Bovine In vitro Embryo Production : An Overview

V. S. Suthar*[1] and R. G. Shah[2]

College of Veterinary Science and Animal Husbandry
Anand Agricultural University, Anand- 388001.
* Corresponding author E-mail: vsuthar28@gmail.com

Abstract

Dairy industry perfected the application of the first reproductive biotechnology, i.e. artificial insemination (AI) - a great success story and also remains the user of embryo transfer technology (ETT). In addition, recently the researchers taking interest to embraced the field of Transvaginal Oocyte Recovery (TVOR) and *in vitro* production (IVEP) of embryos. IVF provides the starting point for the generation of reproductive material for a number of advanced reproduction techniques including sperm microinjection and nuclear transfer (cloning). In several countries commercial IVF facilities are already being employed by cattle ET operators. Various research groups have reported on modification of TVOR technique to give greater efficiency. Much research is still needed in domestic animal (Especially Indian species) on mechanisms controlling embryo development and on development of totally *in vitro* system for embryo culture.

Keywords: Reproductive Biotechnology, Transvaginal Oocyte Recovery, I*n Vitro* Embryo Production.

Introduction

Compared with conventional superovulation and ET, production of embryos in the laboratory has several advantages. First, IVEP can be used on problem bovines such as females that fail to respond to superovulation treatment. Second, IVEP can be used to salvage the genetic potential of terminally ill females that would not be expected to respond to conventional ET. Third, semen from different bulls can be used to fertilize oocytes harvested from a cow resulting in embryos with different sires being produced at the same time. Fourth, oocytes for IVEP can be obtained from the ovaries of live donor using Transvaginal Oocyte Recovery (TVOR), or from the slaughter ovaries. In countries like India where oocytes of cattle are not available, as the cow remains a holy animal and there is ban over slaughter of cow and that's why the technique like TVOR is one of the alternatives to get developmentally competent oocytes.

Low efficiency of superovulation and high cost of FSH, *in vitro* embryo production technology has been researched in the last decade as an efficient alternative to *in vivo* system to produce embryos for faster propagation of elite germplasm as well as for research in the field of developmental biology and emerging biotechnologies.

IVEP-In vitro Embryo Production

Different laboratories and workers have there own protocol for maturation *in vitro*. The most widely used media employed to perform IVM such as Ham's F 10a, tissue culture medium 199 with and without serum and synthetic oviductal fluid (SOF) are complex and may be supplemented with fetal calf serum (FCS), estrus cow serum (ECS), new born calf serum (NBCS) (Gandhi *et al.,* 2000), superovulated cow serum (SCS), anestrus cow serum (ACS) or bovine serum albumin (BSA). Maturation media are also supplemented with pituitary FSH and/or LH (gonadotrophins) with estrdial-17â or with extra gonadotropin hormones like human chorionic gonadotrophins (hCG) or equine chorionic gonadotropin (eCG). Some laboratories also prefer to add growth factors like epidermal growth factor (EGF) (Nedambale *et al*., 2004), EGF plus fibroblast growth factor (FGF), insulin like growth factor (IGF), insulin, transferrin sodium selenite (ITS) (Galli *et al*., 2001) etc., for improvement of maturation *in vitro*.

For *in vitro* fertilization, generally TALP (Tyrode's modified medium; Parrish *et al.*, 1988) or BO (Brackett

1. Ph.D Student, Department of Animal Reproduction, Gynaecology and Obstetrics, 2. Associate Research Scientist, Reproductive Biology Research Unit

and Oliphant medium) is most widely used medium. For successful fertilization of oocytes, good sperm preparation is the essential and crucial step. Different workers tried different methods for separation of good motile sperm like swim-up (Lopata *et al*., 1976) or percoll based separation system. Sperms used for fertilization should pass through process of capacitation. Capacitation involves alterations of the sperm plasma membrane, which cause it to become unstable and to undergo vesiculation with the outer acrosomal membrane. In bovines, the capacitation occurs basically in the oviduct during the period of estrus and there is evidence that capacitation is caused by a heparin-like glycosaminoglycan in the oviductal fluid (First and Parrish, 1987). High IVF rates have been achieved by addition of heparin (Brackett and Zuelke, 1993) or its combination with penicillamine, hypotaurine and epinephrine, Ca^{++} ionophore A23187 with or without caffeine and high ionic strength media (Brackett et *al*., 1982). Presently the efficiency of fertilization *invitro* is approximately 80% for the cattle (First and Parrish, 1987).

To date there are various systems available for *in vitro* culture of zygotes. These includes co-culture with various types of cells such as bovine oviduct epithelial cells (BOEC) (Eyestone and First, 1989), cummulus cells or tropoblastic vesicles, established cell line, buffalo rat liver (BRL) cells or vero cells etc. But now a day the trend is changed toward the use of the chemically defined media like SOF, CR1a, Chatot Ziomek Bavister medium CZB (Chatot *et al.,* 1989), hamster embryo culture medium-6 (HECM-6), and G1.1/G2.2 (Krisher *et al*., 1999) etc. Of which SOF is the medium used very commonly by different laboratories and workers. These defined media generally require low oxygen tension (5 per cent) to yield higher blastocyst rate (Vanroose *et al*., 2001).

Lonergan *et al*. (1999) observed that culturing bovine oocytes in SOF and in SOF plus BSA using 5 percent oxygen compared to 20 per cent increased the blastocyst yield on Day 8. Secretions of the female reproductive tract have several amino acids that can be used as energetic substrate by the embryo (Bavister, 1995). The use of amino acids in serum-free culture media improves embryo development (Lee *et al*., 2004), probably through an antioxidant action, controlling pH and osmolarity (Gaedner, 1998). Amino acids can also reduce the stress and cell fragmentation caused by *in vitro* embryo culture (Donnay *et al*., 1997).

Above are some of the introductory measures and considerations, but the oocyte yield in OPU, maturation, fertilization and culture *in vitro* depends upon species and breed of animal used for experiment, number of follicles available for aspiration, OPU session interval, stage of estrous cycle, environmental consideration, oocytes handling, quality of media (pH, osmolarity etc), O_2 concentration in incubator and culture conditions of the oocytes need to be taken into account when wanting to rescue genetic material from females. Due to above mentioned factors potential of Indian crossbred cattle to act as oocyte donors can, be expected to differ from that of exotic breeds.

Author has performed Transvaginal Oocyte Recovery (TVOR) and subsequent IVEP in eight HF x Sahiwal crossbred cows. For that he used TCM-199 + eCG + hCG, m-TALP (Parrish *et al*. 1988) and m-SOF as *in vitro* maturation, fertilization and culture media, respectively. They found 94 % maturation 64% fertilization and cleavage rate, respectively (Suthar, 2008).

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