Recovery and Preservation of Goat Follicular Oocytes

Khillare, K.P.

Department of Gynecology and Obstetrics,
College of Veterinary and Animal Sciences, Parbhani

Introduction

Embryo-transfer has become the fastest method of genetic improvement of farm animals. In vitro maturation and in vitro fertilization (IVF) of follicular oocytes are the recent advances of embryo transfer, these are the important tools to study gamete physiology. From these techniques embryos can be obtained in abundant quantity, production of transgenic animal, embryo sexing, embryo splitting and multiplication of embryos in vitro on lines of superior offspring is possible by these methods.

The oocytes can be obtained from living animals as well as from slaughtered animals also. If those are collected from immature living animals and from immature slaughtered animals, in vitro matured, in vitro fertilized and transferred to the recipient the generation interval can be reduced. If the oocytes collected from varies of slaughtered animal the utility of that animal even after slaughter is improved. This also formulate low cost supply of follicular oocytes which can be matured, cultured and fertilized in vitro.

Material and Methods

Thirty three pairs of goat ovaries were obtained from' local slaughter house Parbhani immediately after slaughter: Paired ovaries were brought to the laboratory in a thermos flask containing 0.9 per cent normal saline at a room temperature: Normal saline is supplemented with Inj-Benzyl penicillin - 400 IU per m1' of saline: Inj-Streptomycin 200 mg/ml and 0.25 mg Nystatin. The pair of ovaries in various stages of oestrous cycle were classified as per (Zemjanis, 1970) into early luteal stage, luteal state and follicular stage. After recovery of oocytes, the good quality oocytes were selected and 65 oocytes were preserved in Ham's F-10 medium with 10 per cent and 73 oocytes were preserved in 15 percent serum level at 5°C temperature for 24 hours. The ovaries were wahsed with normal saline and placed in a sterile petridish containing medium. The follicles measuring above 3mm in diameter were punctured with. The help of needle (19 guage) and contents were allowed to *flow* freely *into* the medium. The whole pertridish containing culture medium was observed under streoscopic microscope at 25 x in order to locate occytes.

Result and Discussion

The average numbers of follicles between 3-5 mm size in early luteal, luteal, and follicular stages were 4.30 $\pm$ 0.37, 6.00 $\pm$ 0.57 and 5.20 $\pm$ 0.40; 3.00 $\pm$ 1.00, 4.00 $\pm$ 0.40 and 5.50 $\pm$ 0.22 respectively for 10 percent serum level and 15 percent serum level present findings are in agreement with those of Parkale (1987) an d Giri (1992) who reported them as 4.70, 4.95 and 4.32, 3.28, 4.33 and 4.02 respectively for corresponding stages of oestrous cycle in buffaloes. The present findings for early luteal and luteal stages are lower and for follicular stage in agreement with those. of Thakre (1993) who reported them as 5.60$\pm$ 0.35, 5.52$\pm$ 0.40 and 5.24 $\pm$ 0.28 the corresponding stages of *estrous cycle in goat.

The overall average number of follicles per pair of ovaries irrespective of oestrous' stages and follicular sizes were 6,00$\pm$1.07 and 5.27$\pm$0.83 respectively, which are in agreement with those reported by Thakre (1993). These findings are higher than those reported by Parkale (1987) and Giri (1992) as 4.65 and 4.04 respectively.

Differences in the number of follicles may be due to differences in species, breeds, climatic conditions and endocrine profile etc of animals studied by different workers.

The average recovery rate follicular oocytes in early luteal, luteal and follicular stage for 10 per cent and 15 per cent serum levels was 73.33, 63.18 and 72.42] 66.66, 72.22 and 78.84 per cent respectively which are found to be higher than observations made by Giri (1992) and are in agreement with Thakare (1993) who reported them as 47.83, 58.24 and 47.20 and 76.84 per cent respectively which is in accordance with Lambert (1983) who reported 72-79 per cent by laproscopy method. The present

findings are significantly higher than that reported by Leibfred and First (1979), Parkale (1987) and Giri (1992) who reported lower recovery rate of follicular oocytes as 50.00, 50.00 and 50.92 per cent respectively.

In the present study in Ham's F-10 medium 65 medium 65 oocytes for 10 per cent serum level and 73 oocytes for 15 per cent serum level were preserved at 5°C for 24 hours, it was observed that there was no significant change recorded in the morphology of oocytes.

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Poultry Virus As Human Cancer Treatment

Researchers on the Blacksburg and College Park, Md., campuses of the Virginia-Maryland Regional College of Veterinary Medicine have been awarded a major new grant from the National Institutes of Health to support innovative work that seeks to develop a treatment for cancer from a common avian virus. The National Institutes of Health $430,000 R21 grant will allow Drs. Elankumaran Subbiah; assistant professor in the Department of Biomedical Sciences and Pathobiology at Virginia Tech; and Siba Samal, associate dean of the college's University of Maryland campus, to build upon existing work that is focused on the use of reverse genetics to alter the Newcastle Disease virus to treat prostate cancer. Reverse genetics is the process of generating a recombinant virus from cloned complimentary DNA or cDNA copy, explains Subbiah. Through the reverse genetics system, recombinant viruses can be designed to have specific properties that make them attractive as biotechnological tools, live vaccines, and cancer therapies. The change is achieved through the introduction of the desired changes in the cDNA, which are then transferred faithfully to the recombinant virus. "This differs from the previous work in that the recombinant [Newcastle Disease virus] will be targeted against different types of proteases," said Subbiah. "Different types of cancer cells secrete different types of proteases. We are tailoring the virus to match the type of protease secreted by the cancer cells." Normal, healthy cells have an interferon antiviral system that activates upon infection with the virus, thereby preventing replication of the virus, explains Subbiah. Cancer cells, however, have defective interferon antiviral systems, he said. [Newcastle] utilizes these defects to replicate specifically in the diseased cells. The replication of the virus generates apoptosis - also known as programmed cell death or cell suicide - in the diseased cell. According to Subbiah, the use of poultry viruses as cancer therapy poses no threat to humans and several other oncolytic viruses are currently being explored to treat cancer. However, Subbiah's work is the first to alter the Newcastle disease virus through a reverse genetic system for selective protease targeting. Oncolytic virus therapy has gained much attention recently as a result of the progress in understanding virus-host interactions and because currently available chemotherapy is not entirely satisfactory for several reasons, including the possibility of an individual's development of resistance to drugs. "We are excited about the endless possibilities that Newcastle disease virus offers to treat cancer," said Subbiah. This is the second major grant awarded to the researchers for the work aiming to create a cancer therapy from genetically altered Newcastle Disease virus. Subbiah received his bachelor's degree in veterinary science in 1984, master's degree in veterinary science in 1989, and Ph.D. in veterinary microbiology in 1996 from the Madras Veterinary College in Madras, India; Samal received his bachelor's degree in veterinary science from Orissa Veterinary College (India) in 1976, his master's degree in veterinary science from the Indian Veterinary Research Institute, and master of science and Ph.D. degrees from Texas A&M University.