RPF – II

(PROFORMA FOR SUBMISSION OF ANNUAL PROGRESS REPORT OF RESEARCH PROJECT)

PART- I : General Information.

600 Project Code No. :
6001 Institute Project Code No. **: XI / PRSM-1.2**
6002 ICAR Project Code No. :
601 Name of the Institute and Division
6011 Name and Address of Institute : CIRG. Makhdoom , P.O. Farah-281122 Dist: Mathura, UP India

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6012 Name of Division/Section : Physiology, Reproduction and Shelter Management Division

6013 Location of the Project : PR&SM Division, CIRG. Makhdoom, Farah (Mathura) U.P.
602 **Project Title** : **Improvement in Productivity of Goat through Reproductive Biotechnologies including refinement of Frozen Semen, Strengthening of Semen Bank & Augmentation of Prolificacy**

Sub Project ***AUGMENTATION OF PROLIFICACY BY USING BIOTECHNOLOGICAL TOOLS IN GOATS***

603 Priority Area AUGMENTATION OF REPRODUCTION
6031 Research Approach : **Applied Research**/ Basic Research./Tech./ Transfer / Develop. of technology
01 02 03 04

604 Specific Area : **REPRODUCTIVE BIOTECHNOLOGY**

605 Duration of Project: Five years

6051 Date of Start of Project :April, 2007

6052 Likely date for completion of Project : Marche, 2012

6053 Period for which report submitted : **April, 2007 to March, 2008**

606 Total cost of the project : **Rs. 81.75 lakhs**

6061 Expenditure to date : **Rs.1166500.00**

607 Summary of Achievements:

The reproductive status of thirty adult cyclic Jamunapari goats were observed. The estrus detection was done daily in the morning and evening by using aproned buck. Twenty four goats were bred naturally. Out of which nineteen goats (79.16%) were kidded. Eleven goats produced single kid and eight goat produced twins kid. After kidding the goats were observed for the onset of estrus It was observed that only 26.31% goats were came in to estrus within 6 months of kiddig, indicating long postpartum interval in Jamunapari goats.

Sponges of different sizes and shapes were prepared in the laboratory. The types of sponges were square, circular and cylindrical shapes with a diameter of 25 and 30 mm. These sponges were tied with one feet long thread. They were inserted in to the vagina of Sirohi goat and tested for their retention in to vagina for 12 days. The highest percentage (87.5%) of retention was observed in cylindrical shapes of 25 mm diameter sponge.

In order to prepare progesterone impregnated sponges, it was dissolve by using variety of solvents viz. Acetone, ethanol, DMSO, Gum acacia and Twin 20 etc.

a). Two ml of acetone or DMSO dissolved 300mg of progesterone within five minutes at room temperature.

b) Three ml of ethanol was required for completely dissolving 300mg of progesterone in a overnight period.

c) Steroids are water insoluble and therefore to improve their water solubility, gum acacia and twin 20 were added at the 25% and 50% level in water containing progesterone. However they were unable to dissolve the progesterone in water.

Fifty three sponges impregnated with different doses of progesterone were prepared. The sponges were inserted in to the vagina of Sirohi goats up to the external os of the cervix by using glass speculum on day 17 of the estrous cycle. The sponges were kept for 12 days in to the vagina to prolong the estrous cycle. The goats were observed for estrus daily in the morning and evening using teaser buck. The percentage of goats responded to sponge for prolongation of estrous cycle was 40.00, 71.42, 75.00, 66.66, 55.44, 80.00 and 100% for the sponge containing 25, 50, 75, 100, 200, 300 and 350 mg of progesterone, respectively.

The progesterone was estimated in the serum of nine Sirohi goats inserted with sponge using commercially availably ELISA kit (Omega Diagnostics Ltd. U.K.). The absorbance of standard and test samples were taken at 450 nm with a micro titre well reader within 10 minutes. The serum progesterone was maintained more effectively by sponge containing 350 mg of natural progesterone than 300 mg of natural progesterone.

Twenty estrus does were scanned by employing curvilinear trans-rectal (TR) scanners of variable frequency ranging between 3 to 7. The scanning surface of probe was rotated ventrally and laterally and advanced slowly for locating ovary. The uterus was located in all animals but ovaries could be located only in 50% cases.

The average recovery of oocytes using follicle puncture was 1.82 per ovary. The maturation rate of goat oocytes on morphological evaluation was 85.36%. In vitro produced and in vivo produced embryos were transfer surgically in to two and one recipient, respectively.

