

IN VITRO FERTILIZATION

A New Option for Embryo Production

For 10 years researchers have been developing the technology of in vitro fertilization (IVF) and in vitro embryo production for cattle. The procedure involves removing oocytes (eggs) from the ovary, fertilizing the eggs in the laboratory and transferring the resulting "test tube" embryos into a recipient animal.

The technology has long been used to circumvent human infertility problems. In the dairy industry, IVF technology has only been commercially available to breeders since the summer of 1991.

Dr. Charles Looney, director of technical services at Trans Ova Genetics, Sioux Center, Iowa, says the commercial application of this technology is not yet efficient enough to replace conventional superovulation and flush schemes in all situations.

"Any female can be a candidate for oocyte retrieval and IVF. However the most prevalent category is comprised of cows that have been enrolled in a conventional ET program and have produced less pregnancies or embryos than are required by their owners," explains Looney.

"Many of the enrolled IVF donors have produced embryos in the past, but for some unknown reason have ceased embryo production. Others respond to stimulation but due to fertilization failure, no good embryos are produced. These donors are usually successful in IVF, probably because of the number of follicles present on their ovaries. Even cystic donors can become successful in an IVF program. The perfect candidate would be one with blocked oviducts or with some uterine abnormality. Donors not suitable would be ones that have both ovaries adhered such that they can't be retracted to the vaginal wall," Looney says.

In vitro fertilization and production of embryos is a multi-step process which requires a sophisticated lab, specialized equipment and skilled technicians. Trans Ova, like others in the field, maintains facilities for donor cows adjacent to their lab. A herd of recipient animals is also maintained at the facility.

The IVF procedure is non-surgical and semi-invasive. It is considered very safe. "Repeated use of the technique has been found not to be detrimental to the genital tract, ovaries, oviduct, or the vagina of heifers and cows," says Looney.

To retrieve oocytes for IVF, a trained technician inserts an ultrasound guided needle into the cow's vagina. With his other hand in the rectum, he manipulates the cow's ovary against the ultrasound transducer in order to locate follicles which contain the oocytes. Once located, the needle is inserted through the vaginal wall and into the follicle. The contents of the follicle are aspirated out and taken to the lab where technicians search for eggs. The procedure is repeated for all follicles on both ovaries.

Brad Lindsay, coordinator and director of in vitro

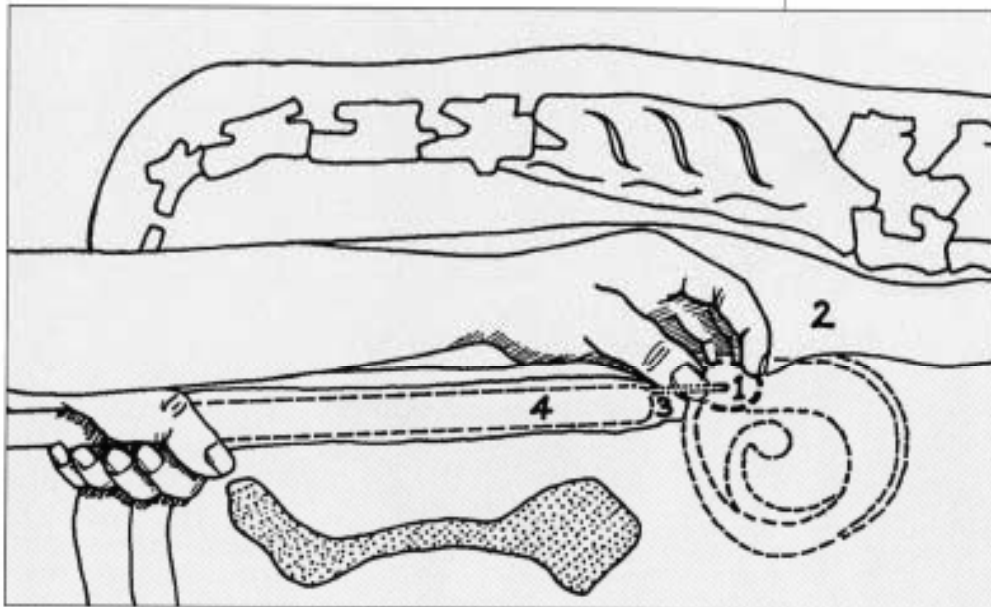


Diagram showing position of ovary (1), rectum (2), cervix (3) and transducer (4) during transvaginal oocyte retrieval.

But, IVF procedures do offer a viable alternative to breeders who own cows of high genetic merit that for some reason fail to produce embryos through traditional flushing and embryo transfer (ET) programs.

