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Dr. Jyoti Jyotsna
Faculty of Science (Zoology), Government Inter College, Zila School, Darbhanga, Bihar, India

Corresponding Author:
Dr. Jyoti Jyotsna
Faculty of Science (Zoology), Government Inter College, Zila School, Darbhanga, Bihar, India

Biotechnology as applicable to animal science: Relevance of animal cell culture

Dr. Jyoti Jyotsna

Abstract
The foundation of animal cell and tissue culture was laid in the beginning of the 20th century was laid (1903). He showed that the animal cells could be survive and divide *in vitro*. Rose inaugurated and devised tissue culture technique for the first time. Animal cell and tissue culture offer a large scope and provide a large area of research in biotechnology. These areas are production of antiviral vaccines, cancer research, cell fusion techniques, genetic manipulation monoclonal antibodies production of pharmaceutical drugs, use of artificial skin etc.

Keywords: Tissue culture, animal cell, biotechnology, vaccines, cancer, antibody

Introduction
Many highly valuable substances used in medicine and research are based on naturally occurring biomolecules that are produced by animal cells or cells genetically engineered using recombinant DNA techniques. The complex structure of these biomolecules, resulting from special post-translational modifications such as formation of glyco-and lipoproteins, carboxylation and phosphorylation, correct folding or disulfide bonding, etc. determines their biological activity. Such modifications can only be performed by the natural producers; the mammalian cells. The success of genetic engineering of mammalian cells has led to increased productivity of cells as well as to new products. The use of mammalian cell products in the medical field can be summarized as follows:

Animal cell culture products	Applications
Plasminogen Activators. Urokinase type plasminogen activator recombinant plasminogen activator.	Acute myocardial infarction, acute stroke pulmonary embolism, deep vein thrombosis.
Erythropoietin's Erythropoietin-a Erythropoietin-b	Anemia resulting from corner and chemotherapy. Anemia secondary to Kidney disease.
Vaccines HIV Vaccines (gp 129) Malaria Vaccines Polio Vaccines (Salk, Sabin)	AIDS prophylaxis and treatment Malaria prophylaxis. Polio myelitis prophylaxis.
Human growth hormones LGH Somatotropin	Human Growth deficiency in children, trauma related infections, renal cell carcinoma Chronic renal insufficiency, Turner's syndrome.
Monoclonal antibodies therapeutic Anti-lipopolysaccharide Murine anti-idotype/human B-cell lymphoma.	Treatment of sepsis B-cell lymphoma
Monoclonal antibodies Diagnostic Anti-fibrin 99 Tcm-FAB(breast) PR 356 CYT-356-in-111	Blood clot (imaging) Breast cancer. Prostate adenocarcinoma.

In vitro fertilization (IVF) and embryo transfer in humans and livestock

The technique of *In vitro* fertilization (IVF) and embryo transfer (ET) assumes great significance in animal biotechnology. These techniques involve collection of oocytes *in vitro* fertilization, sexing of embryos and embryo transfer.

In vitro fertilization (IVF) in humans

IVF involves the technique of recovery of human oocytes, their *in vitro* fertilization and embryo culture.

Causes of infertility

IVF is needed for a couple who may have in fertility due to any one of a variety of reasons:

a. Tubal infertility in female,
b. Nonfunctional or in accessible ovaries,
c. Nonfunctional uterus or absent,
d. Idiopathic infertility,
e. Male infertility: For normal fertility the sperm concentration in human semen should be 15-20 million per ml. However, for IVF as little as 10,000-50,000 motile spermatozoa per mil are adequate.

Assessment of patients

The female partner inclined for IVF programme should fulfill the following criteria:

1. She should be physically fit to carry a resultant pregnancy.
2. Her ovaries should be accessible for oocytes.
3. Her uterus should be capable of accepting a fertilized ovum, and of sustaining pregnancy for full term of 9 months.
4. She should have easily negotiable cervical canal (cervix) for ET, failing which an alternative route through fungus is used

Treatment of patients for IVF

The female patients for IVF are required to record the commencement for each of their menstrual cycles for at least six months. This is needed so that decision may be taken for:

1. Admitting the patient in the hospital
2. Date of urine sampling for luteinizing hormone (LH) surge and
3. Date of administration of human chorionic gonadotropin (hCG) to control the final stages of follicular and oocyte formation.

