COMPARATIVE STUDY ON SUPEROVULATORY RESPONSE AND VIABILITY OF EMBRYOS IN PERIPUBERTAL AND ADULT MALABARI GOATS

By

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THESIS

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DECLARATION

I hereby declare that the thesis, entitled **"COMPARATIVE STUDY ON SUPEROVULATORY RESPONSE AND VIABILITY OF EMBRYOS IN PERIPUBERTAL AND ADULT MALABARI GOATS"** 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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Certified that the thesis, entitled **"COMPARATIVE STUDY ON SUPEROVULATORY RESPONSE AND VIABILITY OF EMBRYOS IN PERIPUBERTAL AND ADULT MALABARI GOATS "** is a record of research work done independently by **Dr. Metilda Joseph**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Dr. Metilda Joseph

To Almighty

who helped me to experience

him more deeply through the

bleating and wagging of tails

of these beautiful

creatures

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LIST OF ABBREVIATIONS

ACP	Acid phosphatase
ALP	Alkaline phosphatase
BL	Blastocyst
Cel.	Celsius
CL	Corpus luteum
CM	Compact morulae
CO_2	Carbon dioxide
dl	Deciliter
DMSO	Dimethyl sulphoxide
DPBS	Dulbecco's phosphate buffered saline
EB	Early blastocyst
EG	Ethylene glycol
ELISA	Enzyme-Linked Immunosorbant Assay
ER	Embryo recovery
ETT	Embryo transfer technology
EXB	Expanded blastocysts
FCL	Functional corpora lutea
FSH	Follicle stimulating hormone
h	Hour
HB	Hatched blastocyst
hCG	Human chorionic gonadotrophin
Inj.	Injection
IM	Intramuscular

IU	International unit
KAU	King and Armstrong Units
kg	Kilogram
LDH	Lactic dehydrogenase
LH	Luteinising hormone
M	Morulae
mg	Milligram
ml	Milliliter
MOET	Multiple ovulation and embryo transfer
ng/ml	Nano gram per ml
oFSH	Ovine FSH
P_4	Progesterone
pFSH	Porcine FSH
$PGF_2\alpha$	Prostaglandin $F_2\alpha$
PMSG	Pregnant mare serum gonadotrophin
PRCL	Premature regression of corpus luteum
SOR	Superovlulatory response
TER	Transferable embryo recovery
U/L	Units / litre
UFO	Unfertilized oocytes
μ	Microns

Introduction

1. INTRODUCTION

Goat is considered as a convenient domestic species for commerce and research due to its biological and managemental advantages over other farm animals. It provides a cost benefit ratio of 1:11.7 with an investment return rate value of over 30 per cent. India ranks first among the countries of the world in goat population and it addsabout 12000 million rupees to Indian economy each year. It is well known that goat plays a vital role in the rural economy of Kerala. While the state forms only 1.18 per cent of the land area in Indian Union, 14.3 per cent of the goat population of the nation is in Kerala as per the livestock census 1996. This shows that goat production is ideally suited for the farming situations existing in the state.

India has very large and diverse genetic resources of goat. Among this, Malabari (Tellicherry) breed is very popular as a good milk producer and is a native of Kerala. Fragmentation of land consequent to explosion of population and globalisation of economy is gradually paving way to new agricultural production systems in Kerala state in which small ruminants have a major role to play. In this context a rapid multiplication and genetic improvement of the elite Malabari breed is highly imperative for sustainable goat production in the state in this new millennium.

Embryo transfer technique is a well established tool for rapid genetic improvement and propagation of important livestock species. During the last two decades tremendous progress has been made in use of female germplasm through multiple ovulation and embryo transfer (MOET) techniques. Though the first embryo transfer in livestock species was performed in goat (Warwick and Berry, 1949), there has been little commercial embryo transfer activity in goats until recently when compared to cattle. Two factors that have contributed most to this lack of utilization of embryo transfer technique in this species were (a) the

relative cost-benefit ratio and (b) the damage caused to genetically valuable animals, by surgical embryo collection procedures. The interest in international exchange of germplasm and the preservation of endangered breeds and species has altered the cost benefit ratio in favour of the use of embryo transfer technology in goats.

During the past ten years much progress has been made in development of techniques of laparoscopy and non-surgical collection of embryos from goats. Recent advances in modern reproductive biotechnologies like *in vitro* maturation and fertilisation of oocytes, cryopreservation, sexing, cloning of embryos, cloning by nuclear transfer, production of transgenic animals etc. have further stimulated research activities in this area in goats.

The rate of genetic gain achieved through MOET programmes can be further enhanced by using peripubertal goats for superovulation and thereby shortening the generation interval. Nowshari *et al.* (1992) reported that peripubertal Boer goats were just as suitable to produce embryos for transfer purpose as adult animals.

Despite research efforts made during the last two decades, marked variability in superovulatory response is still considered as a major limiting factor in the success of ET programme.

Different methods for cryopreservation of goat embryos have been attempted. Factors that have influenced the rate of survival of embryos included the type and concentration of cryoprotectant used, temperature at which embryos are transferred to liquid nitrogen and the stage of embryos at the time of freezing (Li *et al.*, 1990).

LeGal *et al.* (1993) compared the *in* vivo and *in vitro* survival of goat embryos after freezing with ethylene glycol or glycerol. Development of embryos both *in* vivo and *in vitro* showed ethylene glycol to be better cryoprotectant than glycerol. Endocrine status of the donor goats during superovulatory treatment

was found to influence the ovarian response, embryo recovery and quality of embryos (Armstrong *et al.*, 1983a and Appavu and Holtz, 1992).

The first successful embryo transfer in goats in India was performed by Agrawal *et al.* (1979). Perusal of the literature revealed paucity of information on various factors affecting superovulatory response and viability of embryos in peripubertal and adult goats. There are also no comparative studies on the viability of frozen embryos from these age group of animals.

It was in this background that the present study was undertaken with the objective of comparing the suitability of peripubertal and adult Malabari goats for MOET programme.

Review of Literature

2. REVIEW OF LITERATURE

The number of offsprings that a female can produce in her life time can be greatly increased by application of MOET. The availability of embryos for transfer is further increased by inducing superovulation in peripubertal animals which also shortens the generation interval. If large number of embryos are obtained from donor animals excess embryos can be frozen and then transferred at later time. The viability of fresh and frozen embryos are assessed by *in vivo* and *in vitro* evaluation procedures. Assessment of progesterone (P_4) levels in donor and recipient animals are of high value in predicting the viability of embryos. The ultimate proof for embryo viability is the establishment of pregnancy in recipient animals and birth of live young ones.

2.1 SUPEROVULATION IN GOATS

The ovaries of prepubertal goats contain large number of normal growing and vesicular follicles (Kennedy *et al.*, 1974). Mathai (1984) recorded that large follicles were observed in ovaries of cross-bred Malabari goats at the age of 150 days and above and corpus luteum (CL) was found at the age of 240 days and above. McKelvey and Bhattacharya (1992) opined that doe kids should only be used for superovulation if they had attained at least 60 per cent of their mature body weight.

For inducing superovulation gonadotrophins like Pregnant mare serum gonadotrophin (PMSG) and Follicle stimulating hormone (FSH) were commonly used in combination with progestogens and/or prostaglandin $F_2\alpha$ ($PGF_2\alpha$) as synchronising agents. It was reported that Human chorionic gonadotrophin (hCG), Luteinising hormone (LH) and Gonadotrophin releasing hormone (GnRH) were used for controlling ovulation in superovulated goats (Amoah and Gelaye, 1991; Ishwar and Memon, 1996).

2.1.1 Synchronisation of Oestrus

Synchronisation of oestrus may be achieved through the use of P_4 and $PGF_2\alpha$. Progesterone and synthetic progestogens were administered orally, repeated injections, intravaginal sponges, progesterone releasing intravaginal device (CIDR) or as subcutaneous implants for a period of 9 to 18 days. Prostaglandin and its analogues had been utilized effectively in superovulation programme due to its luteolytic action (Ishwar and Memon, 1996; Cognie *et al.*, 1999).

2.1.1.1 Progestogens (Progesterone and Its Analogues)

Armstrong and Evans (1983) reported that Repromap intra vaginal sponges inserted for 10 to 12 days before starting FSH injections and removed on third day of FSH treatment resulted in successful synchronisation of oestrus and superovulation in goats and Merino sheep of less than one year of age. In Danish and Angora hoggets intravaginal progesterone devices were inserted for a period of 18 days for synchronisation of oestrus (Holm *et al.*, 1990). Nowshari *et al.* (1992) synchronised oestrus in peripubertal Boer goats using 3 mg crestar implants or intravaginal sponges containing 40 mg Fluogestone acetate (FGA). Kathiresan (1993) and Dutta *et al.* (2001) superovulated prepubertal goats after synchronising oestrus using progesterone injections and oral preparations respectively.

Adult goats were treated with intravaginal pessaries such as 4 mg FGA, CIDR devices, sponges or 60 mg medroxy progesterone acetate for a period of 9 to 19 days. (Peebles and Kidd, 1994; Fieni *et al.*,1995; Pintado *et al.*, 1996; Kuhholzer *et al.*, 1998; Goel and Agrawal, 1998; Kareta *et al.*, 1999 and Riesenberg *et al.*, 2001).

Oral progestogens were used to synchronise oestrus in adult Barbari goats (Sarmah *et al.*, 1996). Progesterone was administered in the form of injections at the dose rate of 10 to 12.5 mg per day for 16 to 22 days for synchronising oestrus (Moore and Eppleston, 1979; Doijode *et al.*, 1992; Pargonkar *et al.*, 1992; Benjamin, 1994; Pargonkar *et al.*, 1994).

Ear implants containing either 6 mg, 3 mg or 1.5 mg norgestomet were used for synchronisation of oestrus in adult goats (Nowshari *et al.*, 1992; Senn and Richardson, 1992; Akinlosotu and Wilder, 1993; Nowshari and Holtz, 1993; Nowshari *et al.*, 1995).

2.1.1.2 Prostaglandins

Angora kids which had began cycling (based on observation of oestrus or laparoscopic examination of ovaries) were treated using estrumate 50 to 100 μg as a single injection between days five and 15 of oestrous cycle, two days after beginning of FSH treatment regime (Armstrong and Evans, 1983). Yuswiati and Holtz (1996) accomplished synchronisation of oestrus in FSH superovulated peripubertal goats by administering $PGF_2\alpha$ on day 10 of their oestrous cycle.

Armstrong *et al.* (1983a) injected 50 μg cloprostenol 48 h after the initiation of superovulatory treatment in adult goats to trigger luteolysis. Further, double injection schedules of $PGF_2\alpha$ or its analogues at 11 to 12 days interval (Benjamin, 1994; Goel and Agrawal, 1998) or a single injection schedule during the mid to late luteal phase (Agrawal and Goel, 1991; Mahamood *et al.*, 1991; Pandey *et al.*, 1992; Goel *et al.*, 1993; Goel *et al.*, 1995; Deshpande *et al.*, 1997; Tiwari *et al.*, 1998 and Dutta *et al.*, 2000) were found to be effective in synchronising oestrus in superovulated adult goats.

2.1.1.3 Progesterone Plus Prostaglandin

Nubian doelings from 6.5 to 8 months of age were oestrus synchronised by subcutaneous implantation of 6 mg norgestomet ear implants for nine days. Follicle stimulating hormone was started on day seven and at implant removal on day nine a single injection of 5 mg $PGF_2\propto$ (lutalyse) was administered (Pendleton *et al.* 1992; Senn and Richardson, 1992).

Cloprostenol @ 100 µg on day nine of 11 day vaginal sponge (45 mg FGA) insertion was tried to induce synchronisation of oestrus by many investigators (Chemineau *et al.*, 1986; LeGal *et al.*, 1993; Fieni *et al.*, 1995). In a similar manner, $PGF_2\propto$ or its analogues in combination with FGA or Medroxy progesterone acetate (MAP) vaginal sponges were used for synchronisation of heat in superovulation programmes (Fieni *et al.*, 1995; Pintado *et al.*, 1996; Pintado *et al.*, 1998; Salles *et al.*, 1998; Terzano *et al.*, 1999).

Synchronisation of oestrus was accomplished in FSH superovulated goats using 1.5 mg or 2 mg norgestomet implants and $PGF_2\propto$ (Nowshari *et al.*, 1995). Yuswiati and Holtz (1996) used 1.5 mg crestar ear implant for 10 days and intramuscular injection of $PGF_2\propto$ was administered at the end of norgestomet treatment for synchronisation of oesrus in Boer cross-bred goats. Prostaglandin $F_2\propto$ was administered in two equally divided doses at 12 h interval. Whereas, Dhandapani (1998) and Senthilkumar *et al.* (1998) also used 3 mg norgestomet ear implant and $PGF_2\propto$ for synchronisation of oestrus in superovulated Barbari and Tellichery goats respectively.

2.1.2 Superovulatory Agents

Pregnant mare serum gonadotrophin (PMSG) and FSH were usually administered either near the end of the luteal phase of the cycle (day 11 to 13) or

around day one or two before the end of the synchronisation treatments. The first widely used gonadotrophin for superovulation was PMSG or Equine chorionic gonadotrophin (eCG) which was given as a single intramuscular injection due to its long biological half life *in vivo*. Follicle stimulating hormone was commonly administered in six to eight injections at 12 h. interval over a three to four day period due to its short biological half life. A decreasing dose regime was followed for administration of FSH as high LH content in FSH preparations had been shown to affect ovarian response adversely. A combination of PMSG and FSH were also tried for inducing superovulation in goats (Ishwar and Memon, 1996).

2.1.2.1 Pregnant Mare Serum Gonadotrophin (PMSG)

Prepubertal animals were superovulated using a single or double injection of PMSG (Majumdar *et al.*, 1990; Kathiresan, 1993; Dutta *et al.*, 2001). Nowshari *et al.* (1992) and Nowshari and Holtz (1993) and Yuswiati and Holtz (1996) administered PMSG at the rate of 30 IU/kg body weight both in peripubertal and adults goats.

Several workers reported the superovulation of adult goats using PMSG @ 500-1500 IU as a single injection (Benjamin, 1994; Senthilkumar, 1996; Dhandapani, 1998; Goel and Agrawal, 1998; Pintado *et al.*, 1998; Dutta *et al.*, 2000; Risenberg *et al.*, 2001; Dutta *et al.*, 2001).

2.1.2.2 Follicle Stimulating Hormone

Multiple injections of FSH at varying dose rates of 14 mg to 20 mg at 12 h. interval for four days, were administered for superovulation of prepubertal and peripubertal goats (Armstrong and Evans, 1983; Majumdar *et al.*, 1990; Holm *et al.*, 1990; Nowshari *et al.*, 1992; Kathiresan, 1993; Yuswiati and Holtz, 1996).

In adult goats, 10 to 30 mg FSH of porcine origin in six to eight divided step down doses over three to four days at 12 h interval was administered (Chemineau *et al.*, 1986; Tsunoda and Sugie, 1989; Nowshari *et al.*, 1992; Nowshari and Holtz, 1993; Riha *et al.*, 1994; Kuhhlozer *et al.*, 1998; Goel and Agrawal 1998; Pintado *et al.*, 1998). Bessado *et al.* (1988) and Goel and Agrawal (1998) administered a total dose of 15 to 16 mg FSH-P, a priming dose of 4 mg was injected on day four of sponge insertion and the balance quantity was administered as six injections over a period three days towards the end of progestagen treatment.

Purified FSH preparations of porcine or ovine origin administered as multiple injections were used for induction of superovulation (McNatty *et al.*, 1989; Taneja *et al.*, 1991; Senthilkumar 1996; Deshpande *et al.*, 1997; Salles *et al.*, 1998; Tiwari *et al.*, 1998; Kareta *et al.*, 1999; Terzano *et al.*, 1999). Peebles and Kidd (1994) and Terzano *et al.* (1999) induced superovulation in adult goats by administering a single subcutaneous injection of highly purified porcine FSH. Risenberg *et al.* (2001) superovulated German Improved nanny goats using a single dose of pFSH (FSH-P) 36 h before the end of synchronisation treatment using FGA.

Fieni *et al.* (1995) administered six decreasing doses of FSH (total 16 Armour units) along with increasing doses of purified LH to superovulate Alpine and Saanen dairy goats. Nowshari *et al.* (1995) compared the superovulatory response of does to purified FSH supplemented with 30, 40 or 50 per cent pLH. Pereira *et al.* (1998) administered 16 mg pFSH containing 40 per cent LH for superovulating adult goats.

2.1.2.3 Combination of FSH and PMSG

Some of the investigators examined the use of superovulation protocols based on multiple injections of FSH combined with PMSG in adult goats

(Armstrong *et al.*, 1987; Peebles and Kidd, 1994; Goel and Agrawal, 1998; Pintado *et al.*, 1998).

2.1.4 Ovulation Inducing Agents

Senn and Richardson (1992) and Kathiresan (1993) administered 500 to 1000 IU of Human chorionic gonadotrophin (hCG) for inducing ovulation in doelings and prepubertal goats respectively.

Li *et al.* (1990) and Akinlosotu and Wilder (1993) administered Luteinising hormone releasing hormone (LHRH) or Gonadotrophin releasing hormone (GnRH) 24-48 h after removal of progestogen releasing source. Human chorionic gonadotrophin was administered at varying dose rate of 500 to 1500 IU during the early part of the superovulatory heat in adult goats (Taneja *et al.*, 1991; Goel and Agrawal, 1998; Tiwari *et al.*, 1998; Dhandapani, 1998; Dutta *et al.*, 2000). Baril *et al.* (1995) developed a method of controlling the time of LH peak so that AI could be carried out in predetermined time. Ovulation was induced using gonadotrophin releasing hormone antagonist (Antrelix) and purified LH administered after a further 24 h.

2.2 RESPONSE TO SYNCHRONISATION AND OESTROUS CHARACTERISTICS

Oestrous detection and study of oestrous characteristics are very important for successful breeding in a superovulatory programme.

Majumdar *et al.* (1990) reported that prepubertal animals treated with FSH-P or PMSG alone exhibited strong oestrous signs on day two onwards, while steroid hormone pretreated animals showed weak symptoms on days four and five from start of the gonadotrophin treatment. Kathiresan (1993) reported that the mean time taken for onset of oestrus was 64 h. in PMSG group as against

62 h in FSH group. The mean duration of oestrus in PMSG and FSH group were 33 h. and 42 h. respectively.

Senn and Richardson (1992) treated peripubertal Nubian doelings using crestar plus $PGF_2\alpha$ and FSH. Hundred per cent of the doelings responded to synchronisation treatment during breeding season, whereas, during non-breeding season the corresponding figure was 66.66 per cent. It was recorded that the oestrus associated behavior included red and swollen vulvas, mucus discharge, tail twitching, riding over other doelings, increased bleating and vocalization, seeking of bucks and loss of appetite. Yuswiati and Holtz (1996) reported that 88.50 per cent and 92.00 per cent of the peripubertal goats exhibited oestrus response after synchronisation treatment with $PGF_2\alpha$ and norgestomet plus $PGF_2\alpha$ respectively. The mean interval from end of synchronisation treatment to the onset of oestrus was longer in $PGF_2\alpha$ group than in norgestomet plus $PGF_2\alpha$ group (55.30 ± 36.50 h vs 35.70 ± 15.80 h).

East and Row (1989) reported that most of the superovulated does exhibited oestrus between 12 and 72 h. after sponge or implant removal and the synchronisation treatment did not affect the oestrus behaviour. McNatty *et al.* (1989) found that mating activity in superovulated does began between 12 to 24 h and 48 to 60 h and ended within 60 and 84 h respectively after CIDR and MAP vaginal sponge withdrawal. Baril and Vallet (1990) observed that the onset of oestrus was 27.60 ± 3.50 h and 29.60 ± 1.90 h after sponge removal during breeding and non breeding season respectively.

Selgarth *et al.* (1990) reported that the onset of oestrum was within 24 and 30 h in 20 mg FSH and 24 mg FSH treated goats respectively and 24 to 48 h in PMSG treated goats. Pendleton *et al.* (1992) recorded that the mean time taken for onset of oestrum was 33.30 ± 2.70 h and 32.00 ± 3.50 h in norgestomet plus PMSG and norgestomet plus FSH treated goats respectively. Rosina *et al.* (1992) stated that 60 per cent of the does treated for superovulation had less than 12 h

oestrus duration due to premature luteal regression occurring after gonadotrophin treatment. Thilagar *et al.* (1992) stated that the onset of oestrum in Tellicherry goats treated with norgestomet plus PMSG and $PGF_2\propto$ plus PMSG was 26.70 ± 1.12 and 32.00 ± 1.22 h respectively.

Benjamin (1994) reported that all the goats treated with $PGF_2\propto$ and PMSG came to oestrus in 57.80 ± 5.65 h after the $PGF_2\propto$ administration and the duration of heat was 48.00 ± 8.76 h, but in animals treated with P_4 and PMSG, only 83.33 per cent were in oestrus at an interval of 101.60 ± 6.11 h after the last P_4 injection and the duration of oestrus was 28.00 ± 1.41 h. While Dhandapani (1998) reported that good oestrus response was noticed in Barbari goats treated with norgestomet plus $PGF_2\propto$ and PMSG. The overall mean time from implant removal to onset of oestrus and duration of oestrus were 23.33 ± 0.62 h and 37.83 ±1.73 h respectively. The intensity of oestrus in majority of animals was very good (75%) while 16.67 per cent showed good intensity and 8.33 per cent fair.

Salles *et al.* (1998) reported that Saanen does treated with 60 mg medroxy progestrone plus 50 mg cloprostenol and superovulated using 200 mg or 37.50 unit of NIH-FSH-SI did not show significant difference between treatments with respect to onset of oestrus and duration of oestrus.

Senthilkumar *et al.* (1998) studied the oestrous characteristics in Tellicherry goats treated with crestar ear implant plus $PGF_2\propto$ and superovulated using porcine or ovine FSH or PMSG. The onset of oestrus was earlier in PMSG group (15.66 ± 1.31 h) and FSH-P group (17.66 ± 0.80 h) than FSH-0 group (24.66 ± 1.33 h). The duration of oestrus in these three groups were 44.66 ± 1.20 h, 34.33 ± 2.49 h and 24.66 ± 1.33 h respectively.

Ionica and Derivata goats treated with 45 mg FGA plus 3 mg luprostiol and four different superovulatory injection regime using 250 IU of porcine FSH

did not exhibit any difference with respect to onset of heat between breeds. Goats superovulated using six injections and single injection of FSH showed later onset of oestrus than animals treated with eight injections ($P<0.05$). Oestrus was detected within 36 h of sponge removal in four injections and single injection group, 56 h in six injection group and 24 h in eight injection group (Terzano *et al.*, 1999).

2.3 BREEDING OF SUPEROVULATED GOATS

Time of breeding during oestrum following superovulatory treatment should be precise to have maximum fertilization rate and to recover more number of good quality embryos.

Intraperitoneal AI was done with 1 ml of semen at the time of hCG administration and six h later in prepubertal goats. (Majumdar *et al.*, 1990). Kathiresan (1993) carried out surgical insemination with 0.05 ml of freshly collected semen into the uterus, in superovulated prepubertal goats after two and a half days of last P_4 injection.

Nowshari *et al.* (1992) reported that peripubertal doelings were bred or inseminated with fresh semen until they were no longer receptive. Senn and Richardson (1992) bred superovulated Nubian doelings two to four times using two bucks of proven fertility. Yuswiati and Holtz (1996) hand mated superovulated peripubertal goats twice over 12 h interval.

High fertilization rates were reported by natural mating of superovulated does than hand mating or artificial insemination (Moore and Eppleston, 1979). It was postulated that if fertile bucks are available, natural mating should be used in superovulation programme and does should be mated every six hours during oestrus (Trevit *et al.*, 1986). Benjamin (1994) inseminated all the superovulated goats using good quality buck semen four to six h after the onset of oestrus and

therafter at 12 h interval till the end of oestrum. Nowshari and Holtz (1995) mated the does naturally or inseminated them with fresh semen once daily throughout the standing oestrus.

The donors were inseminated by laparoscopic intrauterine injection with 10^8 frozen thawed spermatozoa, 24 h after onset of heat (LeGal *et al.*, 1993). Riha *et al.* (1994) carried out intrauterine insemination of donors by means of laparoscopy some 46 h following progestogen sponge removal.

2.4 OVARIAN RESPONSE

In goats a major and unresolved problem in superovulation is the extreme variability in ovulatory response that is evident among animals treated with similar agents. It is shown that some part of the variability arises from genetic factors, age, season, stage of the cycle at which treatment is applied and type of gonadotrophin employed in stimulating ovarian response (Gordon, 1997).

2.4.1 Anovulatory Follicles

Lohan *et al.* (1989) administered 1000IU PMSG to goats 48h before norgestomet implant removal and reported that the number of anovulatory follicles were 6.00 ± 4.00 during breeding season and 6.75 ± 1.31 during non-breeding season. Pargonker *et al.* (1992) superovulated the goats with 1000 IU PMSG and administered 500 IU hCG at oestrus. The authors reported that the overall mean number of anovulatory follicles were 1.85. Pendleton *et al.* (1992) administered either 750 IU PMSG or 20mg FSH to superovulate the goats and observed that the mean number of anovulatory follicles were 3.10 ± 1.80 in PMSG treated animals and 0.90 ± 0.80 in FSH treated goats. The highest number of anovulatory follicles were observed in goats treated using 1500 IU PMSG and lowest in 15 mg and 20 mg FSH treated animals (Rosina *et al.*, 1992).

The number of anovulatory follicles were much fewer in LHRH or GnRH treated goats than in control group when FSH was used for superovualtion (Akinlosotu and Wilder, 1993 and Krisher *et al.*, 1994).

Dhanda and Lohan (1994) observed that the number of anovulated follicles were low during breeding season compared to non-breeding season in Black Bengal goats.

Deshpande *et al.* (1997) reported that the number of anovulatory follicles were 19 in three goats superovulated using 133 mg of NIH-FSH-P1. Tiwari *et al.* (1998) recorded that the number of anovulatory follicles were higher in animals treated with hCG on day seventh of oestrous cycle prior to gonadotrophin treatment than in control animals (3.12 ± 1.05 vs 5.12 ± 0.93)

2.4.2 Superovulatory Response

The percentage of ovulation was higher in PMSG treated prepubertal goats than in FSH treated kids which were pretreated with P_4 (Ryot and Vadnere, 1989; Majumdar *et al.*, 1990). The latter author reported that the average superovulatory response in PMSG and FSH treated Barbari goats were 9.50 ± 1.70 and 7.75 ± 2.10 respectively. The corresponding values reported by Kathiresan (1993) were 6.67 ± 2.20 and 4.00 ± 0.37 in the same age group of animals.

Average ovulation rate in peripubertal Angora goats treated with $PGF_2 \alpha$ and FSH was 6.70 ± 1.50 (Armstrong and Evans, 1983). While Holm *et al.* (1990) recorded that the superovulatory response in Angora hoggets treated with CIDR and FSH was 9.70 ± 5.10. The average superovulatory response in peripubertal goats treated with PMSG or P-FSH were 3.80 ± 3.30 and 9.20 ± 8.10 respectively (Nowshari *et al.*, 1992). Senn and Richardson (1992) reported that Nubian doelings (6.5 months to 8 months of age) which were treated with 6 mg

crestar implant and P-FSH exhibited a superovulatory response of 28.71 ± 2.30 (range 17 to 47) and 9.30 ± 3.70 (range 0 to 25) during breeding and non-breeding season respectively.

Wolf and McDougal (1994) reported that in eight month old ewe lambs superovulated using ovine FSH plus progestogen and $PGF_2\alpha$ combination, the ovulation rates were similar to those found in older sheep and that the quality of embryos were acceptable. Peripubertal Boer cross bred goats superovulated either using PMSG, pFSH or pFSH plus 40 per cent LH exhibited an ovulation rate of 3.80 ± 3.30, 9.20 ± 3.10 and 19.30 ± 9.00 respectively which showed a significant difference ($P<0.01$) (Yuswiati and Hotlz, 1996).

McNatty *et al.* (1989) administered purified ovine FSH (NIH-FSH-S16) in eight divided equal doses at 12 h interval and 180mg of porcine FSH (NIH-FSH-P1) in step-down doses at 12 h interval in adult goats. The authors reported that superovulatory response (mean number of CL) in these groups were 16.20 ± 2.10 and 16.30 ± 1.80 respectively. Taneja *et al.* (1991) recorded the superovulatory response in goats treated with 12 mg, 14 mg and 16 mg NIH-FSH-P1 as 14.83 ± 4.14, 19.63 ± 2.40 and 10.33 ± 1.60 respectively. Batt *et al.* (1993) reported that the mean ovulation rate was higher with ovine FSH treatment than with porcine FSH. Goel *et al.* (1993) recorded that ovulation rate in Jamunapari goats treated with FSH of equine origin was 13.38 ± 1.62. Superovulatory response in Tellicherry goats treated with porcine FSH, ovine FSH and PMSG were 16.16 ± 3.07, 21.83 ± 1.99 and 11.33 ± 2.67 respectively (Senthilkumar, 1996).

Baril and Vallet (1990) reported that the superovulatory response in FSH treated goats during breeding season and non-breeding season were 12.70 ± 5.20 (5 to 23) and 14.50 ± 7.70 (1 to 24) respectively. Rosina *et al.* (1992) recorded that does responded to gonadotrophins throughout the year with more than 50 per cent of does responding during rainy months compared to less than 35 per cent

responding during dry months. Pintado *et al.* (1998) reported that in Murciana goats treated with PMSG or PMSG plus anti PMSG antibodies, superovulatory response during fall was significantly lower regardless of the treatments adopted.

Puls-Kleingeld *et al.* (1991) compared the efficacy of purified FSH (PFSH) combined with either 40 or 80 per cent purified LH (PLH) and recorded that 40 per cent LH was superior in bringing about higher ovulation rates and increased yield of transferable embryos in goats. A subsequent study by Nowshari *et al.* (1995) who compared the effect of 30, 40 or 50 per cent PLH for superovulation of goats combined with PFSH confirmed finding by the former authors.

Seven injection schedule of FSH treatment caused an increase in ovulation rate in Garganica and Maltese breed when compared to four injection schedule (Martemucci *et al.,* 1992). Peebles and Kidd (1994) reported that administration of a single subcutaneous dose of 200mg NIH-FSH-P1 48h before the end of P_4 synchronisation, resulted in an ovulation rate of 19.90 ± 12.40 in Cashmere goats. Terzano *et al.* (1999) opined that ovulation rate was higher in Ionica and Derivata breeds of goats treated with eight injections of P-FSH.

Akinlosotu and Wilder (1993) studied the effect of LHRH in FSH treated anoestrus goats synchronised using 6 mg norgestomet. The authors found that administration of LHRH improved the ovulation rate and reduced the number of anovulatory follicles. Krisher *et al.* (1994), recorded that FSH plus GnRH treated goats showed a superovulatory response of 18.50 ± 0.70 as against 5.30 ± 4.10 in the control group. Baril *et al.* (1995) who controlled the ovulation time using GnRH antagonist (Antrelix) in FSH superovulated goats, reported a superovulatory response of 13.00 ± 5.40 and 15.90 ± 10.70 respectively in 0.50 mg and 1 mg Antrelix treated groups.

Peebles and Kidd (1994) reported that in Cashmere goats treated using 180 mg NIH-FSH-P1 and PMSG the superovulatory response was 20.20 ± 11.60. Superovulatory response in Jamunapari goats treated with PMSG plus FSH were reported as 7.25 (Goel and Agrawal, 1998). Pintado *et al.* (1998) recorded a superovulatory response of 9.22 ± 1.13 in FSH plus PMSG treated Mauriciana goats as against 10.20 ± 1.82 in the control FSH groups.

Goel and Agrawal (1998) reported that administration of priming dose of 3 mg FSH-P during the initial stage of P_4 synchronisation treatment and administration of 15 mg in six divided doses two days before the end of P_4 treatment resulted in an average superovulatory response of 7.33 in Jamunapari goats.

Salles *et al.* (1998) observed that 200 mg NIH-FSH-P1 was better for inducing superovulation in Saanen goats than 37.50 units of NIH-FSH-S1 when administered as six decreasing doses.

2.4.3 Premature Regression of Corpus Luteum

McIntosh *et al.* (1975) reported that the reasons for premature luteal regression in goats were unknown but might be related to excessive stimulation of follicles and high circulating levels of oestrogen during the early luteal phase which could exert a luteolytic effect. Pendleton *et al.* (1992) attributed this condition to altered endocrine patterns associated with superovulation. In contrast, Pintado *et al.* (1998) opined that PRCL could not be soley attributed to follicular persistence, stress might play a role.