IVF can also be used to salvage the genetics of terminally ill or deceased cows, if the ovaries can be delivered to the lab within a few hours after the animal's death. And, the technology can be used safely with pregnant cows (during the first trimester) and with prepuberal heifers to speed up the genetic progress of the breed.

fertilization at Trans Ova Genetics, says that each retrieval results in about six oocytes with about 30 percent viability. The resulting pregnancy rate is about 60 percent plus or minus, making it comparable to fresh embryos.

Once the oocytes have been retrieved, they must be matured in vitro (IVM). This is accomplished by culturing the oocytes along with the cumulus cells that surround them in a medium containing, among other things, FSH and LH hormones. The culture is incubated for approximately 24 hours at 39 degrees C (101.5 degrees F). At this point, technicians examine the culture for mature eggs.

The next step of the procedure is fertilization. Mature eggs are placed in a fertilization media along with precharacterized sperm for a period of approximately 18 hours.

The process of precharacterizing sperm for capacitation is critical to the success of IVF procedure. Raw sperm is not capable of fertilization. When fertilization takes place in the cow, the sperm are capacitated inside the cow's reproductive tract and only after that are they able to penetrate the egg.

With IVF, it is critical to imitate the process in the lab. To do so, oocytes from ovaries obtained at a slaughterhouse are used to determine the appropriate concentration of sperm and the appropriate concentration of heparin (the capacitation agent). This is done ahead of time and must be done for each bull and for each collection of semen. Not all semen can be used for IVF.

Once the fertilization period is over, technicians remove the cumulus cells surrounding the fertilized egg. The remaining one-cell embryo must then be grown or cultured in vitro (IVC) up to a stage of development suitable for transfer into a synchronous recipient animal. At this point, the embryo is handled in much the same way as any other live embryo. The IVF process, from oocyte retrieval to embryo transfer, usually takes about seven days.

The benefits of IVF to the breeder are numerous. Though not yet considered a replacement for conventional superovulation and flushing programs, the applications for IVF are increasing. Most apparent at this time, is the opportunity for a breeder to overcome

infertility problems in genetically valuable but non-productive donor cows; cows that for some reason do not produce embryos through traditional superovulation and ET procedures.

Some breeders are using IVF in conjunction with superovulation and flushing programs, and a few, even now, prefer IVF. With IVF the potential exists for more embryos to be produced in a shorter period of time because the procedure can be repeated three or four times a month. Also with IVF, there is the advantage of using semen from more than one bull to fertilize oocytes collected at any given time.

The use of IVF on prepuberal heifers will have perhaps the greatest impact on the industry as a whole. By obtaining the best genetics from elite females during their prepuberal stage, genetic progress will occur at an accelerated pace.

Of course, there is no guarantee that every cow will produce viable embryos through IVF and IVC and procedures. But, early successes with the technology make it a viable option, especially for breeders who own valuable cows no longer producing offspring. At Trans Ova, where IVF services have been offered for more than a year, 90 percent of the donors have produced at least one pregnancy. Some have produced many pregnancies in a short period of time to several different sires. Only about 10 percent have been non-productive.

"In the future the IVF technology will become a larger part of cattle breeders' tools in embryo production," says Dr. Looney. "It is likely the techniques can be used to produce embryos from animals that are healthy, but not cycling (i.e. prepuberal heifers, post-partum anestrous cows and gestating females in their first trimester).

"It is also likely to produce pregnancies in this new manner without the use of superovulatory drugs. These rates could one day overshadow our present production in conventional superovulation and flush schemes and lead to a more consistent level of embryo production."

Editor's note: Article courtesy of Stephanie Swenson Field and Trans Ova Genetics

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IVF DEFINITIONS

In Vitro

Latin for "in glass;" live events taking place in the laboratory

Oocytes

Unfertilized egg or ovum as harvested directly from the follicle

Ovum

Latin for egg; usually indicates one unfertilized egg

Follicle

A blister-like structure on the ovary which contains the immature ovum

Ovulation

Natural release of an ovum from the follicle

Embryo

A fertilized egg

Fetus

The phase of development following the embryo stage

Viability

Quality or state of being capable of growing or developing

Donor

The female from which oocytes or embryos are recovered

Recipient

The female that will receive an embryo

Characterization

Series of trials performed on two-to-three units of semen to determine the appropriate concentrations of sperm and capacitating agent for optimum fertilization of oocytes in vitro

Capacitation

The process by which spermatozoa become capable of fertilizing an ovum

Anestrus

The period between two periods of sexual activity in cyclically breeding mammals

IVF

In vitro fertilization

IVC

In vitro culture

IVM

In vitro maturation