RPF-II

(PROFORMA FOR SUBMISSION OF ANNUAL PROGRESS REPORT OF RESEARCH PROJECTS.)

PART -I General Information.

600 Project Code

6001 Institute Project Code No. : PRSM **XI.1.1**

6002 ICAR Project Code No. :

601 Name of the Institute and Division:

6011 Name and Address of Institute: CIRG. Makhdoom , P.O Farah-281122, Mathura, U.P.

6012 Name of Division/Section : PR&SM Division

6013 Location of the Project : CIRG. Makhdoom

602 Project Title: **STUDIES ON REFINEMENT OF FROZEN SEMEN TECHNOLOGY AND STRENGTHENING OF GOAT SEMEN BANK.**

603 Priority Area: Augmentation of Reproduction in goats

6031 Research Approach: Applied Research/Basic Res./Process or Tech.Devel./Transfer of Tech.

01	02 √	03	04

604 Specific Area : Reproductive Biotechnology

605 **Duration of Project: 5 Years**

6051 Date of Start of Project: April 2007

6052 Likely date for completion of Project: March 2012

6053 Period for which report submitted: 2009-2010

606 **Total cost of the project: 126.92 lacs**

6061 Expenditure to date: lakhs

607 **Summary Achievements.**

- An experiment was conducted to test the effect of antioxidant fortification on the motility of buck spermatozoa at Glutathione(7mM),Ascorbic acid (9mM)and Vitamin E(4.5mM) concentration respectively. Semen was assayed for motility, HOST, live & dead and acrosome integrity before and after freezing. It was found

that reduced Glutathione at 9mM showed 39.72±0.321 post thaw motility which was the highest among the three antioxidants. Addition of antioxidants had a beneficial effect on the cryopreservation of buck spermatozoa.

- An experiment was carried out to investigate the cryo-protective potential of combination of different cryo-protectants [Glycerol (G), DMSO(D) and propylene glycol(P)] in the laboratory at G+D concentration 3:5, for G+P Concentration 4:2 and for P+D concentration 1:4 respectively. Semen was assayed for motility, HOST, live & dead and acrosome integrity before and after freezing. The combination of glycerol and propylene glycol was the best with post thaw motility of 34.79±0.67 as compared to other combinations.
- Dextran was proved to be a poor cryo protectant as compared to glycerol for the cryo preservation of goat spermatozoa.
- A workshop was organized at CIRG Makhdoom, Farah (Mathura) UP on 13.4.2009 on Ovine and Caprine AI and ET in collaboration with IMV India Pvt. Limited

608 Key words : Goat semen, cryobiology , frozen semen.

PART -II Investigators Profile

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

610 Principal Investigator:

6101 Name	**: *Dr. S.K. Jindal***
6102 Designation	: Principal Scientist (Animal Physiology)
6103 Division/Section	: PR& SM Division
6104 Location	: CIRG, Makhdoom
6105 Institute Address	: CIRG, Makhdoom P.O.Farah-281122,Mathura (U.P.)

611 Co –Investigator

612 Co -Investigator

6121 Name	: **Dr. A.K.Goel**
6122 Designation	: Principal Scientist (Animal Reproduction)
6123 Division/Section	: PR&SM Division
6124 Location	: CIRG, Makhdoom, -Farah, Mathura (U.P.).
6125 Institute Address	: CIRG, Makhdoom P.O.Farah-281122,Mathura (U.P.)

613 Co -Investigator

6131 Name	: **Dr. S.D.Kharche**
6132 Designation	:Senior Scientist (Animal Reproduction)

6133 Division/Section : PR&SM Division
6134 Location : CIRG, Makhdoom, -Farah, Mathura (U.P.).
6135 Institute Address : CIRG, Makhdoom P.O.Farah-281122,Mathura (U.P.)

614 Co -Investigator

6141 Name : **Dr. N.Ramachandran**
6142 Designation : Scientist (LPM)
6143 Division/Section : PR&SM Division
6144 Location : CIRG, Makhdoom, -Farah, Mathura (U.P.).
6145 Institute Address : CIRG, Makhdoom P.O.Farah-281122,Mathura (U.P.)

6141 Name : **Dr. Ravi Ranjan (On study Leave)**

PART-III : Technical Details

620 Introduction and objectives:

6201 Immediate objectives:

1. To improve frozen semen technology through use of various additives, protocols, automatic freezing machines and standardization of various cryo-protectants for better post thaw motility and fertility.
2. To strengthen the semen bank by storing sufficient number of frozen semen doses of elite animals of important goat breeds.
3. To use AI for improving the productivity of goats under farm and field conditions.

6202 Long term objectives:

To augment reproduction in goats

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

1. Semen collection, evaluation, dilution and storage to study motility / survivability of spermatozoa from Sirohi, Barbari and Jamunapari bucks.
2. Artificial insemination of fifty Sirohi / Barbari / Jamunapari goats with liquid semen in their first and second estrous cycle.
3. Experiments on freezing of semen by conventional method using Tris dilutor with different levels of egg yolk.