The maturation rate of nude goat oocytes on granulosa cell co-culture and its monolayer were 32.40% and 31.77%, respectively. Its maturation rate on supplementation of hormone (FSH, LH and Estradiol) and hormone with insulin were 26.00% and 38.09%, respectively.

Crestar ear implant along with 500 IU PMSG resulted in estrus synchronization in 75% goats during low breeding season.

608 Key words: Goat, Prolificacy, Hormones, Immunization, Post-partum interval, Embryo Transfer.

PART - II : Investigators Profile

(Please identify clearly changes, if any in project personnel)

610 Principal Investigator

6101 Name: Dr. S.D. Kharche
6102 Designation: Senior Scientist (Animal Reproduction)
6103 Division/ Section : PR&SM Division
6104 Location : CIRG, Makhdoom Farah
6105 Institute Address : CIRG, Makhdoom, P.O. Farah - 281 122, Mathura (UP)

611 Co-investigator

6111 Name: Dr. A.K. Goel
6112 Designation : Senior Scientist (Animal Reproduction)
6113 Division/Section : PR&SM Division
6114 Location : CIRG, Makhdoom Farah
6115 Institute Address : CIRG, Makhdoom, P.O. Farah - 281 122 , Mathura (UP)

612 Co-investigator

6121 Name: Dr. S.K. Jindal
6122 Designation : Principal Scientist (Animal Physiology)
6123 Division/Section : PR&SM Division
6124 Location : CIRG, Makhdoom Farah
6125 Institute Address : CIRG, Makhdoom, P.O. Farah - 281 122 , Mathura (UP)

PART- III : Technical Details

620. Introduction and Objectives :

Goat as a ruminant species on earth has sustained by virtue of its relatively high reproductive efficiency in all environments. To achieve this goat of superior reproduction, goats have readily adopted, acquired and resorted to rather limited seasonality, early sexual maturity, vigorous sex drive, relatively high ovulation rate (reflected in the form of multiple births) and less reproductive problems. India is naturally gifted with a vast genetically diverse goat germ plasm. Twenty recognized goat breeds are adapted to diverse agro - climatic regions of the country. Reproductive potential of Indian goats is variable. Over the years, vast assays of reproductive biotechnologies are available which have resulted in augmentation of reproductive efficiency in farm animals including goats. The future trends in small ruminant production are expected to be dominated by emerging bio techniques of reproduction. To bridge the gap between the demand and production (milk, meat and fiber), it is mandatory to optimize production of prolific breeds and to augment the reproduction rate of less prolific breeds. These technologies relate to more frequent kidding, increases in litter size in less prolific breeds, extending mating outside the normal breeding seasons, Embryo Transfer and MOET, IVF-ET and associated techniques, early pregnancy determination, application of real time B-mode ultrasonography in animal reproduction and control of kiddings aiming to fertility augmentation.

6201 Immediate objectives:

1). To shorten the post-partum and kidding interval for more frequent kiddings in goats.

2). To study the effect of hormones on prolificacy of less prolific goat breeds.

3). To study the effect of immunization on prolificacy of less prolific goat breeds.

4). To study the frequency of multiple birth through in vitro and in vivo embryo production and transfer.

6202 Long term objectives

To develop a cost effective protocol / package of practices for reducing kidding interval and enhancing prolificacy in less prolific breeds of goat.

6203 Specific objectives for the year as detailed in RPF-I

- Standardization and preparation of hormone delivery system (sponges and injections).
- Testing of hormone delivery system (sponges and injections).
- Standardization of technique for visualization of follicles / corpus luteum using ultrasonograpy in treated goats.
- Estimation of progesterone / estradiol in blood.
- In vitro embryo production.
- Surgical transfer of in vitro produced embryos to study frequency of multiple births.

621 Project Technical Profile

621 Technical programme

(Indicate briefly plan of procedure, techniques, instruments and special materials, organisms, special environment etc.)

Keeping in view the objectives laid down in this project, technical programme was formulated in different phases detailed as under:

1. Selection of Sirohi (50) and Jamunapari (50) female goats from existing flock of the division.
2. Selection of Sirohi and Jamunapari bucks (5 each) from existing flock of the division.
3. Procurement of hormones, chemicals, drugs and miscellaneous laboratory items/equipments.
4. Standardization and preparation of hormone delivery system (sponges and injections).
5. Testing of hormone delivery system (sponges and injections)
6. Estimation of progesterone / estradiol in blood.
7. Standardization of technique for visualization of follicles / corpus luteum using ultrasonograpy in treated goats.
8. In vitro embryo production.
9. Surgical transfer of in vitro produced embryos to study frequency of multiple births.