Premature regression of corpus luteum (PRCL) was reported as a sporadic problem, which began around day three after superovulation. The causes of this problem were not completely known, but had been associated with low recovery

of embryos if flushed after fifth day from onset of oestrus (Armstrong *et al.*, 1983a).

However, Trevit *et al.* (1984) reported that 14 per cent of FSH superovulated Alpine goats in 1983, which did not receive any supplementary feeding during drought showed PRCL and low embryo recovery rates (66% vs 93%) unlike Saanens superovulated during the same period using FSH.

Trevit *et al.* (1986) reported that the prematurely regressing corpora lutea (PRCL) appeared small and white or light pink in colour as distinct from the rich red vascular appearance of functional corpus luteum (FCL). This phenomenon was associated with an early return to oestrus before the normal time of embryo collection. Embryo recovery rates were often reduced and this was thought to be due to abnormal tubal transport of embryos in does. Battye *et al.* (1988) reported that premature release of prostaglandin might be the cause for PRCL in female goats induced to superovulate using PMSG. Treatment of goats using flunixin meglumine a PG synthetase inhibitor from days three to seven of the superovulatory heat resulted in an increase in number of FCL as indicated by plasma progesterone profile and endoscopic examination.

Premature luteal regression had been reported to occur more frequently in goats superovulated with PMSG than FSH (McNatty *et al.*, 1989; Pendleton *et al.*,1992; Rosina *et al.*, 1992). McNatty *et al.* (1989) further opined that premature luteal failure was less common in goats treated with porcine FSH and appeared to be rare when ovine FSH was used for superovulation.

Martemucci *et al.* (1992) reported a high incidence of PRCL (31 to 46 %) in FSH superovulated Garganica and Maltese goats when different synchronisation and superovulation injection schedules were used. Rosina *et al.* (1992) attributed low embryo recovery rate in non-seasonal Kambing Kakang goats to premature regression of CL. The total number of embryos collected

from Murciana breed was low due to high incidence of PRCL (27% in autumn vs 44% in spring (Pintado *et al.,* 1996) The authors further reported that synchronisation regime did not affect superovulatory response. A high percentage of luteal regression was associated with FSH superovulation (Pintado *et al.,* 1998). The authors opined that gonadotrophin treatment had no effect on incidence of PRCL. A higher incidence of PRCL was observed during fall (45%) than during spring (17.50%) when FSH was used for superovulation.

2.5 EMBRYO COLLECTION

2.5.1 Embryo Collection Media

Menezo medium was used for flushing of uterus for embryo collection in goats (Chemineau *et al.,* 1986). The commonly used flushing medium for embryo collection is Dulbecco's Phosphate Buffered Saline (DPBS) solution with pH 7.2 to 7.4. This media was enriched with either heat inactivated vasectomised buck serum at 10 per cent level. (Trevit *et al.,* 1983) or fetal calf serum @ five to ten per cent level (Martemucci *et al.,* 1992; Senthilkumar, 1996; Dhandapani, 1998) or heat inactivated goat serum @ five to ten per cent level (Goel *et al.,* 1993; Nowshari *et al.,* 1995; Gogai, *et al.,* 2001) or new born calf serum at 10 per cent level (Krisher *et al.,* 1994) to prevent sticking of embryos to the collection petridishes and to facilitate easy handling and transfer of embryos.

Antimicrobial solution containing streptomycin, penicillin, fungizone or amphotericin-B was added to the flushing medium (Nuti *et al.,* 1987 and Pendleton *et al., 1*992) to avoid bacterial and fungal growth. The flushing medium consisted of modified PBS supplimented with 1 g per litre of sodium pyruvate, 10,000 IU/L pencillin 50 mg/litre streptomycin and 2 per cent V/V heat inactivated goat serum (Nowshari *et al.,* 1992). Phosphate buffered saline containg 2 per cent heat inactivated goat serum was used for embryo flushing by many other investigators (Dietrich *et al.,* 1992; Nowshari and Holtz, 1993;

Nowshari *et al.* 1995; Pintado *et al.,* 1998). Benjamin (1994) used PBS containing antibiotics for oviduct flushing of superovulated cross-bred goats. Deshpande *et al.* (1997) used 5 per cent Ringer's solution as flushing medium. Modified DPBS containing 0.20 per cent BSA fraction -V was used for uterine flushing (Tiwari *et al.,* 1998).

2.5.2 Techniques of Embryo Collection

In most instances surgical procedures have been used for recovery of embryos from goats. However, alternative techniques like laparoscopic and transcervical embryo collection had been developed. Embryos were collected after slaugther of superovulated goats and flushing the uterus thereafter (Chemineau *et al.,* 1986; Nowshari *et al.,* 1992; Nowshari and Holtz, 1993).

2.5.2.1 Surgical Method

In surgical method, the collection technique depends on the day that the embryos are to be collected

Three procedures involving laparotomy had been described by Flores-Foxworth *et al.* (1992) for surgical collection of embryos, all of which involve general anaesthesia exteriorisation of the uterus through the mid ventral incision and examination of ovaries for assessing superovulatory response. Techniques of collection involved oviductal stage, oviductal uterine stage and uterine stage embryos.

Oviductal Stage Embryos

Embryos were collected by flushing the oviduct of prepubertal goats (Kathiresan, 1993). Embryos were recovered surgically from Nubian doelings 60 to 72 h after the administration of hCG, via midventral incision and retrograde

flushing of anterior one third of each uterine horn and adjacent oviduct using approximately 15-20 ml of PBS (Senn and Richardson, 1992).

By adopting surgical procedures embryos were recovered from fallopian tubes of adult goats (Agrawal *et al.*, 1982; Thilagar *et al.*, 1992; Goel *et al.*, 1993; Krisher *et al.*, 1994; Deshpande *et al.*, 1997). A vertical incision of 6 cm was made on left flank and oviducts were catheterised and flushed using a smooth edged 22 gauge hypodermic needle attached to a syringe containing flushing medium, inserted through utero-tubal junction (Benjamin, 1994). By adopting midventral laparotomy on day three after breeding the oviductal embryos were collected through a tomcat catheter cannulated in the fimbriated end of the oviduct by flushing from utero tubal junction towards fimbria (Senthilkumar 1996; Goel and Agrawal, 1998).

Oviductal-uterine Stage Embryos

Embryos were collected from both oviduct and uterus through a Foley's catheter inserted in the uterine horn near the bifurcation by flushing the medium from fimbria towards uterus (Armstrong and Evans, 1983).

Several other investigators recovered embryos from both oviduct and uterus (Martemucci *et al.*, 1992; Pendleton *et al.*, 1992; Senn and Richardson, 1992; Gogai *et al.*, 2001).

Uterine Stage Embryos

Uterine stage embryos were collected through a Foley catheter inserted in the uterine horn near the bifurcation and flushing from uterotubal junction towards the body of the uterus (Nowshari *et al.*, 1992; Nowshari and Holtz 1993; Fieni *et al.*, 1995; Yuswiati and Holtz, 1996; Pintado *et al.*, 1998; Dhandapani *et al.*, 2001).

Tiwari *et al.* (1998) fixed an intestinal clamp cranial to the bifurcation of the uterine horn to prevent leakage of flushing medium from that point. A polyethylene catheter was fixed by making a stab wound cranial to the clamp. Media was flushed from uterotubal junction using a 20 gauge needle.

2.5.2.2 Non-surgical Embryo Collection

Embryo transfer is limited in its usefulness for genetic improvement or preservation of endangered species as long as embryos are collected from reproductive tract by conventional surgical methods as it has detrimental effect on fertility of female animal (Kraemer, 1989). Hence alternative techniques like laparoscopic and transcervical methods of embryo collections are being practiced now a days.

Laparoscopic collection

Sucessful laparoscopic collection of uterine stage embryos in goats were reported by many investgators (Brebion *et al.,* 1992; LeGal *et al.,* 1993; Baril *et al.,* 1995). Dhandapani (1998) collected six day old caprine embryos from the uterus of superovulated Barbari goats using laparoscopy.

Transcervical collection

Bessado *et al.* (1988) collected embryos from Angora goats by transcervical method. Gilbert *et al.* (1990) opined that the embryo recovery rate on uterine collection by the transcervical method, from goats which exhibited premature regression of CL was almost similar to the recovery rate from animals treated with flunixine meglumine or CIDR and collected by surgical method.

Flores-Foxworth *et al.* (1992) compared transcervical and laparscopic collection of embryos in goats. Goel *et al.* (1995) described a procedure for

successful non-surgical recovery of goat blastocysts six days after breeding from superovulated Jamunapari goats. For trasnscervical passage of catheter mechanical dilatation of cervix was done. In most of the reports of transcervical collections intracervical treatment with prostaglandin with or without oestradiol benzoate, made the embryo recovery possible (Gordon, 1997).

Injection of $PGF_2\alpha$ and oxytocin helped the introduction of the catheter into the uterus of standing unanaesthetised goats and facilitated embryo collection transcervically. The recovery rate and morphology of such embryos were comparable to that achieved by surgical collection. Healthy kids were born by transfer of such embryos (Pereira *et al.,* 1998; Suyadi *et al.,* 2000).

2.6 GRADING OF EMBRYOS

Embryo evaluation is an important determinant to the success of embryo transfer. Hence embryos should be evaluated as early as possible (Linder and Wright, 1983).

With the advent of increasing technologies to manipulate the mammalian embryos *in vitro* increased emphasis is being placed on predictive assessment of embryo viability *in vitro*. The viability tests help to evaluate embryo morphology, development *in vitro,* blastomere membrane integrity and embryo metabolism. (Overstorm, 1996).

2.6.1 Embryo Morphology

Morphological evaluation has been widely used to delineate embryo quality in bovine (Shea *et al.,* 1976; Linder and Wright, 1983).

Another widely used criteria for embryo evaluations is whether an embryo has attained appropriate stage of development for its chronological age

(Elsden *et al.*, 1978). Cell spreading method or DNA specific dyes were used to assess the embryonic development in goats (Sakkas *et al.*, 1989; Baril *et al.*, 1995). Fluorescence diacetate (FDA) reveals both membrane integrity and cytoplasmic esterase activity. These dyes readily permeate blastomeres where active cytoplasmic esterases cleave the acetate moieties from fluorescein which exhibit green fluorescence when exposed to UV light. Fluoresence diacetate was used to study the viability of goat embryos cultured *in vitro* (Sakkas *et al.*, 1989).

Chemineau *et al.* (1986) and Tsunoda and Sugie (1989) classified caprine embryos as unfertilized, degenerate and normal. Embryos were graded as good, early degenerate and degenerate (McNatty *et al.*, 1989). A three grade system of excellent, good and poor was used to describe caprine embryos (Martemucci *et al.*, 1992). Nowshari *et al.* (1992) recorded that embryos with no visible imperfections and those with a few extruded blastomeres were graded as transferable and those with severe imperfections (blastomeres of varying size or showing signs of degeneration) as non transferable. Several investigators classified the embryos based on their morphological characteristics as excellent, good, fair and poor or degenerate (Pendleton *et al.*, 1992; Akinlosotu and Wilder, 1993; Kathiresan, 1993; Senthil kumar, 1996; Dhandapani, 1998). The authors considered only excellent and good quality embryos as transferable.

LeGal *et al.* (1993) considered the criteria of normalcy as follows: morulae with blastomeres of regular size and similar shape, compacted morulae with one or two blastomeres out of compacted mass, blastocysts and expanded blastocysts with well characterized blastocoele and inner cell mass. Only embryos with cleavage stage appropriate to their age were used for cryopreservation i.e. $\geq$ 16 cells at day six; compact morulae or early blastocysts at day seven, and expanded blastocysts at day eight.

Fieni *et al.* (1995) followed Eldsen's classification and described goat embryos as grade one, two and three in which grade one and two were considered

as viable. Embryos with no visible imperfections were graded as good, those with few extruded blastomeres as fair and those with severe imperfections (blastomeres of various sizes and showing signs of disintegration) as poor. Good and fair were considered as normal (Nowshari and Holtz, 1995).

2.6.3 Developmental Stages of Early Goat Embryos

Baril *et al.* (1988) opined that there could be a great variation in embryo developmental stages among goats collected at the same interval after oestrus as well as within a batch of embryos collected from the individual doe. The first 16-cell (7%) embryo was collected 84h after ovulation and 96h after ovulation 60 per cent of embryos collected had 16-cells or more. The following three cell cycles occurred more rapidly lasting between 10 and 12h. Early blastocysts were first collected 120h after ovulation while after 168h after ovulation all embryos collected were hatched blastocysts (Sakkas *et al.*, 1989).

Tsunoda and Sugie (1989) recorded that the development of the two cell, four cell to eight cell, 16 cell to morula, blastocyst and zona free bastocysts stages were first observed at 1.5, 2.5, 4.5, 6 and 6.5 days respectively after hCG injection.

Rosina *et al.* (1992) recorded that out of 149 fertilized ova collected on day five or six of the oestrous cycle included, 60 per cent morulae, 18 per cent early blastocysts and 14 per cent eight - cell embryos.

Out of the 160 fertilized ova collected on day six after oestrus 31.30, 66.30 and 2.50 per cent were blastocysts, morulae and eight cell stage respectively (Mani *et al.*, 1994). The developmental stages on day two, three, four, five, six, seven and eight after onset of oestrus were described as one to two cells, four to eight cells, eight to 20 cells, 20 cells to early morula, morula, compact morula to expanded blastocyst, expanded blastocyst to hatched

blastocyst respectively (Baril *et al.*, 1995). Goel *et al.* (1995) reported that majority of embryos collected on day seven were compact morula to blastocyst stage.

2.7 EMBRYO RECOVERY RATES

Embryo recovery rates in goats superovulated using FSH was much higher than that from animals treated using PMSG. Luteal failure and early return to oestrus was reported to be a major problem in superovulated goats which affects embryo recovery rates adversely when flushed later than five days after oestrus (Trevit *et al.*, 1983).

2.7.1 Oviductal Embryos

Senn and Richardson (1992) recorded that average viable embryo recovery rate per doeling in Anglo Nubian breed was 15.10 ± 2.00 and 3.30 ± 3.20 during early breeding and late breeding season respectively. Kathiresan (1993) reported that number of cleaved, uncleaved and total ova recovered from PMSG and FSH treated prepubertal goats were 2.50 ± 0.85, 1.83 ± 0.31 and 4.33 ± 1.09 and 1.33 ± 0.49, 1.50 ± 0.56 and 2.83 ± 0.70 respectively when collected on day three from oviducts.

Embryo recovery rate was higher in adult goats treated with FSH than those treated with PMSG (Mohamood *et al.*, 1991; Nowshari *et al.*, 1992; Rosina *et al.*, 1992; Senthilkumar, 1996; Goel and Agrawal, 1998; Pintado *et al.*, 1998).

Taneja *et al.* (1991) treated goats using 12 mg, 14 mg and 16 mg purified FSH (Folltropin-V) and recorded a total embryo recovery rate of 12.67 ± 3.46, 13.56 ± 1.65 and 10.17 ±1.27 and transferable embryo recovery rate of 11.00 ± 0.47, 12.50 ± 2.06 and 9.83 ± 1.08 respectively. Maximum embryo recovery rate was observed on day four following oestrus (64.51 %) and a slightly lower rate

on day three (57.14%) but the recovery rate sharply declined (22.01%) after day six following oestrus on flushing the fallopian tubes (Pandey *et al.*, 1992). Goel *et al.* (1993) recorded that the embryo recovery and number of transferable embryos were 7.46 ± 1.60 and 2.17 ± 0.78 by oviductal flushing of goats superovulated using equine FSH. Embryo recovery rate in goats treated with PMSG plus $PGF_2\alpha$ and PMSG plus P_4 were 57.14 per cent 50.64 per cent respectively of which 70.83 per cent and 74.60 per cent were of transferable quality (Benjamin, 1994).

Embryos were collected on day three post oestrus from Tellicherry goats treated with FSH-P, FSH-O and PMSG. Embryo recovery rates of 81.96 per cent, 74.05 per cent and 44.12 per cent were observed in FSH-P, FSH- O and PMSG treated does respectively of which 7.16 ± 1.96, (43%), 13.16 ± 1.74 (79%) and 4.15 ± 1.14 (25%) were of transferable quality (Senthilkumar, 1996). Embryos were collected from Jamunapari goats treated with PMSG, FSH-P, 3 mg FSH-P priming prior to FSH-P and PMSG plus FSH-P. The embryo recovery rates were 3.00 ±1.00, 7.00 ± 1.52, 5.66 ± 1.86 and 2.25 ± 1.43 of which 2.00 ± 0.05, 5.11 ± 1.60, 3.33 ± 1.45 and 0.75 ± 0.75 were of transferable quality, respectively (Goel and Agrawal, 1998).

Pronuclear stage caprine embryos were recovered laparoscopically at 75-86 h after $PGF_2\alpha$ injection and an embryo recovery rate of 72 per cent was recorded at first collection (Kuhholzer *et al.*, 1998).

2.7.2 Uterine Stage Embryos

The average embryo recovery rate from Angora kids superovulated using FSH was 3.90 ± 1.20 (Armstrong *et al.*, 1982). Holm *et al.* (1990) superovulated Danish Angora and dairy hoggets using 23 mg FSH-P or 17 mg Folltropin-V (Vetrepharm, Canada) and reported a mean egg and embryo recovery rates of 9.70 ± 5.10 and 8.60 ± 5.00 respectively, when collected on days 6.5 to 8 after

breeding. Majumdar *et al.* (1990) recorded that embryo recovery rates from prepubertal goats (Pretreated with P_4 and oestradiol) superovulated using, PMSG and FSH-P were 7.25 ± 2.00 and 4.75 ± 2.60 respectively.

The average embryo recovery rate in peripubertal Boer goats superovulated using PMSG and FSH-P were 3.10 ± 3.90 and 6.90 ± 5.80, respectively. Out of these 32.30 per cent and 53.60 per cent were recorded to be of transferable quality when collected from uterus on days six to seven after last mating (Nowshari *et al.,* 1992). The embryo recovery rate on day seven after breeding in peripubertal goats treated with PMSG, PFSH and PFSH plus 40 per cent LH were 3.10 ± 3.90, 6.90 ± 5.80 and 7.90 ± 7.60 respectively of which 29 per cent, 56 per cent and 84 per cent were of transferable quality. A significantly, higher number of ovulation and transferable quality embryos were obtained when animals were treated with FSH plus 40 per cent LH (Yuswiati and Holtz, 1996).

Brebion *et al.* (1992) reported that surgical collection of embryos six to seven days after fertilization in adult goats resulted in an embryo recovery rate of 72 to 75 per cent, but repeatability was low. The authors further reported that embryo recovery rate by laparoscopic collection although less in first collection, (63%) was successful upto seven collection. Martemucci *et al.* (1992) recorded that egg recovery rate was significantly higher in Maltese (81.10%) than in Garganica goats (59.40%) as well as with 72 h FSH-P treatment than 36 h treatment (81.10% vs 46.90%, $P<0.01$). The authors further reported that a high incidence of PRCL contributed to the reduced embryo recovery rates. Significantly higher number of embryos were obtained by FSH treatment than PMSG superovulations when collected on six to seven days after last mating (4.60 vs 0.40), (Nowshari *et al.,* 1992). Egg recovery was better in FSH treated goats than in PMSG treated ones (6.80 ± 5.30 vs 3.00 ± 3.80 per doe). Higher dose of PMSG treatment caused premature regression of CL and 10 animals which had exhibited PRCL did not yield any embryos (Rosina *et al.,* 1992).

The embryo recovery rate per doe in FSH plus LH-RH treated anoestrus goats were significantly higher than the control group. (10.10 ± 0.84 vs 1.80 ± 0.40). The percentage of viable embryos were 82.20 and 72.20 respectively in these groups (Akinlosotu and Wilder, 1993). Caprine embryos collected on day six post oestrus were found to be of 69.90 per cent fertilized ova and 30.10 per cent non-fertilized (Mani *et al.*, 1994).

Baril *et al.* (1995) recorded an embryo recovery rate of 64.80 per cent of which 62.20 per cent were cleaved. The mean number of transferable embryos per FSH treated goat and per collected goat was 4.50 ± 4.40 and 5.30 ± 4.50 respectively while 14.60 per cent of treated goats exhibited PRCL. Feini *et al.* (1995) reported that the embryo recovery rate was 7.10 per goat superovulated using FSH. The fertilization rate was fairly high (78%), grade three embryos were low (8%). Pintado *et al.* (1996) opined that fewer embryos were recovered from goats with regressed copora lutea (1.20 ± 0.60) than from those with normal luteal structures (8.90 ± 0.90) when superovulated using 16 mg FSH-P and collected after day-six.

Dhandapani (1998) recorded that in goats superovulated using PMSG the embryo recovery rate was 61.36 per cent by surgical collection on day six. The author further reported that embryo recovery rate was 18.92 per cent on laparoscopic collection. Pintado *et al.* (1998) compared the effect of PMSG alone or in combination with anti PMSG antibodies for inducing superovulation in Mauriciana goats and reported that anti PMSG administration was beneficial in improving viability of embryos. Anti PMSG treatment resulted in recovery of almost twice the number of viable embryos. (5.75 vs 2.74). The total embryos collected were lower than other breeds due to high incidence of PRCL which affected 32 per cent of goats superovulated, from which no embryos could be collected.

Tiwari *et al.* (1998) compared the effect of hCG administration prior to start of basic superovulatory treatment using FSH of porcine origin and found that embryo recovery rate per doe in control and treatment group were not significantly different (2.87 ± 0.51 vs 2.87 ± 0.26).

Gilbert *et al.* (1990) recorded an embryo recovery rate of 75.70 per cent by non-surgical collection of embryos even though 35.90 per cent of the 23 does exhibited PRCL, the percentage of degenerate embryos were low in these goats (3.30 %). The author further reported that in does treated with Flunixin meglumine (Finadyne) and CIDR the total egg recovery rate was 79.00 per cent and 86.90 per cent respectively of which 37.50 and 7.60 per cent were degenerate embryos. Flores-Foxworth *et al.* (1992) compared laparoscopic and transcervical embryo collection in goats and concluded that laparoscopic collection had significantly higher, embryo recovery than transcervical method. Goel *et al.* (1995) carried out non-surgical collection of embryos from Jamunapari goats. The average number of embryos, collected on day seven from successful goats was two.

Pereira *et al.* (1998) and Suyadi *et al.* (2000) compared the effect of administration of $PGF_2\alpha$ and oxytocin at various intervals before transcervical collection on embryo recovery rates in unanesthetised Boer goats controlled in a standing position. The recovery rate was estimated as percentage of embryos recovered relative to the number of CL counted endoscopically. Injection of $PGF_2\alpha$ six or eight hours before transcervical embryo collection resulted in a significant increase in embryo recovery (-6 h: 91% with oxytocin, 85% without; -8 h: 91% with oxytocin, 80% without).

2.8 CRYOPRESERVATION

In past quarter of a century, since the first successful freezing of mouse embryos (Whittingham *et al.*, 1972), basic and applied research has resulted in

cryopreservation of embryos of many mammalian species. (Willadsen *et al.*, 1976; Willadsen *et al.*, 1978; Maria *et al.*, 1985). First successful freezing of the goat embryo was carried out by Bilton and Moore (1976).

2.8.1 Methods of Freezing

The conventional controlled freezing and thawing procedures of embryo cryopreservation included initial exposure to an equilibration with cryoprotectants, cooling to subzero temperatures, induction of crystalization (seeding), slow cooling, storage in liquid nitrogen thawing and finally dilution and removal of cryoprotectants and return to physiological environment that will allow further development. Ultra rapid freezing, one step procedure and vitrification are other techniques for cryopreservation of embryos. One step procedure is a modification of controlled freezing and thawing that omits cryoprotectant dilution and microscopical evaluation (Niemann, 1991).

Recently Jainudheen *et al.* (2000) classified the freezing methods into two, namely conventional or "equilibrium cryopreservation" and "non-equilibrium" cryopreservation or vitrification .

2.8.1.1 Conventional or "Equilibrium" Cryopreservation

In this technique the embryos were placed in a cryoprotectant solution in PBS supplemented with serum or BSA at room temperature and the embryos were allowed to equilibrate for 15-20 minutes. After equilibration embryos were loaded in straws and subjected to cooling process. During the cooling process straws were seeded (-4° to -7°C) and cooling was continued at the rate of 0.3 to 0.5°C per minute to -30°C to -40°C when embryos were plunged into liquid nitrogen. A major portion of research work on freezing of goat embryos had been carried out using this procedure (Chemineau *et al.*, 1986; Holm *et al.*, 1990; Li *et al.*,1990; Brebion *et al.*, 1992; LeGal *et al.*, 1993; Riha *et al.*, 1994; Fieni *et al.*,

1995, Hsu, 1995). Goat demi-embryos were cryopreserved using conventional technique (Tsunoda *et al.*, 1987; Udy, 1987; Wang *et al.*, 1988; Yong and Wang, 1990; Nowshari and Holtz 1993). Rao *et al.* (1988) and Rong *et al.* (1989) tried a technique referred as rapid freezing.

Freezing medium

In most of the freezing procedure the medium used was modified PBS (Bilton and Moore, 1976; Li *et al.*, 1990; Nowshari and Holtz, 1993; Fieni *et al.*,1995; Hsu, 1995) which contained 10-20 per cent serum (Fetal calf serum or heat inactivated goat serum) or 0.4 per cent BSA (Fieni *et al.*, 1995) and cryoprotectants.

Chemineau *et al.* (1986) used Menezo medium containing steer serum for freezing of goat embryos. Whereas, TCM-199 containing Hank's Salts was tried by Mani and Vadnere (1988b). Riha *et al.* (1994) carried out freezing of goat embryos using ovum culture medium (OCM) containing 5 per cent heat inactivated goat serum.

Cryoprotectants

The cryoprotectants generally fall into two categories. Intracellular and extra cellular. Common intracellular cryoprotectants used for embryo cryopreservation are glycerol, ethylene glycol (EG), dimethyl sulphoxide (DMSO) and propylene glycol. The extracellular cryoprotectants are larger molecules, such as sugars (monosacharides or disacharides) and proteins (eg. BSA and hyaluronic acid) (Gordon, 1994).

Goat embryos were frozen using either 1.4 or 1.5 M glycerol (Rao *et al.*, 1988; Plus Kleingeld *et al.*, 1992; LeGal *et al.*, 1993; Nowshari and Holtz, 1993; Fieni *et al.*, 1995) or, 1.5 M or 1.8 M Ethylene glycol (Brebion *et al.*, 1992;

LeGal *et al.,* 1993; Riha *et al.,* 1994; Fieni *et al.,*1995; Hsu *et al.,*1995) or, 1.4 M or 1.5 M DMSO (Fieni *et al.,* 1995).

A comparison of cryoprotectants revealed that EG was better for cryopreservation of goat embryos than glycerol or DMSO (LeGal *et al.,* 1993; Fieni *et al.,* 1995).

Cryoprotectant exposure and equilibration

The embryos were exposed to increasing concentration of cryoprotectants (0.5M, 1.0M, 1.5M EG, DMSO or glycerol) at an interval of seven to 10 minutes in two or three steps. (Bilton and Moore, 1976; Li *et al.,* 1990; LeGal *et al.,* 1993, Riha *et al.,* 1994; Fieni *et al.,* 1995).

In contrast, Chemineau *et al.* (1986) and Rao *et al.* (1988) exposed the embryos to 1.4M or 1.5M glycerol in a single step for a period of 30 minutes and 15 minutes respectively. Plus-Kleingeld *et al.* (1992) compared one step procedure of cryoprotectant addition with three step procedure to obtain final concentration of 1.4M glycerol in the freezing media. The author observed that one step procedure was superior to three step procedure for freezing of blastocysts from goats.

Freezing Programme

Li *et al.* (1990) loaded embryos in 0.25 ml insemination straws and cooled at the rate of 2°C/minute to -7°C, held at this temperature for 10 minutes during which formation of ice crystals was induced by touching the straws with precooled forceps (seeding) and then further cooled to at the rate of 0.3°C per minute -36°C before being transferred to liquid nitrogen.

Riha (1994) started the freezing procedure at bench temperature (20°C) and cooled the straws at the rate of -1°C per minute to 15°C, then gave a pause for five minutes for stabilization of freezing chamber. From 15°C to -7°C the cooling was done in five minutes. The straws were held for 10 minutes at -7°C and seeded after 10 minutes. Then the straws were cooled at the rate of 0.3°C minute to -37°C and there after plunged into liquid nitrogen.

Fieni *et al.* (1995) placed the straws horizontally in the cooling chamber of a programmable freezer precooled to -7°C. After an equilibration period of five minutes, manual seeding was done, and the straws were maintained at this temperature five more minutes. The temperature was then reduced to -35°C at the rate of 0.4°C minute, held for five minutes at this temperature and then the straws were stored in liquid nitrogen.

Thawing and cryoprotectant removal

In conventional multistep dilution method, goat embryos were exposed to decreasing concentration of protective agents which usually requires a microscope and a minimum of 1-2 h to be carried out under laboratory conditions (Chemineau, 1986; Li *et al.*, 1990; PlusKleingeld, *et al.*, 1992; Nowshari and Holtz, 1993).

For thawing, straws were placed into a water bath at 30°C for 40 seconds. Then the cryoprotectant was removed in three steps by decreasing concentration of glycerol in 0.3 M sucrose solution at eight minutes interval, embryos were washed three times in culture medium and evaluated (PlusKleingeld, *et al.*, 1992; Nowshari and Holtz, 1993; LeGal *et al.*, 1993).

Exposing the straws in air for seven seconds and water bath at 35°C for 25 seconds thawing was effected. Embryos were exposed to a solution of 0.75 M ethylene glycol together with 0.5 M sucrose in OCM (10 minutes) and 0.5 M

sucrose in OCM (10 minutes). Then embryos were washed in OCM and evaluated microscopically (Riha, *et al.*, 1994).

Fieni *et al.* (1995) thawed the straws rapidly by immersing in a water bath at 37°C for one minute. Removal of cryoprotectants was done by three methods: the classic three-step procedure (Cryoprotectant 1 M-10 minutes; 0.5 M-10 minutes; PBS alone 10 minutes), the same procedure but with 0.25 M sucrose added to the first two steps and a two-step procedure with sucrose alone. The 3-step procedure with sucrose gave the best development rate *in vitro* and differed significantly from the classic three-step procedure.

Hsu (1995) who reported a simple one-step procedure thawed the straws containing embryos in a water bath at 20°C. Two embryos with the cryoprotectant (1.8 M EG) were surgically transplanted directly into the uterus of recipient goats, which resulted in the birth of two kids.

In cattle, embryos in 0.5 ml straws were thawed in air for 20 seconds followed by 20 seconds in water (37°C) while those in 0.25 ml straws were thawed for 15 seconds in air and 20 seconds in water (37°C). The exposure to air reduced damage to zona pellucida (Jainudeen *et al.*, 2000).

2.8.1.2 Vitrification

Vitrification, an innovative freezing and thawing procedure minimizes procedure time and eliminates the need for a programmed freezing (Friedler *et al.*, 1988).

First successful transfer of vitrified goat embryos were reported by Yuswiati and Holtz (1990) in Germany. Morphologically intact blastocysts were vitrified by open pulled straw method (OPS) (Vajta *et al.*, 1998). El-gayar and

Holtz (2001) reported very high success rate on transfer of vitrified goat blastocysts by OPS technique.

In India Agrawal *et al.* (1994) successfully vitrified goat embryos using glycerol and 1-2 propanediol, which resulted in the birth of live kids.

2.9 *IN VITRO* CULTURE

Collection and maintenance of viable mammalian embryos were the key factors for the successful transfer and study of normal embryos. The culture of embryos was of two types, long term (days) and short term (hours). Long-term culture was used to study the effect of media atmosphere and embryo handling system. It was carried out to develop the one to two cell stage embryos produced by *in vitro* fertilization (IVF) to the blastocyst stage. Short term culture systems helps to eliminate the dead and defective embryos before transfer. The effect of freezing and thawing of embryos could be assessed using short-term cultures (Mani and Vadnere, 1988b; Sakkas *et al.*, 1989).

2.9.1 Culture Media

Several culture and co-culture media had been used for *in vitro* culture of goats embryos with varying results. Routinely sera or albumin were added to the media. Types of media that were used for culture of 5-7 day old embryos include simple media like PBS with 10-25 per cent goat serum (Bilton and Moore, 1976; Nowshari and Holtz, 1995).