4. Experiments on freezing of semen by conventional method using Tris dilutor with different equilibration time.
5. Experiments on freezing of semen by controlled freezing using Tris dilutor with different levels of egg yolk.
6. Experiments on freezing of semen by controlled freezing using Tris dilutor with different equilibration time and rate of cooling.
7. Evaluation of frozen semen for post thaw motility, acrosome reaction, HOST, sperm abnormalities and live & dead percentage of spermatozoa.

621 Project Technical Profile
April 2009 to March 2010:

Assessment of combination of cryoprotectants

A series of experiments were carried out to investigate the cryoprotective potential of combination of different cryoprotectants [Glycerol (G), DMSO(D) and propylene glycol(P)] in the laboratory at G+D concentration viz 3:5, 5:3, 7:1, 6:1 &3:2, for G+P Concentration viz 1:4, 4:2 & 6:4 and for P+D concentration viz 2:5, 1:4, 3:5 & 5:5 respectively. A total of ten observations were observed for all the combination. Semen was collected by AV method and assayed for motility, HOST, live & dead and acrosome integrity before and after freezing using a phase contrast microscope. Among all the combination observed the three combination M1 (0.3G+0.5D), M2 (0.4G+0.2P) & M3 (0.1P+0.4D).M2 & M3 showed better motility 40-45% also HOST, live & dead and acrosome integrity as compared to M1. Among these combination of glycerol and propylene glycol was the best with post thaw motility of 34.79±0.67 as compared to other combination.

Effect of Dextran alone and combination with glycerol on the post thaw motility of buck semen.

A study was conducted to study the effect of cryoprotectants;Dextran,Glycerol and the combination of Dextran and Glycerol. Among these Gycerol showed best post thaw motility 36.66±1.67 at 6% level standardized in the lab. Dextran was found to have the post thaw recovery of 23.57±2.4.

Effect of Antioxidant fortification on Sirohi goat buck spermatozoa

An experiment was conducted to test the effect of antioxidant fortification on the motility of buck spermatozoa at Glutathione (7mM), Ascorbic acid (9mM) and Vitamin E (4.5mM) concentration respectively. A total of thirty observations were observed for all the combination. Semen was collected by AV method and assayed for motility, HOST, live & dead and acrosome integrity before and after freezing using a phase contrast microscope. It was found that reduced Glutathione at 9mM showed 39.72±0.321 post thaw motility, which was the highest among the three antioxidants.

6211 Technical programme

1. Semen collection, evaluation, dilution and storage to study motility / survivability of spermatozoa from Sirohi, Barbari and Jamunapari bucks.
2. Experiments on freezing of semen by conventional method using Tris dilutor with different additives like cryoprotectants (eg Dextran), antioxidants and combination of cryoprotectants.
3. Evaluation of frozen semen for post thaw motility, acrosome reaction, HOST, sperm abnormalities and live & dead percentage of spermatozoa.

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

Principal Scientist Dr.S.K.Jindal, 50%, Dr.A.K.Goel 25%	16400-22800	9
Sr. Scientist Dr.S.D.Kharche 25%	12000-18000	3
Scientist Dr. N.Ramachandran 50%	8000-13500	6
T.I-3 Sh.Hari Om	4500-7000	12

Supporting 12
Sh.Lalllan

622 Progress of work
As per Technical Programme

6221 Achievement in terms of targets fixed for each Activity

Generation of facilities i.e procurement of essential inputs like equipments, chemicals, biochemicals, glasswares etc.	Facilities created/upgraded and equipments procured (BOD incubator, pH meter and water bath.)
Review of literature.	done
Refinement of semen freezing protocol using various modifications.	Work in Progress (see technical programme)

6222 Questions- Answered

Combination of cryo-protectants and addition of anti oxidants have favourable effects on cryopreservation at the levels tested.

6223 Process/Product/Technology developed during the year: In progress

6224 Utility of results obtained so far : Not yet.

623 Publications and Material Development
(One copy each to be supplied with this proforma)

6231 Research Papers:

- **Book** : Biotechnology of Animal Health and Production, S.K.Jindal and M.C.Sharma, New India Publishing House, New Delhi 2010, pp 1-246.

- Goel AK, Kharche SD and Jindal SK. 2009. Efficacy of Progestin Implant – eCG to Increase Kidding Rate in Post Partum Anoestrus Jakhrana Goats. Indian Journal of Animal Sciences 79 (5): 473 – 475.
- Goel AK, Kharche SD and Jindal SK. 2009.Determination of Early Pregnancy and Embryonic Development by Trans rectal Ultrasonography. Indian Journal of Animal Sciences 79(5): 476 - 478.