Nature of cycle for follicular development

The nature of the treatment prescribed for the female partner will depend upon which of the following three cycles is utilized for the oocyte recovery for IVF.

1. Natural cycle,
2. Stimulated cycle,
3. Controlled cycle,
4. Ovarian stimulation

a. Monitoring ovarian stimulation

The following parameters are used as indicators for ovarian stimulation to help collection of oocytes at the optimum stage of development:

1. Statistical prediction of the day of LH surge on the basis of data on lengths and dates of menstrual cycles.
2. Temperature chart
3. Changes is cervical mucus score indicating follicular development.
4. Plasma or urinary oestrogen levels, which can be estimated at 24h urine collections and are used for timing the LH surge and hCG injection.
5. Ultrasonic determination of follicular size.
6. Detection of LH surge and progesterone determination.

b. Spontaneous LH surge

For accurate prediction of ovulation time, LH peak is most useful. LH can be assayed in urine (3hrs intervals) either by haemagglutination or by radio immunoassay to allow correct prediction of time for laparoscopy. Plasma progesterone concentrations are also measured since they begin to increase 24h prior to LH surge in stimulated cycles.

c. Administration of hCG for controlled ovulation

hCG is administered so that ovulation is controlled. hCH administration can be made on the basis of:

1. The day of the menstrual cycle.
2. Absolute levels and changes in the levels of oestrodial 17B.
3. Size and number of follicles estimated through ultrasonic examination, and
4. Cervical mucus score.

The size of follicles (at least 1.9cm in diameter) should be confirmed by ultrasonic scan.

Oocyte recovery (laparoscopy)

Oocyte can be recovered by following ways:

Earlier methods

This includes following methods:

1. Follicular fluid is totally aspired and oocytes identified in these follicular aspirates.
2. The excused ovary is minced for oocyte recovery.
3. Intact follicles are dissected prior to puncture to study the developmental stage.
4. Follicles are dissected under the microscope and oocytes are recovered.

Laparoscopy method

The method involves the aspiration of ovulating follicles by aspirating apparatus. The ovarian ligament is grasped gently and follicles are selected for aspiration. Follicles that may have ova capable of undergoing fertilization are smaller than 1.5cm. The follicular fluid (4ml-12ml) is aspirated completely from ovulating follicles. As soon as blood appears aspirating tube, heparinised culture medium is added to aspirate to avoid clotting. As the fluid is aspired, walls of the follicle collapse. The aspirate may be examined and oocyte identified.

Oocyte culture and IVF

The oocyte is identified under microscope and incubated for 5-10hrs in any one of the following four culture media.

1. Modified Hams F10 medium
2. Earl's solution
3. Modified Whitten's culture medium,
4. Whittingham's T6 medium.

Preparation of semen

Semen is collected at site by masturbation, 60-90 minutes before insemination. After liquefaction, the semen is

centrifuged and the resultant sperm pellet is resuspended in culture medium. After recentrifugation, the washed spermatozoa are incubated in culture medium at 37c for 30-60 minutes.

***In vitro* fertilization (IVF)**

A total of 10,000 to 50,000 motile spermatozoa are added to 10ml to 1ml of culture medium containing the oocyte. After insemination, the oocytes left for 12-13hrs and then inspected in a petridish containing culture medium.

The first cleavage takes place 24-30hrs after insemination. The best embryos are selected through fluoresce in diacetate for transfer.