Li *et al.* (1990) and LeGal *et al.* (1993) cultured 6-7 day old frozen goat embryos in bicarbonate buffered saline containing 5 per cent heat inactivated goat serum and B_2 medium supplemented with 10 per cent heat inactivated calf serum respectively.

Senn *et al.* (1993) cultured two cell to early morula stage fresh embryos in TCM-199 containing a commercially produced serum substitute Nu-serum (Nu-S) at concentrations of 2.5 per cent, 5 per cent, 10 per cent, 20 per cent TCM 199 alone or TCM 199 supplemented with FBS. All media contained 0.0450 mg/ml streptomycin and 0.0675 mg/ml penicilling-G.

Izquierdo *et al.* (2002) studied the development of *in vitro* produced embryos (from prepubertal goat ovaries) for eight days using co-culture with oviduct epithelial cells (OEC) and cumulus cells of both bovine and caprine origin in TCM-199. Culture was also carried out in presence of serum and OEC. Four culture media, TCM-199, Ham's F_{10}, CZB (Chatot Zionek Bavister) and synthetic oviduct fluid (SOF) were compared for culture of OEC used for co-culture. Conditional medium prepared using TCM-199 and OEC was also tested for culture of early stage goat embryos.

2.9.2 Culture Environment

The embryos were placed in pyrex glass tubes, (Bilton and Moore, 1976) during culture. Covered culture dishes containing 2 to 5 ml media served as culture Vessels (Mani and Vadnere 1988a; Nowshari and Holtz, 1995). Recently microdroplets of culture media (25 µl to 500 µl) under gas-medium equilibrated paraffin oil in petridishes, multiwell plates or concavity slides were utilized for embryo culture and co-culture (Li *et al.*, 1990; LeGal *et al.*, 1993; Fieni *et al.*, 1995; Izquierdo *et al.*, 1999).

The Culture of fresh and frozen embryos were carried out at 37°C under air (Mani and Vadnere 1988a&b). Sakkas *et al.* (1989) used a culture atmosphere of 37°C and 5 per cent CO_2 for culture and co-culture of early goat embryos. Short term culture of fresh and frozen emrboys were carried out in a moisture saturated atmosphere of five per cent CO_2 in air at 37 to 38.5°C (Li *et al.*, 1990; LeGal *et al.*, 1993; Nowshari and Holtz, 1995; Fieni *et al.*, 1995).

Co-culture of *in vitro* produced goat embryos with OEC was carried out at 38.5°C in five per cent CO_2 and humidified air (Crozet *et al.,* 1995). Izquierdo *et al.* (1999) carried out co-culture at 38.5°C in an atmosphere of five per cent CO_2 and 20 per cent O_2.

Some of the investigators, while carrying out culture, exchanged the medium under microdroplets for fresh medium at regular intervals of 24 or 48 h (Fieni *et al.,* 1995; Izquierdo *et al.,* 1999).

2.9.3 Development in Culture

The studies of Senn *et al.* (1993), indicated that goat embryos may not exhibit the stage specific block characteristic of cattle and sheep embryos. The author further reported that, in comparison with other farm ruminants, there was no great difference in goats in the timing of shift in the control of gene transcription from the maternal to the embryonic genome.

2.9.3.1 Fresh and Chilled Embryos

Bilton and Moore (1976) successfully cultured fresh and chilled caprine embryos. Thirteen of the 15 fresh embryos showed normal development in culture, but only 20 of the 38 embryos stored at 5°C developed in culture. Mani and Vadnere (1988a) cultured embryos of morule and early bastocyst stage for 24 h. Out of the 12 morulae and early blastocyst stage, six developed to the next stage (50%). Of the morulae, four developed into early blastocysts (80%) and of the seven early blastocysts two developed in to blastocysts (28.50%).

Senn *et al.* (1993) evaluated the effect of commercially produced serum substitute (Nus) on *in vitro* development of two cell to early stage goat embryos. Embryos developed in all concentration of Nus to the morula, blastocyst and expanded blastocyst stages. The TCM 199 plus 10 per cent Nus showed

significantly higher percentages of embryos developing to the expanded blastocyst stage than TCM 199 plus FBS (62.00 ± 6.20% vs 44.60 ± 6.40%).

Nowshari and Holtz (1995) cultured morulae to the blastocyst stage before freezing. Out of the 133 good and fair quality morulae 107 (80.50%) developed to blastocysts within 24 h of culture, of which 90.7 per cent were suitable for cryopreservation.

2.9.3.2 Frozen Embryos

Bilton and Moore (1976) recorded that 12.50 percentage of 5-7 day old caprine embryos frozen using 1M glycerol or 3M DMSO developed to the next stage during 24 to 48 h of culture.

Mani and Vadnere (1988b) carried out *in vitro* culture of frozen goat embryos for 24 h and opined that 2M DMSO and 1M glycerol gave better cryoprotection to embryos (75% vs100%) than 1.5 M DMSO and 1.33 M glycerol (25% vs 50%). Embryos were deemed viable if they developed from morula to blastocyst or from blastocyst to hatching and hatched blastocyst during 48 h or if hatched blastocyst became reexpanded during 24 h culture *in vitro*. It was observed that the rate, of survival increased as stage of development of embryos increased (Li *et al.*, 1990).

LeGal *et al.* (1993) compared the *in vitro* survival rate of cryopreserved morulae and blastocysts. *In vitro* development of frozen thawed blastocyst was always higher (40.30%) than that of frozen morulae (14.30%) irrespective of the cryoprotectant used. Development *in vitro* showed ethylene glycol to be a better cryoprotectant than glycerol at both developmental stages (23% vs 0%, 45% vs 35%) for morulae and blastocysts respectively.

Fieni *et al.* (1995) evaluated the viability of goat embryos frozen using glycerol, ethylene glycol and DMSO by *in vitro* culture for 48 h. The *in vitro* viability of morulae was significantly higher when they were frozen with EG than DMSO or glycerol ($P<0.05$). The blastocysts frozen with ethylene glycol or DMSO showed an *in vitro* survival rate significantly better than that of the morulae (54% vs 16% with EG, 41% vs 9% with DMSO). The developmental rates of blastocysts was significantly low when glycerol used as cryoprotectant ($P<0.05$).

Traldi (2000) recorded an embryo survival rate of 60 per cent when vitrified *in vitro* produced goat embryos were cultured either in synthetic oviduct fluid medium or granulosa cell monolayer.

2.10 EMBRYO TRANSFER

Although the first surgical goat embryo transfer date back to the early 1930S, the increasing importance of goat ET in breeding improvement programmes around the world in recent decades has led to the use of laparoscopy as an alternative transfer procedure.

2.10.1 Surgical Transfer

Agrawal *et al.* (1982) transferred embryos collected 72 h after breeding into the fallopian tubes and later stage embryos into uterus. Embryos were transferred using a Pasteur pipette or unopette in 0.01 ml of holding media (Drost, 1986; PulsKleingeld *et al.*, 1992).

Majumdar *et al.* (1990) transferred fresh emrbyos from prepubertal goats into the uterus of adult recipient goats. Several other investigators reported surgical transfer of fresh embryos into uterus. (Agrawal and Goel, 1991; Dietrich *et al.*, 1992; Mani *et al.*, 1994; Deshpande *et al.*, 1997).

Several research workers recorded intra uterine transfer of conventionally frozen embryos surgically in goats (Holm *et al.,* 1990; PulsKleingeld *et al.,* 1992; Nowshari and Holtz, 1995).

Surgical uterine transfer of vitrified goat embryos into the uterus were recorded by Yuswiati and Holtz (1990) and Agrawal *et al.* (1994).

2.10.2 Laparoscopic Transfer

Reproductive tract was visualized via a laparoscope and uterus held by grasping forceps. Stab wounds were made in the uterine wall and embryos were deposited using flexible tubings (Kraemer, 1989). A report by Vallet *et al.* (1989) showed that comparable results could be achieved by laparscopy in comparison to surgery; the authors listed several advantages to laparoscopy under field conditions, including speed of transfer (5 vs 15 minutes) and freedom from genital tract adhesions.

Laparoscopic intrauterine transfer of frozen embryo were reported by many investigators (Li *et al.,* 1990; Flores-Foxworth *et al.*, 1992; LeGal *et al.,* 1993; Riha *et al.,* 1994).

Besenfelder *et al.* (1994) reported laparoscopic transfer of early stage embryos into the oviducts of goats. The authors opined that if used routinely, this technique would take less than five minutes for transfer of embryos. After manipulation of reproductive organs, they recorded no visible alterations or injuries.

2.10.3 Transcervical Transfer

Agrawal and Battacharya (1982) successfully transferred goat embryos non-surgically. Flores-Foxworth *et al.* (1992) compared the transcervical and

laparoscopic techniques of embryo transfer in goats. The authors did not observe any significant difference in terms of kidding between non-surgical and laparoscopic transfer of embryos.

2.11 PREGNANCY AND EMBRYO SURVIVAL RATES

There was no difference in the pregnancy rates, which resulted from transfer of oviduct stage embryos, as opposed to uterine stage embryos (Armstrong and Evans, 1983). The authors further reported that optimum pregnancy rates could result when the time of onset of oestrus in the donor animal coincided as closely as possible with that of recipients.

Factors influencing the rate of survival of frozen thawed embryos included the type and concentration of cryoprotectant used, the rate of cooling, thawing and the developmental stages of embryos used for freezing. (Leibo, 1986; Li *et al.,* 1990; PulsKleingeld *et al.,* 1992; LeGal *et al.,* 1993) and origin (Han *et al.*, 2001) Ethylene glycol was reported to be superior cryoprotectant for freezing of goat embryos with higher survival rates approaching those achieved with fresh embryos and possibility to transfer directly after thawing (Brebion *et al.,* 1992).

2.11.1 Fresh Embryos

Six good quality embryos collected from prepubertal superovulated Barbari goats were transferred to two recipients, both conceived and gave birth to five live kids (Majumdar *et al.,* 1990). Goel *et al.* (1993) transferred 13 morphologically normal embryos (eight cell to blastocysts) from Sirohi goats to six recipient goats and recorded a pregnancy rate of 83.33 per cent, resulting in the birth of five kids. Riha *et al.* (1994) reported that fresh Angora goat embryos transferred to white Sannen type horned recipients during breeding season

resulted in a pregnancy rate of 71.40 per cent compared to an out of season pregnancy rate of 10 per cent.

Besenfeldor *et al.* (1994) carried out enodscopic tubal transfer of eight cell goat embryos from Boer goats. Out of nine recipients five became pregnant with an overall embryo survival rate of 36 per cent.

The effect of under nutrition either before or after embryo transfer on pregnancy rate and embryo survival rate was studied by Mani *et al.* (1994). The authors opined that the pregnancy rates and embryo survival rates were considerably reduced in goats undernourished after ET.

Beckett *et al.* (1999) reported a pregnancy rate of 38 per cent and embryo survival rate of 25 per cent on transfer of 95 fresh demi embryos to 55 recipients. The authors further reported that with exogenous progestin support, transfer of 29 demi embryos to 15 recipients resulted in a pregnancy rate of 62 per cent and an embryos survival rate of 34 per cent.

2.11.2 Frozen Embryos

Chemineau *et al.* (1986) transferred 63 morphologically normal embryos after thawing. The percentage of kids born on transfer of hatched blastocysts was higher (64%) than young and expanded blastocysts (36%). Rao *et al.* (1988) reported that on transplantation of 16 embryos frozen using glycerol and 39 embryos frozen using DMSO into foster mothers, six and 16 kids were born respectively. Both cryoprotectants afforded a similar cryoprotection in this rapid freezing procedure.

Holm *et al.* (1990) recorded a pregnancy rate of 75 per cent and embryo survival rate of 54 per cent for Angora goat embryos. Li *et al.* (1990) reported that the survival rate of frozen thawed seven day old embryos was 59 per cent

(64/109) on transfer to recipients. The authors further opined that the rate of survival increased as the stage of development of the embryos increased.

Brebion *et al.* (1992) reported that when embryos were frozen using 1.5 M ethylene glycol as a cryoprotectant with 20 per cent fetal calf serum (FCS) in the medium, 85 per cent and 65 per cent survived freezing and transfer respectively. PlusKleingeld *et al.* (1992) reported that blastocysts were more suitable for cryopreservation than morulae and 1-step equilibration using glycerol was superior to 3-step equilibration during the freezing procedure. LeGal *et al.* (1993) carried out laparoscopic transfer of frozen thawed morulae and blastocysts and opined that unlike *in vitro,* morulae and blastocysts developed equally *in vivo*. Development *in vivo* showed ethylene glycol to be a better cryoprotectant than glycerol (34.50% vs 21%, 35% vs 23%) for morulae and blastocysts respectively.

Agrawal *et al.* (1994) transferred 12 normal vitrified goat embryos in five recipients (2-3 embryos/recipient). Three recipients conceived but only one survived to term resulting in the birth of a healthy kid. Traldi *et al.* (1999) compared the pregnancy rate and embryo survival rate of *in vivo* and *in vitro* produced vitrified goat embryos. The authors recorded a pregnancy rate and embryo survival rate of 56 per cent and 37 per cent, 50 per cent and 30 per cent respectively for *in vivo* and *in vitro* produced embryos.

Riha *et al.* (1994) transferred six-day-old frozen thawed embryos to recipients by endoscopic method. Those recipients received embryos during season had a pregnancy rate of 85.70 per cent; this was significantly higher ($P<0.05$) than the pregnancy rate, which resulted from transfer of embryos out of season (50%).

Transfer of 24 frozen thawed embryos collected as blastocysts to 12 recipients resulted in a pregnancy rate of 83 per cent (10/12) and an embryo

survival rate of 67 per cent. Corresponding results for frozen thawed blastccysts that had been cultured from morulae prior to freezing and transferred to 11 recipients were 54 per cent (6/11) and 41 per cent respectively (Nowshari and Holtz, 1995).

EI-Gayar and Holtz (2001) proved that OPS vitrification was superior to conventional freezing for preservation of embryos from goats. Out of 12 recipients, which received 24 conventionally frozen embryos seven (58%) became pregnant and gave birth to 10 kids (kidding rate 42%). The corresponding figures were 100 per cent and 64 per cent when OPS vitrified embryos used for transfer.

2.12 BIOCHEMICAL PROFILE

2.12.1. Glucose Level in Donor Goats

Level of blood glucose reflects the various oxidative processes and the role of hormones in maintaining blood sugar (Dixit and Nagia, 1969). The concentration of blood glucose varied depending on the age, stage of oestrous cycle, pregnancy, lactation, season, environmental and nutritional status of animals (McClare, 1972). Kaneko *et al.* (1997) reported that the normal blood glucose levels in adult goats ranged from 40 to 75 mg/dl.

Jana *et al.* (1991) reported that the average glucose level in the blood in pregnant goats reared under extensive management was 42.64 ± 2.64 mg/dl.

2.12.2 Serum Enzymes in Donor Goats

Significant changes in enzymatic activity due to influence of sex steroids depends on metabolic need, for bringing about a specific biochemical change in the tissues (Singh and Madan, 1986).

2.12.2.1 Lactic Debydrogenase (LDH)

Jain *et al.* (1995) reported that the specific activity of LDH (IU/dl) in normal goats was 57.29 ± 2.19 (range 36.85 to 80.83).

Lehninger (1978) observed a high LDH activity in animals, which were in follicular stage. The author attributed this to the stress on body during oestrus and ovulation. Jindal and Ratten (1992) observed that plasma LDH level was high in buffaloes during various changes of synchronised oestrous cycle. Behra *et al.* (1993) recorded that the, LDH activity ranged from 16.87 to 25.62 IU/dl in serum of Black Bengal goats. These authors further observed that there was a significant difference in the LDH activity between different age groups. The authors reported that peak activity was at nine months (25.62 ± 0.62 IU/dl), which showed a decrease at 12 months (21.25 ± 1.80 IU/dl) and the lowest level was observed after 24 months (17.44 ± 0.48 IU/dl).

Ghosh (1998) reported a significant difference in the LDH activity of ovaries between GnRH responded does and non-cyclic control does.

2.12.2.2 Acid Phosphatase (ACP)

Mathai and Nirmalan (1992) observed no significant variation in the ACP level in serum, ovarian and uterine tissue during different phases of the oestrous cycle in crossbred Malabari goats. The authors reported that serum ACP level (IU/L) was 3.80, 5.59 and 6.68 and 4.72 respectively on days 0, 6, 14 and 18 of oestrous cycle.

The level of ACP (KA units/dl) in the serum of Black Bengal goats at nine months, 12 months and 24 months and above were 0.38 ± 0.05, 0.35 ± 0.02 and 0.46 ± 0.07 respectively (Behra *et al.*, 1993).

Ghosh (1998) reported a non-significant increase in the level of ACP in ovarian and uterine tissue of GnRH responded does as compared to non-cyclic controls.

2.12.2.3 Alkaline Phsophatase (ALP)

Mathai and Nirmalan (1992) reported that the serum ovarian ALP did not vary significantly during different phases of oestrous cycle in goats, however the values were higher on day 14 and 18 of oestrous cycle when compared to the other days.

Patel *et al.* (1992a) reported that serum alkaline phosphatase activity was 11.82 and 14.08 KA units/dl in Surti and marwari kids respectively on forty ninth week. Patel *et al.* (1992b) noticed that the level of ALP was low upto day 20 of gestation period but later on tended to increase upto term in Surti and Marwari goats. The author recorded that ALP (KA units/dl) was 25.85 ± 7.78 in Marwari goats on day 40 of gestation.

Behra *et al.* (1993) reported that ALP activity showed an upward trend upto six months in female after which there was sudden decline in activity. The author recorded that ALP activity was 4.65 ± 0.31 IU/dl, 3.11 ± 0.18 IU/dl and 4.14 ± 0.35 IU/dl respectively in goats of nine months, 12 months and 24 months and above.

Mean serum ALP levels before oestrus, at the time of oestrus and 48 h after oestrus were recorded as 144.00 ± 6.63, 187.75 ± 14.31 and 197.88 ± 14.00 U/L respectively in $PGF_2\alpha$ or P_4 synchronised superovulated goats (Ishwar and Pandey, 1994)

Jain *et al.* (1995) estimated ALP activity in normal goats by calorimetry. The specific activity (μmol of product formed per litre of serum per minute at 37°C) of ALP was 11.31 ± 1.13 (range 1.07 to 39.13).

Dutta and Baruah (1999) recorded the effect of hCG treatment on serum level of alkaline phosphatase during oestrous cycle in goat. The values on day of oestrus and day 12 of oestrous cycle were 9.18 ± 0.22 and 15.04 ± 0.18 KA units/100 ml respectively.

2.13 PROGESTERONE PROFILE IN DONOR AND RECIPIENT GOATS

Goat belongs to a group of eutherian mammals in which functional CL is essential for maintenance of pregnancy. Estimation of serum progesterone concentration is of high value in assessing the life span of CL and for early pregnancy diagnosis (Thorburn and Schneider, 1972).

2.13.1 Progesterone Level in Various Stages of Oestrous Cycle

EL-Hommosy *et al.* (1991) observed the lowest P_4 concentration on the day of oestrus as 0.70 ± 0.20 and 0.50 ± 0.40 ng/ml for Baladi and Anglo-Nubian goats respectively. The highest P_4 level was reported on day 14 in Baladis (5.40 ± 0.80 ng.ml) and on the day 10 in Anglo-Nubian goats (3.10 ± 1.50 ng/ml).

Dutta *et al.* (1993) recorded that the P_4 level on twelth day of oestrous cycle was 5.40 ± 0.25 ng/ml, 6.28 ± 0.18 ng/ml and 6.25 ± 0.15 ng/ml in animals treated with 550IU, 650IU and 750IU hCG respectively.

Hwang *et al.* (1994) observed a significantly higher serum P_4 level after day 10 of oestrus in Anglo-Nubian X Taiwan goats. The serum P_4 levels in cross bred goats were <0.01 ng/ml on the day of oestrus, increased to 7.8 ng/ml on day 10 and decreased rapidly during the last three days of the cycle.

Progesterone concentration of Korean native goats averaged 0.40 ng/ml on the day of oestrus, increased gradually to 4.03 ng/ml by day 14 (Na *et al.*, 1994). Leyva-Ocariz *et al.* (1995) observed that the highest P_4 concentrations were detected on day 12 (10 ng/ml) and 15 (12 ng/ml) in crossbred and native goats in semi arid areas of Venezuela. Progesterone concentration in both native an cross-bred does on day 19 (2.10 ± 1.20 ng ml^{-1} and 1.80 ± 1.00 ng ml^{-1}) indicated that luteal regression had started.

Ghosh (1998) recorded that the mean serum progesterone concentration for two consecutive oestrous cycle in cyclic does were 0.304 ± 0.087, 1.294 ± 0.382, 2.531 ± 0.758, 3.619 ± 0.794, 2.456 ± 0.430 and 0.871 ± 0.246 ng/ml on day one, four, six, ten, fourteen and eighteen respectively.

2.13.2 Progesterone Level in Superovulated Goats

Armstrong *et al.* (1983 b) reported that the goats treated with PMSG plus MAP sponge, PMSG plus cloprostenol and FSH plus cloprosternol had plasma concentrations of 3.60 ± 0.60, 7.70 ±1.20 and 7.10 ± 1.00 ng/ml at the start of gonadotrophin treatment, 6.20 ± 1.20, 11.30 ± 1.70 and 7.30 ± 1.40 ng/ml at cloprostenol treatment and 1.50 ± 0.80, 1.60 ± 0.30 and 0.80 ± 0.10 ng/ml at the onset of oestrum, respectively. The authors concluded that the circulating P_4 levels were significantly increased by PMSG treatment and not by FSH treatment.

Stubbings *et al.* (1986) reported that due to premature luteal regression, decrease in serum progesterone concentration was noticed three days after ovulation in goats superovulated using PMSG.

Armstrong *et al.* (1987) assessed the duration of the luteal phase in superovulated donor goats by daily heat checks and serum P_4 determinations. Seven of eight PMSG treated and six of eight anti-PMSG treated does had short

luteal phases with mean P_4 levels above 1ng/ml for 5.60 ± 2.10 and 6.80 ± 2.10 days respectively; significantly longer durations of elevated P_4 were observed in control and FSH treated does on 14.40 ± 0.50 and 12.50 ± 2.30 days.

Battye *et al.* (1988) assessed the P_4 level in superovulated does treated with flunixin meglumine, a PG synthetase inhibitor from day three to seven of the synchronised oestrous cycle using RIA. The authors reported that the corpora lutea of the treated females appeared to be functional as indicated by plasma P_4 profile in comparison to untreated control animals.

Appavu and Holtz (1992) stated that there was a distinct linear relationship between serum progesterone concentration and number of corpora lutea in superovulated does. The authors were of the opinion that determination of progesterone may serve as a tool to predict the extent of superovulatory response in does prior to embryo flushing. Cordova *et al.* (1992) found that the blood progesterone concentration on the day of embryo recovery was significantly correlated with the number of corpora lutea, total number of embryos recovered and number of transferable embryos. They also stated that there was no significant difference in serum P_4 concentration during oestrus or the post luteal phase between goats superovulated using PMSG alone, PMSG plus anti PMSG and FSH treatment. But P_4 level was significantly higher during luteal phase in goats treated with PMSG alone.

Studies reported by Borque *et al.* (1993) in Spain with superovulated Murciana goats recorded that does which failed to yield viable embryos on day six (Post ovulation) had shown a significant decrease in P_4 concentration as a consequence of premature luteal regression. The authors not only found that regression of the corpora lutea was evident on day four, but day three progesterone levels were lower.

Mutiga and Baker (1994) found that the plasma progesterone concentration following superovulation in goats was high and the concentration reached a peak of 10 to 35 ng/ml.

Senthilkumar (1996) superovulated Tellicherry goats using FSH-P, FSH-O and PMSG and reported that, the mean P_4 concentration on the day of oestrum was 0.41 ± 0.09 ng/ml, 0.31 ± 0.03 ng/ml and 0.59 ± 0.11 ng/ml respectively. The progesterone on the day of embryo collection (day three) was significantly higher than other treatment days in all the groups.

Tiwari *et al.* (1998) studied the progesterone profile in nondescript goats following hCG administration on day seventh of oestrus cycle prior to superovulation treatment with a view to eliminate dominant follicle. The mean P_4 level in ng/ml on day of superovulatory heat was 0.69 ± 0.09 and 0.97 ± 0.16 (ng/ml) respectively for in control and treatment groups. The corresponding values on day six after superovulation were 5.38 ± 2.08 and 8.46 ± 1.78 respectively. The authors reported that the correlation between P_4 on day of superovulatory heat and number of copora lutea on the day of embryo collection was negative (r = -0.26).

2.13.3 Progesterone Levels in Pregnancy

Thorburn and Schneider (1972) reported that plasma profile of P_4 during early pregnancy was 2.50 to 3.50 ng/ml which was similar to luteal phase concentrations. Abusa *et al.* (1989) also recorded similar concentrations of P_4 in goats during the initial stages of pregnancy.

Jarrell and Dziuk (1991) showed that goats with multiple CL had a significantly higher P_4 concentration from days 7 to 30 than goats with one CL.

In recipients goats the P_4 levels remained significantly low (0.14 to 0.60 ng/ml) on the day of oestrus. However, from the day of embryo transfer onwards, till day 55, the P_4 levels remained high in recipients, which ultimately turned out pregnant. On the other hand, in non-pregnant animals, the P_4 levels remained low or irregular after day 20 post-oestrus (Deshpande and Mehta 1992; Deshpande *et al.*, 1997).

Kaushik *et al.* (1992) assessed the P_4 concentration in plasma of Black Bengal goats using radio immuno assay at two weeks interval during early (2,4 and 6 weeks); mid (8, 10, 12 and 14 weeks) and late (16, 18 and 20 weeks) pregnancy. Mean plasma P_4 levels increased significantly ($P<0.01$) from 4.51 ± 0.37 ng/ml during early to 6.87 ± 0.87 ng/ml at mid to 7.26 ± 0.37 ng/ml at late pregnancy. Plasma P_4 values for goats with single or twin fetuses did not vary significantly during different stages of gestation.

Na *et al.* (1994) recorded P_4 concentration during early pregnancy (day 40) as 4.03 ng/ml, which peacked, at 8.73 ng/ml on day 80 and decreased there after to 0.40 ng/ml on day 150 in Korean native goats.

Beckett *et al.* (1999) assessed the P_4 level in recipient goats at five day intervals upto day 30 in which demi embryos were transferred with P_4 support in form of progestin implants. Serum progesterone level in three pregnant does that showed CL regression subsequently, were 3.80, 5.50 and 6.80 ng/ml on day 15 of pregnancy, which were comparable to values for the five pregnant demi-embryo recipients that maintained their CL throughout the pregnancy. By day 20 of pregnancy P_4 concentrations for the three does were 0.20, 4.10 and 4.00 ng/ml respectively suggesting that CL had regressed in one doe but still were functional in the other two. Progesterone concentrations on day 25 were 0.00, 0.50 and 0.40 ng/ml respectively, indicating that CL in all three does had regressed.

Materials and Methods

3. MATERIALS AND METHODS

3.1. EXPERIMENTAL ANIMALS

Thirtyseven Malabari goats maintained in the Network Project on Embryo Transfer, Department of Animal Reproduction belonging to the following two pre-assigned categories were utilized for the study.

Peripubertal Goats

They were of nine to twelve months of age and of 16 to 18 kg body weight. They were identified just prior to or just after first oestrus. In all, 19 animals formed this group.

Adult Goats

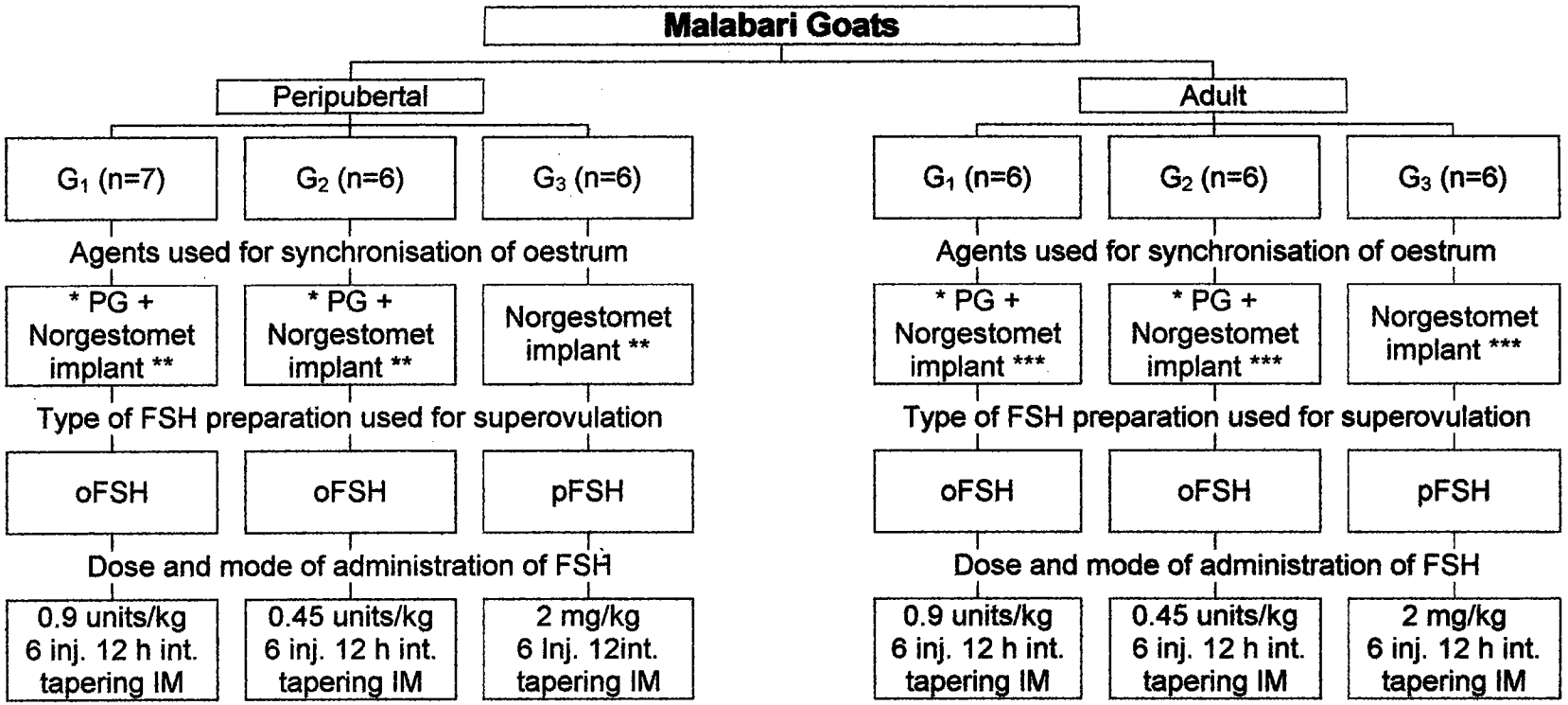
They were above one year and below three years of age with a body weight of 21 to 35 kg. They included both parous and non-parous goats. Altogether 18 animals were there in this group.

All the goats were maintained under uniform managemental conditions and fed with adequate quantity of feed and water.

3.2 DESIGN OF EXPERIMENT

3.2.1 Treatment Groups

The above two categories of goats were distributed at random into three groups of six or seven animals namely G_1, G_2, G_3. (Fig.1).



* → Luprostiol 7.5 mg (prosolvin 1 ml) ** →Crestar implant 1.5 mg + 0.5 ml Crestar injection

*** → Crestar implant 1.5 mg + one ml Crestar injection

oFSH → NIH-FSH-S17 (Sigma Chemicals, USA)

pFSH → NIH-FSH-P1 (Folltropin-V, Vetrepharm Inc. Canada)

Breeding- Goats of all groups which exhibited oestrus were bred with fertile buck/inseminated using fresh semen at 36, 48 and 60 h of implant removal.

hCG @500 IU and 750 IU as IM injection in peripubertal and adult goats respectively within 6 h after the onset of heat.

Fig. 1 Superovulation schedule followed in the study

3.2.1.1. *Oestrous Synchronisation Treatment*

All the goats were treated for synchronising oestrum using a combination of progestogen (Norgestomet–Crestar) and prostaglandin $F_2\alpha$ analogue (Luprostiol-Prosolvin) or progestogen alone.