- Ranjan R, Ramachandran N, Jindal SK, Sinha NK, Goel AK, Kharche SD and Sikarwar AKS. 2009. Effect of Egg Yolk Levels on Keeping Quality of Marwari Buck Semen at Refrigeration Temperature.. Indian Journal of Animal Sciences 79(7): 662 – 664.
- Ranjan R., Ramachandran N., Jindal S. K., Sinha N. K., (2009). Hypo osmotic swelling test in frozen thawed goat spermatozoa. *Indian journal of animal science*. 79 (10):56-
- Ranjan R., Ramachandran N., Jindal S. K., Sinha N. K., Goel A. K., S. D. Kharche and Sikarwar A. K. S. (2009). Effect of egg yolk levels on keeping quality of Marawari buck semen at refrigeration temperature. *Indian journal of animal science*. 79 (7):662-664.
- S.K.Jindal, S.Yadav, S. Saraswat, Ravi Ranjan, S.D.Kharche, A.K.Goel and N. Ramachandran: Seminal characteristics of Sangamneri goats, Indian Journal of Animal Science. (Submitted)
- Ravi Ranjan , N.Ramachandran and S.K.Jindal : Effect of egg yolk levels and equilibration period on freezability of goat semen, Indian Journal of Animal Sciences (submitted),
- Ravi Ranjan, N.Ramachandran and S.K.Jindal: Comparison between normal and dual staining technique for evaluating acrosome status and viability in frozen thawed buck spermatozoa spermatozoa Indian Journal of animal Sciences. (Submitted)

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6232 Popular articles

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- Kharche SD, Goel AK and Jindal SK. 2009. Uni parental Embryos: An Emerging Tool in Reproductive Biotechnology for Multiplication of Superior Germplasm.Goat News 3(1):2-3.

6233 Reports : -

- Kharche, S.D., Ramachandran, N, Ranjan, R., Goel, A.K. Jindal, S.K., Sinha, N.K. and Sharma, M.C. (2009) Semen technology and artificial insemination in goats. Laboratory Manual, CIRG Publication, pp 1-84.

- Sinha, N.K., Ramachandran, N. and Ranjan R. (2009). Nasal Sudar ke liye bakrio me krithrim garbadhan. In: Vivasayik bakri palan book, CIRG Publication. (In Hindi). P18-21.

- Jindal, S. K. and Ranjan, R. (2009). Bakri palan ke adhunik sidhant avam naye ayam. In: Vivasayik bakri palan book, CIRG Publication. (In Hindi). P 58-60.

6234 Seminars and workshops (relevant to the project) in which the scientists have participated/organised.

Organised and participated in Workshop on Ovine and Caprine AI and ET 2009. Sponsored by IMV India Pvt. Limited at CIRG Makhdoom,Farah (Mathura) UP on 13.4.2009.

- Annual Workshop of NAIP Project entitled : Developmental potency of parthenogenetic goat embryos".at IVRI, Izatnagar (Bareilly) UP on 14 to 15.4.2009

- Edited and Compiled Institute's Publication "Advances in Production and Reproduction in Goats".

- Hindi Workshop (First Quarterly Workshop) at CIRG Makhdoom,Farah (Mathura) UP on 25.6.2009.

625 Infrastructural facilities developed
It is in process

PART-IV. Project Expenditure.
(Summary)
Year : 2009-2010

630 Recurring Expenditure
6301 Salaries (Designation with pay scale)

i.	Designation	Pay Band	Man-months	Amount Rs.Lakh
1.	Scientific			
1.1	Principal Scientist	Pay Band IV	9	4.5
I.II	Sr. Scientist	Pay Band IV	3	2.4
I.III	Scientist	Pay Band III	6	1.3

II.	T.I-3	4500-7000	12	2.8
III	Supporting		12	1.4
IV	Wages			nil
	Sub total			12.4

6302 Consumable (Rs in Lakhs)

i. Chemical	0.42
ii. Glassware	0.44
iii. Others (LN_2, Misc.items etc)	0.22
Sub total	1.1

6303 Travel Nil

6304 Miscellaneous (other costs)

i. Feed and fodder	0.50
ii. Other items	0.20

6305 Sub total (Recurring) 14.2 lakhs

631 Non recurring expenditure : (Equipments) 0.0 lakhs

632 Total (630 and 631) 17.2 lakhs

Signature of Project Investigator Dr. S.K. Jindal

Co-investigators:

1. Dr. A. K. Goel
2. Dr. S.D.Kharche
3. Dr.N.Ramachandran

Signature & Comments of the Head of the Division/Section

Signature & Comments of the Director.

Dated

I/C PME
CIRG

Enclosed please find two copies of the RPF-II in respect of Institute Project entitled "Studies on refinement of frozen semen technology and strengthening of goat semen bank." for the period of 2009-2010 for further necessary action plese.

(S.K.Jindal)
Principal Scientist and
For Head, PR&SM Division