



EMBRYO PLUS

POWERED BY  Vytelle™

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BASIC INFORMATION REGARDING EMBRYO TRANSFERS

Embryo transfer is acknowledged globally as the best way for innovative breeders to accelerate the multiplication of superior genetics within a herd. There are 2 methods of producing embryos:

IVF-embryo production

A procedure that has now overtaken conventional embryo flushing worldwide in terms of embryos produced and is following the same trend at Embryo Plus.

Embryo Plus has proudly partnered with Vytelle, a renowned company with more than 14 laboratories worldwide at this stage.

Through Vytelle's development of superior media (containing no foetal calf serum) and innovative new techniques and procedures, problems initially associated with IVFEP, such as large calves at birth or poor conceptions with frozen embryos, were eliminated. The Vytelle system also requires no hormones being used on the donor animals.

In Vitro Embryo Production (IVEP) in cattle consists of the following components:

- Aspiration of oocytes (eggs) from the donor animals (Ovum Pick Up OPU)
- Fertilization of oocytes in laboratory (IVF)
- Culturing of embryos in laboratory (IVC)
- Transfer OR freezing of embryos

Aspiration of oocytes:

The donors are aspirated (OPU) in order to harvest the oocytes from the ovaries. Ultrasound is used to visualize the ovaries and a guided needle is then used to puncture the follicles on the ovaries in order to retrieve the oocytes with suction.

Fertilization of oocytes in laboratory:

The oocytes are matured for 24 hours after the OPU's, before they are fertilized in the laboratory.

- Some batches or specific bulls' semen do not fertilize well with IVF, even though the semen may be of good quality when used for normal AI's.
- If possible, it is a good idea to test semen intended for future use in IVF programs, using trial runs on oocytes obtained from abattoirs.
- If possible, semen from more than one bull may also be used at the same time in order to minimize the risk of poor fertilization, especially in cows with a lot of oocytes.
- A straw of semen can also be used to fertilize up to 7 donor's oocytes, depending on the quality of the semen.
- IVFEP allows for the use of sex-sorted semen, even though lowered embryo production is sometimes seen.

Culturing of embryos in laboratory:

After fertilization, the embryos are cultured for 7 more days in incubators in the laboratory under very specific and strictly sterile conditions.

Transfer or freezing of embryos:

The embryos are graded and the grade 1 embryos may be transferred "fresh" into recipients, or frozen. Recipients are synchronized prior to transfer according to a hormone program supplied to the client by Embryo Plus. Any extra embryos will be frozen and stored for later use.

IVF-embryo production – current standards using Vytelle's system

- Embryos obtained per aspiration done 4-5
- Conception with fresh embryo transfers 50 – 60%
- Conception with frozen embryo transfers 40-50%
- Can be repeated every 2 weeks
- Currently mostly invoiced per embryo successfully produced

Advantages

- Eggs can be harvested from donors that are 2-3 weeks after calving, up to 3.5 months pregnant, so can fit in easier within a normal breeding season
- No hormones required for donors
- Can be repeated more frequently than conventional programs
- Donors with functional ovaries, but with other problems related to the reproductive tract, such as blocked fallopian tubes or pathology of the uterus, could still do well with IVF programs.
- Ovaries of cows that were sent to the abattoir, or just recently died, can still be used to produce embryos for a last time
- Sex sorted semen can be used
- Less semen required; 1 straw of good quality can fertilize the eggs of up to 7 donors
- Less "labour intensive" for the breeder, in terms of the donors, since a hormone program and AI is not necessary with IVF programs.

Potential disadvantages

- Expensive, sensitive laboratory required, mostly non-mobile, which makes the logistics challenging for doing the OPU's and lab work on-farm in remote areas or in countries without IVF labs
- Some countries currently do not allow imports of IVF produced embryos.