Group I (G_1)

Peripubertal Goats

Crestar implants containing 3mg of synthetic progesterone norgestomet was divided into two equal halves. One half of the implant was inserted subcutaneously in the middle third of the outer surface of ear of each doeling by using an applicator. At the time of implant insertion all the peripubertal goats were administered 0.5ml Crestar injection containing 1.25mg oestradiol valerate and 0.75 mg norgestomet. The day of implant insertion was considered as day '0' of the experiment. The inserted implant was left in situ for 10 days. The implant was removed on day eleven. The insertion and removal of implant was done as per standard procedures. Immediately after withdrawal of the implant 1ml Prosolvin containing 7.5 mg luprostiol was administered intramuscularly.

Adult Goats

In this group oestrus synchronisation was accomplished by administering 1.5 mg norgestomet ear implant and 1 ml Crestar injection containing 2.5 mg oestradiol valerate and 1.5 mg norgestomet as intramuscular (IM) injection. The implant was kept subcutaneously in the ear for ten days and on day 11 immediately after withdrawal of implant 1 ml Prosolvin containing 7.5 mg luprostiol was administered intramuscularly.

Group II (G_2)

Peripubertal Goats

The synchronisation regimen adopted in this group was same as that of G_1 consisting of PG and P_4 implant.

Adult goats

The method and duration of synchronisation treatment adopted in this group was same as in G_1.

Group III (G_3)

Peripubertal goats

Oestrous synchronisation in this group was same as that of G_1 and G_2 except for PGF_2 α administration. No Prosolvin was administered in this group at the time of implant removal.

Adult goats

Oestrous synchronisation in this group was same as that of the G_1 and G_2 except for $PGF_2\alpha$ administration. No Prosolvin was administered in this group at the time of implant removal.

3.2.1.2. *Superovulation Treatment*

For superovulation in peripubertal and adult goats two purified FSH preparations were used.

(a) NIH- FSH – S_{17} derived from sheep pituitary (oFSH.)

(b) NIH-FSH-P_1(Folltropin–V) derived from porcine pituitaries (pFSH).

Group I (G_1)

Peripubertal goats

Superovulation in this group was induced by administering oFSH, (NIH-FSH-S_{17}) at the rate of 0.9 units/kg body weight in six divided step down doses at 12 h interval starting on day nine of the implant insertion. The vial containing 50 units of oFSH was diluted using 10 ml millipore water before use. The weight of each animal in this group was 18 kg and the total dose of FSH administered per animal was 16.2 units. The percent of FSH used in each injection was respectively 25, 25, 16.67, 16.67, 8.33 and 8.33 of the total dose. Human chorionic gonadotrophin (hCG) was administered at the rate of 500 IU as IM injection within six hours after the onset of heat for induction of ovulation.

Adult goats

Superovulation was induced by administering oFSH @ 0.9 units/kg body weight in six step down doses at 12 h interval. The percentage of FSH used in each injection was respectively 25, 25, 16.67, 16.67, 8.33 and 8.33. The minimum total dose administered was 18.9 units and maximum was 24.3 units as the weight of the animals ranged from 21 to 27 kg in this group. Time of initiation of FSH treatment was on day nine of implant insertion. Human chorionic gonadotrophin was administered @ 750 IU as intramuscular injection within six hours after the onset of heat.

Group II (G_2)

Peripubertal goats

Superovulation was induced in six peripubertal goats belonging to this group by intramuscular injection of oFSH @ 0.45 units/kg body weight in six divided step down doses at 12 h interval starting on day nine of the implant insertion. The percentage of FSH used in each injection was same as in G_1. The

weight of each animal in this group and the total dose of FSH administered in this group were 18 kg and 8.1 units respectively. Administration of hCG was carried out as in G_1.

Adult goats

Ovine FSH was administered as intramuscular injection @ 0.45 units/kg body weight in six divided step down doses at 12 h interval. The percentage of FSH used in each injection was same as in G_1. The total dose of oFSH per goat ranged from 10.4 to 15.8 units as the weight of the animal ranged from 23 kg to 35 kg. Administration of hCG was carried out as in G_1.

Group III (G_3)

Peripubertal goats

Six peripubertal goats in this group were treated with pFSH (NIH-FSH-P_1) @ 2 mg/kg body weight as intramuscular injection in six divided step down doses at 12 h interval. The percentage of FSH used in each injection was same as in G_1 and G_2. The minimum total dose of pFSH administered per doeling was 32 mg with a maximum of 36 mg as the weight of the animals ranged from 16 to 18 kg in this group. Human chorionic gonadotrophin was administered in the same way as in G_1 and G_2.

As the incidence of premature regression of corpus luteum was very high in superovulated goats in the first two groups in order to improve embryo recovery rate progesterone was administered @ 20 mg twice daily as intramuscular injection from latter half of day four of superovulatory heat until the day of embryo collection.

Adult goats

Goats in this group were administered pFSH @ of 2 mg/kg body weight as intramuscular injection in six divided step down doses at 12 h interval. The per

cent of FSH used in each injection was same as in G_1 and G_2. The minimum and maximum dose administered per goat were 50 mg and 70 mg respectively. Human chorionic gonadotrophin was administered in the same way as in G_1 and G_2.

In adult goats P_4 was administered @ 25 mg twice daily in a similar manner as in peripubertal goats.

3.3 ASSESSMENT OF OESTROUS CHARACTERISTICS

After the end of synchronisation treatment (implant removal and PGF2α administration) all the goats were observed individually for oestrous signs at four hours interval during daytime using an aproned buck. The oestrus was detected by observing reaction of the does to the aproned buck. The does were recorded as being in oestrus if mounted by buck.

3.3.1. Onset of Oestrum

First standing of the doe to be mounted by the aproned buck was recorded as the time of onset of oestrum. The time from the end of synchronisation treatment to onset of oestrum was recorded in hours for each goat in peripubertal and adult group.

3.3.2. Duration of Oestrum

In all the goats duration of oestrum was determined in hours as the period between first and last mounting by the aproned buck.

3.3.3. Intensity of oestrum

The intensity of oestrum in each female was measured by assessing the behavioral signs on approach of the buck and by observing physical changes in the external genitalia. The score adopted for measuring the intensity of oestrum is furnished in the Table 1.

Table 1. Intensity of oestrum in superovulated goats

Intensity of heat	Score	Description
Very good	4	Behavioral signs obviously very intense and changes in the external genitalia very prominent
Good	3	Behavioral signs intense and changes in external genitalia less prominent
Fair	2	Behavioral signs mild, changes in external genitalia evident
Poor	1	Absence of behavioral signs, changes in external genitalia evident

3.4 BREEDING

Both peripubertal and adult goats of all groups, which exhibited oestrus were bred with a fertile buck or inseminated using fresh semen at 36, 48 and 60 h of implant removal.

3.5 ASSAY OF BIOCHEMICAL FACTORS

Biochemical estimation of glucose and enzymes in the serum of donor peripubertal and adult goats were carried out on the day of superovulatory heat.

3.5.1 Glucose

Glucose level was estimated soon after the collection of serum by O-Toluidine method using the kit supplied by Dr. Reddy's Laboratories, Hyderabad.

3.5.2 Serum enzymes

3.5.2.1 Acid phosphatase (ACP)

Acid phosphatase was estimated by modified King's method (King and Jagatheesan, 1959).

3.5.2.2 Alkaline phosphatase (ALP)

Estimation of Alkaline phosphatase in the serum of peripubertal and adult goats were carried out using the method of Kind and King (1954).

3.5.2.3 Lactic dehydrogenase (LDH)

Lactic dehydrogenase was assessed by photometric determination using modified King's method (King and Jagatheesan, 1959).

3.6 ASSAY OF SERUM PROGESTERONE

Blood samples were collected from jugular vein of peripubertal and adult donors on days of superovulatory heat and embryo collection for P_4 estimation. Also blood was collected from recipients on day of embryo transfer and thereafter at weekly intervals upto day twenty eight. The serum was separated as per standard procedures and samples were stored at –20°C until assay (Prakash et al., 1992).

The serum P_4 was determined by enzyme–immunological test for the quantitative determination of progesterone using the test principle Enzyme-linked Immunosorbant Assay (ELISA) or competition using streptavidin technology (Morgenthaler, 1987). The sensitivity of the test ranged from 0 to 30.7 ng/ml.

3.7. ASSESSMENT OF SUPEROVULATORY RESPONSE

3.7.1 Surgical Method

The superovulatory response in peripubertal and adult goats were evaluated by carrying out laparotomy under general anaesthesia and observing the ovaries on any day starting from six to eight after the onset of heat. Pre-operative preparations, surgical procedure and postoperative care were carried out as per the methods described by Nowshari et al. (1992). The goats were sedated using xylazine at the dose rate of 0.2 mg per kg body weight intramuscularly. Ten miutes later ketamine hydrochloride at the rate of 10 mg/kg body weight was administered as IM injection. Anaesthesia was maintained during course of operation by administering ketamin @ 5 mg/kg body weight.

3.7.2 Ovarian Response

Ovaries of donor animals were examined immediately after operation and number of functional copora lutea, prematurely regressing white avascular corpora lutea and follicles of more than 5 mm diameter were recorded.

3.8. EMBRYO COLLECTION

After observing the ovarian response, embryo flushing was carried out from the uterine horns of peripubertal and adult goats as per the method of Nowshari et al. (1992).

3.8.1 Embryo Flushing Media

Flushing media consisted of modified Dulbecco's phosphate buffered saline (DPBS) supplied by Sigma Chemicals, USA (Cat.No.D-6650). Heat inactivated goat serum (Chang, 1949) was added to this media at the rate of ten percent. It was filtered through a cellulose acetate filter of pore size 0.45 microns

(μ) and warmed to body temperature before use. Final pH of media was adjusted to 7.2-7.4.

3.9 EMBRYO RECOVERY

Immediately after flushing the embryos were recovered from the flush under stereo zoom microscope at 20-40x. All embryos from both the horns were pooled and transferred into a sterile petridish (35 x 10 mm,) containing two ml fresh holding medium (culture medium) consisting of modified DPBS from Sigma Chemicals, USA (Cat.No. 4031) and 20 per cent heat inactivated goat serum. The culture medium was filtered using 0.22 μ syringe filter before use. The embryos were washed ten times by serially transferring them into fresh drops of culture medium and was held in the medium until freezing at room temperature (20-25°C) and during grading before that.

3.10 GRADING OF EMBRYOS

Morphological appearance of embryos in culture medium was examined at 60 X of stereo zoom microscope and they were graded as per standard procedures.

3.10.1 Based on Fertilisation Status

The flushed out ova were classified as fertilized and unfertilized as per the method described by Agarwal and Tomer (1998) and transferred to separate sterile petridishes (35 X 10 mm).

3.10.2 Based on morphological status

The fertilized embryo were further evaluated based on their morphological characteristics and graded as good, fair and poor as described by Nowshari et al. (1992). Both good and fair quality embryos were considered as transferable and poor quality were discarded. All transferable embryos were stored in a third petridish containing fresh culture medium.

3.10.3 Based on developmental stages

The developmental stages of transferable embryos on various days of collection were assessed as morulae and blastocysts using stereozoom microscope at 60 X as per the method of Gordon (1994).

3.11. CRYOPRESERVATION OF EMBRYOS

Thirty two good quality embryos each from peripubertal and adult goats were subjected to freezing within 45 minutes after collection.

3.11.1. Freezing media

After washing ten times in culture medium the embryos were suspended in sterile freezing medium which was prepared by adding 1.5 M ethylene glycol (EG) in holding medium (culture medium) and held at room temperature for twenty minutes. The loading of straw with embryos and placing them in the freezing machine was carried out as per the method described by Hafez (1993).

The embryos were loaded in groups of two to four in 0.25 ml sterile plastic straw. Before loading of embryos the straw was rinsed and filled half way with freezing media. An air bubble of 3 to 4 mm was created followed by another column of freezing medium containing embryos, so that the straw was 90 per cent full when cotton plug was wetted. Finally 1.5 to 2 mm of mineral oil was added and straw was sealed using heat/sealing and identification plug and then placed into the chamber of the vertical freezing machine with the sealed end down so that embryo sank and rested on the mineral oil.

3.11.2. Freezing protocol

Freezing protocol was started at bench temperature (20 to 25°C) and proceeded as shown below as per the method of Riha *et al.* (1994) with minor modifications (Table 2).

Table 2. Freezing protocol

Steps	Rate of freezing	Cooling rate per minute	Time taken for cooling
1	20 to 25°C to 15°C	1°C per minute	5 to 10 min in total depending on bench temperature
2	Pause 5 min for stabilization of the freezing chamber	-	5 min
3	15°C to -7°C	4.4°C per minute	5 min
4	Pause 10 min at –7°C, seeding after 6 minutes	-	10 min
5	-7 to -37 ° C	0.3°C per minute	100 min

Immediately after the freezing the straws were transferred into a goblet filled with liquid nitrogen and was subsequently transferred into a labelled canister of a liquid nitrogen container of 20 litre capacity for final storage.

3.11.3. Thawing and Cryoprotectant Removal

After one month of storage straws were removed from liquid nitrogen container. Thawing and cryoprotectant removal was carried out as per the method of Riha *et al.* (1994) with minor modifications i.e. change of culture medium to PBS containing 20 per cent heat inactivated goat serum.

3.12. ASSESSMENT OF VIABILITY OF FROZEN EMBRYOS *IN VITRO*

3.12.1. Morphological Examination

After thawing and cryoprotectant removal embryos were equilibrated in culture medium for 30 minutes at 37.5°C. Then they were examined for morphological characteristics under stereo zoom microscope at 60 X. Good and fair quality embryos from peripubertal and adult goats were used for transfer or *in*

vitro culture. The post thaw morphological changes of all frozen embryos were recorded (Nowshari *et al.*, 1992).

3.12.2. *In vitro* Culture of Frozen Embryos

In order to study the viability, half of the thawed good and fair quality embryos (pooled blastocysts from different treatment groups) each from peripubertal and adult goats were subjected to *in vitro* culture for a period of 24 h.

3.12.3 Culture Medium

Embryos from peripubertal and adult goats were cultured individually in 150 µl sterile culture medium (modified DPBS + 20 per cent heat inactivated goat serum) under gas and medium equilibrated mineral oil in 35 X 10 mm sterile petridishes.

3.12.4 Culture Procedure

Culture conditions were maintained at five per cent CO_2 in humidified air and 37.5°±1°C using a carbon dioxide incubator. Embryonic development and morphological characteristics were assessed under stereo zoom microscope (60 X). Embryos were considered to be viable if they developed from early blastocysts to blastocysts, blastocysts to expanded blastocysts and expanded blastocysts to hatched blastocysts after 24 h.

3.13 EMBRYO TRANSFER

The other half of the frozen, thawed good and fair quality embryos (morulae) each from peripubertal and adult goats were transferred into the uterus of synchronous recipients surgically as per the method described by Puls-Kleingeld *et al.* (1992).

3.14. ASSESSMENT OF PREGNANCY

3.14.1 Based on P_4 Level

Pregnancy was assessed by observing the animals for heat signs and by assessing the progesterone level at 21 and 28 days after embryo transfer.

3.14.2 Based on Ultrasonography

Conformation of pregnancy was made by ultrasonogrpahy on day 60 (Arthur *et al.*, 1996).

3.15. DETAILS OF RESEARCH MATERIALS AND EQUIPMENTS

Details of research materials and equipments used in this study are furnished in table 3

3.16 STATISTICAL METHODS

The data were analysed statistically by using the methods of Snedecor and Cochran (1980). Techniques of analysis of variance, correlation and students 't' test were used for drawing inferences.

Table 3. Details of hormones, drugs, chemicals, glasswares and equipments

Sl. No	Name of item	Patent name and/ catalogue no.	Manufacturer
1	**Hormones & drugs**		
a.	oFSH(FSH from sheep pituitary)	Follicle stimulating hormone, F-4520	Sigma Chemicals, St. Louis, USA
b.	p-FSH(FSH from porcine pituitary)	Folltropin – V,	Vetrepharm Canada Inc.
c.	Prostaglandin F2α analogue (Luprostiol)	Prosolvin	Intervet International Boxmeer, Holland

Table 3 (Contd.)

Sl.No	Name of item	Patent name and/ catalogue no.	Manufacturer
d.	Norgestomet ear implant with injection	Crestar	Intervet International, Boxmeer, Holland
e.	HCG	Chorulon	"
f.	Progesterone	Uniprogestin - 50	Unichem laboratories Ltd. Mumbai
g.	Xylazine	Xylocad	Cadila, India
h.	Ketamine Hydrochloride	Ketmin - 50	Themsis, Hyderabad
i.	Streptopencillin	Dicrysticin- S	Sarabhai Chemicals, Baroda, India

2	**Media & chemicals**		
a.	Modified DPBS	Modified DPBS, D 6650	Sigma Chemicals, St. Louis, USA
b.	Modified DPBS	Modified DPBS, D 4031	"
c.	Goat Serum	Goat Serum, 11-035	PAA Laboratories GmbH, Austria
d.	Ethylene glycol	Ethylene glycol, E 9129	Sigma Chemicals, St. Louis, USA
e.	Sucrose	Sucrose, S 1888	"
f.	Mineral oil	Mineral oil, M 8410	"
g.	Glucose Kit	Glucose reagent	Dr. Reddy's Laboratories Diagnostic Division, Hyderabad

Table 3 (Contd.)

Sl.No	Name of item	Patent name and/ catalogue no.	Manufacturer
h.	Acid phosphatase kit	Acid phosphatase kit	Dr. Reddy's Laboratories Diagnostic Division, Hyderabad
i.	Alkaline phosphatase kit	Alkaline phosphatase kit	"
j.	LDH kit	Lactic dehydrogenase kit	"
k.	Progesterone kit	Enzymun – Test progesterone, 1204475	Boehringer Mannheim, Immunodiagnostics, Germany

3	**Disposable wares**		
a.	Foley's Catheter No.8	Foley's Catheter No.8	Rush, Stuttgart, Germany/TTK Medicare LTd. Chennai, India
b.	100 X 15 mm Square style petridishes	Falcon, 351112	Becton Dickinson, USA
c.	Petridish (35mm)	Tarsons, 460035	Tarsons Products Pharma Trust, Laboratory Instruments, Mumbai
d.	Syringe filters	Millex, SLGV 013 SL & SLGS 025 OS	Millipore Corporation Bedford, MA 01730 U.S.A
e.	0.25 ml sterile plastic straw	0.25 ml sterile plastic straw	IMV India Ltd., Guragon, Haryana

Table 3 (Contd.)

Sl.No	Name of item	Patent name and/ catalogue no.	Manufacturer
4.	**Equipments**		
a.	Carbon dioxide (CO_2) incubator	Labline	Labline, USA
b.	Millipore water purification system	Millipore water purification system	Millipore India, Bangalore
c.	Stereo Zoom Microscope	MZ6	Leica Microsystems Wetzlar, GmbH, Germany.
d.	Programmable freezer	PTC 1000	Apex Instruments, Calcutta
e.	Spectrophotometer	Photometer – 5010	Boehringer Mannheim, Immunodiagnostics, Germany
f.	Spectrophotometer	Spectronic –20	Baush and Lomb, Germany
g.	Ultrasonograph	Symphony	L&T, Mumbai

Results

4. RESULTS

4.1. RESPONSE TO SYNCHRONISATION AND OESTROUS CHARACTERISTICS

The percentage of animals responded to synchronisation treatments and oestrous characteristics (Mean ± SE) in peripubertal and adult goats are furnished in the Table 4.

4.1.1. Response to Synchronisation

Out of the 19 peripubertal goats treated, 18 (94.74%) showed oestrus. All the animals in group G_1 and G_2 exhibited oestrus whereas only 83.33 per cent in G_3 responded to oestrous synchronisation.

Out of the 18 adult goats 17 (94.45%) showed superovulatory heat. All the animals in G_1 and G_2 showed oestrus response whereas only 83.33 per cent in G_3 responded to synchronisation treatment.

4.1.2. Interval from the End of Synchronisation to Oestrum

The interval from the end of synchronisation to heat (h) was more in G_3 followed by G_1 and then G_2 with an overall mean of 25.06 ± 1.52 in peripubertal animals.

The interval from the end of synchronisation to heat was shortest in G_3 followed by $G_1$and then G_2 with an overall mean of 29.90 ± 3.05 in adult goats. No significant difference was observed between groups.

4.1.3. Duration of Oestrum

The overall duration of heat (h) in peripubertal goats was recorded as 42.83 ± 2.55. The duration of oestrus was more in G_2 followed by G_3 and then G_1.

Table 4. Response to synchronisation and oestrous characteristics in peripubertal and Adult goats

Sl. No	Treatment groups	Synchronisation & superovulation treatment	No. of animal treated		No. responded		Interval from end of synchronisation to heat (h)		Duration of heat (h)		Intensity of heat (h)	
			P	A	P	A	P	A	P	A	P	A
1	G_1	PG plus norgestomet implant & oFSH 0.9 units/kg body weight	7	6	7 (100.00)	6 (100.00)	25.60 ± 1.89$^{a.x}$	33.00 ± 3.81$^{a.x}$	36.70 ± 1.47$^{a.x}$	40.80 ± 13.60$^{a.x}$	3.85 ± 0.14	4.00 ± 0.00
2	G_2	PG plus norgestomet implant & oFSH 0.45 units per kg body weight	6	6	6 (100.00)	6 (100.00)	21.30 ± 1.84$^{a.x}$	33.20 ± 3.97$^{a.y}$	52.00 ± 5.32$^{b.x}$	43.00 ± 1.39$^{a.x}$	3.17 ± 0.16	2.60 ± 0.30
3	G_3	norgestomet implant and pFSH 2 mg per kg body weight	6	6	5 (83.33)	5 (83.33)	28.80 ± 3.90$^{a.x}$	22.40 ± 1.88$^{a.x}$	40.40 ± 3.58^{ax}	40.00 ± 4.26$^{a.x}$	3.20 ± 0.37	2.80 ± 0.37
	Overall		19	18	18 (94.74)	17 (94.45)	25.06 ± 1.52^{x}	29.90 ± 3.05^{x}	42.83 ± 2.55^{x}	41.35 ± 4.40^{x}	3.44 ± 0.15	3.18 ± 0.21

Figures in parenthesis denote percentage
Values bearing different superscripts in the same column and row differ significantly ($P<0.05$)
P = Peripubertal, A = Adult

The duration of heat in G_1 was significantly different from other two groups ($P<0.05$).

The overall duration of heat (h) in adult goats was recorded as 41.35 ± 4.40. The duration of heat was more in G_2, followed by G_1 and then G_3 and no significant difference was observed between groups.

4.1.4. Intensity of Oestrum

Intensity of heat (score) was highest in G_1 followed by G_3 and then G_2, with an overall mean of 3.44 ± 0.15 in peripubertal goats.

The score on intensity of heat was maximum in G_1 Followed by G_3 and then G_2 with an overall score of 3.18 ± 0.21 in adult goats.

4.1.5. Signs of Oestrum

All peripubertal goats exhibited physical changes like congestion of vaginal mucosa, vulval oedema and mucus discharge from vulva. Wagging of tail was the most prominent behavioural sign exhibited by all the animals. Few animals exhibited bleating in G_1 and G_3. Frequent urination, reduction in feed and water intake etc. were also exhibited by some of the animals in this age group. Circling with the buck was not prominent in all the groups except G_1.

All physical signs of heat exhibited by peripubertal goats were observed in adult goats. Circling with the buck homosexual behaviour and bleating was very prominent in goats belonging to G_1. One animal belonging to this group exhibited very intense signs like biting the ears of the buck and mounting over the buck. The heat signs were less prominent in G_2 and G_3. Frequent urination was observed in most of the adult goats.

4.2. OVARIAN RESPONSE

The ovarian response was assessed by counting the number of corpora lutea both functional and regressing ones and anovulatory follicles of >5 mm diameter. The number of CL in right and left ovaries was counted separately and the total number of ovulations was expressed as superovulatory response. The result of ovarian response in peripubertal and adult goats are furnished in Table 5 and Fig. 2.

4.2.1. Anovulatory Follicles

The average number of anovulatory, follicles of >5mm diameter was highest in G_1 (2.57) followed by G_2 (2.00) and lowest in G_3 (0.16). The overall average number of anovulatory follicles in peripubertal goats was recorded as 1.63.

The average number of anovulatory follicles was highest in G_1 (3.00), followed by G_2 (1.50) and no anovulatory follicles could be observed in G_3. The overall average number of anovulatory follicles in adult goats was recorded as 1.50.

4.2.2. Superovulatory Response

No significant difference was observed in superovulatory response between right and left ovaries in peripubertal goats in all the groups. But the response was found to be more in left ovary than in right. Total superovulatory response was highest in G_1 (12.20±1.64), followed by G_2 (6.96±1.45) and lowest response was recorded in G_3 (4.66±1.50). Even though there was no significant difference in total superovulatory response between G_1 and G_2 it was observed that G_1 was significantly different from G_3 ($p<0.05$). The overall mean superovulatory response in peripubertal goats was recorded as 8.16 ± 0.84.

In adult goats also there was no significant difference in superovulatory response between right and left ovaries in all the groups though, the left ovary

Table 5. Ovarian response in peripubertal and adult goats

Sl. No	Treatment groups	No. of animals treated		Average no. of anovulatory follicles >5mm		No. of CL/superovulatory response					
		P	A	P	A	P		A		P	A
						Rt ovary	Lt ovary	Rt ovary	Lt ovary	Total	Total
1	G_1	7	6	2.57	3.00	4.50 ± 0.62^{x}	7.70 ± 1.47^{x}	8.00 ± 1.09^{x}	8.16 ± 1.85^{x}	12.20 ± 1.64^{ax}	16.16 ± 2.30^{ax}
2	G_2	6	6	2.00	1.5	3.16 ± 0.80^{x}	3.80 ± 0.87^{x}	6.30 ± 0.88^{x}	8.00 ± 1.80^{x}	6.96 ± 1.45^{abx}	14.30 ± 2.60^{ay}
3	G_3	6	6	0.16	0.00	2.16 ± 0.60^{x}	2.50 ± 1.14^{x}	2.80 ± 1.35^{x}	3.60 ± 1.38^{x}	4.66 ± 1.50^{bx}	6.40 ± 2.70^{ax}
	Overall	19	18	1.63	1.5	3.36 ± 0.86^{x}	4.80 ± 1.02^{x}	5.70 ± 0.61^{x}	6.60 ± 1.30^{x}	8.16 ± 0.84^{x}	12.30 ± 1.40^{y}

Values bearing different superscripts in the same column and row differ significantly ($P<0.05$)
P = Peripubertal, A = Adult

was found to be more active. The total superovulatory response in G_1, G_2 and G_3 were recorded as 16.16 ± 2.30, 14.30 ± 2.60 and 6.40 ± 2.70 respectively which did not differ significantly. The overall mean superovulatory response was recorded as 12.30 ± 1.40.

4.2.2.1 Degree of Superovulatory Response

The degree of superovulatory response in peripubertal and adult goats are furnished in Table 6 and plates 1 to 3.

In peripubertal goats, the overall percentage of animals that exhibited low response (1-8CL), medium response (9-16CL) and high response (>16CL) were 44.44, 55.56 and 0.00 respectively. Out of the seven animals responded in G_1, six showed medium response. In G_2, number of animals which showed low and medium response were equal, while in G_3 out of the five animals responded four showed low response.

In G_1 the number of adult goats, which showed low, medium and high response were one, zero and five respectively. The corresponding values in G_2 and G_3 were one, three and two and, three, two and zero respectively. The overall percentage of low, medium and high response recorded in adult goats were 29.41, 29.41 and 41.18, respectively.

4.2.2.2 Premature Regression of Corpus Luteum

Premature regression of corpus luteum (PRCL) was predominantly noticed in this study. The functional corpora lutea (FCL) appeared as cherry red elevations of 3-10 mm diameter and protruded prominently from surface of superovulated ovaries. Whereas regressing copora lutea were white avascular structures with less diameters and the protrusion was not prominent as in FCL (Plate 4 and 5). Some of the goats showed corpora lutea in varying stages of regression whereas in some other animals the regression was almost complete. These animals had one to two large follicles in their ovaries. Most of these

Table 6. Degree of superovulatory response in peripubertal and adult goats

Sl. No	Treatment group	No. of animals treated		No. of animals responded		Number (%) of animals responded to superovulation treatment and degree of response					
						Low (1-8CL)		Medium (9-16 CL)		High (>16 CL)	
		P	A	P	A	P	A	P	A	P	A
1	G_1	7	6	7	6	1	1	6	0	0	5
2	G_2	6	6	6	6	3	1	3	3	0	2
3	G_3	6	6	5	5	4	3	1	2	0	0
	Overall	19	18	18	17	8 (44.44)	5 (29.41)	10 (55.56)	5 (29.41)	0	7 (41.18)

Figures in parenthesis denote percentage
P = Peripubertal, A = Adult

Plate 1. Superovulatory response – high (*in situ*)

Plate 2. Superovulatory response – high (excised genitalia)

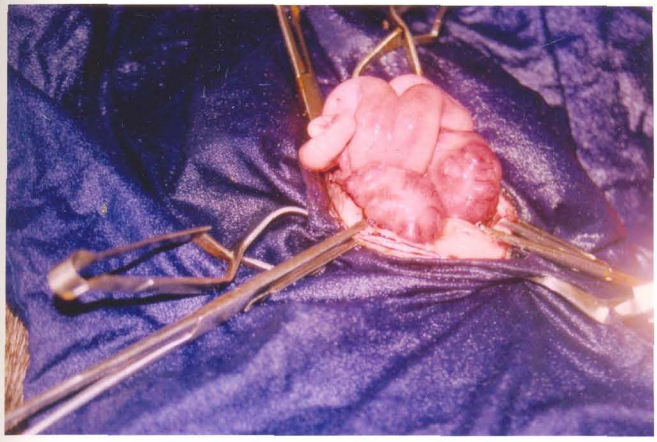


Plate 1

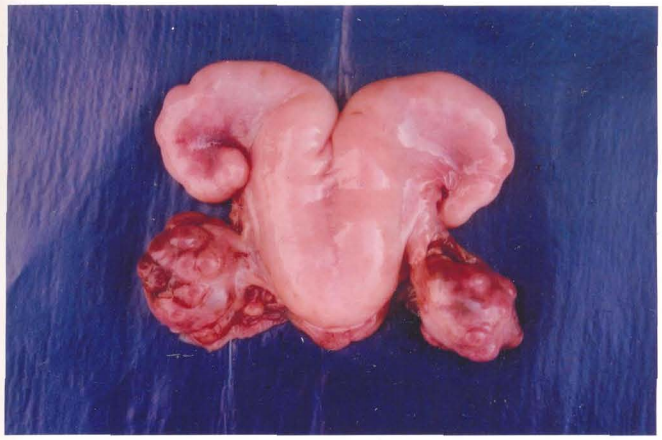


Plate 2

Plate 3. Superovulatory response – medium (*in situ*)

Plate 4. Premature regression of CL (*in situ*)

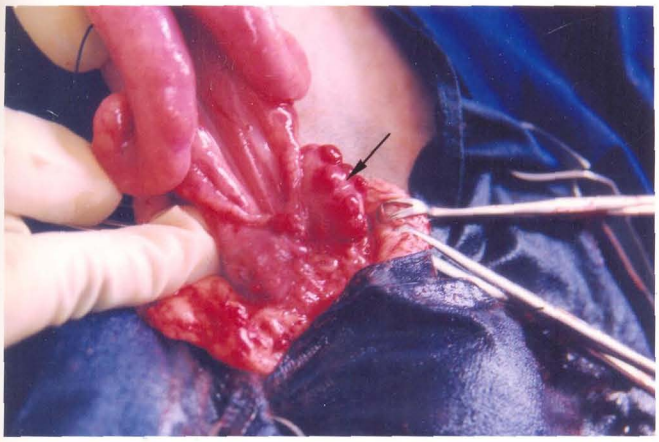


Plate 3

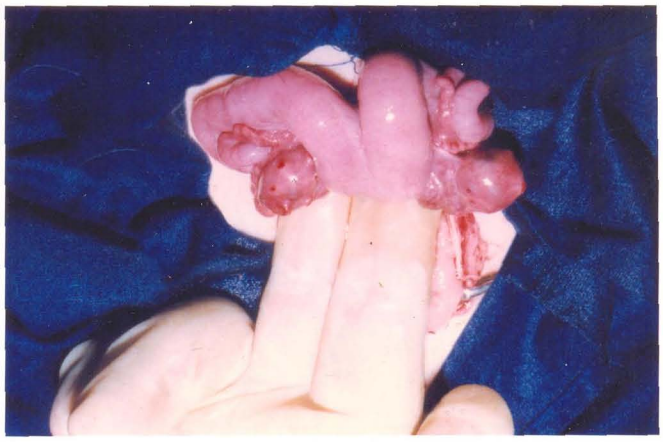


Plate 4

Table 7. Incidence of premature regression of corpus luteum in peripubertal and adult Malabari goats

Sl. No	Treatment groups	No. of animals treated		No. of animals responded		No. (percentage) of animals that exhibited PRCL	
		P	A	P	A	P	A
1	G_1	7	6	7	6	4 (57.14)	5 (83.33)
2	G_2	6	6	6	6	2 (33.33)	4 (66.67)
3	G_3	6	6	5	5	3 (60.00)	0 (00.00)
	Overall	19	18	18	17	9 (50.00)	9 (52.94)

Figures in parenthesis denote percentage
P = Peripubertal, A = Adult

Plate 5. Premature regression of CL (excised genitalia)

Plate 6. Flushing of uterus for embryo recovery

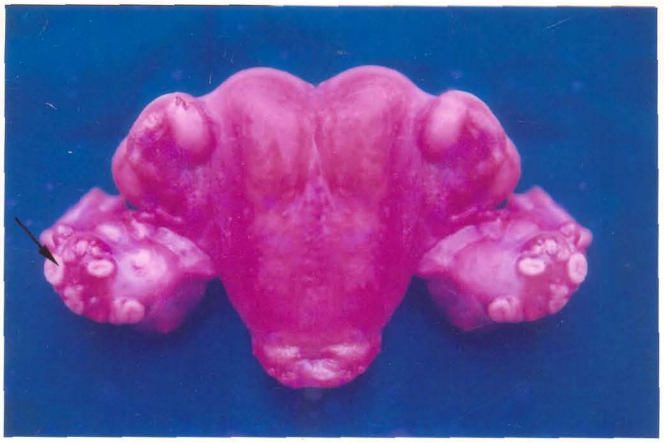


Plate 5

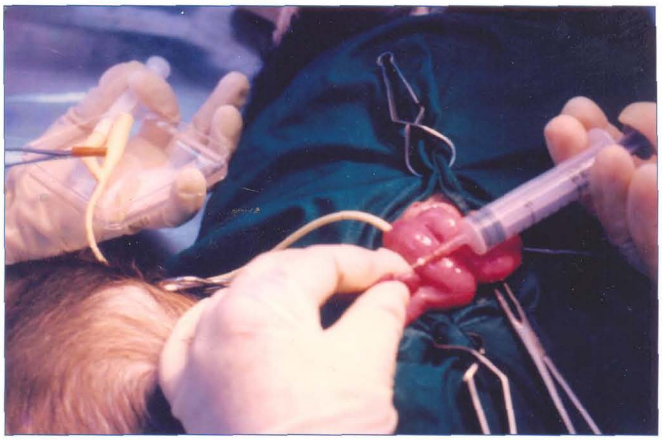


Plate 6

animals exhibited prominent signs of oestrum on the day of embryo collection. Such animals could be identified before laparotomy as they exhibited vaginal discharge and wagging of tail indicating short oestrous cycle. The uterine horns of these animals were very rigid and vascular indicating the presence of high levels of oestrogen in the blood. These animals were very nervous and needed more anaesthetics to maintain the anaesthesia during laparotomy.

