5.1 h) after the PMSG treatment.

In control group of Newzealand White rabbits, the number of corpora lutea in the right and left ovaries averaged 2.5 ± 0.86 and 2.2 ± 0.5 respectively, with a total of 4.7 ± 1.2

ovulations from both the ovaries. The number of anovulatory follicles from both the ovaries averaged 2.3 ± 0.94. The total number of embryos recovered from this group averaged 2.5 ± 0.7. The embryo recovery rate was 47.22 per cent. Among the 15 embryos recovered, 13 were (86.7%) fertilized and 2 were (133%) unfertilized. Out of the 13 fertilized embryos 92.3 per cent were transferrable and 7.7 per cent were non-transferrable.

In superovulated Newzealand White rabbits, the number of corpora lutea averaged 11.2 ± 0.82 and 10.8 ± 1.2 from right and left ovaries respectively. The total number of ovulations averaged 22.0 ± 1.35. The mean number of anovulatory follicles from both the ovaries was 5.0 ± 1.6. Average number of embryos harvested from this group was 6.8 ± 3.14 with an embryo recovery rate of 31.67 per cent. All the 41 embryos collected from this group were fertilized (fertilization rate 100%), of which 36 were (87.8%) transferrable and 5 were (12.2%) non-transferrable.

Soviet Chinchilla rabbits in control group responded with 3.8 ± 0.16 and 2.8 ± 0.65 ovulations from the right and left ovaries respectively, while the group average was 6.7 ± 0.65. The number of anovulatory follicles from both the ovaries averaged 1.5 ± 0.2. An average of 3.5 ± 0.8 embryos were

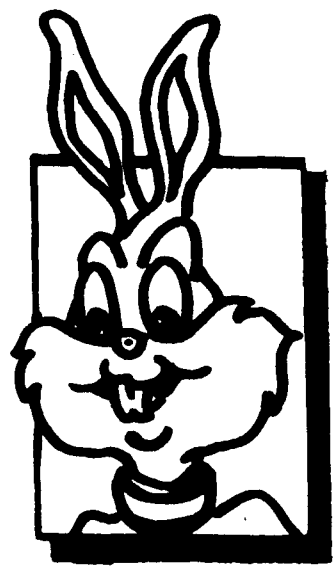
recovered from this group, with a recovery rate of 53.33 per cent. Out of the 21 embryos recovered in total, 16 were (77.8%) fertilized and 5 were (22.2%) unfertilized. Among the fertilized embryos 93.8 and 6.2 per cent were transferrable and non-transferrable respectively.

In superovulated Soviet Chinchilla rabbits, the mean number of corporalutea from both the ovaries put together were 20.0 ± 3.2 with individual values of 11.7 ± 2.0 and 8.3 ± 1.35 for the right and left ovaries respectively. The mean number of anovulatory follicles from both the ovaries were 7.7 ± 1.27. The average number of embryos recovered from this group was 8.3 ± 3.35, with a recovery rate of 36.27 per cent. Out of the 51 embryos collected, 29 were (69.82%) fertilized and 22 were (31.2%) unfertilized. Among the fertilized embryos, 93.1 and 6.9 per cent were transferrable and non-transferrable respectively.

Statistical analysis of the data revealed significant difference ($P<0.01$) in the ovulation response between treatment and control groups of both the breeds. However, there was no significant difference in the embryo recovery rate, fertilization rate and the percentage of transferrable embryos between the groups.

To sum up it could be stated that with the present superovulation protocol of 150 IU PMSG followed by double

mating at the induced heat and 150 IU HCG post coitum, satisfactory superovulation response was achieved in rabbits. In the present investigation, the percentage of embryos recovered from treatment groups were lower than from control animals, which could be attributed to the interference with ovum transport mechanism due to a probable high dose rate of PMSG leading to an altered oestrogen-progesterone profile and the detrimental effects of gonadotrophin treatment on the embryos. Both the Newzealand and Soviet Chinchilla breeds responded similarly to the superovulation treatment and statistically no significant difference was observed in ovulation rate, recovery rate, fertilization rate and percentage of transferrable embryos between the two breeds. Thus breed influence in rabbits on the above parameters could not be appreciated. Further detailed investigations on dose rate of PMSG and factors hindering embryo recovery are warranted to ensure high embryo crop from rabbits.



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SUPEROVULATION AND EMBRYO RECOVERY IN RABBITS

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ABSTRACT

Superovulation was induced in Newzealand White and Soviet Chinchilla breeds of rabbits by administration of a single dose of 150 IU PMSG followed by double mating at induced cycle and 150 IU HCG soon after second mating to induce ovulation. The onset and intensity of oestrum, number of ovulations, embryo recovery and quality of embryos were studied and compared with those of the controls of the respective breeds.

The mean interval from PMSG administration to onset of oestrus in both the breeds was 56.0 ± 5.1 h. It was further observed that most of the treated animals showed intense oestrum when compared to controls.

The ovulation rate based on the number of corporalutea in control animals of Newzealand White breed was 4.7 ± 1.2 as against 22.0 ± 1.35 in the treated group. There was significant difference ($P<0.01$) in the ovulation rate between the groups. The percentages of embryo recovery, fertile embryos and transferrable embryos in the control group were 47.22, 86.7 and 92.3 while those of the treatment group were 31.67, 100 and 87.8 respectively. There was no statistically significant difference between the groups.

While the control animals in Soviet Chinchilla breed had an ovulation rate of 6.7 ± 0.65, the treated rabbits showed a

higher ovulation rate of 20.0 ± 3.2. There was significantly higher ovulation rate ($P<0.01$) in treated group when compared to controls. The embryo recovery rate, fertilized embryos and transferrable embryos in the control group were 53.33 per cent, 77.8 per cent and 93.8 per cent respectively. The corresponding values in the treatment group were 36.27 per cent, 69.82 per cent and 93.1 per cent respectively. There was no significant difference between the groups. No breed influence on the above parameters could also be noticed in this study

It may be concluded that superovulation could be successfully induced in Newzealand White and Soviet Chinchilla breeds of rabbits with single dose of 150 IU PMSG, followed by 150 IU HCG soon after second mating. Eventhough there was superovulation, the embryo recovery rate was comparatively lower in the treated group probably on account of an altered oestrogen-progesterone profile interfering with the transport of the zygote, however the fertilization rate and the quality of the embryos were unaffected with the superovulation treatment.