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6212 Man-months involvement of component project workers for the specified year.

Scientific	:	15
Technical	:	12
Supporting	:	24

622 Progress of work

6221 Achievement in terms of targets fixed for each Activity

a) Procurement of essential inputs (Dr S.D. Kharche & Dr A.K. Goel):

i) Chemicals glassware, plasticware and miscellaneous items (except cell culture tested chemicals & culture media) were purchased.

b) Scientific achievements:

1. Preparation of sponges of different sizes and shapes to study their retention (Dr S.D. Kharche)

Sponges of different sizes and shapes were prepared in the laboratory. The types of sponges were square, circular and cylindrical shapes with a diameter of 25 and 30 mm. These sponges were tied with one feet long thread. They were inserted in to the vagina of Sirohi goat and tested for their retention in to vagina for 12 days.

Table. 1 Effect of size and shapes of sponges on its retention in to vagina of goats.

S. No.	Type of sponge	Total goats	Sponge retained	Sponge lost	Sponge retained (%)
1.	Square (25mm)	7	4	3	57.14
2.	Circular (25mm)	6	4	2	66.66
3.	Circular (30mm)	6	5	1	83.33
4.	Cylindrical (25mm)	8	7	1	87.5
5.	Cylindrical (13mm)	7	4	3	57.14

2. Study on solubility of natural progesterone using different solvents(Dr S.D. Kharche):

In order to prepare progesterone impregnated sponges, it was dissolve by using variety of solvents viz. Acetone, ethanol, DMSO, Gum acacia and Twin 20 etc.

a). Two ml of acetone or DMSO dissolved 300mg of progesterone within five minutes at room temperature.

b) Three ml of ethanol was required for completely dissolving 300mg of progesterone in a overnight period.

c) Steroids are water insoluble and therefore to improve their water solubility gum acacia and twin 20 were added at the 25% and 50% level in water containing progesterone. However they were unable to dissolve the progesterone in water.

3. Preparation and testing of hormone delivery system (Dr S.D. Kharche & Dr A.K. Goel):

Fifty three sponges impregnated with different doses of progesterone were prepared. The required quantity of progesterone was weighed in to a glass vial and dissolved in ethanol and kept for overnight. The dissolved progesterone then taken in to a 5 ml syringe and impregnated in to sponge by pricking the needle in to the sponge. The sponges were inserted in to the vagina of sirohi goats up to the external os of the cervix by using glass speculum on day 17 of the estrous cycle. The sponges were kept for 12 days in to the vagina to prolong the estrous cycle. The goats were observed for estrus daily in the morning and evening using teaser buck.

Table 2 Effect of different doses of progesterone impregnated sponges on prolongation of estrous cycle in goats.

S. No.	Progesterone (mg)	No. of animals treated (n)	Prolonged estrous cycle (%)
1.	25	5	40.00

2.	50	7	71.42
3.	75	4	75.00
4.	100	9	66.66
5.	200	9	55.44
6.	300	10	80.00
7.	350	4	100.00

4. Estimation of progesterone in blood (Dr S.D. Kharche & Dr S.K. Jindal):

The blood samples of nine Sirohi goats were collected at different interval before and after sponge insertation to test the hormone delivery through sponge in to the blood serum of goats. The samples were kept overnight in slanting position and serum was taken from these samples. The serum was centrifuge at 5000 rpm and stored in a 5 ml vial at -40^{o}C till analysis. The progesterone was estimated in the serum using commercially availably ELISA kit (Omega Diagnostics Ltd. U.K.). The absorbance of standard and test samples were taken at 450 nm with a micro titre well reader within 10 minutes.

Table 3. The average serum progesterone (ng/ml) concentration in goats inserted with sponges.

S.No.	P4 Sponge (ng/ml)	Day 17 Before sponge insertion	Day 18 At sponge insertion	Day 20	Day 22	Day 24	Day 26	Day 29 At sponge withdrawal	Day 31
1.	350	4.15 ±1.11	6.0 ±1.28	4.32 ±1.53	1.8 ±0.41	3.32 ±1.45	1.77 ±0.26	2.35 ±0.24	1.87 ±0.20
2.	300	5.3 ±2.5	10.36 ±4.68	4.56 ±2.14	5.76 ±2.88	8.24 ±4.22	5.38 ±2.30	3.75 ±2.02	3.27 ±1.60

5. Reproductive status of Jamunapari Goats (Dr S.D. Kharche):

The reproductive status of thirty adult cyclic Jamunapari goats were observed. The estrus detection was done daily in the morning and evening by using aproned buck. Twenty four goats were bred naturally. Out of which nineteen goats (79.16%) were kidded. Eleven goats produced single kid and eight goat produced twins kid. After kidding the goats were observed for the onset of estrus It was observed that only 26.31% goats were came in to estrus within 6 months of kidding, indicating long postpartum interval in Jamunapari goats.