The incidence of PRCL in peripubertal and adult goats are furnished in Table 7.

In peripubertal goats belonging to G_3, regression of corpora lutea was evident, but there were no large follicles in the ovaries, the genitalia was quiescent and the animals did not exhibit heat signs unlike those in $G_1$and G_2. Highest incidence of PRCL was noticed in G_3 (60%) followed by G_1 (57.14%) and the lowest was in G_2 (33.33%) with an overall incidence of 50 per cent in peripubertal goats.

In adult goats, incidence of PRCl was highest in G_1 (83.33%) followed by G_2 (66.67%) and no regression of CL could be observed in G_3. The overall incidence of PRCL in adult goats was recorded as 52.94 per cent.

4.3 EMBRYO RECOVERY RATE

Embryo collection was carried out by uterine flush (Plate 6). The total ova and embryo recovery as percentage of superovulatory response, average ova or embryo recovery per goat, percentage of fertilized ova or embryo recovery, average fertilized ova or embryo recovery per goat and overall values for peripubertal and adult goats are furnished in Table 8 and Fig. 2. The details of embryo recovery on progesterone support and without progesterone support are furnished in Table 9.

4.3.1 Total Ova and Embryo Recovery

The total ova and embryo recovery rate was lowest in G_1 (26.74%) followed by G_2 (64.29%) and highest rate was recorded in G_3 (75.00%) with an overall recovery rate of 45.51 per cent in peripubertal goats. The average ova and embryo recovery per goat was 3.29, 4.50 and 3.50 respectively in G_1, G_2 and G_3 with an overall recovery rate of 3.94 (Fig. 2).

In adult goats the percentage ova and embryo recovery in G_1, G_2 and G_3 were respectively 30.93, 38.37 and 46.15 with an overall recovery rate of 36.48 per cent. The average ova and embryo recovery rate per goat in G_1, G_2 and G_3 were recorded as 5.00, 5.50 and 3.00, respectively with an overall recovery rate of 4.5 (Fig. 2).

4.3.2 Fertilized Ova or Embryo Recovery

Fertilized ova or embryo recovery rate was 100 per cent in G_1 and G_3, while it was 70.37 per cent in G_2 with an overall value of 88.73 per cent in peripubertal goats. Average fertilized ova or embryo recovery per goat was highest in G_3 followed by G_1 and then G_2, with an overall value of 3.32 (Fig. 2). None of the peripubertal goats in G_1 and G_2 which exhibited PRCL yielded ova or embryos. In G_3 all the animals, which exhibited PRCL and FCL yielded embryos. In G_2 one animal with FCL yielded seven unfertilised oocytes (UFO) (Plate 7f).

In adult goats, the fertilization rate (%) in G_1, G_2 and G_3 were respectively 66.67, 75.76 and 83.33 with an overall rate of 74.07. The corresponding values for fertilized ova or embryo recovery per goat were 3.33, 4.17 and 2.50 with an overall recovery rate of 3.33 (Fig. 2). In G_1 only one adult goat with FCL yielded embryos. Out of five animals, which exhibited PRCL, three yielded UFO. In G_2 two animals, which had functional CL yielded a total of 25 embryos while altogether eight UFO was recovered from four animals with PRCL. In G_3, one animal, which had 15 functional CL yielded three UFO only.

Table 8. Embryo recovery in peripubertal and adult goats

Sl. No	Treatment groups	No. of animals treated		Total ovulations (n)		Average super ovulatory response per goat		Total (%) embryo and ova recovery		Average ova and embryo recovery per goat		Total (%) fertilized ova/ embryo recovery		Average fertilized ova/ embryo recovery per goat	
		P	A	P	A	P	A	P	A	P	A	P	A	P	A
1	G1	7	6	86	97	12.29	16.17	23 (26.74)	30 (30.93)	3.29	5.00	23 (100.00)	20 (66.67)	3.28	3.33
2	G2	6	6	42	86	7.00	14.23	27 (64.29)	33 (38.37)	4.50	5.50	19 (70.37)	25 (75.76)	3.17	4.17
3	G3	6	6	28	39	4.67	6.50	21 (75.00)	18 (46.15)	3.50	3.00	21 (100.00)	15 (83.33)	3.50	2.50
	Overall	19	18	156	222	8.21	12.33	71 (45.51)	81 (36.48)	3.94	4.50	63 (88.73)	60 (74.07)	3.32	3.33

Figures in parenthesis denote percentage

P = Peripubertal, A = Adult

Table 9. Embryo recovery on progesterone support in peripubertal and adult Malabari goats

SL No	FSH treatment groups	No of animals treated		No. of animals responded		Total super-ovulatory response		PRCL(%)		Ova/embryo recovery			
										Total (%) ova and embryo recovery		Total (%) fertilized ova/embryo recovery	
		P	A	P	A	P	A	P	A	P	A	P	A
1.	G_3 (P4 treated)	6	6	5	5	28	39	12 (42.85)	Nil (0)	21 (75)	18 (46.15)	21 (100)	15 (83.33)
2.	$G_1 + G_2$ (no P4 treatment)	13	12	13	12	128	183	59 (46.09)	125 (68.85)	50 (39.06)	63 (34.42)	42 (84)	45 (71.43)

Figures in parenthesis denote percentage

P = Peripubertal, A = Adult

4.3.3 Embryo Recovery on Progesterone Support

In peripubertal goats belonging to G_3 where embryo collection was carried out under P_4 support, the values for percentage ova and embryo recovery and fertilized ova or embryos recovered were 75 and 100 respectively. The corresponding values for peripubertal goats in G_1 plus G_2 not supported with P_4 were 39.06 and 84 per cent.

In adult goats in G_3 where embryos were collected on P_4 support, the total ova and embryo recovery and fertilized ova or embryo recovery were 46.15 and 83.33. The corresponding values for adult goats in G_1 plus G_2 where no P_4 support was given were 34.42 and 71.43 per cent.

4.4 EMBRYO QUALITY

The embryo quality in peripubertal and adult goats belonging to G_1, G_2 and G_3 and the overall values are furnished in Table 10, Fig. 2 and 3. The fertilized ova or embryos were divided into good (Plate 7a & g), fair (Plate 7b & d) and poor (Plate 7c, e & h). The good and fair embryos were considered as transferable and poor quality was denoted as non-transferable.

4.4.1 Transferable Embryos

The percentage of transferable embryos in G_1, G_2 and G_3 in peripubertal goats was 95.65, 89.47 and 100 respectively, of which 68.18, 88.24 and 71.43 were of good quality and the remaining ones were fair quality. The overall percentage of transferable good and fair quality embryos in peripubertal goats were recorded as 95.23, 73.00 and 25.00.

The average number of transferable embryos recovered per peripubertal goat in G_1, G_2, G_3 and overall values were 3.14, 2.83, 3.50 and 3.15, respectively. The corresponding values for good and fair quality embryos were 2.14, 2.50, 2.50 and 2.37 and, 1.16, 0.33, 1.00 and 0.78 respectively.

Table 10. Embryo quality in peripubertal and adult goats

Sl. No	Treatment groups	Transferable												Non transferable embryos			
		Total (%) transferable embryos recovered as percentage of fertilized ova		Average transferable embryo recovery per goat		Total (%) good embryos recovered		Average good embryo recovery per goat		Total (%) fair embryo recovery		Average fair embryo recovery		Total (%) poor embryo recovery as percentage of fertilized ova		Average poor embryo recovery per goat	
		P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A
1	G1	22 (95.65)	19 (95.00)	3.14	3.16	15 (68.18)	12 (63.16)	2.14	2.00	7 (31.82)	7 (36.84)	1.16	1.17	1 (4.35)	1 (5.0)	0.14	0.17
2	G2	17 (89.47)	23 (92.00)	2.83	3.83	15 (88.24)	8 (34.78)	2.50	1.33	2 (11.76)	15 (65.02)	0.33	2.50	2 (10.53)	2 (8.0)	0.33	0.33
3	G3	21 (100.00)	15 (100.00)	3.50	2.50	15 (71.43)	13 (86.67)	2.50	2.16	6 (28.57)	2 (13.33)	1.00	0.33	00.00	00.00	00.00	0.00
	Overall	60 (95.23)	57 (95.00)	3.15	3.17	45 (73.00)	33 (57.89)	2.37	1.83	15 (25.00)	24 (42.11)	0.78	1.33	3 (4.76)	3 (5.00)	0.17	0.18

Figures in parenthesis denote percentage

P = Peripubertal, A = Adult

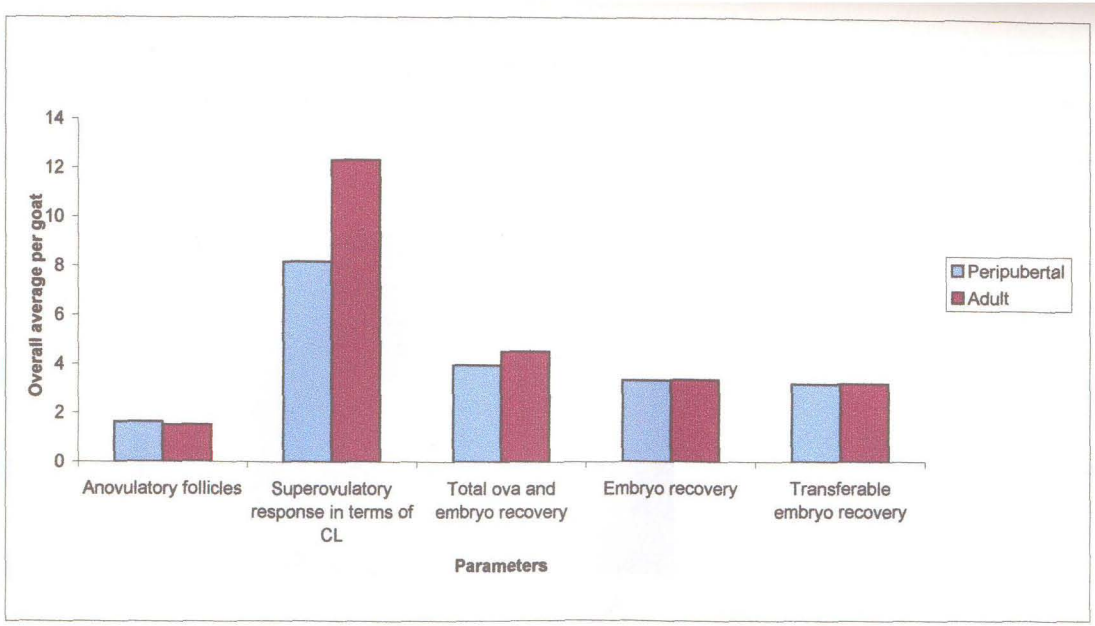


Fig. 2 Ovarian response, embryo recovery and quality in peripubertal and adult goats

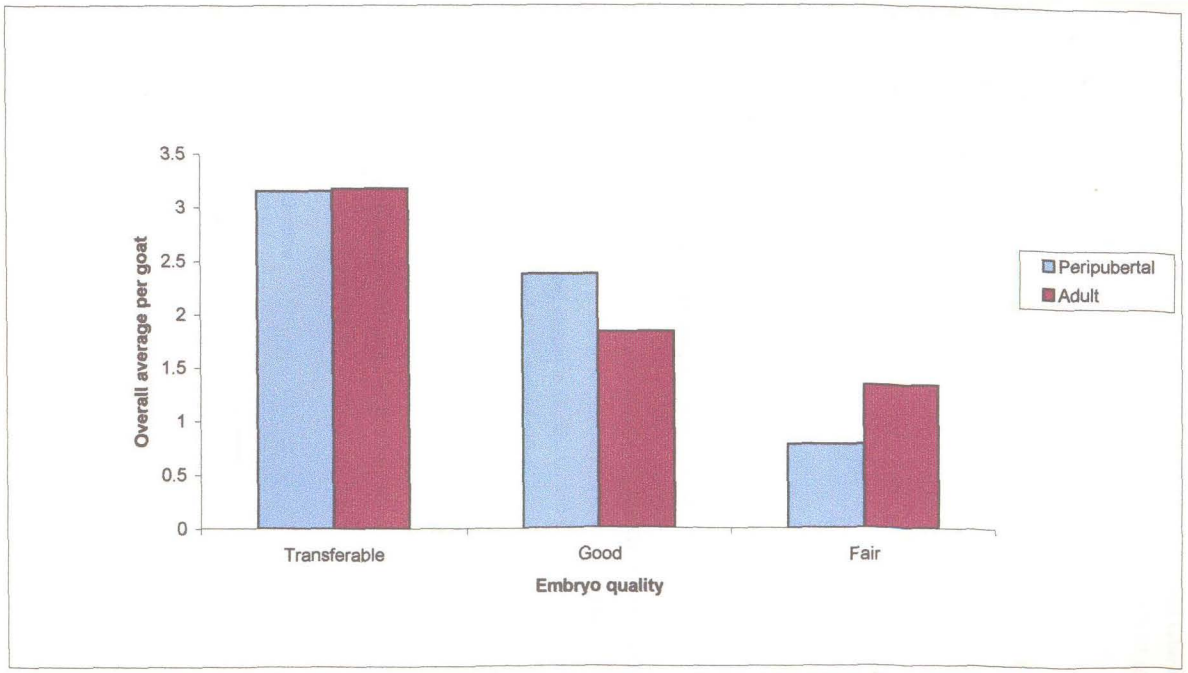


Fig 3. Transferable embryo recovery in peripubertal and adult goats

The percentage of transferable embryos in G_1, G_2, G_3 and overall value in adult goats were recorded as 95, 92, 100 and 95 respectively of which 63.16, 34.78, 86.67 and 57.89 were of good quality and the remaining were fair quality. The average number of transferable embryos recovered per goat and overall value for adult goats were respectively 3.16, 3.83, 2.50 and 3.17 of which 2.00, 1.33, 2.16 and 1.83 were good quality, the remaining were of fair quality. In G_2 an animal, which yielded 16 embryos except one, all were of fair quality. This particular animal showed a high superovulatory response (21 CL) but all the CL were pale pink in colour.

4.4.2 Non-Transferable or Poor Quality Embryos

The percentage of poor quality embryos in G_1, G_2 and G_3 were respectively 4.35, 10.53 and 0.00 with an overall value of 4.76 for peripubertal goats. The average number of poor quality embryos per goat in G_1, G_2 and G_3 were recorded as 0.14, 0.33 and 0.00 with an overall value of 0.17 per animal.

The percentage of poor quality embryos in G_1, G_2 and G_3 and the overall value in adult animals were 5, 8, 0 and 5, respectively. The average number of poor quality embryos recovered per animal in G_1, G_2, G_3 and the overall value in adult goats were 0.17, 0.33, 0.00 and 0.18, respectively.

4.5 DEVELOPMENTAL STAGES OF EMBRYOS ON DIFFERENT DAYS OF COLLECTION

The details on developmental stages of embryos on days six, seven and eight are furnished in Table 11 and Fig. 4.

4.5.1 Sixth Day of Collection

Out of the 38 embryos collected from peripubertal goats, morulae and compact morulae were 30 (78.95%) and eight (21.05%) respectively.

Table 11. Developmental stages of embryos on different days of collection in peripubertal and adult goats

Sl. No.	Day of collection	Total embryos (%)		Developmental stages of embryos															
				Morulae						Blastocysts								Total	
				M		CM		Total		EB		BL		EXB		HB			
		P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A
1	6th day	38 (100)	27 (100)	30 (78.95)	18 (66.67)	8 (21.05)	8 (29.63)	38 (100)	26 (96.30)	0	1 (3.70)	0	0	0	0	0	0	0	1 (3.70)
2	7th day	9 (100)	15 (100)	0	3 (20.00)	4 (44.44)	10 (66.67)	4 (44.44)	13 (86.67)	5 (55.56)	2 (13.33)	0	0	0	0	0	0	5 (55.56)	2 (13.33)
3	8th day	13 (100)	15 (100)	0	0	0	0	0	0	0	0	6 (46.15)	5 (33.33)	6 (46.15)	9 (60.00)	1 (7.70)	1 (6.67)	13 (100)	15 (100)

M = Morulae, CM = Compact Morulae, EB = Early Blastocysts

BL = Blastocysts, EXB = Expanded Blastocysts, HB = Hatched Blastocyst

Figures in parenthesis denote percentage

P = Peripubertal, A = Adult

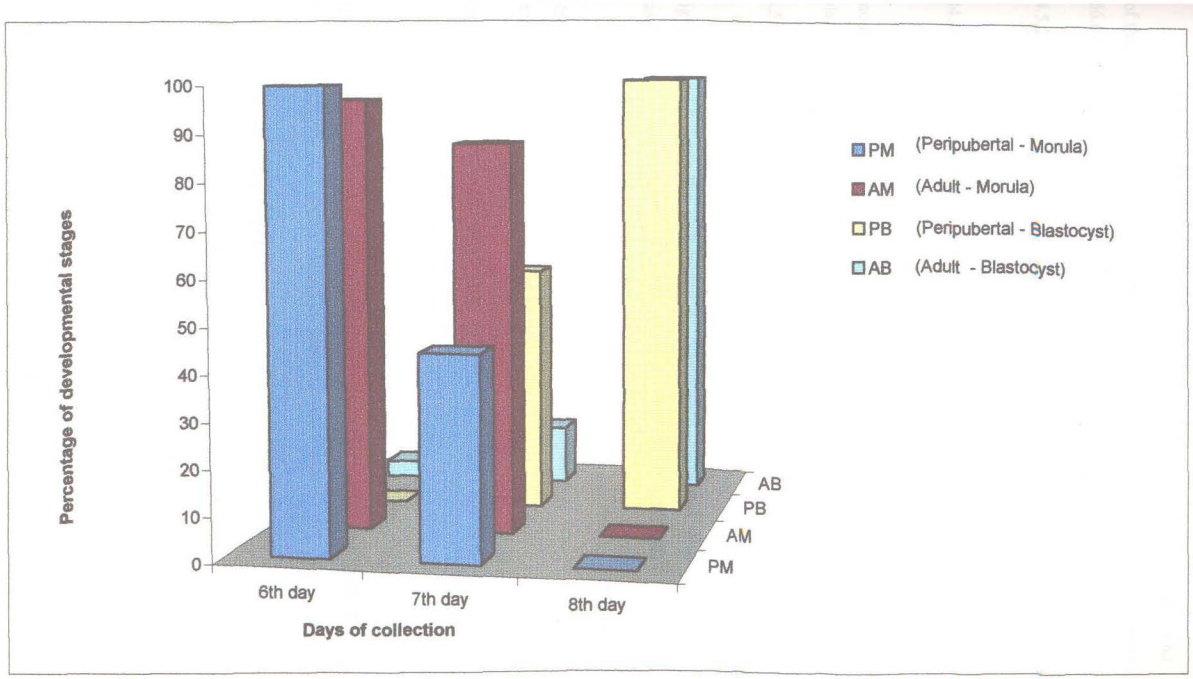


Fig 4. Developmental stages of embryos on different days of collection

On sixth day a total of 27 embryos were collected from adult goats. Out of these the percentage of morulae, compact morulae and early blastocysts were 66.67, 29.63 and 3.70, respectively.

4.5.2 Seventh Day of Collection

Out of the nine embryos collected from peripubertal goats on day seven 44.44 per cent were compact morulae and 55.56 per cent early blastocysts.

In adult goats three (20%) were morulae while 10 (66.67%) were compact morulae on day seven. Out of the 15 embryos collected, two (13.33%) were early blastocysts.

4.5.3 Eighth Day of Collection

A total of 13 embryos were collected from peripubertal goats on day eight. Out of these the percentage of blastocysts, expanded blastocysts, and hatched blastocysts recovered were 46.15, 46.15 and 7.70 respectively.

Out of the total 15 embryos collected from adult goats five (33.33%) were blastocysts, while nine (60%) were expanded blastocysts and one (6.67%) was hatched blastocyst.

4.6 EMBRYO QUALITY AFTER THAWING AND CRYOPROTECTANT REMOVAL

Embryos were frozen by conventional method (Plate 8). The results on quality of frozen embryos from peripubertal and adult goats after thawing and cryoprotectant removal are furnished in Table 12 and Fig. 5.

4.6.1 Transferable Embryos

Out of the total 32 embryos frozen, 30 were recovered after freezing in peripubertal goats of which 22 (73.33%) were recorded as transferable. The

Table 12. Embryo quality after thawing and cryoprotectant removal

Sl.No	Parameters	Developmental stages of embryos					
		Peripubertal goats (P)			Adult goats (A)		
		Morulae (%)	Blastocysts (%)	Total (%)	Morulae (%)	Blastocysts (%)	Total (%)
1.	Total embryos frozen	16	16	32	16	16	32
2.	Total embryo recovered after freezing	14 (87.50)	16 (100.00)	30 (93.75)	15 (93.75)	15 (93.75)	30 (93.75)
3.	Transferable embryos	9 (64.29)	13 (81.25)	22 (73.33)	9 (60.00)	12 (80.00)	21 (70.00)
	a) good	7 (77.78)	10 (76.92)	17 (77.27)	6 (66.67)	8 (66.67)	14 (66.67)
	b) Fair	2 (22.22)	3 (23.08)	5 (22.73)	3 (33.33)	4 (33.33)	7 (33.33)
4	Non-transferable embryos	5 (35.71)	3 (18.75)	8 (26.67)	6 (40.00)	3 (20.00)	9 (30.00)

Figures in parenthesis denote percentage

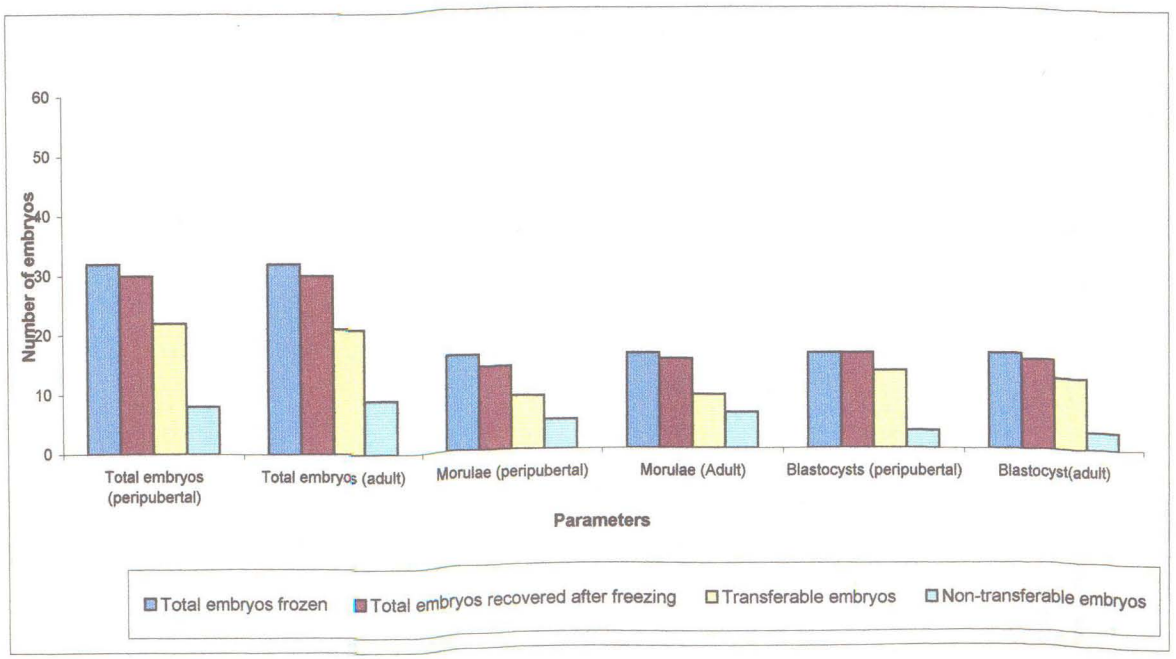


Fig.5 Embryo quality after thawing and cryoprotectant removal

number (%) of good and fair quality embryos were 17 (77.27) and five (22.73), respectively.

In adult goats, the number (%) of embryos recovered after freezing was same as in peripubertal goats. Out of the 30 embryos recovered 21 (70%) were found to be transferable. Out of these 14 (66.67%) were of good quality and seven (33.33) per cent were fair quality embryos.

4.6.2 Non-transferable or Poor Quality Embryos

Embryos with cracked zona and dispersed and degenerated cell mass were considered as non-transferable (Plate 7m).

Out of the 30 embryos frozen, eight (26.67%) were found to be non-transferable in peripubertal goats.

A total of nine (30%) were recorded as non-transferable embryos in adult goats.

4.6.3 Effect of Developmental Stages on Quality of Frozen Embryos

The results on effect of developmental stages of embryos on freezability are furnished in Table 12 and Fig. 5.

4.6.3.1 Morulae

Out of the 16 morulae frozen in peripubertal goats, 14 (87.50%) were recovered after freezing, of which nine (64.29%) were considered as transferable. The number (%) of good and fair quality morulae were recorded as seven (77.78) and two (22.22) respectively. The number (%) of poor quality (non transferable) morulae were found to be five (35.71) in peripubertal goats.

The number (%) of total morulae frozen in adult goats, morulae recovered after freezing and transferable quality morulae were respectively 16 (100), 15 (93.75) and nine (60). Out of which six (66.67%) and three (33.33%) were found

Plate 7. Fresh and frozen embryos of various quality of and developmental stages

a) Morula – good quality (160x)
b) Morula – fair quality (40x)
c) Morula – poor quality and retarded embryonic development (60x)
d) Fair quality compact morula (100x)
e) Poor quality compact morula (100x)
f) Unfertilized oocytes (60x)
g) Blastocysts and expanded blastocysts – good quality (60x)
h) Early blastocysts and blastocysts – poor quality (60x)
i) Expanded blastocysts collapsed inside zona pellucida (60x)
j) Expanded blastocyst collapsed inside zona pellucida (160x)
k) Hatching blastocyst (zonalysed) (60x)
l) Hatched out blastocysts (60x)
m) Good and poor quality (cracked zona and dispersed cell mass) embryos after freezing (60x)
n) Hatched blastocyst (160x)
o) Hatched re-expanding blastocyst (160x)

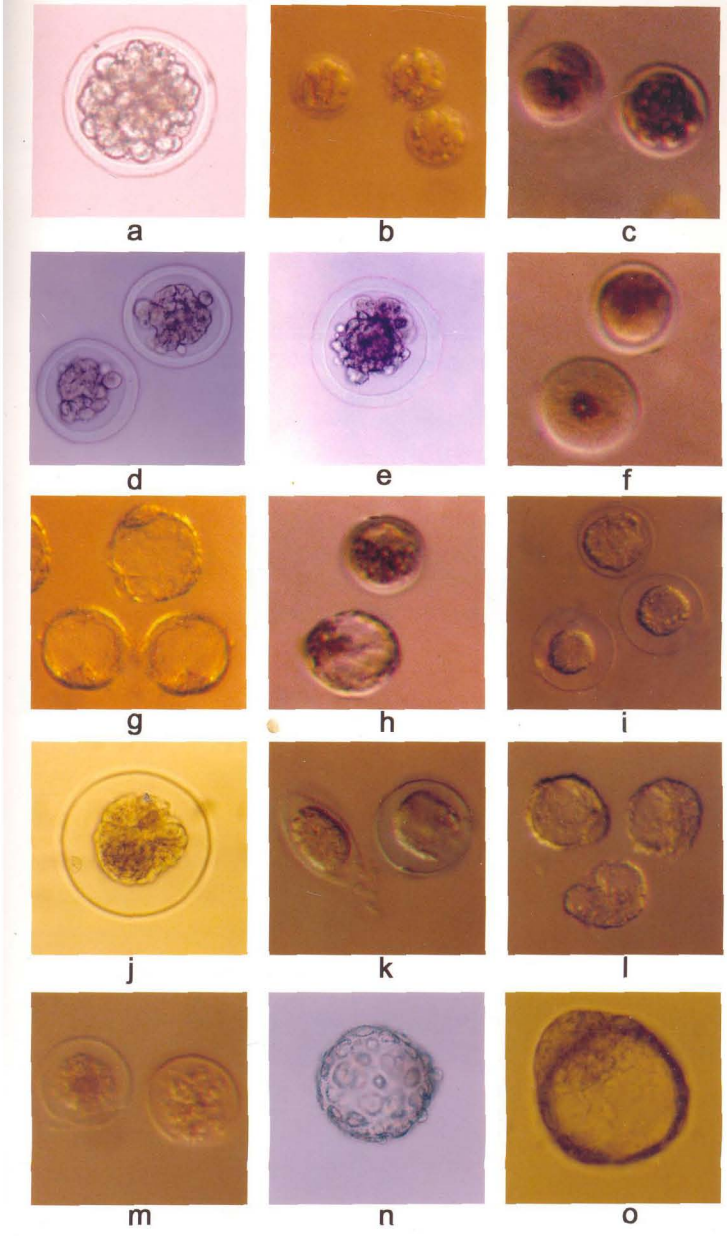


Plate 7

to be of good and fair quality respectively. The number of poor quality or non-transferable embryos was recorded as six (40%).

4.6.3.2 Blastocysts

Out of the 16 blastocysts frozen in peripubertal goats, 16 (100%) were recovered, of which 13 (81.25%) were recorded to be of transferable quality. The number (%) of good and fair quality blastocysts were 10 (76.92) and three (23.08), respectively. The number of non-transferable blastocysts was (poor quality) recorded as three (18.75%) in peripubertal goats.