Embryo transfer (ET) in humans

Usually 2-4 celled cultured embryos are used for the transfer to uterus. The technique involves the following steps:

1. The patient is given 10mg diazepam (valium) orally before embryo transfer.
2. The patient is placed in the knee chest or lithotomy position with enough head down tilt to ensure that the fundus is lower than the cervical canal.
3. A sterile bivalve vaginal speculum is gently inserted to visualize the cervix.
4. The uterus is manipulated to align the cervical canal and uterine cavity.
5. When the cervix is exposed, the embryo is drawn into a Teflon catheter in 10ml of tissue culture medium.
2. The Tefloncatheter is then passed down an outer Teflonsheath, which protects the inner catheter form vaginal contamination.
3. The catheter is inserted into the uterine cavity
4. The embryo is culture medium is gently infected and the catheter and cannula are gently withdrawn.
5. Catheter is examined under the microscope to ensure that the embryo has been expelled.

After embryo transfer, patient is advised to rest for 2-7h and to abstain from intercourse for at least a week. No hormonal support is advised after embryo transfer.

Artificial insemination (AI) and embryo transfer in livestock

The introduction of semen (sperm) in the vagina of females by artificial means is called artificial insemination. This method is comparatively better and economical. Several cows can be inseminated by semen of a single bull.

Artificial insemination involves

1. Semen collection and storage

Artificial Insemination has been the single most effective factor in increasing the productivity of cattle. The semen ejaculate is collected, diluted (extended), and examined by microscope to determine the number and motility or sperm. The extended semen is dispensed into small plastic 'straws' that are plugged at each end. The properly diluted semen can be used fresh within a few days. For most applications a cryoprotectant like 'glycerol' us added to preserve the nitrogen (-196 c) for long term storage and transport.

Commercial producers extend bull semen up to 100-fold and some 10 million motile sperm 0.2ml is used for insemination. For cattle, AI is done on standing animals without anaesthetic using a procedure known as "rectal palpation". Each ejaculate can be to inseminate as many as 500 cow's prolonged storage of sperm in liquid nitrogen allows a single male to inseminate many thousands of females. The genetics influence of a single superior male can be profound.

2. Oestrous detection and synchronisation

Females are inseminated after ovulation is indicated by behavioural oestrus (heat). In many breeds it is very difficult to detect oestrous, since it may last only a few hours and occurs mostly at night. The ovulation in females can be induced be administration of progesterone and/or prostaglandin. Total synchrony of oestrous is never achieved but some 80% of females usually respond.

3. Sperm 'sexing'

All livestock production industries would prefer most of their animals to be at one sex. In dairy and beef cattle females are usually more desirable than males. Sperm 'sexing' can be determined by analysing X or Y chromosome containing gametes (sperm). Because half the sperm is an ejaculate contain the X and half contain the X and half contain the Y, the sex ratio of pregnancy is usually very close to 1:1. The fluorescent dye Hoechst 33342 stains the DNA of X- and Y-bearing sperm with different intensities. The two population of sperms can be separated with this dye using a fluorescent activated cell sorter (FACS): This instrument breaks a suspension of sperm into micro droplets each one containing a single sperm cell, and passes the droplets through a laser beam. The fluorescence of the dye is measured electronically and droplets of different intensities are deflected into separate collection tubes. The procedure is very expensive and very slow, taking 24hrto process one ejaculate. The yield of viable sperm is insufficient to allow AI, but sperm separated in this way have been used to produce pre-sexed calves by *in vitro* fertilization.

Conclusion

Biotechnology has given to humans several useful products by using microbes, plant, animals and their metabolic machinery. Animal/Plant tissues can be dissociated into their component cells, from which individual cell types can be purified and used for biochemical analysis or for the establishment of cell cultures. Many animal and plant cells survive and proliferate in a culture dish if they are provided with a suitable medium containing nutrients and specific protein growth factors.

DNA technology has made it possible to engineer microbes, plants and animals such that they have novel capabilities. Genetically Modified Organisms have been created by using methods other than natural methods to transfer one or more genes from one organism to another, generally using techniques such as recombinant DNA technology. Transgenic animals are also used to understand how genes contribute to the development of a disease by serving as models for human diseases, such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's. Genetherapy is the insertion of genes into an individual's cells and tissues to treat diseases especially hereditary diseases. Adopting biotechnology has resulted in distinct benefits in terms of animal improvement and economic returns.

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