Conventional flushing (MOET)

This older technique has been commercially popular since the early 1980's. It involves the following:

- The injection of certain hormones (FSH), which causes a female donor animal to super-ovulate, that is to ovulate more than the customary one egg or ovum they normally do when on standing heat.
- The cow is then AI'ed when on heat and the eggs are fertilized, as during a normal heat, by the sperm in the fallopian tubes.
- Recipients or surrogate animals are synchronized with a hormone program to come on heat on the same day as the donor animal, but they are not AI'ed or mated.
- 7 days after fertilization, a catheter is placed in the uterus of the donor animal, and the embryos are flushed out with fluid, retrieved and classified under a microscope in a laboratory. The 7-day old embryos can then be transferred right away or be frozen for later use.

Conventional flushing – current industry standards

- Embryos obtained per flushing 6-8
- Conceptions with fresh embryo transfers 60%
- Conceptions with frozen embryo transfer 40-50%
- Can be repeated every 6-8 weeks, mostly for 2-3 cycles
- Usually invoiced per flushing done, regardless of number of embryos obtained

Potential disadvantages:

- Can only be done on non-pregnant animals, so sometimes the donor's ICP is compromised if she has to wait for a flushing program before falling pregnant again.
- Donors with pathology to the uterus or both fallopian tubes cannot be used.
- Requires more input from the breeder, such as the injecting of the donors during the program, that needs to be accurate and on time, as well as the AI of the donors that is critically important.
- Donors receives certain hormones that, if not administered in the right way as prescribed by an expert, could lead to fertility problems later on, if used at excessive dosages or for too many programs in a cycle.
- Only semen of a very high quality can be used (poor fertilization usually achieved with sub-optimal semen or sex sorted semen in mature cows) and requires at least 3 straws of semen per donor per flush.

Advantages

- Easier to implement currently in very remote areas or neighbouring countries without access to IVF laboratories.
- Some countries would only consider conventional embryos for imports.

RECIPIENTS

Regardless of whether we do IVFEP or conventional flushing, the management of the recipients remains a most critical aspect for success and is unfortunately often neglected by lesser experienced clients.

1) Selection of animals to be recipients

There are 3 basic criteria to select recipients:

- 1) They must be able to cycle normally and become pregnant
- 2) They must be able to maintain the pregnancy and have a normal calving
- 3) They must be able to raise the calf well (beef animals)

Keeping this in mind all the time, one then has to decide between the following options:

Heifers vs cows

Heifers have the advantage that they do not have any lactational stress, therefore they usually cycle better than cows. They usually have a very low incidence of clinical or subclinical genital tract infections, compared to multiparous cows. Because of the lower metabolic maintenance requirements, they are relatively cheaper to feed than adult cows.

When using mature cows, there are definitely also some advantages, especially if the policy in that specific herd has been to cull strictly on reproductive performance and the ability to properly raise the calve. In such herds, cows that have had between 3 and 6 calves are functionally fertile animals. Mature cows will also be better suited when transferring embryos of breeds with relatively large calves, and they usually have more milk than heifers to raise the calf.

Animals used successfully before as recipients tend to repeat this in a next breeding cycle, making them very valuable. The owners should try and retain their successful recipients.

In the case of beef animals, we usually start synchronizing them to transfer at 3 months after calving. There will always be exceptions where animals reconceive earlier, but at the current drug fees for the program and the value of the embryo, it is cheaper to wait. If you wait too long, the nutritional needs of the calves becomes too high, and the cows go onto a negative plane of condition.

We prefer using recipients between 3 to 4.5 months after calving, alternatively letting them wean first before using them. With adequate nutritional assistance, calves can be weaned at 5 months without problems in order to get their mothers ready for embryo transfers.

In the case of heifers, we use the same guidelines as for normal breeding, that is about 65% of mature weight at the time of transfer. Breed differences and nutritional management on the farm determine the age at which heifers will be ready for transfer.

In our opinion, the worst recipients will be the first calvers with calves at foot. These animals are still growing out, have added lactational stress and are the most difficult group to get pregnant, even by normal means. I would not recommend using first calvers, except if you're prepared to wait until the calves are weaned.

Different breeds of recipients

There are many different factors to consider when choosing a breed of recipient. Fertility, temperament, adaptability, calving ease and adequate milk should all be considered.