6. Standardization of technique for visualization of follicles / corpus luteum using ultrasonograpy in goats (Dr S.D. Kharche & Dr A.K. Goel).

Twenty estrus does were scanned by employing curvilinear trans-rectal (TR) scanners of variable frequency ranging between 3 to 7. The does were kept off feed 12 hour prior to scanning. The examinations were carried out in standing posture after proper restraining. After evacuating the rectum with fingers, a small amount of ultrasound gel was infused in to the rectum o get distinct image. After proper restraining of the animal, the gel lubricated scanner was introduced in to the rectum and urinary bladder was located as non-echoic black area. The scanning surface of probe was rotated ventrally and laterally and advanced slowly for locating ovary. The uterus was located in all animals but ovaries could be located only in 50% cases.

7. In vitro embryo production and transfer:

i) Collection of estrus goat serum (Dr S.D. Kharche):

Goats were observed for the occurrence of estrus daily twice at 12 hr intervals in the experimental herd. Estrous serum from these goats were collected 12 hr following the onset of estrus and filtered through 0.22 µm millipore syringe filter. The estrus goat serum was heat inactivated at 56^0C for 30 min. in a water bath, dispensed in to 1 ml and 10 ml aliquots and stored at -20^0C until used.

ii) Oocyte collection (Dr S.D. Kharche & Dr A.K. Goel)

Oocytes were recovered by follicle puncture technique for in vitro maturation, fertilization and culture from goat ovaries collected from slaughter house located at Agra. A total of 1874 goat ovaries were used for recovery of oocytes using follicle puncture technique for IVMFC. The recovery of oocytes using follicle puncture was 1.82 per ovary.

iii) In-vitro maturation of goat oocytes (Dr S.D. Kharche) :

Goat ovaries obtained from a abattoir situated at Agra. Oocytes from 2-6 mm follicle (tertiary) of goat ovaries were collected by puncturing the follicles with a 18 gauge needle and placed in modified phosphate buffered saline containing sodium pyruvate (36 mg/l), glucose (1gm/l), penicillin (60 mg/l) and streptomycin (50 mg/l). The oocytes surrounded in a compact cumulus mass with an evenly granulated cytoplasm were selected under stereozoom microscope.

The oocytes collected from goat ovaries were cultured in tissue culture medium (TCM-199) containing 20% EGS or 10% NCS with FSH, LH, 1µg/ml E_2, and bovine serum albumin, (pH: 7.2-7.4) supplemented with insulin (50ng/ml) or EGF (10ng/ml) in 50 µl drops of maturation medium covered with mineral oil. The cumulus oocyte complexes (COCs) were cultured for 27 h at 38.5 $\pm$ 1^0C, 5% CO_2 in humidified air. The matured oocytes were evaluated under stereo zoom microscope and the maturation rate of oocytes on morphological evaluation was 85.36%.

iv) In-vitro maturation of nude goat oocytes (Dr S.D. Kharche) :

During recovery of oocytes from ovaries by different technique, a large number of nude oocyte will also be collected along with the good quality oocytes. These nude oocytes will be thrown out due to absence of cumulus investment. Utilization of these oocytes will help in increasing the number of in vitro mature oocytes required for in vitro embryo production and various biotechnological experiments.

a). Effect of granulosa cell co-culture and monolayer on IVM of nude oocytes

The selected nude oocytes were assigned at random in following treatment groups:

Group 1. Approximately 115 selected oocytes were matured in maturation medium comprising of TCM-199 (sigma) containing L-glutamine (1mg/10ml), sodium pyruvate (0.25mmol) and Gentamycin (50μg/ml) supplemented with 10% FBS in 50μl droplets covered with sterile mineral oil for 27 h in humidified atmosphere of 5% CO_2 at 38.5 $\pm$ 1^0C in CO_2 incubator.

Group 2. Approximately 108 selected oocytes were matured in maturation media comprising of TCM – 199 (sigma) containing L–glutamine (1mg/10ml), sodium pyruvate (0.25mmol) and Gentamycin (50μg/ml) supplemented with 10% FBS and granulosa cell co-culture in 50μl droplets covered with sterile mineral oil for 27h in humidified atmosphere of 5% CO_2 at 38.5 $\pm$ 1^0C in a CO_2 incubator.

