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Kennady Vijayalakshmy
Department of Veterinary Physiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Meenakshi Virmani
Assistant Professor, Animal Physiology Division, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, India

Ranjeet Verma
Research Scholar, Department of Animal Reproduction, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Manimegalai J
Research Scholar, Department of Animal Biotechnology, Lala Lajpat Rai University of Veterinary and Animal Sciences

Upendra Lambe
PhD Scholar, Department of Animal Biotechnology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Vikas Choudhiry
Department of Veterinary Gynecology and Obstetrics, NDUAT, Faizabad, Uttar Pradesh, India

Correspondence
Kennady Vijayalakshmy
Department of Veterinary Physiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Micromanipulation of embryos: An overview

Kennady Vijayalakshmy, Meenakshi Virmani, Ranjeet Verma, Manimegalai J, Upendra Lambe and Vikas Choudhiry

Abstract
Manipulation of embryos, especially micromanipulation includes various microscopic treatments or techniques that helps in achieving greater fertilisation potential that helps in achieving greater pregnancy rates. Micromanipulation techniques are not really simple, but it requires specialised instruments and equipments. Also the person associated with micromanipulation techniques should be highly experienced and knowledged. The present review helps in understanding the basic concepts associated with various techniques of micromanipulation.

Keywords: Embryo, micromanipulation, oocyte, sperm, zona pellucida

1. Introduction
Micromanipulation indicates various techniques where there is a treatment given to sperm, eggs or embryos which aids in fertilisation and to improve pregnancy rates. Micromanipulation of the embryos requires specific equipment's which are specialised for micromanipulation techniques. Apart from that, micromanipulation techniques are not easily done and it requires a specific trained personnel who has a very good experience in handling eggs, sperm and embryos [1].

2.1. Microsurgery
Most of the microsurgery experiments on the group of domestic animal s are mainly aimed at increasing the production of multiplets, chimeras and identical twins, which ultimately helps in improving the production status of the animal. Splitting of embryos up to sixth day and transferring the split halves subsequently to the recipient animal that are oestrus synchronised will results in the production of identical twins [2].
Surgical collection of Pre compaction embryos at different stages like two cell stage, four cell stage or eight cell stage have to be done. After division, each halves should be placed into a surrogate zona pellucida or zona pellucida which is made out of agar [3].
Collection of post compaction embryos can be done either surgically or non-surgically. Non-surgical collection of post compaction embryos that are used for splitting has to be collected from the superovulated donor cows on sixth day after insemination [3]. The embryos that are collected are placed into a petri dish containing Foetal Calf Serum (FCS) and Phosphate Buffered Saline Medium (PBS) and they are manipulated using a stereo zoom-microscope (100x enlargement). Compacted morulae that are classified as excellent grade, should be only considered for splitting experiments [2].
The zona pellucida is separated by using a sharp edge of a blade and the embryo is split into two almost approximately equal halves with a glass needle or very fine knife. Out of the two halves, one should be removed from the zona and they are placed in a foreign zona. It is always advised to transfer the half-embryos within few hours of splitting [3]. A skilled and experienced person requires only l0 to 15 minutes for splitting an embryo. Transfer of these embryos can be done either non-surgically or surgically. Differences in the pregnancy rates mainly depend on the embryo's. However, under certain experiments and conditions all embryos are considered to be transferable can be used for splitting. Whereas, in case of other experiments only the embryos that are considered to be of best grade only will be selected [2].

2.2. Chimeras
Production of chimeras is an experimental technique that is sometimes considered to be the opposite of embryo splitting into halves.

In this case, removal of zona pellucidae from two (in some cases, there are more) preimplanted embryos which will be then aggregated within a single zona [4]. After the *in vitro* compaction, the chimeric embryos will be transferred to the recipients. Having four times more cells per embryo as like with half-embryos may be the reason for probable influence for the success of this technique. The embryo splitting and aggregation of the two separate half-embryos within the single zona is the other way of production of chimeras [5].

Injection of certain blastomeres of single morula into the inner-cell-mass of an another embryo will result in the production of chimeras that are termed as 'Injection chimeras' [5]. There are only few reports available on the successful production of chimeras in case of sheep, goats and cattle, and the chimeras between sheep and goats are done by aggregating halves or quarters of embryos from both sheep and goat [4].