Out of 16 blastocysts frozen in adult goats 15 (93.75%) were recovered. Out of these eight (66.67%) were found to be of good quality and four (33.33%) were fair quality. Thus a total of 12 (80%) transferable blastocysts could be obtained from adult goats after freezing. The number (%) of non-transferable blastocysts recovered after freezing in this age group were three (20).

4.7 VIABILITY OF FROZEN THAWED BLASTOCYSTS ON *IN VITRO* CULTURE

The results on viability of frozen thawed blastocysts collected from peripubertal and adult goats are furnished in Table 13 and Plate 7i, j, k, l, n, and o.

Out of the 13 blastocysts (10 good quality and three fair quality) subjected to *in vitro* culture in peripubertal goats, seven (53.85%) developed after twentyfour hours, while six (46.15%) degenerated. The number (%) of good and fair quality blastocysts, which showed development was six (60) and one (33.33), respectively.

A total of 12 embryos were subjected to *in vitro* culture in adult goats, which included eight good quality and four fair quality blastocysts. Out of these, five (62.50%) good quality and one (25%) fair quality blastocysts showed

Table 13. Viability of frozen-thawed blastocysts on *in vitro* culture

Sl. No	Age group	Number of blastocysts subjected to *in vitro* culture			Number of blastocysts developed after twenty four hours			Number of blastocysts degenerated
		Good	Fair	Total	Good (%)	Fair	Total (%)	(%)
1	Peripubertal	10	3	13	6 (60.00)	1 (33.33)	7 (53.85)	6 (46.15)
2	Adult	8	4	12	5 (62.50)	1 (25.00)	6 (50.00)	6 (50.00)

Figures in parenthesis denote percentage

Expressed as percentage of morphologically normal embryos after thawing

Plate 8. Freezing protocol

Plate 9. Technique of embryo transfer

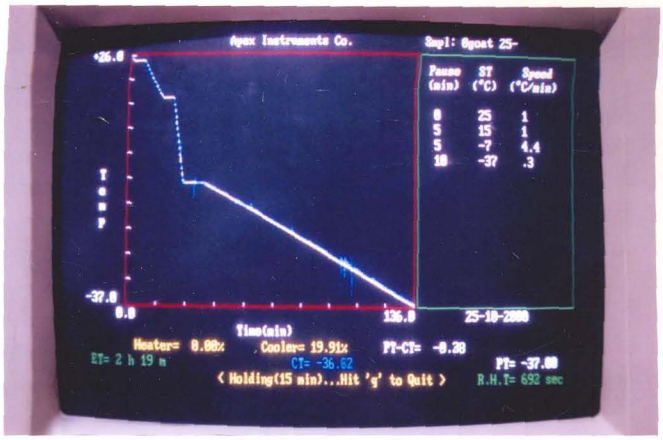


Plate 8

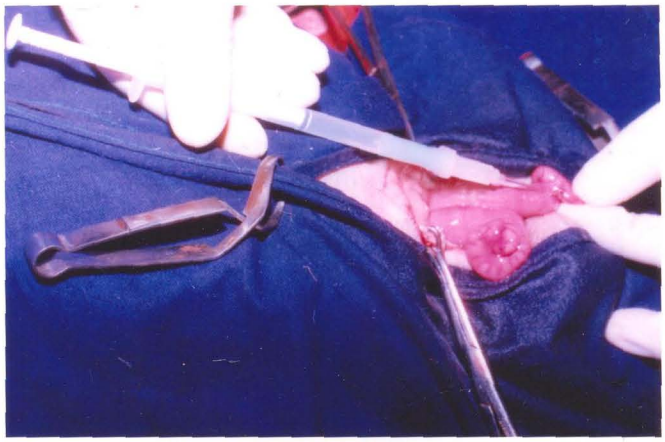


Plate 9

development. The total number and percentage of blastocysts, which developed and degenerated in culture, were equal (6 and 50) in adult goats.

4.8 PREGNANCY RATES IN RECIPIENTS ON TRANSFER OF FROZEN EMBRYOS

Embryos were transferred surgically into the uterus of recipients (Plate 9). The results on rate of pregnancy on transfer of frozen embryos from peripubertal and adult goats are furnished in Table 14.

Out of three recipients, which received embryos (three each) from peripubertal goats two (66.67) were found to be pregnant based on P4 assay on day 21. The non-pregnant goat showed heat signs on day 21. Again another recipient which received embryos from this age group showed heat on day 27. Progesterone assay on day 28 revealed that one (33.33%) animal which received embryos from this age group was pregnant. This was further confirmed by ultrasonography on day 60.

Out of three recipients which received embryos (three each) from adult goats one showed heat on day 21 which was confirmed to be not pregnant on P_4 assay. Another recipient in this group showed heat on day 26. Progesterone assay on day 28 revealed that this animal was not pregnant. The ultrasonography on day 60 confirmed that pregnancy rate was 33.33 per cent in animals, which received embryos from adult goats.

4.9 ASSAY OF BIOCHEMICAL FACTORS

The mean ± SE of serum glucose on the day of superovulatory heat in peripubertal and adult goats are furnished in Table 15.

Table 14. Pregnancy rate in recipient goats on transfer of frozen thawed morulae

Sl. No	Parameter	Source of embryos	
		Peripubertal goats	Adult goats
1	Total embryos (morulae) transferred	9	9
2	No. of recipients used for transfer	3	3
3	No. of embryos transferred per recipient	3	3
4	No. (%) of recipients identified as pregnant based on P4 assay on day 21	2 (66.67)	2 (66.67)
5	No. (%) of animals identified as pregnant based on P4 level on day 28	1 (33.33)	1 (33.33)
6	No. (%) of animals in which pregnancy was confirmed based on ultrasonography	1 (33.33)	1 (33.33)

Table 15. Serum glucose and enzyme levels in peripubertal and adult goats on the day of superovulatory heat

Sl. No.	Treatment groups	No. of samples collected		Glucose mg/dl		LDH (U/L)		ACP (KA units/dl)		ALP (KA units/dl)	
		P	A	P	A	P	A	P	A	P	A
1	G_1	6	6	60.32 ± 5.84^{ax}	$60.44^{ax} \pm 8.74^{ax}$	289.83 ± 34.11^{bx}	447.00 ± 57.79^{ax}	0.94 ± 0.21^{ax}	0.63 ± 0.06^{ax}	24.37 ± 12.62^{ax}	21.61 ± 9.92^{ax}
2	G_2	6	6	63.50 ± 8.21^{ax}	61.54 ± 6.49^{ax}	450.00 ± 50.31^{ax}	541.50 ± 47.16^{ax}	0.85 ± 0.33^{ax}	0.53 ± 0.07^{ax}	39.84 ± 13.49^{ax}	26.81 ± 13.06^{ax}
3	G_3	5	5	49.36 ± 4.13^{ax}	52.49 ± 3.06^{ax}	467.00 ± 42.6^{ax}	467.00 ± 31.85^{ax}	1.06 ± 0.30^{ax}	1.07 ± 0.30^{ax}	16.05 ± 5.62^{ax}	11.53 ± 2.89^{ax}
	Overall	17	17	57.73 ± 6.43^{x}	58.16 ± 6.30^{x}	402.28 ± 52.42^{x}	485.28 ± 47.17^{x}	0.95 ± 0.27^{x}	0.74 ± 0.20^{x}	26.75 ± 11.26^{x}	20.00 ± 9.42^{x}

Values bearing different superscripts in the same rows and columns differ significantly ($P<0.05$)

P = Peripubertal, A = Adult

4.9.1 Serum Glucose

Serum glucose (mg/dl) was highest in G_2 followed by G_1 and lowest in G_3 with an overall value of 57.73 ± 6.43 in peripubertal goats. No significant difference was observed in serum glucose levels between groups.

The pattern of serum glucose in G_1, G_2 and G_3 were same as in peripubertal goats with an overall value of 58.16 ± 6.30 mg/dl in adult goats. No significant difference was observed between G_1, G_2 and G_3 in glucose levels.

4.9.2 Serum Enzymes

The level of enzymes in the serum of peripubertal and adult goats during superovulatory heat are furnished in the table 15.

4.9.2.1 Lactic Dehydrogenase (LDH)

Mean of serum LDH (U/L) in peripubertal goats was lowest in G_1 (289.83 ± 34.11) followed by G_2 (450.00 ± 50.31) and highest in G_3 (467.00 ± 42.67) with an overall value of 402.28 ± 52.42 in peripubertal animals. A significant difference in LDH level was observed between G_1 and G_2 and G_1 and G_3 ($P<0.05$).

In adult goats LDH level was highest in G_2, followed by G_3 and lowest level was observed in G_1, with an overall value of 485.28 ± 47.17 U/L. However, there was no significant difference ($P>0.05$) in the LDH levels between groups.

4.9.2.2 Acid Phosphatase (ACP)

The level of ACP (KA units/dl) did not show any significant difference ($P>0.05$) between three groups. The level of ACP was highest in G_3 followed by G_1 and lowest in G_2, with an overall value of 0.95 ± 0.27 in peripubertal goats.

In adult goats, the level of ACP (KA units/dl) was recorded to be highest in G_3 followed by G1 and lowest in G_2 with an overall value of 0.74 ± 0.20. No

significant difference (P>0.05) was observed in the level of ACP between three groups in adult goats.

4.9.2.3 Alkaline Phosphatase (ALP)

The level of alkaline phosphatase in KA units/dl in G_1, G_2 and G_3 did not show any significant difference (P>0.05) in peripubertal goats. The ALP level was lowest in G_3, followed by G_1 and then G_2 with an overall value of 26.75 ± 11.26 KA units/dl in peripubertal goats.

The overall level of ALP in adult goats was recorded as 20.00 ± 9.42 KA units/dl. The ALP levels in G_1, G_2 and G_3 exhibited a similar pattern as in peripubertal goats and did not show any significant difference (P>0.05) between groups.

4.10 SERUM PROGESTERONE LEVEL IN DONORS DURING SUPEROVULATORY HEAT

The progesterone level in the serum of peripubertal and adult goats during superovulatory heat is furnished in Table 16 and 17 respectively.

The mean serum progesterone level (ng/ml) in G_1, G_2 and G_3 in peripubertal goats were recorded as 0.29 ± 0.04, 0.45 ± 0.05 and 0.42 ± 0.06 respectively with an overall value of 0.38 ± 0.03.

The progesterone level in the serum of adult goats in G_1, G_2 and G_3 were 0.40 ± 0.09 ng/ml, 0.27 ± 0.04 ng/ml and 0.38 ± 0.05 ng/ml respectively with an overall value of 0.32 ± 0.05.

4.10.1 Correlation of Progesterone Profile during Superovulatory Heat with Superovulatory Response

A significant negative correlation was observed between P4 level and total superovulatory response in peripubertal goats belonging to G_1 (P<0.05,

Table 16. Progesterone level in peripubertal goats on day of superovulatory heat and embryo collection

Sl. No.	Group name	Animal No.	P_4 level (ng/ml)			
			On the day of breeding		On the day of embryo collection	
			Individual value	Group average	Individual value	Group average
1	G_1	PD_1	0.20	0.29 ± 0.04	20.10	7.1 ± 3.65
2		PD_2	0.30		22.20	
3		PD_3	0.50		3.50	
4		PD_4	0.20		1.00	
5		PD_5	0.30		0.90	
6		PD_6	0.20		0.90	
7		PD_7	0.30		1.10	
8	G_2	PD_8	0.30	0.45 ± 0.05	15.00	10.55 ± 3.09
9		PD_9	0.60		0.80	
10		PD_{10}	0.50		14.90	
11		PD_{11}	0.40		15.80	
12		PD_{12}	0.30		16.00	
13		PD_{13}	0.60		0.80	
14	G_3*	*PD_{14}	0.40	0.42 ± 0.06	> 30.70	
15		*PD_{15}	0.30		> 30.70	
16		*PD_{16}	0.60		> 30.70	
17		*PD_{17}	0.50		> 30.70	
18		*PD_{18}	0.50		> 30.70	
Overall			0.38 ± 0.03			

* - P_4 supported group

Table 17. Progesterone level in adult goats on day of superovulatory heat and embryo collection

Sl. No.	Group name	Animal No.	P4 level (ng/ml)			
			On the day of breeding		On the day of embryo collection	
			Individual value	Group average	Individual value	Group average
1	G_1	AD_1	0.50	0.40 ± 0.09	1.00	5.82 ± 4.80
2		AD_2	0.20		1.10	
3		AD_3	0.20		29.80	
4		AD_4	0.70		1.20	
5		AD_5	0.20		0.90	
6		AD_6	0.20		0.90	
7	G_2	AD_7	0.30	0.27 ± 0.04	18.50	8.72 ± 5.23
8		AD_8	0.10		0.90	
9		AD_9	0.40		0.80	
10		AD_{10}	0.30		0.80	
11		AD_{11}	0.20		30.50	
12		AD_{12}	0.30		0.80	
13	G_3*	AD_{13}	0.20	0.38 ± 0.05	> 30.70	
14		AD_{14}	0.40		> 30.70	
15		AD_{15}	0.40		> 30.70	
16		AD_{16}	0.50		> 30.70	
17		AD_{17}	0.40		> 30.70	
Overall			0.32 ± 0.05			

*P_4 treated group

r = -0.85), G_2 ($P<0.05$; r = -0.93) and G_1 plus G_2 ($P<0.01$; r = 0.91). Similarly P_4 level and number of FCL and P_4 level and number of PRCL showed a significant negative correlation in G_2 ($P<0.05$; r = -0.91 and $P<0.05$; r = -0.82) (Table 18).

A significant negative correlation was observed between P_4 level on the day of superovulatory heat and total number of CL ($P<0.05$; r = -0.94) in adult goats belonging to G_2. The correlation between P_4 level and PRCL and FCL during superovultory heat was also negative, but not significant ($P>0.05$) (Table 18).

4.10.2 Correlation of Progesterone Profile during Superovulatory Heat with Embryo Quality

A negative non-significant correlation ($P>0.05$) was observed between P_4 level on day of superovulatory heat and transferable embryo recovery in peripubertal goats (Table 18).

The correlation between P_4 level on day of superovulatory heat and transferable embryo recovery was negative and non-significant ($P>0.05$) in adult goats (Table 18).

4.11 PROGESTERONE LEVEL ON DAY OF EMBRYO COLLECTION

The P_4 level on the day of embryo collection in peripubertal and adult goats are furnished in Table 16 and 17, respectively.

The progesterone level in G_1 and G_2 on days of embryo collection ranged from 0.80 to 22.20 ng/ml in peripubertal goats. The animals with PRCL showed a low P_4 level (range = 0.80 to 1.10). In animals which were supported with exogenous progesterone the P_4 level was >30.70 ng/ml. The mean progesterone level in G_1 and G_2 were recorded as 7.10 ± 3.65 ng/ml and 10.55 ± 3.09 ng/ml respectively on day of embryo collection.

Table 18. Correlation of progesterone (P4) profile with superovulatory response and embryo quality

Treatment group*** Name	Correlation coefficient (r) of P_4 profile with No. of FCL, PRCL, Total CL and TER															
	On the day of superovulatory heat								On the day of embryo collection							
	P_4 & No. of FCL		P_4 & No. of PRCL		P_4 & Total CL		P_4 & No. of TER		P_4 & No. of FCL		P_4 & No. of PRCL		P_4 & Total CL		P_4 & No. of TER	
	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A
G_1	NS -0.08 (7)	NS -0.30 (6)	NS -0.43 (7)	NS -0.07 (6)	-0.85* (7)	NS -0.42 (6)	NS -0.09 (7)	NS -0.30 (6)	0.99** (7)	0.99** (6)	-0.78* (7)	-0.81* (6)	NS 0.26 (7)	NS 0.67 (6)	0.99** (7)	0.99** (6)
G_2	-0.91* (6)	NS -0.22 (6)	-0.82* (6)	NS -0.47 (6)	-0.93* (6)	-0.94* (6)	NS -0.40 (6)	NS -0.20 (6)	0.99** (6)	0.99** (6)	-0.97** (6)	NS -0.74 (6)	0.98** (6)	NS 0.38 (6)	NS 0.68 (6)	0.99** (6)
$G_1 + G_2$	NS -0.25 (13)	NS -0.28 (12)	NS -0.45 (13)	NS -0.06 (12)	-0.91** (13)	NS -0.50 (12)	NS -0.17 (13)	NS -0.27 (12)	0.99** (13)	0.99** (12)	-0.69** (13)	-0.77* (12)	NS 0.29 (13)	NS 0.48 (12)	0.88** (13)	0.98** (12)

Figures in parenthesis denote number of observation
FCL – Functional CL
PRCL – Prematurely Regressing CL
TER – Transferable embryos recovered
* - $P < 0.05$, ** - $P < 0.01$
*** G_3 excluded as P_4 support was given.
P – Peripubertal, A - Adult

The progesterone level in adult goats in G_1 and G_2 ranged from 0.80 to 30.50 ng/ml. Progesterone level in animals with PRCL ranged from 0.80 to 1.20 ng/ml in this age group. The three animals with FCL showed a high level of P_4 (29.80 ng/ml, 18.50 ng/ml and 30.50 ng/ml). Animals which were supported with exogenous P_4 the level was >30.70 ng/ml. The mean P_4 level in G_1 and G_2 were recorded as 5.82 ± 4.80 and 8.72 ± 5.23 respectively on the day of embryo collection.

4.11.1 Correlation of P_4 Profile on Day of Embryo Collection with Superovulatory Response

A significant positive correlation was observed between P_4 profile on day of embryo collection and number of FCL in G_1, G_2 and G_1 plus G_2 ($P<0.01$; $r = 0.99$) in peripubertal goats. A significant negative correlation was observed between P_4 profile and number of PRCL in G_1 ($P<0.05$; $r = -0.78$), G_2 ($P<0.05$; $r = -0.97$) and G_1 plus G_2 ($P<0.01$; $r = -0.69$). The P_4 level and total number of CL showed a positive correlation, which were significant only in G_2 ($P<0.05$; $r = 0.98$) (Table 18).

A significant positive correlation ($P<0.01$; $r = 0.99$) was observed between progesterone profile and number of FCL in adult goats belonging to G_1, G_2 and G_1 plus G_2. In contrast, a significant negative correlation ($P<0.05$) was observed between P_4 level and number of PRCL in G_1 ($r = -0.81$) and in G_1 plus G_2 ($r = -0.77$). The correlation between P_4 profile and total CL was positive but not significant (Table 18).

4.11.2 Correlation of P_4 Profile on the Day of Embryo Collection with Embryo Quality

A significant positive correlation ($P<0.01$) was observed between progesterone profile and transferable embryo recovery in peripubertal goats belonging to G_1 ($r = 0.99$) and G_1 plus G_2 ($r = 0.88$) (Table 18).

A significant correlation ($P<0.01$) was observed between progesterone profile and transferable embryo recovery in adult goats belonging to G_1 ($r = 0.99$) and G_2 ($r = 0.99$) and G_1 plus G_2 ($r = 0.98$) (Table 18).

4.12 PROGESTERONE PROFILE IN RECIPIENT GOATS AT WEEKLY INTERVALS

The P_4 level in recipient goats at weekly intervals after embryo transfer are furnished in Table 19, Fig.6a and 6b respectively. All recipients showed high P_4 level on day of transfer and on day 14. But on day 21, two recipients PR3 and AR3 had basal levels of P_4. Again on day 28 the P_4 level in two goats (PR2 and AR1) was higher compared to the goats which showed heat signs on day 26 and 27 (AR2 and PR1).

4.12.1 Correlation of Progesterone Profile of Recipient with Pregnancy

Out of the six recipients four showed a high P_4 level on day 21, while the other two (PR3 and AR3) had basal levels of P_4, indicating pregnancy in four recipients. Subsequently one recipient which received embryos from adult donor and another from peripubertal donor exhibited heat signs, on day 26 and 27 respectively. On analysis of P_4 level on day 28 both these animals (PR1 and AR2) showed a low P_4 level (<1 ng/ml) indicating non-pregnancy. Pregnancy in the other two recipients (PR2 and AR1) was confirmed by ultrasonography on day 60.

Table 19. Progesterone level in recipient goats at weekly intervals after embryo transfer

Sl. No	Age group of donor	Recipients No.	P4 level at weekly intervals (ng/ml)				Remarks
			Day of transfer	Day 14	Day 21	Day 28	
1	Peripubertal goats	PR_1	3.50	5.00	5.60	0.70	2CL showed heat on day 27
		PR_2	4.00	5.60	6.00	6.50	3CL
		PR_3	3.00	4.50	0.50	4.20	2CL showed heat on day 20
2	Adult goats	AR_1	4.5	6.5	6.80	6.95	3CL
		AR_2	3.00	3.9	4.5	0.80	2CL Showed heat on day 26
		AR_3	4.50	4.00	0.6	3.40	2CL showed heat on day 21

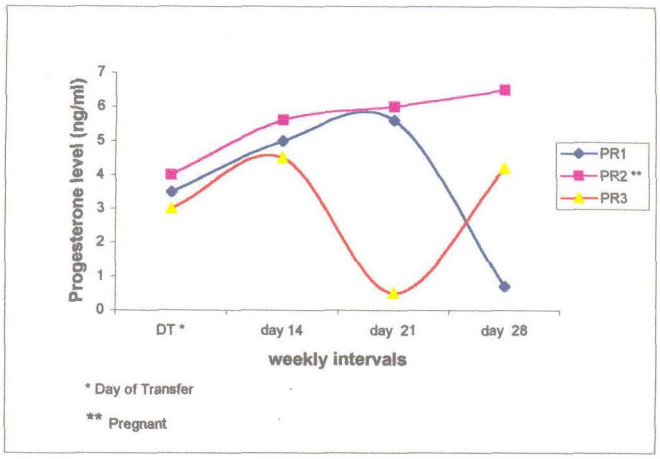


Fig. 6(a) Progesterone level in recipient goats PR1, PR2 and PR3 at weekly intervals after embryo transfer

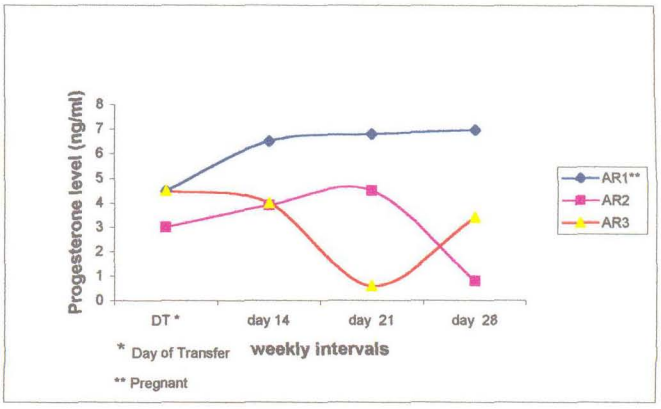


Fig. 6 (b) Progesterone level in recipient goats AR1, AR2 and AR3 at weekly intervals after embryo transfer

Discussion

5. DISCUSSION

The study was designed and conducted with the objective of evaluating the superovulatory response (SOR), recovery of embryos and viability of fresh and frozen embryos in peripubertal and adult Malabari goats.

5. 1 RESPONSE TO SYNCHRONISATION AND OESTROUS CHARACTERISTICS

5.1.1 Response to Synchronisation

The comparative response of different groups of peripubertal and adult goats to synchronisation treatment and their superovulatory oestrous characteristics are shown in table 4. While all (100 %) peripubertal and adult goats in G_1 and G_2 showed oestrus, in G_3, oestrum was exhibited by only 83.33 per cent. The overall response of peripubertal and adult goats to oestrous synchronisation was almost equal. (94.74 % vs 94.45 %).

The values reported in G_1 and G_2 in peripubertal goats are in agreement with the finding of Senn and Richardson (1992) who treated Nubian doelings in a similar manner during breeding season. The same authors reported a low synchronisation rate when treated during non-breeding season. Yuswiati and Holtz (1996) recorded slightly lower response (92%) in peripubertal Boer goats treated using PGF2 α and 1.5 mg Crestar implants.

Kathiresan (1993) reported that all prepubertal goats exhibited oestrous signs, while standing to be mounted by the teaser buck was exhibited by only 83.33 per cent of the P_4 primed and FSH treated goats, which is in agreement with the finding in G_3 in the present study. In this study, the reaction of goats to norgestomet and pFSH is lower both in peripubertal and adult groups than the report of Nowshari *et al.* (1992). The oestrous response reported in adult goats in G_3 is slightly lower than the report of Pendleton *et al.* (1992). The response to synchronisation can vary depending on season breed as well as agents used for synchronisation and superovulation. The response of adult goats in G_1 and G_2 are

in agreement with the finding of Martemucci *et al.* (1992); Thilager *et al.* (1992) and Senthilkumar *et al.* (1998). The latter authors used 3 mg norgestomet implants for oestrous synchronisation in Malabari goats. The present study indicates that 1.5 mg norgestomet ear implants can be effectively used for oestrous synchronisation in adult Malabari goats.

5.1.2 Interval from End of Synchronisation to Oestrus

The interval from end of synchronisation to heat (h) did not differ significantly between groups in peripubertal and adult goats. The interval from the end of synchronisation to heat (h) was shorter in peripubertal goats of G_1, but there was no significant difference between peripubertal and adult goats. In G_2 the interval from the end of synchronisation to heat was significantly longer ($p<0.05$) in adult goats (33.20 ± 3.97 h) than in peripubertal goats (21.30 ± 1.84h). It might be due to early development of follicles after gonadotrophin administration in the G_2 in peripubertal goats. In G_3 there was no significant difference between peripubertal and adult goats but the interval from end of synchronisation to heat was longer in peripubertal goats when compared to adult goats. The overall interval from end of synchronisation to heat (h) did not vary significantly between peripubertal and adult goats, even though it was longer in adult goats (25.06 ± 1.52 h vs 29.90 ± 3.05 h).

The overall values recorded in peripubertal goats in the present study is in agreement with the report in adult Malabari goats treated in a similar manner (Thilagar *et al.*, 1992 and Senthilkumar *et al.*, 1998). The overall mean interval reported in peripubertal goats in the present study is shorter than the reports of Kathiresan (1993) and Yuswiati and Holtz (1996).

In adult goats the onset of oestrum in G_1 and G_2 were later when compared to G_3 which is in agreement with the report of Baril and Vallet (1990). While Senthilkumar (1998) reported that this interval was much shorter in Malabari goats in which 180 mg pFSH was administered as eight injections. The FSH injection schedule might be a reason for the deviation as opined by Terzano

et al. (1999). Moreover in the present study prostaglandin was administered at the time of removal of implant. While Senthilkumar *et al.* (1998) reported administration of $PGF_2\alpha$ 12 h before the implant removal. The delay thus caused in regression of CL in the present study might be a reason for the longer interval from end of synchronisation to heat in the same breed. However, adult goats in G_3 exhibited heat within 24 h after removal of implant, which corroborates with the findings of Akinlosotu and Wilder (1993) and Senthilkumar *et al.* (1998).

5.1.3 Duration of Oestrum

The duration of superovulatory heat was significantly longer (52.00 ± 5.32 h) in peripubertal goats belonging to G_2 when compared to G_1 and G_3 (36.70 ± 1.47 and 40.40±3.58h) while no significant difference was observed between different groups in adult goats. Rosina *et al.* (1992) reported that 60 percentage does treated for superovulation using PMSG, which exhibited premature regression of CL, had less than 12h oestrus duration. However, shortest duration of heat was observed in G_1 among peripubertal goats. Further studies are required to assess the relation between PRCL and duration of heat. Individual variation might be a reason for this significant difference.

The duration of heat between peripubertal and adult goats did not show any significant difference in any of the groups. The overall average duration of heat (h) in peripubertal and adult goats were almost same (42.83 ± 2.55 vs 41.35 ± 4.40). These values are in close agreement with the report of Baril and Vallet (1990), and Kathiresan (1993). While shorter duration was reported by Benjamin (1994) in P_4 and PMSG treated goats. Senthilkumar (1998) also reported shorter duration in adult Tellicherry goats treated using crestar implants and $PGF_2\alpha$ and pFSH or oFSH. This variation seems to be due to difference in the agents used for oestrous synchronisation and source and dose of gonadotrophins used in the above studies. It is also reported that LH content in the gonadotrophin preparations may directly induce ovulation resulting in shorter duration of heat (Batt *et al.*, 1993).

5.1.4 Intensity of Oestrum

The mean of intensity of heat (score) was highest in G_1 followed by G_3 and least in G_2 both in peripubertal and adult goats. Adult goats in G_1 showed a score of 4.00, while in G_2 and G_3 the intensity of heat was found to be higher in peripubertal goats than in adult. The overall mean intensity of heat (score) was slightly higher in peripubertal goats than in adult goats. The highest intensity of heat in G_1 both in peripubertal and adult goats indicated that high dose of ovine FSH resulted in recruitment of larger number of antral follicles for superovulation and the high oestradiol concentration in both these age groups. The present study is in agreement with the report of Arthur *et al.* (1996) in cattle that there were great variations among individual cattle in intensity of heat and that the manifestations of heat tend to be more marked in heifers than in cows. Perhaps younger animals exhibited heat signs with more intensity than adult goats due to the high sensitivity of their nervous system to changes in oestradiol levels than in adult animals, even though superovalatory response was less in this group.

5.1.5 Signs of Oestrum

All peripubertal and adult goats exhibited physical signs like congestion of vaginal mucosa and vulval oedema. While, majority in G_1 showed mucus discharge from vulva. Wagging of tail was noticed in all the animals in both the age groups. But in peripubertal goats it was more prominent than in adult goats. A few animals belonging to G_1 and G_3 in peripubertal group exhibited bleating, while, most of the animals in G_1 showed bleating in adult goats. Adult animals belonging to G_1 exhibited frequent urination and reduction in feed intake. Circling with the buck was not prominent in peripubertal goats except in G_1. While in adult goats this sign was noticed in all the groups and very prominent in G_1. Also homosexual behaviour and bleating was highly prominent in adult goats belonging to this group. Frequent urination was noticed in all the adult goats but this sign was less prominent in G_2 and G_3.

Majumdar *et al.* (1990) reported weak signs in P_4 primed and FSH treated prepubertal goats. The observation that none of the peripubertal and adult goats exhibited poor oestrous signs is in agreement with the finding of Senthilkumar (1998) in the same breed of adult goats. Frequent bleating was not observed in all the peripubertal goats as observed by Senn and Richardson (1992) in Anglo-Nubian doeling. But the present study does not agree with the observation of Dhandapani (1998) who recorded no bleating in superovulated Barbari goats.

The age, breed agroclimatic conditions, management practices and dose and type of gonadotrophin might have probably contributed to the variation in the exhibition of heat signs in the present study.

5.2 OVARIAN RESPONSE

Armstrong *et al.* (1983b) opined that in common with most other species in which superovulation and embryo transfers have been carried out, the goat exhibited a high degree of variability in ovulation rate and in the number of embryos recovered after superovulation.

In the present investigation purified preparations of ovine and porcine FSH were administered based on the body weight of animals in peripubertal and adult goats. No attempt was made to compare the LH content in these preparations as reported by McNatty *et al.* (1989).

5.2.1 Anovulatory Follicles

Perusal of the table 5 revealed that both in peripubertal and adult goats number of anovulatory follicles were found to be high in G_1 and G_2 when compared to G_3. The high level of P_4 in the circulation from day four until the time of embryo collection might have suppressed further development of follicles in G_3 by inhibiting the release of GnRH from hypothalamus. In contrast, in G1 where the incidence of PRCL was high and where no P_4 administration was carried out average number of anovulatory follicles were highest due to low P_4 level. A similar pattern was observed in G_2 also in both these age groups. Most

of these animals with PRCL exhibited heat signs on day of embryo collection indicating follicular phase.

The average number of anovulatory follicles was higher in peripubertal goats when compared to adult goats in all the groups except G_1. The overall average number of anovulatory follicles recorded in peripubertal goats in the present study was also found to be slightly higher (1.63 vs 1.50). Nowshari *et al.* (1992) reported lower values in peripubertal Boer goats when compared to adult animals. Nowshari *et al.* (1992) assessed the superovulatory response of animals with FCL only after conducting the P_4 assay and as such there is no indication of animals with PRCL in that study. The number of anovulatory follicles in adult goats in each group and the overall average in adult goats were lower than the values reported by the above authors in adult Boer goats and Senthilkumar (1996) in the same breed. Tiwari *et al.* (1998) who administered hCG in non-descript adult goats on day seven of oestrous cycle prior to start of superovulatory treatment using FSH reported a higher number of anovulatory follicles. The present study indicates that administration of hCG in Malabari goats during early part of superovulatory heat probably ensured ovulation and reduced the number of anovulatory follicles. It is reported that use of hCG and GnRH or its analogues with superovulatory drugs resulted in higher ovulation rates and reduced the number of anovulatory follicles (Krisher *et al.*, 1994; Biswas *et al.*, 2001).

