Ultrasound and the production of genetically superior embryos

The quest for elite breeding credentials drives breeders to invest and experiment with different emerging technologies. Ovum Pick Up (OPU) and its associated in-vitro embryo production is one such technology. Its development over many years has reached a stage that the process has entered the commercial world.

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Collection in progress.

Research into OPU started about 28 years ago and has developed to the point where there are now laboratories and collection centres in most of the major bovine areas of the world. It has changed and developed greatly with help from developments in the human field.

OPU utilises the combination of the practical collection of the cow's egg with the laboratory culture, fertilisation and maturation of the bovine embryo. The bovine reproductive egg, the oocyte, is collected in the form of a cumulus oocyte complex (COC).

The COC is the bovine egg surrounded by the bank of cells (cumulus cells) in which it sits on the ovary. These cells are vital for the embryo production process.

The donors and sires

OPU collection can be performed in heifers as young as 3-4 months. Collection from such an early age speeds the production of the next generation of superior offspring. Including the genomic testing technology to identify the superior donor and sire further increases the rate of genetic progress through this embryo production technique. Lastly, by fertilising, where possible, with sex sorted semen even more advantages are gained. Many of the high scoring genomic bulls are now having their semen sexed, allowing greater choice for fertilisation.

Another donor group to benefit are the cows where conventional embryo collection is unyielding or has become non-productive. These animals, for various reasons, do not produce in vivo embryos and this can be overcome by the OPU method. The technique also allows the collection of oocytes from a bovine female when they are up to 4-5 months pregnant.

Only a small amount of semen is required to fertilise all of the COCs from one collection. Half a straw is required for one batch of COCs. In addition the COCs can be divided into batches fertilised by different bulls.

Problems overcome

IVF has been utilised in commercial enterprises, especially in the South American countries, for many years. Initially, production efficiency was very poor with small numbers of collected oocytes developing through to the transferable embryo stage.

Quality was also an issue with very low pregnancy rates resulting at transfer. Further complications occurred once pregnancy was established, which included, increased risk of abortions, monster calves born, an increased rate of Hydrops and increased rates of other calf abnormalities were associated with the IVF calf. However, these problems and increased risks have largely been overcome with modification of the IVF laboratory process.

The Large Offspring Syndrome (monster calves) was a problem found to be associated with the culture media. The culture media has been modified to overcome this problem.

Ultrasound equipment

The collection equipment consists of the ultrasound machine, a vacuum pump, tubing and a temperature controlled water bath or warming block plus collection pots.

The ultrasound machine used for OPU is a modified version of the one in daily use by the veterinary surgeon who performs reproductive examinations during routine fertility visits.

The ultrasound probe, which houses the crystals producing the image, is housed in a ridged handle to accommodate a needle guided tubing apparatus. The ultrasound

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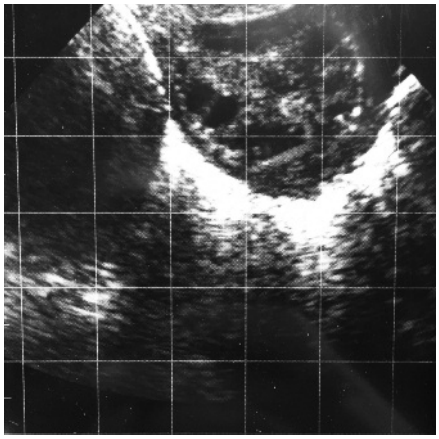

Ovary with follicles. Images taken with an Easi-Scan Microconvex.

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machine along with its aspiration apparatus and collection pots have been perfected over time allowing collection of good quality COCs.

Three steps to good quality embryo production

The production of good quality IVF embryos depends on excellent donor preparation, super-stimulation, excellent OPU technique, excellent oocyte handling and an excellent laboratory process.

● Step 1. Preparation of the donor
All donors need to be in good health and on a well balanced nutritious diet. Donors are usually housed in collection centres where the apparatus is located and the care, including the nutritional input, of the donor can be optimised and consistent. Before the OPU process starts the donors are primed with a drug called Follicle Stimulating Hormone (FSH). FSH stimulates the maturation of the oocytes and increases the yield of embryos. It does not increase the number of COCs collected but does increase the conversion to a viable embryo with a better chance of subsequent survival after transfer. Multiple injections of FSH are given over a period of days for maximum production.