2.3. *In vitro* fertilization

In vitro fertilisation (IVF) procedure includes the recovery of oocytes at the near time of ovulation, then fertilization with the help of in vitro capacitated sperm, culturing of the embryos and then the embryo transfer that lead to the production of normal offspring [6]. For facilitating the greater recovery of natural oocytes and for the improvement of in vitro conditions to insure the good rate of development of viable embryos which can able to continue to develop normally after following non-surgical uterine transfer, additional and advanced research are very much required [7].

In vitro fertilisation is a most promising technique for various reasons that includes it can overcome certain types of infertility, able to simultaneously produce more numbers of half-siblings, aids to greatly extend the valuable semen, accurate assessment of the functional performance of female and male gametes and provision of pronuclear stage ova for the gene injection and nuclear transfer that are synchronously developed [6].

2.4. Parthenogenesis

Development of a gamete without any chromosomal participation of the opposite sex is termed as parthenogenesis. Natural parthenogenesis are sometimes noticed amongst reptiles and insects. Many factors are induced by artificial parthenogenesis. Suppression of second polar body with the help of cold shock is another successful way of parthenogenesis. This particular procedure works well tolerably in fish. Damaging or removal of one of the pronuclei may leads to a product i.e. haploid egg that can be made diploid with the help of cytochalasin [8].

2.5. Cloning

Cloning is the procedure where there is a development of an enucleated egg after somatic cell nuclear transplantation. In case of mice, there is a successful transfer of an embryonic cell nucleus. After dissociation and isolation of inner cell mass, embryonic nuclei will be transplanted into the fertilized eggs after removing the pronuclei. Collection of blastocysts from the donor females on fourth day and they will be kept in a culture medium. After the surgical removal of zona pellucida, the blastocysts will be manually dissected into inner cell mass (ICM and trophectoderm), both were then dissociated enzymatically into single cells [9].

An ICM or TE cell will be mechanically disrupted by means of suction by using a small glass pipette and the cell nucleus with surrounding cytoplasm, will be then subsequently injected into fertilized egg. Following the nuclear injection, the genome of recipient egg will be removed by means of sucking the male and female pronucleus into the glass micropipette. The nuclear transplant embryos were cultured in vitro to the blastocyst stage and then transferred together with some control embryos into the uterus of a pseudopregnant female in order to allow full term development [9].

2.6. Gene transfer

Some bacterial plasmid and a eukaryotic gene will be injected into the pronucleus of the fertilized egg with a selected DNA sequences. There are various reports valuable that the mice and frogs that get injected with these eggs are containing the injected gene sequences. The technique of directly injecting the selected genes into the eggs acts as a promising technique for selective alteration in the genetic makeup of the animals [9].

The main aim and the practical application of the gene transfer is that the selective or specific gene of interest will be injected to the productive large farm animals to further increase their production strategies and make the animal to produce valuable products. The best example to understand this concept is that growth hormone gene insertion under the control of the promotor metallothionein into the genetic pool of the specific animal or livestock which helps them to increase their efficacy in milk production and also in their growth [10].

2.7. Intra cytoplasmic sperm injection (ICSI)

This procedure is as like an *in vitro* fertilization procedure where a selective single sperm will be injected directly into an egg. Generally, multiple micromanipulation devices that includes microinjectors, micropipette and micromanipulator can be used to do this microscope under microscope [11]. The mature oocyte will be hold with the help of holding pipette that hold oocyte with a gentle suction from the microinjector. On the other side, a hollow, thin glass micropipette with the sperm collected and the sperm is immobilised by cutting the tail of the sperm with the help of micropipette. Piercing of the oocyte through oolemma and then directed into the inner part of oocyte, i.e., cytoplasm, after which the sperm is released into oocyte. After all above mentioned procedure, the placement of oocyte into the cell culture will be done and it will be checked for signs of fertilization on the following days [11].

3. Conclusion

Micromanipulation techniques can do wonder in the world of cellular biology. These techniques can be utilized in an essential way to achieve greater success in achieving higher fertilization rates and pregnancy rates. But the greater disadvantage with micromanipulation techniques is that it needs a technically skilled person. Otherwise, it will end up in a failure. Another major disadvantage associated with micromanipulation is that it is very costly and not quite economical.

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