Group 3. Approximately 107 selected oocytes were matured in maturation medium comprising of TCM-199 (sigma) containing L-glutamine (1mg/10ml), sodium pyruvate (0.25mmol) and Gentamycin (50μg/ml) supplemented with 10% FBS and granulosa cell monolayer in 50μl droplet covered with sterile mineral oil for 27 h in humidified atmosphere of 5% CO_2 at 38.5 $\pm$ 1^0C in a CO_2 incubator.

Table 4 : Effect of granulosa cell co-culture and monolayer on IVM of nude goat oocytes.

Groups	Total no. of oocytes (n)	Total no. of oocytes matured	Maturation rate (%)
Gr 1	115	27	23.47
Gr 2	108	35	32.40
Gr 3	107	34	31.77

The values given in a column do not differ significantly (P> 0.05).

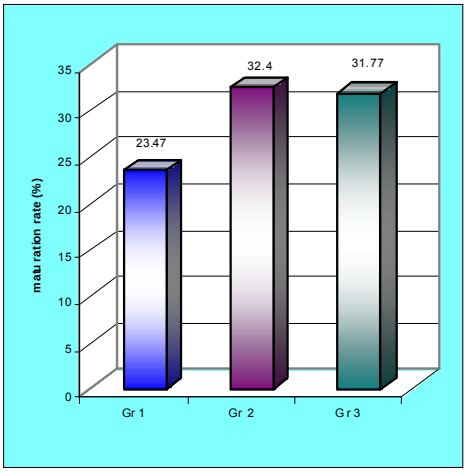

Fig. 1 Effect of granulosa cell co-culture and monolayer on IVM.

b). Effect of hormones on IVM of nude oocytes

Group 1. Approximately 107 selected oocytes were matured in maturation medium comprising of TCM-199 (sigma) containing L-glutamine (1mg/10ml), sodium pyruvate (0.25mmol) and gentamycin (50μg/ml) supplemented with 10% FBS in 50μl droplets covered with sterile mineral oil for 27h in humidified atmosphere of 5% CO_2 at 38.5 $\pm$ 1^0C in a CO_2 incubator.

Group 2. Approximately 100 selected oocytes were matured in maturation media comprising of TCM-199 (sigma) containing L-glutamine (1mg/10ml), sodium pyruvate (0.25mmol) and gentamycin (50μg/ml) supplemented with 10% FBS, FSH (5μg/ml), LH (5μg/ml), estradiol-17β (1μg/ml) in 50μl droplets covered with sterile mineral oil for 27h in humidified atmosphere of 5% CO_2 at 38.5 $\pm$ 1^0C in a CO_2 incubator.

Group 3. Approximately 105 slected oocytes were matured in maturation media comprising of TCM-199 (sigma) containing L-glutamine (1mg/10ml), sodium pyruvate (0.25mmol) and gentamycin (50μg/ml) supplemented with 10% FBS and FSH (5μg/ml), LH (5μg/ml), estradiol-17β (1μg/ml) and insulin (50ng/ml) in 50μl droplets covered with sterile mineral oil for 27h in humidified atmosphere of 5% CO_2 at 38.5 $\pm$ 1^0C in a CO_2 incubator.

After 27h of maturation of oocytes, the oocytes were washed by vortexing. The oocytes were then fixed for 15 minutes in 2.5% (w/v) glutaraldehyde in phosphate buffered saline, washed twice with PBS, stained with 0.1μg/ml 4, 6 – diamidino – 2 phenylindole (DAPI) in PBS and mounted on slides. The evaluation of nuclear status was done by epifluorescence microscopy.

Table 5 : Effect of hormones on in vitro maturation of nude goat oocytes.

Groups	Total no. of oocytes (n)	Total no. of oocytes matured	Maturation rate (%)
Gr 1	107	26	24.29
Gr 2	100	26	26.00
Gr 3	105	40	38.09

The values given in a column do not differ significantly (P> 0.05).