The number of COCs recovered per OPU varies from cow to cow. However, cows and heifers have repeatable numbers of follicular structures in their follicular waves. More fertile females simply have bigger ovaries with more follicles visible.

These individuals are likely to be more fertile and yield more embryos with normal embryo transfer or more COCs with OPU. Numbers of COCs collected is dependable upon the individual donor which has as variable an oocyte production as the conventional embryo collection procedure.

Oocyte numbers collected from some donors will be as low as 3-4 oocytes, whilst from a productive donor it can be over 20. Embryo production from oocytes collected varies depending on the skill of the OPU operator and the quality of the laboratory culture, maturation and conception of the oocyte. It can get as high as 60% of the oocytes collected.

● Step 2. The collection technique
The ultrasound probe and handle with its puncture needle and aspiration guide needs precise alignment with the follicles and optimal suction pressures to ensure collection without spillage or damage of the oocyte.
The sensitivity of the ultrasound equipment is extremely important for maximising the number and quality of oocytes recovered.

For the OPU collection method, the left hand is inserted into the rectum and the right hand introduces the transducer into the vulva until the convex probe is positioned against the anterior vaginal wall.

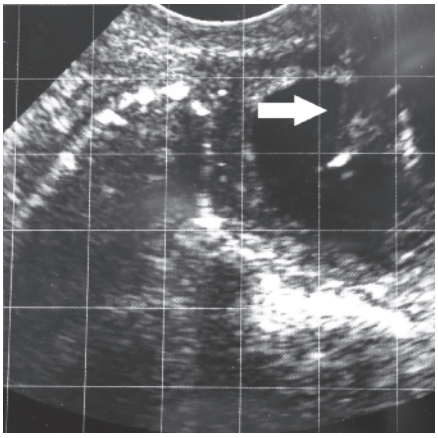
The transducer is tilted to the side corresponding to the ovary being aspirated. The left hand elevates the ovary to the abdominal side of the anterior vaginal wall aligning it with the transducer so that it appears on the ultrasound image. The first follicle is presented to the area where the needle will puncture the vaginal wall.

The whole technique requires a very high skill level as alignment of consecutive follicles whilst the needle remains in the ovary is difficult. With every additional follicle punctured and collected the ovary becomes smaller making holding and positioning for the subsequent follicles more difficult. It is usual to leave the largest follicle to last as this makes holding the ovary easier.

● Step 3. Culture/fertilisation and maturation of the embryo
The COC is transported to the laboratory either on site or in some cases hundreds of miles away. It is matured during the first 24 hours then fertilised.

Semen is then introduced to the oocytes for fertilisation to take place. Once fertilisation has been successful the embryo is matured in media for a further seven days, reaching what is called the late morula stage of embryo development. The day seven embryo is then either transferred fresh into a synchronised recipient or frozen for future transfer.

Laboratory manipulation.



Arrow showing the needle puncturing a follicle in the ovary.

Benefits of OPU and IVP

One of the main benefits for OPU and subsequent in-vitro embryo production is successful embryo production irrespective of the reproductive status of the donor.

It can be applied in pregnant and acyclic (non-bulling) animals, in animals which have blocked fallopian tubes and genital tract infection or other pathology. Oocytes can be collected from pregnant cows until pregnancy advances to a point where the ovary cannot easily be brought in close apposition with the vaginal probe. Breeders are therefore able to produce calves from donors otherwise destined to go barren.

Additional uses include:

● Treatment of cystic ovarian disease.
The OPU unit can be used to physically eliminate an ovarian cyst. This form of cystic treatment is very effective especially for cysts which have not responded to other forms of treatment. It has been successfully done repeatedly in some cows.

● Biopsy of the ovary and uterus.
Some reproductive examinations reveal unusual structures on the ovary and around the reproductive tract. These can be biopsied with the OPU needle to be evaluated.

In the present depressed economic climate the use of this technology is perhaps questionable. However, it remains a successful method for genetic improvement and will be used by the elite producers to enhance their superior genetic lines.

Looking ahead, this technology could be used to easily generate large numbers of embryos of a given genomic score to establish new elite herds either in their country of origin or abroad. ■