The most Continental or European breeds have good fertility. Dual purpose type of animals usually raises beautiful calves because of the extra milk. Most dairy breeds have good temperaments. All of this means nothing if they die of tick-borne diseases or suffer because of extremes in temperatures or parasite burdens.

In most cases the recipients will be genetically inferior animals from the same breed as the embryo donor or be from the commercial part of the farming enterprise.

Use recipients that are suited for the specific farm's management and for that specific area, such as hardy animals for extensive, semi desert type conditions, with the Nguni breed as a very good example. Allow heifers enough time to mature properly before using them as recipients. It is better to have at least 50% Bos Taurus in the recipient instead of using pure Brahman.

More early maturing, European type animals can be used in mild climates with adequate nutrition or artificial pastures.

Some breeders use dairy heifers to transfer embryos into. Once they have calved down, they let one cow rear 2 or 3 calves on her own and sell the other recipients as fresh in milk dairy cows, with obvious economic advantages.

Own animals vs. buying in

Using animals that were raised by the breeder himself is a far better option than buying in animals to be used for recipients, because you know the real age, nutritional state, vaccination status, calving history etc.

If you have to buy animals, try buying from respected breeders that you trust and try and buy from total dispersal sales. Another alternative is to buy young heifers and raise them on the farm. If you buy a group of open heifers at breeding age from an unknown source, they may very well be his most infertile animals that did not conceive after running with the bull for various reasons. Heifers that were neglected while growing up as far as nutrition goes are very often stunted for life and will always be of lower fertility. Buying open cows without young calves at foot is buying someone else's problems.

2) Preparation of recipients

Animals brought from another area or from a different nutritional system requires at least 3 months adaptation period on the farm where the transfers will be done.

When receiving a new group of animals onto the farm or getting a group of animals ready for transfer, liaise with your local vet and get the following in place:

- Test for TB and Brucellosis at least.
- Do pregnancy diagnosis on every animal, even if she has "never been close to a bull". At the same time examine the recipient for breeding soundness and ovarian activity. Cows with calves at foot can be routinely doused at a month post-partum to make sure she does not carry any subclinical uterine infection into the embryo program.

- Vaccinate against relevant diseases, especially diseases directly affecting fertility like BVD. If they are going to be moved to a Heartwater, Redwater or Gallsickness area later after receiving embryo's, make sure that the immunization occurs before the transfer synchronization starts.

Proper **identification** of the recipients is essential. Ear tags must be clearly visible from a distance. Put ear tags into both ears, as they get lost regularly. Make sure there is no duplication of recipients with the same number. A permanent hot iron brand would be ideal, tattooing is also a good option. Do this well in advance so as not to stress the recipients too close to the transfers.

Nutrition is obviously extremely important and warrants a separate discussion on its own, please consult your nutritionist to assist. It is better to start with animals slightly down in condition and get their condition to improve during the program, than to start with fat animals that only maintain their condition or even lose condition. An animal with a condition score of 2 to 2.5 at the start of the program that gains condition to a 3 to 3.5 at the time of transfer is ideal.

Correct any micromineral imbalances or deficiencies prior to the program. We give Vit A (Ocean Gold) and Multimin as part of our synchronization as well.

Guard against high levels of NPN in the diet. Sudden changes in diet close to time of transfer is not advisable and after the transfers the recipients should stay in exactly the same grazing conditions (same camp if possible) and on the same nutritional program at least until 2 months after transfers, when the pregnancy diagnosis can be performed. Cows with small calves at foot can be night-weaned if possible and the calves receive additional concentrates during the night.

Take care not to get the recipients in an over fat condition at the time of calving, with the resultant dystocia's etc.

Animals with **temperament** problems should be removed from the transfer program. If you're dealing with recipients managed under a very extensive conditions, take them through the handling facilities where the transfers will take place at least once a day for a month before the actual transfer takes place, to get them accustomed to the setup and bring their stress levels down. Work quietly and calm, no dogs/electrical prodders/whips etc.