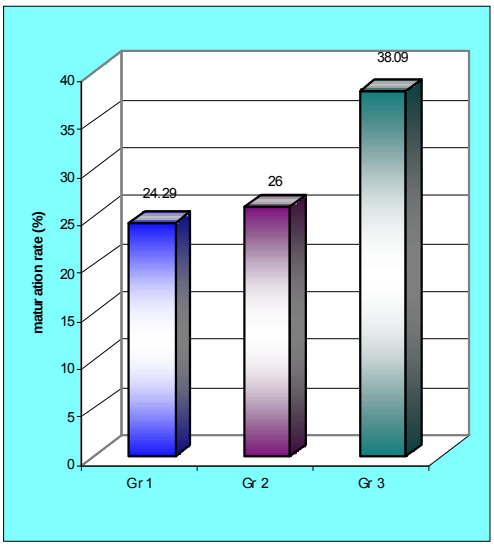

Fig. 2 Effect of hormones on in vitro maturation of goat oocytes.

v) Preparation of granulose cell monolayers (Dr S.D. Kharche)

Ovaries were tirimmed and washed several times in warm sterile NSS and DPBS. They were then exposed to 70% ethyl alcohol for 20 seconds and finally washed in oocytes collection medium (OCM).

Follicular contents were aspirated from large (>4mm diameter) follicle by a syringe and 22 guage needle from the apparently non atretic surface follicles. Aspirated cells were pooled in centrifuge tubes, mixed with OCM and centrifuged at 500 rpm for 10 minutes. Supernatant was discarded and cells were reconstituted with fresh OCM and again centrifuged at the same rate. The process was repeated for 3-4 times. These washings were essential to get rid of bacterial load.

Finally the cells were reconstituted in OHM with 10% EGS. A small sample of this was used for estimation of concentration of granulosa cells. Cell number was counted with the help of hemocytometer and concentration was adjusted around 1-2 x 10^6 live cells/ml. Seeding was done @ 0.5 ml of cell suspension per well of a 24 well culture plate, and were cultured upto confluence (5-6 days) to established monolayers at 38.5±1^0 C and 5% CO_2 in humidified air. Half of culture medium was replaced with fresh medium every 48h.

vi) Preparation of oviductal epithelial cells (OECs) (Dr S.D. Kharche)

The oviducts epsilateral to ovary having corpus haemorhgium were collected from abattoir and were dissected free from adjacent connective tissue. The oviducts were washed 5 to 8 times with normal saline containing 100IU/ml penicillin-G and 100µg/ml streptomycin and finally washed with DPBS. The oviducts were then squeezed from isthumus towards fimbriated end in a petridish with a pair of forceps and the whitish thick fluid oozed out of the opening of the oviducts was mixed with OCM containing 10mg/ml Gentamicin in a centrifuge tube. Extruded cells clumps were aspirated and expelled several times through a 26 guage needle attached to a

syringe to break up the sheets of cells into smaller clumps and then left for 1 h. Then the oviductal epithelial cells (OEC) were washed three times by centrifugation at 200 rpm for 10 minutes. The oviductal epithelial cells then washed 8-10 times by drop washing in OHM. The cells were cultured for 48 hr in 24 well plates in TCM-199 containing 10% EGS covered with mineral oil.

The media was refreshed at every 48 h by removing half of their medium and replacing the same volume of fresh equilibrated medium.

vii) In-vitro fertilization of in vitro matured goat oocytes (Dr S.D. Kharche) :

The matured oocytes were separated from cumulus cells by treating them with PBS containing 0.1% hyaluronidase and by passing through a fine pipette and kept for fertilization in 100ul fertilization drop.

Fresh semen samples were obtained by an artificial vagina from a fertile purebred Sirohi bucks. The capacitation medium for spermatozoa consisted of TALP medium supplemented with heparin, BSA or 10% or 20% EGS and antibiotics. First and second ejaculates were virtually examined for volume, colour, consistency and gross motility, then 50 ul of neat semen was diluted with 5 ml of capacitation medium and wash by centrifugation at 1800 rpm for 5 min. The supernatant was discarded and the pellet again washed with 5 ml of medium and the supernatant was discarded. The pellet was diluted with 5 ml of medium and kept for incubation at 38.5^0C in a CO_2 incubator for 30 minutes. After incubation sperm suspension was centrifuge and 50 µl of sperm pellet was diluted with 750 µl of fertilization medium. Fertilization drop containing oocytes were inseminated with 25 to 50 µl of final diluted semen ($1x10^6$ sperm / ml). The oocytes were washed after 18-24 hr of co-incubation with spermatozoa at 38.5^0C in an atmosphere of 5% CO_2 in humidified air.

viii) In-vitro culture of In-vitro fertilized goat oocytes (Dr S.D. Kharche & Dr S.K. Jindal) :

Following 18-24 hr of co-incubation with spermatozoa at 38.5^0C in an atmosphere of 5% CO_2 in humidified air, oocytes were washed in culture medium (EDM) and co culture with OEC or culture on the GCM for further development of in vitro fertilized oocytes. The oocytes were evaluated at 48-72 hour after insemination for cleavage rate and embryo development.