5.2.2 Superovulatory Response

The total superovulatory response (number of ovulations) in right and left ovaries and total response per animal was found to be highest in G_1, followed by G_2 and lowest in G_3 both in peripubertal and adult goats. Even though no significant difference was found between right and left ovaries, left ovary was found to be more active in the present study in all the groups. Pandey *et al.* (1992) reported that there was no difference in the ovarian response between left and right ovaries in Black Bengal goats superovulated using PMSG and flushed on day six. In contrast, Sarmah *et al.* (1996) reported a significantly higher

ovulation rate from right ovary than left. The role of breed as a probable cause for variability in superovulatory response is well established (Nuti *et al.*, 1987).

The superovulatory response in G_1 in peripubertal goats was found to be significantly higher than in G_3, ($P<0.05$) while in adult goats no significant difference ($P>0.05$) was observed between groups. The fact that both in peripubertal and adult goats the superovulatory response was higher in G_1 than in G_2 indicate that administration of high doses of oFSH increased the superovulatory response in both these age groups. However, the response in peripubertal and adult goats were comparatively lower in G_3 when compared to G_1 and G_2. Batt *et al.* (1993) opined that pFSH group might have achieved higher ovulation rate as in oFSH group if the dose of pFSH was sufficiently increased.

The superovulatory response of peripubertal Malabari goats in G_1 (12.20 ± 1.64) was found to be higher when compared to the reports of Armstrong and Evans (1983) and Nowshari *et al.* (1992) where pFSH was used for inducing superovulation. Armstrong and Evans (1983) reported lower values than those recorded in G_2 (6.96 ± 1.45) in the present study. This might be due to the fact that oFSH is superior in inducing superovulation than pFSH in Malabari breed as opined by McNatty *et al.* (1989).

The ovulation rate in peripubertal goats in G_3 (4.66 ± 1.50) corroborates with the report of Armstrong and Evans (1983) and Kathiresan (1993). But higher values were reported by Majumdar *et al.* (1990), Holm *et al.* (1990), Nowshari *et al.* (1992), Senn and Richardson (1992) and Yuswiati and Holtz (1996). However, the SOR is found to be higher than the report of Nowshari *et al.* (1992) who treated peripubertal Boer goats using PMSG. The higher response of peripubertal goats to pFSH compared to the present study in the reports of above authors can be attributed to the higher dose of pFSH used in those studies as well as the breed and season.

The superovulatory response in the present study in adult goats belonging to G_1 (16.16 ± 2.30) is in agreement with the report of McNatty *et al.* (1989) while Senthilkumar *et al.* (1996) reported much higher response in Malabari goats superovulated using commercial preparation of oFSH, Ovagen. The superovulatory response recorded by Taneja *et al.* (1991), Pendleton *et al.* (1992) and Greyling *et al.* (2002) using pFSH were comparable to the result in G2 (14.30 ± 2.60), in the present study. While Krisher *et al.* (1994) and Peebles and Kidd (1994) reported higher values using pFSH. The lack of correlation between bioactivity and immunoactivity of commercial gonadotrophins and intrinsic ovarian factors are major causes for variability in superovulatory programmes. The ovulation rate in adult goats in G_3 (6.40 ± 2.70) is found to be higher than the reports of Tiwari *et al.* (1998) while Taneja *et al.* (1991) and Senthilkumar (1996) reported higher ovulation rate. The dose of pFSH used in the present study was much lower than the above reports.

There was no significant difference in the total superovulatory response between peripubertal and adult goats in G_1 (12.20 ± 1.64 vs 16.16 ± 2.30) and G_3 (4.66 ± 1.50 vs 6.40 ± 2.70), but significant difference was observed between these age groups in G_2 (6.96 ± 1.45 vs 14.30 ± 2.60). The overall superovlatory response in peripubertal and adult goats were recorded as 8.16 ± 0.84 and 12.30 ± 1.40, which differed significantly ($P<0.05$). This is in agreement with the report of Holm *et al.* (1990) who recorded a lower response in Angora hoggets than in does when superovulated using pFSH. In contrast, Nowshari *et al.* (1992) recorded that there was no significant difference between peripubertal and adult Boer goats in the ovulation rate. These authors administered comparatively higher doses of pFSH to peripubertal goats when compared to adult goats (16 mg vs 18 mg) unlike in the present study. In contrast, Dutta *et al.* (2001) reported significantly higher ovulation rates in prepubertal local goats of Assam than in cyclic does superovulated using PMSG at the rate of 650 IU and 750 IU respectively.

Marked variability in the superovulatory response has been reported to be caused by the type and dose of gonadotrophin preparations used (Selgarth *et al.*, 1990), the ratio of FSH and LH in the preparations (McNatty *et al.*, 1989), the status of follicular development in the animals at the time of initiation of superovulatory treatment (Bo *et al.*, 1996), age (Moore, 1982), body weight, nutrition (Trevit *et al.*, 1984; Mani *et al.* 1994), season (Senn and Richardson, 1992) and breed (Martemucci *et al.*, 1992 and Greyling *et al.*, 2002).

5.2.2.1 Degree of Superovulatory Response

Perusal of table 6 revealed that the percentage of animals that exhibited low response (1-8CL) were 44.44 and 29.41 per cent respectively in peripubertal and adult groups. Medium response (9-16) was shown by 55.56 per cent in peripubertal and 29.41 per cent in adult goats. While 41.18 per cent adult goats showed high response none of the peripubertal goats had >16 CL. This clearly indicates that superovulatory response in adult Malabari goats is much higher than that in peripubertal goats. The body weight of this age group might have influenced the superovulatory response as opined by Trevit *et al.* (1984). The ovulation rate in G_2 and G_3 in peripubertal goats indicates that higher doses of FSH administration might be required in this age group to obtain maximum response as recorded in G1.

5.2.2.2 Premature Regression of CL

Premature regression of corpus luteum was predominantly observed in the present study. The fact that some of the goats showed corpora lutea in varying stages of regression whereas in some other animals the regression was almost complete is in agreement with the report of Rosina *et al.* (1992). Such animals had one to two large follicles in their ovaries. The finding that these animals exhibited prominent signs of oestrum on the day of embryo collection, and they could be identified before laparotomy as they exhibited vaginal discharge and wagging of tail indicating short oestrous cycle is in agreement with the report of Trevit *et al.* (1983). The uterine horns of these animals were very rigid and

vascular indicating the presence of high levels of oestrogen in the blood further made it clear that they were in follicular phase.

The incidence of PRCL was highest in G_3 in peripubertal goats followed by G_1 and then G_2. In adult goats highest incidence was observed in G_1 followed by G_2 while no PRCL could be observed in G_3. The overall incidence of premature regression of CL in peripubertal and adult goats were recorded as 50.00 and 52.94 per cent respectively. In the group I, 57.14 per cent of peripubertal and 83.33 per cent adult goats exhibited premature regression of CL. The corresponding values in peripubertal and adult goats in G_2 and G_3 were 33.33 and 66.67 and 60.00 and 00.00 percent respectively. This is in contrast to the report of McNatty *et al.* (1989) and Pendleton *et al.* (1992) who opined that the incidence of PRCL was common in PMSG and pFSH treated animals while it was rare in oFSH treated goats. In G_3, where SOR was low, only peripubertal goats exhibited PRCL. Perusal of literature did not reveal any comparable studies on incidence of PRCL in peripubertal and adult goats. However, Pintado *et al.* (1998) reported that 51 and 45 per cent of the Murciana goats showed regressive CL during fall when treated with PMSG and pFSH respectively. During spring the corresponding figures were reported to be 14.0 per cent and 17.5 per cent respectively.

The overall incidence of premature regression of CL recorded in both age groups were higher than the report of Trevit *et al.* (1984), McNatty *et al.* (1989), Martemucci *et al.* (1992), Rosina *et al.* (1992), Borque *et al.* (1993), Mani *et al.* (1994), Tiwari *et al.* (1998) and Pintado *et al.* (1998). High superovalatory response in oFSH treated goats might have resulted in high incidence of PRCL due to stress as opined by Pintado *et al.* (1998). The factors like age, breed, season, nutritional status and type and dose of gonadotrophins used might have contributed to the high incidence of PRCL in Malabari goats in the present study. The fact that Malabari goats do not exhibit any seasonality of breeding unlike breeds originated in temperate regions might have also contributed to this finding. Armstrong *et al.* (1983a) opined that it is uncertain whether the

luteal phase was as a result of premature luteolysis or failure of the corpora lutea to become functional following superovulation. However, premature release of prostaglandin has been implicated as the cause for early luteal regression in superovulated goats (Battye *et al.*, 1988). Premature regression of CL was observed in adult animals with high superovulatory response, while all groups of peripubertal goats exhibited this phenomenon irrespective of the type and dose of FSH treatment. This further indicates that in the present study in Malabari goats, stress might be a cause for the high incidence of PRCL.

5.3 EMBRYO RECOVERY

5.3.1 Total Ova and Embryo Recovery

The percentage of ova and embryo recovery was highest in G_3 in peripubertal goats, followed by G_2 and then G_1 with an overall recovery rate of 45.51 per cent. This parameter showed a similar trend in adult goats with an overall value of 36.48 per cent. The average ova and embryo recovery per goat was highest in G_2 (4.50) in peripubertal goats followed by G_3 (3.50) and then G_1 (3.29) with an overall value of 3.94. The corresponding figures in G_1, G_2 and G_3 in adult goats were 5.00, 5.50 and 3.00 with an overall value of 4.50.

Inspite of the higher superovulatory response the percentage of ova and embryo recovery rate was found to be lower in adult goats than in peripubertal goats in all the groups except G_1 (26.74% vs 30.93%, 64.29% vs 38.37% and 75.00% vs 46.15%). The overall percentage ova and embryo recovery rate in peripubertal and adult goats were respectively 45.51 and 36.48. The average ova and embryo recovery per goat in peripubertal and adult goats in G_1, G_2 and G_3 were respectively 3.29 and 5.00, 4.50 and 5.50 and, 3.50 and 3.00. The overall ova and embryo recovery rate per goat in peripubertal and adult goats were recorded as 3.94 and 4.50 respectively.

The low percentage of ova and embryo recovery and average ova and embryo recovery per goat in peripubertal and adult Malabari goats can be attributed to high incidence of premature regression of CL noticed in this breed

attributed to high incidence of premature regression of CL noticed in this breed on days six to eight after breeding. Premature regression of CL causes abnormal embryo transport leading to the expulsion of embryos via cervix as a result of endocrine abnormalities (Armstrong *et al.*, 1983b). The percentage of ova and embryo recovery in G_1 and G_2 in peripubertal and adult goats indicate that as the incidence of premature regression increases there is a proportionate reduction in the recovery rate of embryos.

In G_3 where P_4 support was given there is a clear difference in the percentage of ova and embryo recovery in peripubertal goats inspite of high incidence of PRCL. But in adult goats in G_3 eventhough the CL were functional, the percentage of ova and embryo recovery rate was lower. This was due to the low recovery (three) from a single donor with moderate superovulatory response (15 CL). This might have been caused due to defective pick up of ovum by the fimbriae of this nulliparous animal, as a result of hyperstimulation of ovaries.

The reports of Holm *et al.* (1990) and Kathiresan (1993) in prepubertal goats are higher than the values recorded in peripubertal goats in the present study.

The overall average ova and embryo recovery rate in the present study in adult goats was found to be higher than the reports of Rosina *et al.* (1992) and Pintado *et al.* (1998) in PMSG treated animals. Tiwari *et al.* (1998) reported lower values in FSH treated indigenous goats. The ova and embryo recovery rates using laparoscopic and non-surgical collection (Goel *et al.*, 1995 and Dhandapani, 2001) are lower than the values recorded in the present study. But higher recovery rates were reported by Gilbert *et al.* (1990), Martemucci *et al.* (1992), Akinlosotu and Wilder (1994), Peebles and Kidd (1994), Fieni *et al.* (1995), Dhandapani (1998), Pereira *et al.* (1998) and Greyling *et al.* (2002) by surgical and non-surgical collection from uterus. In addition to the high incidence PRCL, the day and the technique of collection might have influenced the values in the present study. Generally the embryo recovery from uterine horn

Perusal of literature did not reveal any comparable studies on the ova and embryo recovery rate in peripubertal and adult goats.

5.3.2 Fertilized Ova or Embryo Recovery

The percentage of fertilized ova or embryo recovery in peripubertal goats were 100 in G_1 and G_3 while it was 70.37 in G_2, with an overall value of 88.73 percent. In adult goats fertilized ova recovery rate (%) was highest in G_3 (83.33) followed by G_2 (75.76) and then G_1 (66.67) with an overall value of 74.07. The average fertilized ova or embryo recovery per goat in peripubertal group was highest in G_3 (3.50) followed by G_1 (3.28) and then G_2 (3.17) with an overall value of 3.32. While in adult goats lowest value was observed in G_3 (2.50) followed by G_1 and then G_2. The overall average fertilized ova in adult goats were 3.33. The low fertilization rate in G_2 in peripubertal goats was due to recovery of seven UFO from a single animal with FCL. In adult goats the low fertilization rate G_1 and G_2 could be attributed to the recovery of only UFO from animals with PRCL while in peripubertal goats no ova could be recovered from animals with PRCL. Also one adult goat in G_3 with FCL yielded three UFO only inspite of medium superovulatory response. The low fertilization rate in G2 in peripubertal and G1, G2 and G3 in adult goats may be due to abnormal sperm transport as a result of altered hormonal profile in superovulated animals. In animals with PRCL the embryos might have migrated or got transported to the posterior part of uterus due to the unfavourable environment created in the uterus because of low P_4 level and thus making them unavailable for collection. The present finding in peripubertal and adult goats agrees with the report of Rosina *et al.* (1992) and Pintado *et al.* (1998) who stated that no embryos could be recovered from goats, which exhibited PRCL. In contrast, Drost (1986) and Pintado *et al.* (1996) recorded low embryo recovery from animals with PRCL.

Perusal of literature did not reveal any study on percentage embryo recovery in peripubertal goats. The overall fertilization rate (%) in peripubertal goats are higher than the values reported in adult goats by Gilbert *et al.*, (1990), Martemucci *et al.* (1992), Mani *et al.* (1994) and Dhandapani (2001). The

fertilization rate in adult goats in the present study corroborates with the finding of Brebion *et al.* (1992). Higher fertilization rates were reported by Fieni *et al.* (1995), Senthilkumar *et al.* (1998) and Pereira *et al.* (1998) than the values recorded in G_1 and G_2 and the overall values in the present study in adult goats. Also the reports of the above authors are higher than the values reported in G_2 in peripubertal goats.

The average number of fertilized ova recovered per peripubertal goat is found to be higher than the report of Kathiresan (1993) in prepubertal goats and Pintado *et al.* (1996) and Tiwari *et al.* (1998) in adult goats. Whereas Armstrong and Evans (1983), Holm *et al.* (1990) and Majumdar *et al.* (1990) recorded higher number of fertilized ova per goat. The overall average fertilized ova recovery rate per adult Malabari goat in the present study is found to be higher than the reports of Nowshari *et al.* (1992), who treated Boer goats using PMSG, Pintado *et al.* (1996), Pintado *et al.* (1998) and Tiwari *et al.* (1998). Akinlosotu and Wilder (1994), Fieni *et al.* (1995) and Senthilkumar *et al.* (1998) recorded higher values. The fertilization depends upon a number of factors viz. timely breeding with proven sire, ovulation time and altered hormonal profile of animal as a result of exogenous hormonal administration. However the fairly high fertilization rate both in peripubertal and adult goats indicates the effectiveness of techniques of synchronisation and breeding adopted in the present study.

The fertilization rate in G_1 in peripubertal and adult goats was recorded as 100 per cent and 66.67 per cent respectively. The average embryo recovery rate in this group was 3.28 and 3.33 respectively. In G_2 the percentage of embryo recovery and the embryo recovery per goat were 70.37 and 75.76 and, 3.17 and 4.17 respectively in peripubertal and adult goats. In G_3 where P_4 support was given fertilization rate and embryo recovery per goat were found to be higher in peripubertal goats than in adult goats (100% vs 83.33% and 3.50 vs 2.50). The corresponding overall values in these age groups were (88.73% Vs 74.07% and 3.32 Vs 3.33).

Nowshari *et al.* (1992) reported that there was no significant difference in SOR and average embryo recovery rate in peripubertal and adult Boer goats treated using pFSH and PMSG. In the present study overall average embryo recovery and percentage embryo recovery were almost equal, even though significant difference was observed in overall superovulatory response and SOR between these age groups in G2. This can be explained on the basis that a substantial percentage of adult goats in G_1 and G_2 exhibited premature regression of CL, when compared to peripubertal goats, from which no embryos could be recovered, as there was reduced endogenous and no exogenous P_4 support.

5.3.3 Embryo Recovery on Progesterone Support

In the present study among the P_4 treated animals in G_3, adult goats did not show any PRCL. But peripubertal goats which had non-functional CL (42.85%) showed high ova recovery rate (75%) compared to the P_4 non-treated group (G_1 and G_2) which yielded only 39.06 per cent embryos. No comparative study on the effect of P_4 support on embryo recovery rates by surgical collection could be found in the literature in peripubertal goats. However, the adult goats in G_3, which were supported with endogenous P_4, and exogenous P_4 from day four, the yield of ova as percentage SOR was higher than that of G_1 plus G_2 (46.15% vs 34.42%). The reason for low ova recovery in adult animals belonging to G_3, inspite of medium response and FCL can be attributed to abnormal catching of ova by the fimbriae in a donor animal in this group, which yielded only three oocytes. It is clear that P_4 administration helped to prevent the transport of embryos to the posterior part of the reproductive tract by further suppressing follicular development and quietening uterus as in luteal phase. The embryo recovery rate in peripubertal goats is in agreement with the finding of Gilbert *et al.* (1990) and Brebion *et al.* (1992) who collected embryos nonsurgically from adult goats. The fertilized ova recovery in P_4 treated adult and peripubertal goats were 100 per cent and 83.33 per cent respectively when compared to non-progesterone treated group (84% vs 71.43%). This clearly indicates that P_4

administration can be used as a preventive measure to avoid loss of embryos from uterus when collected on days six to eight.

5. 4 EMBRYO QUALITY

5.4.1 Transferable Embryos

Perusal of table 10 revealed that both in peripubertal and adult goats the highest recovery rate of transferable embryos was recorded in G_3 followed by G_1 and then G_2. This clearly indicates that administration of exogenous progesterone do not affect the embryo quality in animals with FCL and PRCL. The percentage recovery of transferable embryos in G_1 in peripubertal and adult goats were respectively 95.65 and 95.00. The corresponding values in G_2 were 89.47 and 92 per cent. In G_3 where P_4 support was given peripubertal and adult goats yielded 100 per cent transferable embryos. The overall percentage transferable embryos in peripubertal and adult Malabari goats were recorded as 95.23 and 95.00 respectively.

The overall percentage transferable embryo recovery reported in the present study in both age groups is almost equal. The report of Deshpande et al (1997) corroborates with the finding in peripubertal and adult goats in the present study. Nowshari *et al.* (1992) recorded lower yield of transferable embryos from peripubertal Boer goats than in adult. Yuswiati and Holtz (1996) recorded lower values in peripubertal Boer goats. Lower transferable embryo recovery was reported in adult goats by many other authors (Gilbert *et al.*, 1990, Martemucci *et al.*, 1992; Akinlasotu and Wilder, 1993; Benjamin, 1994; Mani *et al.*, 1994; Senthilkumar, 1998).

In peripubertal group average embryo recovery per goat was highest in G_3 followed by G_1 and then in G_2, while in adult goats G_2 showed maximum average transferable embryo recovery (TER) per goat followed by G_1 and then G_3. The average transferable embryo recovered per goat in G_1 and G_2 were lower in peripubertal animals (3.14 Vs 3.16 and 2.83 vs 3.83). But in G_3, it was higher in

peripubertal goats (3.50 vs 2.50). The overall transferable embryo recovery per animal in peripubertal and adult goats was almost equal (3.15 Vs 3.17).

Inspite of high ovulation rate, the low average transferable embryo recovery in adult animals can be attributed to high incidence of PRCL and defective catching of ovum by fimbriae in one animal in G_3. The overall values recorded in the present study in both the age groups are found to be in accordance with the report of Martemucci *et al.* (1992) in superovulated Maltese goats. Senn and Richardson (1992) in Anglo-Nubian goats, Nowshari *et al.* (1992) in Boer goats and Goel and Agrawal (1998) in Jamnapari goats. However Goel *et al.* (1993), Akinlosotu and Wilder (1993) and Pintado *et al.* (1998) recorded lower average viable embryo recovery per goat. In contrast, higher values were reported by Nowshari *et al.* (1992), Senthilkumar (1996) and Peebles and Kidd (1994). The differences in the breed, age, reproductive status, day of collection, flushing technique, season, body weight, nutritional status of animals and type and dose of gonadotrophins, used etc. might have influenced these results.

In peripubertal goats highest percentage good quality embryo recovery was recorded in G_2, followed by G_3 and then G_1 with an overall recovery rate of 73. While in adult goats maximum recovery rate was observed in G_3 followed by G_1 and then G_2. The overall percentage recovery rate of good quality embryos in adult goats was 57.89. The percentage recovery rate of good quality embryos in G_1 in peripubertal and adult goats were 68.18 and 63.16 respectively. The corresponding values in G_2 were 88.24 and 34.78 respectively. In G_3 71.43 and 86.67 per cent embryos were of good quality in peripubertal and adult goats, with an overall value of 73.00 and 57.89 in these age groups respectively.

The average good embryo recovery rate per goat is found to be equal in G_2 and G_3 and a low good embryo recovery rate is recorded in G_1 in peripubertal goats. In adult goats average good embryo recovery was found to be highest in G_3 followed by G_1 and then G_2. This further asserts that administration of exogenous P_4 do not affect the embryo quality in presence of endogenous P_4 both in peripubertal and adult goats.

In the present study, the average good embryo recovery rate per goat in all the three groups and the overall values were found to be higher in peripubertal goats than in adult goats. Perusal of literature did not reveal any comparative study on this aspect.

In peripubertal goats highest percentage of fair quality embryos were recovered from G_1 followed by G_3 and then G_2 with an overall recovery rate of 25.00. While in adult goats highest fair quality embryo recovery rate was observed in G_2 followed by G_1 and G_3 with an overall value of 42.10 per cent. The percentage recovery of fair quality embryos in G_1 was almost equal in peripubertal and adult goats (31.82 Vs 36.84). In G_2 the corresponding values were 11.76 and 65.02 while in G_3 it was 28.57 and 13.33 respectively. The overall percentage of fair quality embryos recovered from peripubertal and adult goats were 25.00 and 42.11 respectively.

The average fair quality embryo recovery per goat in all the groups also showed a similar trend as in percentage embryo recovery with an overall value of 0.78 and 1.33 respectively in peripubertal and adult goats.

The high fair embryo recovery rate in adult goats was due to the recovery of more number of embryos of the same quality from a single donor in G_2, which showed high SOR (21 CL). Perusal of literature did not reveal any comparative study on this aspect.

5.4.2 Non-transferable Embryos or Poor Quality Embryos

The percentage of non-transferable embryos was highest in G_2 and then in G_1 in peripubertal goats, while no poor quality embryos could be observed in G_3. The overall percentage of non-transferable embryo recovery rate was 4.76 both in peripubertal and adult goats. In adult goats the percentage recovery rate of non-transferable embryos in G_1, G_2 and G_3 showed a similar trend as in peripubertal goats.

The percentage of non-transferable embryos in peripubertal and adult goats in G_1, G_2 and G_3 were 4.35 Vs 5.00, 10.53 Vs 8.00 and 00.00 Vs 0.00 respectively with overall per cent of 4.76 and 5.00 in these age groups.

Gilbert *et al.* (1990) reported lower values than recorded in G_1 and G_2 when non-surgical collection was done without any treatment. But these authors recovered a higher percentage of degenerate embryos on treatment with Flunixin meglumine and CIDR as compared to the values in G_3 in the present study. This indicates that P_4 in form of injections would be ideal and cheap to maintain embryo quality in animals with PRCL when surgical uterine collection is practised. The treatment with P_4 does not affect the embryo quality in animals with FCL and PRCL. The values recorded in the present study are lower than those reported by Fieni *et al.* (1995) and Senthilkumar (1996) in the same breed by oviductal collection, where higher doses of oFSH were used for superovulation. The average number of poor quality embryos recovered per goat in G1, G2 and G3 in peripubertal and adult groups showed a similar trend as in percentage non-transferable embryo recovery rate.

The average number of non-transferable embryo recovery per goat in peripubertal and adult goats in G_1, G_2 and G_3 were respectively 0.14 and 0.17, 0.33 and 0.33 and 0.00 and 0.00 with on overall value of 0.17 and 0.18.

5.5 DEVELOPMENTAL STAGES OF EMBRYOS ON DIFFERENT DAYS OF COLLECTION

5.5.1 Sixth Day of Collection

On day six, all the embryos collected from peripubertal goats were morulae, while in adult goats 96.30 per cent were morulae and the remaining early blastocysts. The fact that majority of embryos collected on day six is morulae corroborates with the findings of Rosina *et al.* (1992) and Biswas *et al.* (2001) who used PMSG for superovulation. But these authors reported recovery of 2-8 cell embryos also on day six. In the present study there was not much

difference in the developmental stages of embryos collected on day six between peripubertal and adult goats.

5.5.2 Seventh Day of Collection

On day seven, 44.44 per cent of embryos collected were morulae and the remaining were early blastocysts in peripubertal goats, while in adult goats 86.67 per cent were morulae. The developmental stages of embryos collected on day seven is in accordance with the finding of Goel *et al.* (1995). While, Chemineau *et al.* (1986) observed expanded and hatched blastocysts on day seven. The breed, season and endocrine factors might have influenced the time of ovulation and subsequent development of goat embryos.

5.5.3 Eighth Day of Collection

Both in peripubertal and adult goats hundred percent of embryos collected on day eight were blastocysts. This observation is in agreement with the report of Sakkas *et al.* (1989) and Rosina *et al.* (1992).

It is evident that there is not much difference in the developmental stages of embryos collected on day six, seven and eight from peripubertal and adult goats. Human chorionic gonadotrophin was administered in the present study to synchronise ovulation. The minor variability in developmental stages might be due to the occurrence of ovulation over a period of 12 hours in superovulated goats (Baril and Vallet, 1990).

5. 6 EMBRYO QUALITY AFTER THAWING AND CRYOPROTECTANT REMOVAL

5.6.1 Transferable Embryos

Out of the 30 embryos recovered after freezing, 22 (73.33%) and 21 (70.00%) were found to be morphologically normal in peripubertal and adult goats respectively. The percentage of good quality embryos obtained from peripubertal goats after freezing and thawing were comparatively higher. In contrast, higher recovery rate was recorded in adult goats in case of fair embryos.

5.6.2 Non-transferable or Poor Quality Embryos

The morphologically abnormal embryos included broken zona, disintegrated and degenerated cell mass etc. Total number of embryos with abnormal morphology was low in peripubertal goats than in adult goats (26.67% Vs 30.00%). Both in peripubertal and adult goats higher percentage of morulae showed abnormal morphology (35.71 vs 40.00) than blastocysts (18.75 vs 20.00).

The viability of embryos in peripubertal goats in the present study is found to be lower than the report of LeGal *et al.* (1993) in which cryoprotectant removal was carried out in successive dilutions of ethylene glycol. The same authors recorded higher values when sucrose was added in the cryoprotectant removal media.

5.6.3 Effect of Developmental Stages on Quality of Frozen Embryos

5.6.3.1 Morulae

The percentage of morulae which showed normal morphology both in peripubertal and adult goats was also lower than the report of LeGal *et al.* (1993) in which successive dilutions of EG was carried out. But the same authors reported higher values when successive dilutions of cryoprotectant removal carried out using sucrose. The difference in the cryoprotectant removal medium and steps involved in the present study might be the reason for this variation.

5.6.3.2 Blastocysts

Both in peripubertal and adult animals blastocysts were more resistant to freezing procedure (81.25% Vs 80.00%) than morulae (64.29% Vs 60.00%). This is in agreement with the report of Li *et al.* (1990), Puls-Kleingeld *et al.* (1992), LeGal *et al.* (1993), Nowshari and Holtz (1995), Riha *et al.* (1994) and Fieni *et al.* (1995). From the biological point of view, the changes in individual blastomeres at blastocyst stage, which is a critical stage in development, is not fully understood. At that stage there is intense clevage activity formation of intercellular bridges at the beginning of differentiation. Simultaneously new cell

membrane transport mechanism begins to operate (Biggers *et al.*, 1977). In addition to different transport mechanism differences in permeability are expected to exist in morulae and blastocysts. Blastocysts with peripheral trophoblast cells and a distinct blastocoel respond to osmotic changes in a different way than morulae with their compact blastomeres (Niemann, 1991). The difference in survivability of blastocysts and morulae may also be due to a difference in their sensitivity to cryoprotective agents (Leibo and Masur, 1978).

Perusal of the literature did not reveal any comparable studies on the viability of frozen embryos from peripubertal and adult animals. The present study based on morphology of embryos indicates that embryos from peripubertal goats can be utilized for freezing like adult goats.

5.7 VIABILITY OF FROZEN EMBRYOS ON *IN VITRO* CULTURE

Perusal of the table 13 revealed that while 53.84 per cent blastocysts from peripubertal goats developed after 24 h of culture only 50.00 per cent from adult goats showed development. This further indicates that embryos from peripubertal goats can be utilised for freezing and culture as in adult goats. Perusal of the literature did not reveal any comparative study on this aspect. Values reported in the present study in adult goats agree with the finding of Mani and Vadnere (1988a) who cultured fresh embryos. But, LeGal *et al.* (1993) reported lower values. The authors used frozen embryos for *in vitro* culture irrespective of quality. In the present study only blastocysts with normal morphology after thawing and cryoprotectant removal were used for culture. But Fieni *et al.* (1995) reported higher rate of development on *in vitro* culture of frozen embryos from adult goats, which is equivalent to the viability of embryos from peripubertal goats in the present study. Traldi (2000) recorded higher survival rate on in vitro culture of embryos frozen-thawed using open pulled straw method. The method of freezing and cryoprotectant addition and removal techniques might have influenced the results in the present study.

5.8 PREGNANCY RATE IN RECIPIENT GOATS ON TRANSFER OF FROZEN THAWED EMBRYOS FROM PERIPUBERTAL AND ADULT GOATS

Embryos from peripubertal and adult goats were transferred into synchronised recipients to assess viability. Assessment of pregnancy based on the P_4 assay on day 21 and observing the animals for heat signs revealed that two (66.67%) recipients which received embryos each from peripubertal and adult goats were pregnant. Non-pregnancy was confirmed in one (33.33%) recipient each from peripubertal and adult goats, which exhibited heat signs on day 20 and 21 of pregnancy respectively. Subsequently two more animals one each from peripubertal and adult goats showed heat on fourth week of transfer, indicating that only 33.33 per cent was the pregnancy rate both in peripubertal and adult goats on day 28. This was confirmed by P_4 assay, which further indicated that there was no difference in the *in vivo* viability of embryos collected from peripubertal and adult goats. The pregnancy was again confirmed by ultrasonography in both these animals on day 60.

Pregnancy rate of 66.67 per cent reported in the present study was reduced to 33.33 in both these age groups which indicates embryonic death. The report of Mani *et al.* (1994) who transferred fresh embryos to undernourished recipients before ET is in agreement with the present study. But lower values were reported by Yuswiati and Holtz (1990) and Agrawal *et al.* (1994) by transfer of vitrified embryos. Puls-Kleingeld *et al.* (1992) and LeGal *et al.* (1993) also reported lower pregnancy rates in which morulae were frozen using glycerol. But higher rates of pregnancy was recorded by many investigators (Brebion *et al.*, 1992; LeGal *et al.*, 1993; Riha *et al.*, 1994; Hsu, 1995; Yuswiati and Holtz, 1990; Traldi *et al.*, 1999, Beckett *et al.*, 1999 and El-Gayar and Holtz, 2001). Eventhough the synchrony between oestrous cycle of donor and recipient animals was less than plus or minus one day the low rate of pregnancy obtained in the present study can be attributed to adverse factors of uterine or embryonic origin. Moreover only a few frozen morulae were used for transfer in this study. Further

studies using large number of animals are required to draw a definite conclusion. However, it is clear that embryos from peripubertal and adult goats are equally suitable for freezing and transfer.