There are certain minimum requirements for **handling facilities**, such as a proper crush with a side gate to allow direct entry into the crush behind the animal in front that is restrained in a secure neck clamp. For OPU, a solid floor area right next to the open side gate is needed where the ultra-sound machine and suction pumps need to be positioned, with electricity available. Embryos are extremely sensitive to UV light, so a shaded area is required for the OPU/flushing and thawing/transfer of embryos. **Please contact our office for plans for handling facilities.**

Handling facilities also has a direct effect on the temperament and stress levels of the recipients. It obviously also affects the quality of the transfer vet's work if the work has to be done in adverse weather conditions with inadequately restrained recipients.

There are many different ways of **synchronizing** the recipients, each with certain advantages to it. We use progesterone implants, combined with other hormones. Where at all possible, natural heats are the best, but not very practical.

Heat spotting is an extremely important part of the program. If you're dealing with a big group of recipients, divide them up into more manageable groups of not more than 40 animals. It helps to remove the animals that have been seen on proper standing heat to a separate camp so as to get subordinate animals to show heat as well. Get a dedicated person to do heat spotting for the 3 days.

The importance of easily readable ear tags is obvious. Use aids like teaser bulls, heat detection devices or electronic systems such as Sensehub as far as possible, but they must never replace proper

observation time spent with the animals. Good recordkeeping of the time of onset of standing estrus is important.

Usually, about 60% of the recipients which have been placed in a program, should be suitable to receive an embryo, so it makes sense to prepare more recipients than the total of embryos, if available.

3) Handling of recipients after transfers

It is ideal not to tamper with the recipients at all before the pregnancy diagnosis is done. If possible, keep them on same diet and management program as before the transfers. Do not do vaccinations or wean their calves during this time, to avoid the fever reaction or unnecessary stress. If you need to dip, use a water-based spray dip.

Wait 8 weeks before examining the recipients, because some embryos develop slower than normal pregnancies and may be missed, even by an experienced vet, if the examination is done too early.

The use of serum samples to determine pregnancy could be done from 28 days after transfers, is usually very sensitive, non-invasive and a good option if the non-pregnant recipients from a previous transfer are to be used immediately in a follow-up program.

It very often happens that recipients that have received an embryo, show heat again afterwards, even though she is pregnant. Do not AI the recipients on the first heat signs after the transfer, since it may cause a resorption if she was pregnant from the transfer. Rather use a bull instead.

After pregnancy diagnosis at 2 months after transfer, the recipients can be moved, weaned, vaccinated with safe vaccines etc. Take care to continue with a balanced diet, since the fetus is already sensitive at this stage to certain deficiencies and the recipient need to grow sufficiently (in the case of heifers) and have enough reserves to calve normally and raise the calf.

4) Handling of recipients around calving

Remember, the calve is worth much more than the recipient and good observation and timely intervention could help to avoid potential calving problems. Embryo calves from transfers on the same day could be born up to 4 weeks apart.

- Control the recipient's energy intake during the last trimester, to prevent the calf from growing too big.
- Do the necessary vaccinations against scours and lung conditions 1 to 2 months before calving, so that the newly born calf's immunity will be boosted.
- It is ideal to have the recipients calve in a camp not too far from the handling facilities where predators can be kept out and observation is possible.
- The area should be big enough to still allow the recipient the necessary privacy to calve without being disturbed and to avoid infection of the new-born calf with pathogens associated with overcrowded/insufficiently disinfected calving areas.
- Depending on the breed and age of the recipient and the breed of embryo transferred, induction could be considered 285 days after transfer. Please discuss this with your veterinarian and arrange a specific time to do this as to have the vet on standby to assist should there be a problem with calving.
- **Save the calf.** If it is too big, do a caesarean.
- It is extremely important to ensure adequate colostrum intake within 6 hours after birth, have a bank of frozen colostrum available should it be required.

Frequently asked questions

How do I best incorporate ET work into my breeding season?

Some breeders have dedicated recipient herds with adequate nutrition available during the year and could perform smaller ET programs throughout the year.

In most instances though, there is a fixed breeding season or 2 during which all animals need to fall pregnant. In this instance, it would make sense to do one or 2 programs at the start of the breeding season, using all the most fertile recipients that calved first (calves already +/- 3 months old at time of transfer)

A follow-up program can be timed, if need be, 3 weeks later to accommodate all the recipients that calved later or to give those not suitable for transfer at time of first program a 2nd chance.