ix) In vitro produced embryo transfer (Dr S.D. Kharche, Dr A.K. Goel & Dr S.K. Jindal) :

Twelve in vitro produced embryos of 4-16 cell stage were transferred in to two natural synchronized recipients on day 2 or 3 post oestrous surgically at tip of the uterine horn of the genital organ. The recipient was monitored for the oestrus / pregnancy. Following transfer, pregnancy was detected by using ultrasound scanner at 8 weeks. These goats could not sustained pregnancy.

x) In vivo produced embryo transfer (Dr S.D. Kharche, Dr A.K. Goel & Dr S.K. Jindal):

Eight goats having good health were selected from a flock. They were maintained on semi intensive system of management. Each goat was inserted with

half Crestar ear implant (1.5 mg Norgestomet) s/c for 9 days. Simultaneously an injection of 1.5 mg Norgestomet and 2.5 mg estradiol valerate was administered i/m in each goat at the time of implant insertion and an injection of 7.5 mg of Dinoprost tromethamine (Lutalyse, Upjohn, USA) i/m on the day of implant removal. These goats were divided into donor and recipient groups. The recipient goats were given 500IU PMSG on the day of implant removal. The donor goats were injected with 400 IU of PMSG in the morning on day 7 and 8 mg of FSH-P in descending dose schedule (2mg, 2mg, 2mg, 1mg, 1mg) in the evening of day 7, morning and evening on day 8 and 9 of the implant insertion. injected with 400 IU of PMSG in the morning on day 7 and 8 mg of FSH-P in descending dose schedule (2mg, 2mg, 2mg, 1mg, 1mg) in the evening of day 7 , morning and evening on day 8 and 9 of the implant insertion. The percentage of goats responded to oestrus synchronization treatment in recipient and donor groups were 75.00% and 100%, respectively. The onset and duration of oestrus in recipient and donor were 32.00$\pm$10.58/36.0$\pm$12.00 and 28.00$\pm$10.58/32.0$\pm$4.0 hrs, respectively. The average number of follicles and corpus luteum in donors were 7.0$\pm$2.51 and 4.66$\pm$2.60, respectively Three embryos were recovered. Two embryos were transfer surgically at the tip of one uterine horn and remaining one was transfer into another horn of a synchronized recipient goat. The results indicated that

a) Crestar ear implant can be used effectively for oestrus synchronization in donor and recipient goats during non-breeding season.
b) The recipient can not sustained pregnancy and repeated after two months..

6222 Questions- Answered : Not applicable at this stage

6223 Process/Product/Technology developed during the year: Nil

6224 Utility of results obtained so far: Will be known later on with the progress of the project.

623 Publications and Material Development :

6231 Research Papers and Abstracts:

a). **Kharche, S.D**., Goel, A.K., Jindal, S. K. and Sinha, N.K. 2007. Effect of different serum supplementation in fertilization media on cleavage rate of in vitro matured goat oocytes. In XXII Annual convention and National symposium on Challenges in improving reproductive efficiency of farm and pet animals at the Department of Gynaecology, College of Veterinary Science and A.H, Orissa University of Agriculture and Technology, Bhubaneswar from 7th to 9th December 2007.

6232 Popular articles :

1. **Goel, AK** and Kharche SD. 2008. Aasay Hooga Vikas-Bakarion Kaa. **Kheti (ICAR**, **Hindi)** 60(10): 25 -28 (January 2008).
2. **Goel A K,** Kharche SD, Jindal SK and Singh NP. 2007. Diagnosis of Early Pregnancy in Goats. **ICAR News - Promising Technology 13(1):2**(January-March 2007).

3. Kharche SD and **Goel AK.** 2007. Stem Cell Technology - Future prospects. **Goat News** 1(1):3.
4. **Goel AK,** Kharche SD and Jindal SK. 2007. Techniques of Early Pregnancy Diagnosis in Goats by Sonographic Imaging. **Goat News** 1(1): 3 - 4.
5. **Goel AK** and Kharche SD. 2007. Striking Differences in Reproductive Characteristics in Goat and Sheep. **Goat News** 1(1): 6.

