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Guest Editorial

Summary of what is *In Vitro* Fertilization (IVF)

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Introduction

IVF is an integral part of the embryo transfer (ET) process used for the industrial production of bovine embryos; this process involves *in vitro* oocyte maturation, IVF, *in vitro* embryo development, and finally the *in utero* transplantation of the embryo in the recipient dam. IVF is also a valuable means of learning more about the mechanisms involved *in vivo* fertilisation.

In farm animals, IVF presents several important advantages, such as the production of a large number of embryos in the same time period, the ability to use embryos from dead cows or those with reproductive problems, the possibility of producing embryos from a cow during the first third of gestation as well as the production of embryos in pre-pubertal heifers.

This technique is currently used intensively in cattle, on pregnant and non pregnant cows, with or without reproductive abnormalities. Thus IFV is a low cost means of producing embryos on a massive scale from ovaries obtained at the abattoir. in recent years, this biotechnology has been widely used in cattle on a commercial scale.

In practice, the procedure for producing embryos *in vitro* involves four distinct phase:

1. Harvesting the oocytes (via OPU, Ovum Pick Up, ultrasound guided follicular puncture; or via needle aspiration from ovaries obtained post-mortem).
2. *In vitro* maturation (IVM).
3. *In vitro* fertilisation (IVF).
4. *In vitro* development (IVD).

Obtaining immature oocytes via ovum pick up (OPU)

Oocyte can be obtained post-mortem via follicular aspiration, or the oocyte harvested via a needle introduced into the vagina under ultrasound guidance , the animal is sedated, often with additional epidural anaesthesia, the probe is inserted intravaginally and the ovary grasped via the rectum and held against the head of the probe. The follicles are thus visible on the screen of the ultrasound allowing a needle to be guided through the vaginal wall and into follicle. An aspiration system into a specific medium (containing PBS and heparin) allows collection of the follicular fluid and the oocyte surrounded by its cumulus oophorus, composed of follicular cells. This minimally invasive technique enables a high rate of repeated harvest, up to twice a week for several weeks, with no negative effects on the donors. As such up to 5 oocyte can be collected at a time. The OPU technique is also useful for other species such as the bison and horses. In small ruminants, oocyte are usually recovered via laparotomy or laparoscopy.

In vitro oocyte maturation (IVM)

An oocyte blocked at the germinal vesicle stage requires a maturation phase to enable fertilization. This involves a certain number of modifications: nuclear maturation culminates in the emission of the first polar globule and the formation of the second maturation phase.

Membrane maturation, which enables the oocyte to specifically recognise the spermatozoa of its species, and cytoplasmic maturation, which prevents polyspermia via the cortical reaction, and which ensures the synthesis of the proteins needed for fertilization.

During *in vitro* oocyte maturation, the nuclear phase normally occurs spontaneously, whereas cytoplasmic aspects are dependent on the maturation medium. The most widely used medium for maturing oocytes is TCM-199; this medium is buffered with bicarbonate and contains mineral salts, sources of carbon and energy(glucose), and amino acids such as cystine and cysteine, important in the metabolism of glutathione and glutamine, which with glucose and pyruvate provide the principal energy sources of the oocyte. this medium has similar characteristics to the follicular fluid. synthetic polymers are added to this medium such as polyvinyl alcohol (PVA), its surfactant effect prevents the adhesion of the oocyte cumulus complexes.

In addition, the gonadotrophic hormones, notably FSH, have been used in oocyte maturation media in cattle by promoting the expansion of the cells of the cumulus *in vitro*. FSH potentiates the action of growth factors such as EGF or PDGF and stimulates the expression of the LH receptor by the cells of the cumulus; LH has a direct action on the metabolism of oocyte, increasing glycolysis and oxidative phosphorylation. GH accelerates nuclear maturation, stimulates the expansion of the cumulus, and improves the rate of development to the blastocyst stage in the absence of serum and gonadotrophic hormones.

The physical conditions used for *in vitro* maturation are generally those of the core temperature of the species (37°C in primates and in small rodent, 39°C in domestic mammals), in an atmosphere enriched with 5% CO_2 saturated in humidity, for a duration of 24 hours (mice, ruminants) to 44 hours (humans, pigs).

