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Production of cattle embryos in the laboratory (in vitro)

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Definition

In vitro means outside the animal, in the laboratory (i.e. in test tubes). *In vivo*, on the other hand, means inside the animal.

The *in vitro* production of embryos therefore means the production of embryos (including fertilisation) in test tubes in the laboratory. *In vivo* embryos are those that are fertilised and grown inside the animal such as those that are collected and transferred in traditional embryo transfer (ET).

History

The techniques for fertilising cattle eggs and growing (culturing) the resulting embryos in the laboratory were discovered during the 1980s. The first calf produced by *in vitro fertilisation* (IVF) was born in 1982, whereas the first human 'IVF' baby was born in 1978.

One major breakthrough in cattle IVF involved understanding how to treat bovine sperm with chemicals. This enabled the sperm to fertilise eggs in the test tube, instead of inside the cow where naturally produced chemicals give sperm the ability to fertilise eggs.

Techniques used for the *in vitro* production of embryos

Once oocytes (eggs) are collected from the ovaries of valuable animals, there are three separate techniques that must be used to produce embryos: *in vitro maturation*, *in vitro fertilisation* and *in vitro culture*. Once these embryos are about seven days old, they are transferred into suitably prepared recipient (surrogate) cows or frozen for later transfer. These cows carry the pregnancy to term although they have no genetic input into the calf.

In Vitro Maturation (IVM)

The eggs that are collected from the ovaries are immature. This means they are not 'ripe' and cannot be fertilised until they have been matured (ripened).

This maturation process takes 24 hours and is done in a special salt solution which contains hormones. This solution attempts to mimic the events that occur naturally in the cow's ovary in the 24 hours prior to ovulation.

In Vitro Fertilisation (IVF)

Once they are ripe the eggs can accept the sperm and so become fertilised. However before this can happen the sperm themselves must be treated with a special chemical called heparin.

Normally when a cow is mated, either using AI or a bull, the sperm must spend 6 to 8 hours in the reproductive tract of the cow before they can fertilise the egg released from the cow's ovary. During this time natural chemicals (similar to heparin) 'mature' the sperm so that it can penetrate the egg. However in IVF heparin must be added.

Between 2,000 and 20,000 individual sperm are required to fertilise each egg, depending on the bull. Different bulls differ in their fertilising ability in IVF.

In Vitro Culture (IVC)

The sperm are left with the eggs for around 24 hours, after which time the fertilised eggs (now called *zygotes*) are placed into a different salt solution where they develop. *Culture* is the name given to the process of growing the embryos in the special salt solutions.

After fertilisation, when the sperm enters the egg, the zygote is still only one cell. It then divides and after 24 hours a two-cell *embryo* results. Cell division (or *cleavage*) continues, and once every 12 to 24 hours each newly formed cell divides again, so that about seven days after fertilisation the embryo contains 100 to 140 cells (see *page 8* for diagrams). At this stage (*blastocyst* stage) it is ready to be transferred into a recipient cow which showed signs of heat seven days earlier.

The *in vitro* production of embryos therefore involves a series of complex steps. If any one of these steps is not optimum then few or no embryos will result.

One of the most critical components of the whole procedure is the media or special salt solutions used for maturation, fertilisation and culture. The exact composition of these solutions is vital for success.

The present results from the *in vitro* production of embryos are still a long way from optimum. From every 100 eggs put into the system, ten to fifteen calves should result if everything goes smoothly.

This technology is still very new and the techniques and media are constantly being refined. Results will steadily improve making the technology more and more commercially attractive.

The development of techniques to grow embryos in the laboratory from eggs taken from the ovaries of valuable animals (either calves or adult cows), has opened up several new reproductive technologies for commercial use.

The whole process of producing embryos in the laboratory, including IVM, IVF and IVC is referred to as the *In Vitro Production* (IVP) of embryos.

Oocyte Pick Up (OPU)

OPU from adult cows

Oocyte Pick Up in adult cows is carried out using a special ultrasound machine inserted into the vagina. The procedure can be performed several times per week and is described in more detail in *Chapter 11*.

Animals that are large enough to undergo rectal examination can have eggs harvested from their ovaries using this technique. It can be used in older animals that have become sterile and which cannot produce a calf either naturally or through conventional ET techniques. It can also be used on pregnant animals and calves as young as five months old.

OPU from young calves

Over the last three years techniques have been developed for collecting eggs from the ovaries of calves as young as two months old.

In calves which are too small to allow rectal manipulation, the oocytes must be collected by exposing the ovaries by surgery or laparoscopy.

These eggs can be then used to produce embryos *in vitro*. About 20 to 30% of oocytes recovered can develop into embryos. After transfer the calves are born at a time when their genetic mothers are only 11 months old.

In certain circumstances this technology allows significant genetic gains to be made (see *Chapter 7: New technologies*).

The advantage of this technology on young calves compared to conventional ET in older animals is shown in *Table 10.1*.

Oocyte recovery post-mortem

If a valuable female dies, eggs (and subsequently embryos and calves) can be recovered from her ovaries providing the ovaries can be transported to a suitable laboratory within 6 to 12 hours of death.

Generation (G)	*Age (mths)*	*OPU*	*Conventional ET (heifer flush)*
G1	2	OPU from elite heifers and embryos transferred to recipients	
G2	11	Calves from G1 embryos born	
	12		G1 heifers flushed and their embryos transferred to recipients
	13	OPU from 2 month old G2 heifers and embryos transferred to recipients	
	21		Calves from G1 embryos born
G3	22	Calves from G2 embryos born	
	24	OPU from 2 month old G3 heifers and embryos transferred to recipients	

Table 10.1: Comparison of Oocyte Pick Up (OPU) and conventional Embryo Transfer (ET)

Further reading

Gordon I. (1994), *Laboratory production of cattle embryos*, CAB International, Wallingford, UK.