After this, bulls can be introduced ensure that all the recipients could still fall pregnant within the breeding season, even if the embryo transfer was not successful.

The synchronization program also helps some inactive recipients to start cycling, so even if she does not fall pregnant from the ET, she could still shorten her ICP because of that, helping her to wean a heavier commercial calf with obvious economic advantages.

For these big programs, it makes sense to have a bank of frozen embryos that could be used should there not be enough fresh embryos to transfer on the day. Many breeders would send animals to the centre prior to the breeding season to OPU every 2 weeks and freeze embryos to build up this bank, and then time the last OPU to coincide with the fresh transfers at the start of the breeding season.

Which cows should I use for donors?

Using the right donor cow (in terms of genetics) matched with the correct bull is the single most important factor determining the success of the ET program. The process is simply too expensive to get only average quality calves born, even if a very good embryo production and pregnancy rate was achieved.

The quality and quantity of oocytes produced by a donor may be influenced by her environment for 90 days prior to collection. We therefore recommend giving a cow more than one opportunity to prove herself, since there are so many factors that determine the success of the IVF or flushing program. This will enable us and the breeder to sort out any issues, such as possible problems with the animal's health or reproductive system, potential problems with specific batches of semen, stress on the cow, management and environmental factors like climate and rain, nutrition, etc.

Heifers, first calvers or very old cows may produce less embryos than the average, as do dairy cows during peak lactation.

Some animals may produce better when not lactating/nursing a calf.

Can IVF be done on the farm, or must the donors go to a center?

Should a client wish to do IVF on the farm, the following needs to be available:

- Proper crush with neck clamp, under a roof
- Access gate into crush, behind animal caught in neck clamp, with stable floor area
- Electricity
- Since Embryo Plus has a mobile laboratory, there does not have to be a laboratory close to the crush

Should this not be in place, or if the owner wishes to do OPU's every 2 weeks and travel fees become an issue, it is better to send the donor to a center.

The maturation, fertilization and culturing of the embryos have to be done in our laboratory at Brits, so the oocytes obtained during OPU on farm would then be transported back to our center.

8 days after the OPU, we would then visit the farm again for the transfers if recipients were prepared.

There is also the option available of keeping recipients at the center in Brits, where they can be synchronized, and embryos transferred into them every 3 weeks. They would then be kept at the center until PD's can be done, before they're discharged to the client's own farm.

How many donors do I need to do IVF?

There are certain fixed costs involved with the project (e.g., travel fees), which make it relatively more economical to do larger numbers of donors and recipients at a time, but there is certainly no minimum number required.

Should the clients not have frozen embryos in stock and have many suitable donor animals available, it makes sense to calculate the number of donors to be OPU'ed by using the number of recipients available:

Say you have 50 recipients that can be synchronized:

Of these, 60 - 65% is usually suitable for transfer, thus implying you would need about 30-35 embryos.

In order to get 30-35 embryos, you'd need more or less 100 oocytes

In order to get 100 oocytes, you'd need to aspirate about 7-10 donors

Most clients would then, in this case, identify say 15 potential donors and rank them. The best ones are aspirated first, going down the order until you've obtained more or less 100 oocytes of good quality. The lower ranked animals are only aspirated if the better ones do not yield enough oocytes.

What are the costs involved when doing IVF?

The following fees are applicable when doing IVF (please see our fees list for more details)

- Travel fees: 1 trip should the OPU's be done on the farm + 1 trip for the transfers
- Normal hormones and drugs required for the synchronization of recipients
- Normal transfer fees
- Each embryo successfully produced will be invoiced according to our fees list (this fee includes the OPU, fertilization and culturing). Should freezing be required, this would also be additionally invoiced per embryo then according to the fees list
- Should the donor animals be admitted to the center for OPU's (for a series of OPU's every 2 weeks), the above will apply, without any travel fees for the aspiration, but with lodging and disease testing added as per fees list)
- Donors may also come in as day visitors for OPU's, then only fees per embryo produced and for freezing/transferring applicable