Classification of oocyte quality for *in vitro* maturation (IVM)

Immature bovine oocytes can be classified into 4 categories depending on the degree of compaction of the cells of the cumulus and the transparency of the cytoplasm.

Category 1

The cumulus oocyte complex (COC) is transparent. The cumulus is compact and completely surrounds the oocyte. The cytoplasm has homogeneous appearance.

Category 2

The COC has the same appearance as in category 1, but the cytoplasm is more irregular. It has a darker zone visible in the periphery.

Category 3

The entire COC is dark, the cumulus is less compact, the cytoplasm is more irregular and presents darker clumps.

Category 4

The cumulus is completely disorganised or even absent.

In vitro Fertilization (IVF)

Various factors are important during IVF: firstly, the elimination of the seminal plasma, which would normally occur during the passage through the cervix or by resorption in the uterus, *in vitro*, it should be performed by centrifugation using a density gradient, or via spontaneous ascending migration in a diluent (swim up). Next is spermatic capacitation, this is achieved *in vitro* through the presence of specific substances in the medium in which the spermatozoa are suspended.

The most widely used medium for the capacitation of spermatozoa and IVF is FERT-TSLP (Fertilization tyrode's Albumin Lactate Pyruvate medium).

Successful *in vitro* fertilization is dependent on a high concentration of spermatozoa in contact with the oocyte, yet *in vivo* only a few spermatozoa make it to the oviduct. Although the spermatozoal density is high, *in vitro* capacitation is still less effective than *in vivo*. If

capacitation is incomplete when the gametes come into contact, fertilization may not occur until 6 to 8 hours later. This leads to ageing of the oocyte, which prejudices the success of the fertilization and subsequent embryo development.

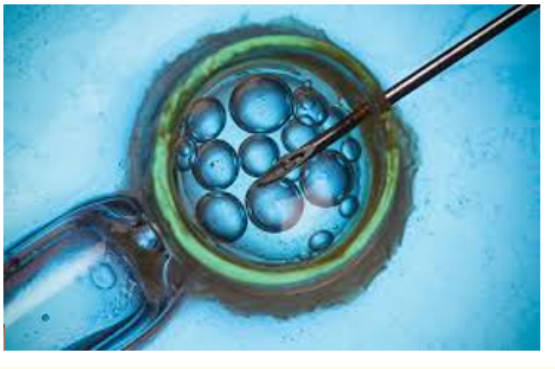
The conditions used for IVF are the same as those used for IVM.

In vitro Development (IVD)

The secretions of the fallopian tubes and uterus play an essential role during *in vivo* embryo development. *In vitro*, the pre-implantation development of the embryo occurs in close relation with the culture medium. This demands specific balanced conditions in the medium to allow embryonic metabolism. With the exception of man and rabbits, there is an *in vitro* blockade of the development of the segmented egg in numerous mammals: at the 2-cell stage in mice, 4-cell stage in pig, 8-16-cell stage in cows. These blockades correspond to poor synchronisation between the fall of maternal mRNA and the start of mRNA transcription from the embryonic genome. This stoppage is linked to inadequate culture conditions for the development of the embryo. The secretions of the fallopian tubes and uterus play an essential role during *in vivo* embryo development. *In vitro*, the pre-implantation development of the embryo occurs in close relation with the culture medium. This demands specific balanced conditions in the medium to allow embryonic metabolism. With the exception of man and rabbits, there is an *in vitro* blockade of the development of the segmented egg in numerous mammals: at the 2-cell stage in mice, 4-cell stage in pig, 8-16-cell stage in cows. These blockades correspond to poor synchronisation between the fall of maternal mRNA and the start of mRNA transcription from the embryonic genome. This stoppage is linked to inadequate culture conditions for the development of the embryo.

Numerous studies have been conducted into culture system to improve the conditions of *in vitro* development. proposed SOF (Synthetic Oviduct Fluid), but this system does not make it possible to remove the blockade of development observed in ruminants at the 8-16-cell stage. The first experiments with embryo co-culture with tubular or uterine cells were conducted in ewes. This system made it possible to eliminate the blockade in ruminant eggs.

Co-culture was performed in the presence of epithelial cell lines of non-genital origin such as BRL (Buffalo Rat Liver) or Vero cells (renal cells of green monkeys) in an atmosphere enriched with 5% CO_2. These cell lines were used successfully for the co-culture of murine, bovine and human embryos.



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