6233 Lead paper / Lectures :

1. **Kharche, S. D.** and Goel, A. K. 2008. Techniques of Artificial Insemination in goats. In Training manual on Semen and Embryo transfer in goats. Eds. N.P. Singh and S.K. Jindal. Pp 119-112.
2. Goel, A. K. and **Kharche, S. D.** 2008. Advances in Female Reproduction in Goats – An Overview. In Training manual on Semen and Embryo transfer in goats. Eds. N.P. Singh and S.K. Jindal. Pp. 122-139.
3. Goel, A. K. and **Kharche, S. D.** 2008. Selection and Management of Embryo Transfer Donors and Recipients. In Training manual on Semen and Embryo transfer in goats. Eds. N.P. Singh and S.K. Jindal. Pp. 145-147.
4. Goel, A. K. and **Kharche, S. D.** 2008. Quality Control Measures in Embryo Technology and Artificial Insemination Programmes. In Training manual on Semen and Embryo transfer in goats. Eds. N.P. Singh and S.K. Jindal. Pp. 148-150.
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11. **Kharche, S.D.** 2008. Recent advances in cryopreservation of embryo and ova in farm animals. In National training programme on Assisted Reproductive and Management Technologies for Genetic improvement of livestock from 25-3-2008 to 14-4-2008 at CAS, animal Genetic breeding NDRI, Karnal, p-118-124.

12. **Kharche, S.D**. 2007. Bakari ka jivan chakra. In National training on commercial goat farming from 12-11-07 to 21-11-07 at CIRG, Makhdoom.
13. **Kharche, S.D.** 2007. Bakari utpadan badane me janan sambandhit jankaria. National training on commercial goat farming from 12-11-07 to 21-11-07 at CIRG, Makhdoom.
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6233 Books ;

6234 Reports :

1. Two half yearly report
2. Four quarterly report
3. Annual report-One

6234 Seminars and workshops (relevant to the project) in which the scientists have participated:

1. XXII Annual convention and National symposium on Challenges in improving reproductive efficiency of farm and pet animals at the Department of Gynaecology, College of Veterinary Science and A.H, Orissa University of Agriculture and Technology, Bhubaneswar from 7th to 9th December 2007.
2. National Goat Fair & scientists-Enterpreneurs-Farmers Interective Meet on Opportunities for commercial goat farming in India, C. I. R. G. Makhdoom, Farah - 281122 (Mathura) U. P., March 1 -3rd ,2008.
3. Attended 7th training programme on '**Developing Winning Research Proposals**" from April, 20-25, 2007 at NAARM, Hyderabad, India..

625 Infrastructural facilities developed: ----------

PART-IV. Project Expenditure.
(Summary)
Year: April, 2004 to March 2005

630 Recurring Expenditure

6301 Salaries (Designation with pay scale)

Designation	Pay scale(Rs.)	Man-months	Amount (Rs.)
Scientific			
Principal Scientist (2)	16400-22800	9	440000.00
Sr. Scientist (1)	12000-18300	6	240000.00
Scientist	--	--	--
Technical			
T.-5 (1)	6500-10500	12	180000.00
Supporting (2)	2550- 3200	24	168000.00
Sub Total			**Rs. 1028000.00**

6302 Consumable (Rs)

(i) Chemicals and hormones:	Rs. 98000.00
(ii) Glasswares and plasticwares	Rs. 28000.00
(iii) Others	----------
Sub Total	**Rs. 126000.00**

6303 Travel	Rs. 10000.00
6304 Miscellaneous	Rs. 2500.00
Sub total	**Rs. 12500.00**
630 Sub total (Recurring)	**Rs. 1166500.00**
631 Non recurring expenditure : (Equipments)	—
632 Total (630 and 631)	**Rs. 1166500.00**

Signature of Project Investigator :

Dr. S.D. Kharche, Sr. Scientist

Co-investigators: 1. Dr. A.K. Goel, Sr. Scientist

2 Dr. S.K. Jindal, P.S.

Signature & Comments of the Head
of the Division/Section

Signature & Comments of the
Joint Director (Research)

Signature & Comments of the
Director.

TECHNICAL PROGRAMME FOR 2006 :

- Continuation of work on collection of ovaries from slaughter house, oocyte recovery from ovaries, evaluation and selection for *in-vitro* maturation.
- *In-vitro* maturation of oocytes under different culture conditions.
- Semen collection, evaluation, preparation of spermatozoa and their capacitation in different media.
- *In-vitro* fertilization of oocytes with fresh, frozen or epididymal spermatozoa.
- *In-vitro* culture of embryos.
- Development of embryos from 2 to 32 cell stage.
- Selection and preparation of recipients, synchronization of oestrus and transfer of *in-vitro* produced embryos in closely synchronized recipients.
- Cryopreservation of *in-vitro* produced embryos.
- Transfer of fresh and frozen embryos in closely synchronized recipients.
- Pregnancy determination in embryo transfer recipients.
- Compilation of research findings.
- Publications of monographs, bulletins and research papers.
- Final report writing.