5.9 ASSAY OF BIOCHEMICAL FACTORS

5.9.1 Serum Glucose

The present study indicated that there was no significant difference in serum glucose level between groups both in peripubertal and adult goats. Also serum glucose level did not vary significantly between peripubertal and adult goats in all the groups. The overall mean serum glucose (mg/dl) in peripubertal and adult goats were 57.73 ± 6.43 and 58.16 ± 6.30 respectively. The serum glucose level in peripubertal and adult goats recorded in the present study is in agreement with the report of Kaneko *et al.* (1997) in normal goats. But the values are found to be lower than the report Angami *et al.* (1997) in Beetal x Assam local crossbred goats of three to six months of age offered with high, medium and low energy feed. The difference in age and nutritional status might have influenced the results. Perusal of the literature did not reveal any report on serum glucose levels in superovulated animals.

5.9.2 Serum Enzymes

5.9.2.1 Lactic Dehydrogenase

The level of lactic dehydrogenase (U/L) showed a significant difference between groups in peripubertal goats. In peripubertal group the LDH level was lowest in G_1 (289.83 ± 34.11 U/L), which was significantly different from G_2 (450.00 ± 50.31 U/L) and G_3 (467.00 ± 42.67 U/L). The difference in the age of animals in G_1 was slightly higher than that of G_2 and G_3. This might be the reason for the significant difference in LDH activity between the groups in peripubertal goats as opined by Behra *et al.* (1993). But the values were well within normal range.

However, no significant difference was observed in LDH activity between groups in adult goats. Also there was no significant difference in the level of LDH between peripubertal and adult goats in any of the groups. The overall values reported in the present study in peripubertal and adult goats were 402.28 ± 52.42 and 485.28 ± 47.17 U/L respectively. However, these values were found to be higher than the reports of Behra *et al.* (1993) and Keneko *et al.* (1997) indicating high metabolic activity in superovulated Malabari goats. Ishwar and Pandey (1994) recorded lower values in superovulated goats.

However, the values reported by Jain *et al.* (1995) in normal goats are found to be higher than the present study both in peripubertal and adult goats. Activity of LDH is liable to vary a lot individually and between various stages of reproduction and pregnancy. Ghosh (1998) reported significant difference in LDH activity of both the ovaries together between GnRH responded does and noncyclic control does.

5.9.2.2 Acid Phosphatase

Perusal of literature did not reveal any comparative study on serum level of ACP in superovulated goats. There was no significant difference in the level of ACP between groups both in peripubertal and adult goats. Also no significant difference could be observed in serum ACP between peripubertal and adult goats. However, the serum ACP was found to be highest in G_3 both in peripubertal and adult goats. The overall values of ACP (KA units/dl) were recorded as 0.95 ± 0.27 and 0.74 ± 0.20 in peripubertal and adult goats respectively, which did not show any significant difference. Behra *et al.* (1993) reported lower values in goats belonging to different age groups. Superovulatory treatment and subsequent increase in oestrogen might have resulted in the rise in enzyme levels in the present study. Whereas Jain *et al.* (1995) recorded higher ACP activity in the serum of normal goats. The difference in the reproductive status, age and breed could have contributed to this variation in the level of ACP.

5.9.2.3 Alkaline Phosphatase

There was no significant difference in the alkaline phosphatase activity between groups in peripubertal and adult goats. Also no significant difference was observed between ALP activity in peripubertal and adult goats in all the groups. The overall alkaline phosphatase activity recorded in peripubertal and adult goats were respectively 26.75 ± 11.26 and 20.00 ± 9.42 KA units/dl. The higher alkaline phosphatase activity observed in peripubertal goats corroborates with the finding of Patel *et al.* (1992a) in Marwari goats on day 40 of gestation and Bharali *et al.* (2002) in crossbred goats of Assam on day seven and 30 of pregnancy. From the higher values of ALP observed both in peripubertal and adult goats in the present study it could be inferred that superovulation increased the demand for nutrients resulting in higher metabolic rate in donor goats as in pregnancy.

5.10 SERUM P4 LEVEL IN DONOR GOATS DURING SUPEROVULATORY HEAT

The study of P_4 profile was carried out using serum collected from superovulated donor animals on day of oestrum. The mean values of serum progesterone in G_1, G_2 and G_3 in peripubertal goats on day of superovulatory heat was recorded as 0.29 ± 0.04 ng/ml, 0.45 ± 0.05 ng/ml and 0.42 ± 0.06 ng/ml respectively with an overall mean of 0.38 ± 0.03 ng/ml. The corresponding values in adult goats in G_1, G_2 and G_3 were 0.40 ± 0.09 ng/ml, 0.27 ± 0.04 ng/ml and 0.38 ± 0.05 ng/ml with an overall mean of 0.32 ± 0.05 ng/ml.

Values recorded in the present study are in agreement with the report of Appavu and Holtz (1992) in Boer crossbred goats and Senthilkumar (1996) in Malabari goats. The latter author reported slightly higher values in animals treated using PMSG. The present finding is in agreement with the observation made on day one of normal heat by Leyva-Ocariz *et al.* (1995) in native and crossbred goats in tropical semi arid zone of Venezuela, Ghosh (1998) in Malabari crossbred goats and Behra *et al.* (2001) in Black Bengal does.

However, Ghosh (1998) reported lower values in acyclic goats, which responded to GnRH treatment. Armstrong *et al.* (1987) who assessed the P_4 levels during superovulatory oestrus recorded higher values (>1 ng/ml) in animals treated with PMSG while animals treated using FSH showed lower values. The authors opined that circulating P_4 levels were significantly increased by PMSG and not with FSH. While Tiwari *et al.* (1998) were of the view that the LH treatment on day seven of oestrous cycle might have influenced the P_4 level on the day of oestrus as it was found to be comparatively higher than control animals. The difference in the FSH preparations used and the synchronisation treatment adopted in this study might be the reason for lower P_4 level during oestrus in the present study.

5.10.1 Correlation of Progesterone Profile during Superovulatory Heat with Superovulatory Response

A negative correlation was observed between progesterone profile during superovulatory heat and number of FCL, PRCL and total superovulatory response both in peripubertal and adult goats, belonging to G_1, G_2 and G_1 plus G_2. The P_4 level in G_2 of peripubertal group showed a significant negative correlation with the number of PRCL and FCL, while the adult goats in this group did not show any significant correlation. Also a significant negative correlation was observed between P_4 level and total SOR in the above groups in peripubertal goats while this parameter was significant only in G_2 in adult goats. Tiwari *et al.* (1998) also observed a negative correlation between P_4 level on day of superovulatory heat and superovulatory response in goats. The author opined that higher levels of P_4 on day of oestrus as a probable cause for lower SOR in that study. The variability in the observations in the present study might be due to the lack of correlation between bioactivity and immunoactivity of commercial gonadotrophins and intrinsic ovarian factors.

5.10.2 Correlation of Progesterone Profile during Superovulatory Heat with Embryo Quality

The progesterone profile during superovulatory heat in peripubertal and adult goats showed a negative non-significant correlation. Perusal of literature did not reveal any comparative study on this aspect.

5.11 PROGESTERONE LEVEL ON DAY OF EMBRYO COLLECTION

The P_4 level in G_1 and G_2 of peripubertal goats ranged from 0.80 to 22.20 ng/ml, while in adult goats the range was 0.80 to 30.50 ng/ml. Both in peripubertal and adult goats belonging to G_1 and G_2 the P_4 level on day of embryo collection was low in animals, which exhibited PRCL (range = 0.80 to 1.10 vs 0.80 to 1.20). In animals with FCL P_4 levels ranged from 3.50 to 22.20 and 18.50 to 30.50 in peripubertal and adult goats respectively. Progesterone level in animals belonging to G_3 was more than 30.70 ng/ml in both these age groups due to administration of exogenous P_4.

The values reported in G_1 and G_2 of peripubertal and adult goats corroborates with the report of Armstrong *et al.* (1987); Stubbings *et al.* (1986); Appavu and Holtz (1992); Borque *et al.* (1993); Senthilkumar (1996) and Tiwari *et al.* (1998).

5.11.1 Correlation of P_4 Profile on Day of Embryo Collection with Superovulatory Response

A significant positive correlation ($P<0.01$) was observed between P_4 profile on day of embryo collection and number of FCL both in peripubertal and adult goats. This observation is in accordance with the finding of Appavu and Holtz (1992) who recorded a correlation coefficient of 0.93 in Boer crossbred goats. Also Santamaria *et al.* (1992) observed a significant correlation between total number of FCL and P_4 level in superovulated goats.

In contrast, a significant negative correlation was observed between number of PRCL and P_4 level (G_1 plus G_2) on day of embryo collection both in

peripubertal and adult goats. The low correlation coefficient obtained in adult animals might be due to the fact that some of the goats showed CL in varying stages of regression in which P_4 level was >1 ng/ml. A positive correlation was observed between P_4 level and total superovulatory response which was not significant except in G_2 of peripubertal goats. The significant correlation in G_2 in peripubertal goats might be due to the low incidence of PRCL in this group unlike in other groups.

Jarrell and Dziuk (1991) also observed a direct relationship between number of CL and serum P_4 in untreated goats on day 45 of gestation. The results clearly indicated that the number and type of corpora lutea determine the P_4 concentration in the serum. The relationship between corpora lutea and P_4 profile in the present study might help to determine good and poor response in animals with FCL as well as animals with PRCL. Despite the distinct linear relationship between serum progesterone concentration and number of FCL prediction of ovulation rate remains to be an approximation. This is mainly due to substantial individual variability observed in this study and others (Appavu and Holtz, 1992; and Senthilkumar, 1996).

Borque *et al.* (1993) opined that regression of CL was evident on day four, but, day three P_4 levels were lower than as they should be if the doe did not possess normally functioning CL. The finding in the present study and the above study indicates the importance of giving P_4 support or developing transcervical collection in Malabari goats which will enable to collect viable embryos from uterus suitable for cryopreservation from animals with PRCL as reported by Gilbert *et al.* (1990).

5.11.2 Correlation between P_4 Profile on Day of Embryo Collection and Embryo Quality

None of the animals with PRCL yielded embryos. A significant correlation was observed between P_4 level on day of embryo collection and transferable embryo recovery in both the age groups except in G_2 of peripubtertal

animals. The low correlation in this group can be explained due to recovery of seven UFO from a single donor with FCL. Santamaria *et al.* (1992) recorded similar results in goats superovulated with FSH or PMSG. The fact that ovary is the main source of P_4 during pregnancy (Bloom and Lyngset, 1971) and Caprine placenta produces small amount of P_4 during late pregnancy (Thorburn and Schneider, 1972) might be the reason for the positive correlation with P_4 level functional CL and TER in superovulated goats unlike other superovulated animals.

5.12 SERUM PROGESTERONE LEVEL IN RECIPIENT GOATS AT WEEKLY INTERVALS

Goats which received embryos from peripubertal and adult donors on day 6 or 7 showed P_4 level ranging from 3 to 4.50 ng/ml of serum which is equal to the normal luteal phase P_4 levels of this particular stage (Jarrel and Dziuk, 1991; Appavu and Holtz, 1992; Kaushik *et al.*, 1992).

On day 14 of pregnancy all the recipients, which received embryos from both peripubertal and adult goats exhibited slightly higher P_4 level. These values are in accordance with the report of Beckett *et al.* (1999).

On day 20 or 21 two recipients in which embryos were transferred from peripubertal and adult goats showed heat and the low P_4 level in these animals indicated that they were in follicular phase and not pregnant. On this day the other recipients showed fairly high P_4 level suggestive of pregnancy in these animals. Progesterone concentrations on day 28 confirmed pregnancy in PR2 and AR1 and luteal phase in PR3 and AR3. In the present study maximum P_4 concentration (6.95 ng/ml) observed in early pregnancy was lower than the values reported by Jarrell and Dziuk (1991). However, values recorded by Kaushik *et al.* (1992) are in agreement with the present study.

Unlike in cattle, there was a high proportion of PRCL in superovulated goats, which was responsible for the low embryo recovery rate in the present study. The obvious reason for the PRCL might be inadequate luteotrophic effect

or premature release of $PGF_2\alpha$. As such it is felt necessary that the treatment regimen for superovulation in goats should be suitably modified, by incorporating either GnRH or prostaglandin synthetase inhibitor in appropriate time and dosage. Surgical collection before day six and transcervical collection are other alternatives to improve embryo yield.

Eventhough the superovulatory response was significantly higher in adult goats ($P<0.05$) the recovery rate and viability of fresh embryos were almost equal in both the age groups consequent to the high incidence of PRCL. The transferable embryo recovery rates in animals with PRCL could be substantially increased by administration of progesterone. The study further revealed that the embryos from peripubertal goats were suitable for freezing *in vitro* culture and transfer as in adult goats. It can be concluded that peripubertal goats are as suitable as adult goats for MOET programme.

Summary

6. SUMMARY

The study was designed and conducted with the objective of evaluating the superovulatory response (SOR), yield and viability of fresh and frozen embryos from peripubertal and adult Malabari goats.

Nineteen peripubertal and eighteen adult Malabari goats were selected and utilised for the study. Animals of each age group were at random allotted to sub groups G_1,G_2 and G_3 consisting of six animals, except G_1 of peripubertal goats which contained seven. The animals in each age group were subjected to three superovulation protocols using ovine FSH (oFSH, NIH – FSH S-17) or porcine FSH (pFSH, NIH–FSH-P1) after synchronising them using 1.5 mg norgestomet ear implants plus PGF2 α (G_1 and G_2) and norgestomet ear implant alone (G_3) for a period of 11 days. The dose of oFSH administered in G_1 and G_2 were 0.9 units /kg body weight and 0.45 units /kg body weight respectively while pFSH was administered @ of 2 mg/kg body weight in G_3. In all the groups FSH was administered as six intramuscular injections at 12 h interval in a tapering dose regime. The animals were bred or inseminated using fresh semen on exhibition of heat signs. Human chorionic gonadotrophin was administered @ 500 IU and 750 IU within six hours after the onset of heat as IM injection in peripubertal and adult goats respectively. The animals belonging to G_3 in both these age groups were supported using progesterone injection (IM) twice daily @ 20 and 25 mg per animal respectively starting from later half of the day four until the day of embryo collection.

The embryos were collected on days six to eight by uterine flushing after midventral laparotomy using Foley's catheter. After grading them the developmental stages of transferable embryos were studied. Thirtytwo good quality embryos (16 morulae and 16 blastocysts) each from peripubertul and adult goats were frozen by conventional technique in a programmable biofreezer using ethylene glycol as cryoprotectant. The quality of frozen thawed embryos was assessed morphologically. All transferable frozen thawed (blastocysts) were

subjected to in vitro culture and the development was assessed after 24 h culture. Nine transferable frozen thawed embryos each from peripubertal and adult goats were transferred into the uterus of three synchronous recipients.

The level of serum glucose, LDH, ACP and ALP on the day of superovulatory heat in donors were assessed. Serum P_4 profile in donors on day of superovulatory heat and embryo collection was studied. Progesterone level in recipients on day of embryo transfer and thereafter at weekly intervals upto day 28 was assessed.

The response to synchronisation was hundred per cent in peripubertal and adult goats belonging to G_1 and G_2 while, it was only 83.33 per cent in both the age groups in G_3 with an overall response of 94.74 and 94.45 per cent respectively. The interval from end of synchronisation to heat (h) did not vary significantly between groups both in peripubertal and in adult goats. The overall mean interval from end of synchronisation to heat (h) was 25.06 ± 1.52 and 29.90 ± 3.05 respectively, in peripubertal and adult goats, which did not show any significant difference. But this interval was significantly different ($P<0.05$) between peripubertal and adult goats in G_2 (21.30 ± 1.84 h vs 33.20 ± 3.97 h). The overall mean duration of oestrum in peripubertal and adult goats were 42.83 ± 2.55 h and 41.35 ± 4.40 h respectively which did not vary significantly. The duration of heat was significantly different ($P<0.05$) between G_1 and G_2 (36.76 ± 1.47 h and 52.00 ± 5.32 h) and G_2 and G_3 (52.00 ± 5.32 h and 40.40 ± 3.58) in peripubertal goats. Intensity of heat was found to be highest in G_1 followed by G_3 and then in G_2 both in peripubertal and adult goats. The intensity of heat in G_2 and G_3 were found to be higher in peripubertal goats than in adult. The overall mean intensity of heat (score) was higher in peripubertal goats than in adult goats (3.44 ± 0.15 vs 3.18 ± 0.21).

Both in peripubertal and adult goats the number of anovulatory follicles were found to be high in G_1 and G_2 when compared to G_3 in which progesterone was administered from day four of superovulatory heat. The overall average

number of anovulatory follicles in peripubertal goats was found to be higher than that in adult goats (1.63 vs 1.50).

No significant difference could be observed between right and left ovary in SOR, but left ovary was found to be more active. The average superovulatory response per animal was found to be highest in G_1, followed by G_2 and lowest in G_3 both in peripubertal and adult goats. The total SOR in G_1 (12.20 ± 1.64) in peripubertal goats was found to be significantly higher than that in G_3 (4.66 ± 1.50), while in adult goats no significant difference was observed between groups. There was no significant difference in the total superovulatory response between peripubertal and adult goats in G_1 (12.20 ± 1.64 vs 16.16 ± 2.30) and G_3 (4.66 ± 1.50 vs 6.40 ± 2.70), but significant difference was observed between these age groups in G_2 (6.96 ± 1.45 vs 14.30 ± 2.60). The overall mean superovlatory response in peripubertal and adult goats were recorded as 8.16 ± 0.84 and 12.30 ± 1.40 respectively which differed significantly ($P<0.05$).

The percentage of animals that exhibited low response (1-8CL) was 44.44 and 29.41 per cent respectively in peripubertal and adult groups. The percentage of animals that exhibited medium response (9-16) in peripubertal and adult goats was 55.56 and 29.41 respectively. While 41.18 per cent adult goats showed high response, none of the peripubertal goats had >16 CL.

The incidence of PRCL was highest in G_3 (60.00%) in peripubertal goats followed by G_1 (57.14%) and then G_2 (33.33%). In adult goats highest incidence was observed in G_1 (83.33%) followed by G_2 (66.67%) while no PRCL could be observed in G_3. The overall incidence of premature regression of CL in peripubertal and adult goats were recorded as 50.00 per cent and 52.94 per cent respectively. In group I, 57.14 per cent peripubertal goats and 83.33 per cent adult goats exhibited premature regression of CL. The corresponding values in peripubertal and adult goats in G_2 and G_3 were 33.33 and 66.67 and, 60 and zero per cent respectively.

The percentage of ova and embryo recovery was highest in G_3 in peripubertal goats, followed by G_2 and then G_1. This parameter showed a similar trend in adult goats also. Inspite of the higher superovulatory response the percentage of ova and embryo recovery rate was found to be lower in adult goats than in peripubertal goats in G_2 and G_3 (64.29 vs 38.37 and 75.00 vs 46.15). The corresponding values in G_1 in peripubertal and adult goats were 26.74 vs 30.93 per cent. The overall percentage ova and embryo recovery rate in peripubertal and adult goats were respectively 45.51 and 36.48. The average ova and embryo recovery per goat in peripubertal and adult goats in G_1, G_2 and G_3 were respectively 3.29 and 5.00, 4.50 and 5.50 and, 3.50 and 3.00. The overall ova and embryo recovery rate per goat in peripubertal and adult goats were recorded as 3.94 and 4.50 respectively.

In peripubertal goats belonging to G_3 eventhough 42.85 per cent of CL had regressed an embryo recovery rate of 75.00 per cent could be obtained in comparison with the non-treated groups (G_1 plus G_2), which yielded only 39.06 per cent. In adult goats also the embryo recovery rate in G_3 was higher than in G_1 plus G_2 (46.15% vs 34.42%). The fertilized ova recovery in P_4 treated adult and peripubertal goats were 100 per cent and 83.33 per cent respectively when compared to non-treated group (84% vs 71.43%).

The percentage recovery rate of transferable embryos in G_1 in peripubertal and adult goats were respectively 95.65 and 95.00. The corresponding values in G_2 were 89.47 and 92. In G_3 where P_4 support was given peripubertal and adult goats yielded 100 per cent transferable embryos. The overall percentage transferable embryo recovery in peripubertal and adult goats were almost equal (95.23% and 95.00%).

In peripubertal group average number of transferable embryos recovered per goat was highest in G_3 followed by G_1 and then in G_2, while in adult goats G_2 showed maximum average transferable embryo recovery (TER) per goat followed by G_1 and then G_3. The average TER per goat in G_1 and G_2 were lower in peripubertal animals (3.14 vs 3.16 and 2.83 vs 3.83). But in G_3, this value was

high in peripubertal goats (3.50 vs 2.50). The overall transferable embryo recovery per animal in peripubertal and adult goats was almost equal (3.15 vs 3.17).

The percentage recovery rate of good quality embryos in G_1 in peripubertal and adult goats were 68.18 and 63.15 respectively. The corresponding values in G_2 and G_3 were 88.23 and 34.78 and 71.43 and 86.67 per cent respectively. With an overall value of 75.00 and 57.89 in these age groups respectively. Average good quality embryo recovery per goat in G_1 and G_2 were higher in peripubertal goats when compared to adult goats (2.14 vs 2.00 and 2.50 vs 1.33). While in G_3 the average good embryo recovery was higher in peripubertal goats (2.50 vs 2.16). Overall number of good embryos recovered per animal was high in peripubertal goats when compared to adult goats (2.37 vs 1.83).

The percentage recovery rate of fair quality embryos in G_1 was almost equal in peripubertal and adult goats (31.81 vs 36.84). In G_2 the corresponding values were 11.76 and 65.02 while in G_3 it was 28.59 and 13.33 respectively. The overall percentage recovery rate of fair quality embryos in peripubertal and adult goats were 25.00 and 42.10 respectively. The average number of fair quality embryos recovered per goat in G_1 and G_2 were lower in peripubertal goats (1.16 vs 1.17 and 0.33 vs 2.50), while in G_3 a higher value was recorded (1.00 vs 0.33) with an overall value of 0.78 and 1.33 in peripubertal and adult goats respectively.

The percentage of non-transferable embryos in peripubertal and adult goats in G_1, G_2 and G_3 were 4.35 and 5.00, 10.53 and 8.00 and 00.00 and 0.00 respectively with an overall value of 4.76 per cent and 5.00 per cent. The overall average nontransferable embryo recovery per goat in peripubertal and adult group was 0.15 and 0.17 respectively.

On day 6, all the embryos collected from peripubertal goats were morulae, while in adult goats 96.30 per cent were morulae and the remaining

early blastocysts. Forty four per cent of the embryos collected on day 7 were morulae and the remaining were early blastocysts in peripubertal goats, while in adult goats 86.67 per cent were morulae. Both in peripubertal and adult goats hundred percent of embryos collected on day 8 was blastocysts.

Out of 30 embryos recovered after freezing, 22 (73.33%) showed normal morphology in peripubertal goats, while in adult goats only 21 (70.00%) were found to be morphologically normal. The percentage of good and fair quality embryos from peripubertal and adult goats after freezing and thawing were almost similar, eventhough slightly higher values were recorded in peripubertal goats.

Morphological evaluation of frozen embryos revealed that both in peripubertal and adult animals, blastocysts were more resistant to freezing (81.25% vs 80.00%) than morulae (64.29% vs 60.00%). While 53.84 per cent of blastocysts from peripubertal goats developed after 24 h of culture, only 50.00 per cent from adult goats showed development. Total number of embryos with abnormal morphology was low in peripubertal goats than in adult goats (26.67% vs 30.00%).

Pregnancy rate in animals in which embryos were transferred from peripubertal and adult goats was found to be 66.67 per cent on day 21 based on P_4 assay, which was subsequently reduced to 33.33 per cent on day 28. Ultrasonography on day 60 revealed that 33.33 per cent recipients, which received embryos each from peripubertal and adult goats were pregnant.

The overall mean serum glucose (mg/dl), ACP (KAU/dl) and ALP (KAU/dl) were recorded as 57.73 ± 6.43 and 58.16 ± 6.30, 0.95 ± 0.27 and 0.74 ± 0.20 and, 26.75 ± 11.26 and 20.00 ± 9.42 respectively in peripubertal and adult goats, which did not show any significant difference.

The level of lactic dehydrogenase (U/L) showed a significant difference between groups in peripubertal goats. In peripubertal group the LDH level was lowest in G_1 (289.83 ± 34.11 U/L), which was significantly lower from G_2

(450.00 ± 50.31 U/L) and G_3 (467.00 ± 42.60 U/L). The overall mean value of LDH (U/L) recorded in peripubertal and adult goats were 402.28 ± 52.42 and 485.28 ± 47.17 respectively.

The mean of progesterone level on the day of superovulatory heat in 19 peripubertal and 18 adult goats were recorded as 0.46 ± 0.02 ng/ml and 0.42 ± 0.02 ng/ml respectively. A negative correlation was observed between P_4 level during superovulatory heat and number of FCL, PRCL (significant $P<0.01$ in peripubertal goats), total CL and TER both in peripubertal and adult goats.

The P_4 level in peripubertal goats belonging to G_1 and G_2 ranged from 0.80 to 22.20 ng/ml, while in adult goats the range was 0.80 to 30.50 ng/ml. Both in peripubertal and adult goats in G_1 and G_2 the P_4 level on day of embryo collection was low in animals which exhibited PRCL (range = 0.80 to 1.10 ng/ml and 0.80 to 1.20 ng/ml) while in animals with FCL P_4 levels ranged from 3.50 to 22.20 and 18.50 to 29.80 in peripubertal and adult goats respectively. Progesterone level in animals belonging to G_3 was more than 30.70 ng/ml in both these age groups due to administration of exogenous P_4. A significant positive correlation was observed between P_4 profile on day of embryo collection and number of FCL and TER both in peripubertal and adult goats. In contrast, a significant negative correlation was observed between number of PRCL and P_4 level on day of embryo collection both in peripubertal and adult goats. None of the animals with PRCL yielded embryos.

Eventhough the overall superovulatory response was significantly higher ($P<0.05$) in adult goats, the total and transferable embryo recovery rates were almost equal in peripubertal and adult goats, consequent to high incidence of premature regression of corpus luteum. The embryo recovery rates in animals with PRCL could be substantially increased by administration of P_4. Progesterone assay on day of embryo collection can be used as a tool for assessing the functional status of CL and predicting the embryo recovery rates. The developmental stages of embryos did not show much variation between these age groups. The study further showed that the embryos from peripubertal goats

were equally suitable for freezing, *in vitro* culture and transfer as in adult goats. The pregnancy rate on transfer of frozen embryos from both the age groups was found to be equal (33.33%). It can be concluded that the peripubertal Malabari goats are as suitable as adult goats for MOET programme.

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COMPARATIVE STUDY ON SUPEROVULATORY RESPONSE AND VIABILITY OF EMBRYOS IN PERIPUBERTAL AND ADULT MALABARI GOATS

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ABSTRACT OF A THESIS

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ABSTRACT

The objective of the study was to compare the superovulatory response and viability of embryos in peripubertal and adult Malabari goats.

Nineteen peripubertal and eighteen adult Malabari goats were selected and utilised for the study. Animals of each age group were at random allotted to sub groups G_1, G_2 and G_3 consisting of six animals, except G_1 of peripubertal goats, which contained seven. The animals in each age group were subjected to three superovulation protocols i.e., oFSH @ 0.9 units/kg body weight (G_1), oFSH @ 0.45 units/kg body weight (G_2) and pFSH @ 2 mg/kg body weight (G_3) as six injections after synchronising the oestrus using 1.5 mg norgestomet ear implant and $PGF_2\alpha$ (G_1 and G_2) and norgestomet ear implant alone (G_3). On exhibition of heat signs animals were bred or inseminated and hCG was administered @ 500 IU and 750 IU in peripubertal and adult goats respectively. The serum glucose, LDH, ACP, ALP, and P_4 were estimated. Peripubertal and adult goats belonging to G_3 were supported with P_4 injection @ 20 mg and 25 mg respectively twice daily from day four to day of embryo collection.

The embryos were collected six to eight days after breeding using uterine flushing technique. Thirty two good quality embryos (16 morulae and 16 blastocysts) each from peripubertul and adult goats were frozen by slow freezing technique using EG as cryoprotectant. The quality of frozen thawed embryos was assessed morphologically and transferable frozen thawed blastocysts from each age group were subjected to *in vitro* culture. A total of nine transferable frozen thawed morulae each from peripubertal and adult goats were transferred into the uterus of three synchronous recipients and P_4 levels at weekly intervals were assessed up to day 28.

The overall response to synchronisation (percentage) was almost same in peripubertal and adult goats (94.74 vs 94.45). The overall mean interval from

end of synchronisation to heat and duration of heat did not show any significant difference between these age groups. The overall mean intensity of heat (score) was slightly more in peripubertal goats (3.44 ± 0.15 vs 3.18 ± 0.21).

A decreasing trend was observed in the average number of anovulatory follicles and in superovulatory response from G_1 to G_3 both in peripubertal and adult goats. While a significant difference ($P<0.05$) in SOR was observed between G_1 and G_3 in peripubertal goats, no such variation could be noted between different groups in adult goats. The overall average superovulatory response (8.16 ± 0.84 and 12.30 ± 1.40) and SOR in G_2 (6.96 ± 1.45 and 14.30 ± 2.60) showed a significant difference ($P<0.05$) between peripubertal and adult goats. As a result of high incidence of PRCL (50% vs 52%) the embryo recovery rate was low from both the age groups.

The overall percentage ova and embryo recovery and fertilized ova recovery rates were higher in peripubertal goats (45.51 vs 36.48 and 88.73 vs 74.07), while overall average values of these parameters were found to be almost equal in both these age groups (3.94 vs 4.50 and 3.32 vs 3.33). In peripubertal and adult goats in G_3 which were supported with exogenous progesterone, the percentage embryo recovery rate was 75 and 46.15 respectively as against 39.06 and 34.42 in nontreated groups. The overall percentage (average) transferable embryo recovery in peripubertal and adult goats were 95.23 and 95 (3.15 and 3.17) respectively.

The developmental stages of embryos collected from peripubertal and adult goats were almost similar on sixth day (100% morulae vs. 96.30% morulae and 3.70% blastocysts) and eighth day (all blastocysts). But on seventh day more advanced stages (morulae and blastocysts) could be recovered from peripubertal goats than adult animals.

Out of 30 embryos recovered after freezing, 73.33 per cent were of transferable quality in peripubertal goats, while, it was 70 per cent in adult goats.

Both in peripubertal and adult goats, blastocysts were more resistant to freezing than morulae (64.29% Vs 81.25% and 60 % Vs 80%). On *in vitro* culture of the frozen blastocysts from peripubertal goats 53.84 per cent showed development while in adult goats it was only 50 per cent. Pregnancy rate in recipient goats on transfer of frozen morulae from peripubertal and adult goats were found to be equal (66.67%) on day 21 while it was 33.33 per cent on day 28 as diagnosed by progesterone assay.

The overall values of serum glucose, LDH, ACP and ALP did not show any significant difference between peripubertal and adult goats on the day of superovulatory heat. The mean serum progesterone (ng/ml) on day of superovulatory heat in peripubertal and adult goats were 0.38 ± 0.03 and 0.32 ± 0.05 respectively. A negative correlation was observed between P_4 level during superovulatory heat and number of FCL, PRCL, total superovulatory response (significant in peripubertal goats ($P<0.01$)) and transferable embryo recovery.

The serum P_4 level (ng/ml) on day of embryo collection was low in animals with PRCL and it ranged from 0.8 to 22.20 and 0.8 to 30.50 in peripubertal and adult goats respectively. In P_4 supported animals the level was >30.70 ng/ml.

A significant positive correlation ($P<0.01$) was observed between P4 level on day of embryo collection and number of FCL and transferable embryo recovery, both in peripubertal and adult goats. While P_4 profile on day of embryo collection and number of PRCL showed a significant negative correlation in both these age groups ($P<0.01$ vs $P<0.05$). In pregnant recipient goats the progesterone level on day 21 and 28 was higher compared to non-pregnant animals.

The study revealed that peripubertal Malabari goats are as suitable as adult goats for MOET programme.