

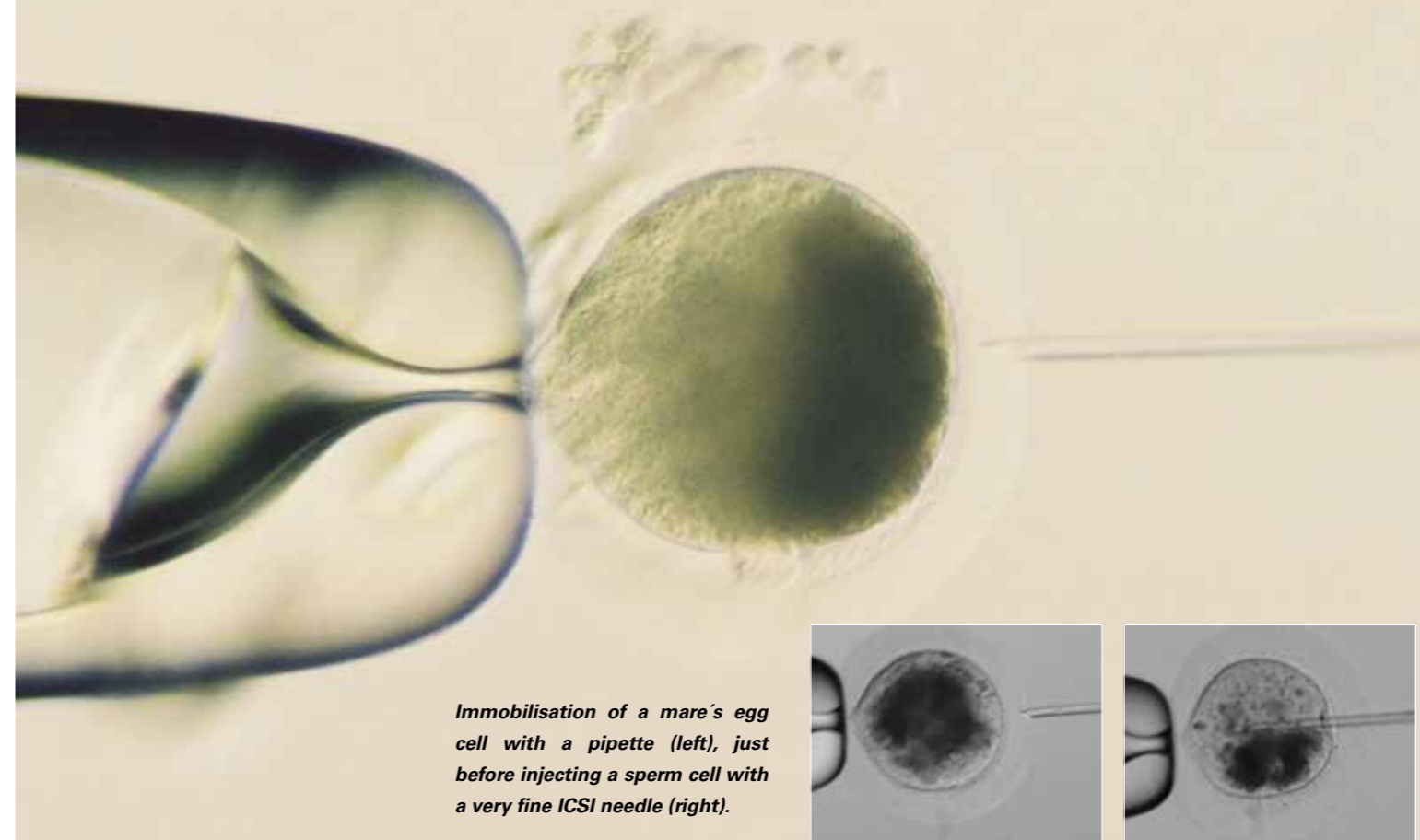
Veterinary

# Modern reproduction techniques: OPU/ICSI

*Uncanto di Villagana with Rachel Steffen, one of the very first ICSI foals and successfully competing at 1.60m level.*



BY: FREDERIK DE BACKER



*Immobilisation of a mare's egg cell with a pipette (left), just before injecting a sperm cell with a very fine ICSI needle (right).*

The reproduction process of horses, it's something we're all interested in: the complete cycle of detecting when a mare's in season, choosing a stallion, ordering semen samples, then the stud service and later scanning for pregnancy. These are procedures many breeders have mastered far better than filling in their tax forms. But those tax forms have evolved to an online service which for some is as clear as running water but for others more of a nuisance. And with this I'm just referring to how those forms should be completed, not the amount payable that emerges below the line. I had never given it much thought that equine reproduction would bear any likeness to our tax declarations. From natural stud services to AI and frozen semen, about flushing embryos, cloning and now also OPU/ICSI.

When I was still living in Belgium I watched this evolution develop from close-by. A veterinarian / stallion owner / researcher / pioneer jumped on the bandwagon of progress and offered the technique of embryo transfer. The short version: the mare is inseminated and after the impregnation, about eight days after the stud service, the uterus is flushed out – hence the much-used term flushing. In case one or more embryos are found in the retrieved fluid these will be transferred to recipient mares, hopefully resulting in healthy pregnancies. Eleven months later, you guessed right, we find the surrogate mare with a healthy foal by its side in the stable. In the mean time, the donor dam has been used for the production of more embryos or shortly after flushing,

continued with the order of the day, the sport. Talking of embryo flushing was such a familiar thing and it was so widely used that it appeared to be the most natural thing in the world to me. When I started embryo flushing myself it was a great success, both the technique and my personal achievements.

That is, until I came to live in the Netherlands where I was often interrogated on the subject of embryo transfer. The first times I hardly registered this but after a while I began to realise the extent of both scepticism and lack of information north of the Moerdijk. 'Fake news', very much the topic of today. My move to the Netherlands happened practically



Early ultrasound pregnancy diagnosis.

simultaneously with the emergence of OPU/ICSI, the next big leap forwards in stud services. The 300km between the green lung of Aalst and the heart of Overijssel meant that I wasn't there to witness this last step. There was little I could say about the technique, its successes, the risks, the advantages and disadvantages. So when I was confronted with a few grim, negative stories I considered the time had come to find out what was going on.

That quest took me to Professor Peter Daels. After his graduation at the University of Gent he left for California in the United States of America in 1984 to take his doctor's degree at the Davis University. Later he swapped the West Coast for the East Coast and became a professor at the Cornell University of New York. In 1997 he worked at the University of Utrecht for the duration of one year and subsequently he moved on to the INRA (Institut National de Recherche Agronomique) in the vicinity of Paris to do research. He has also worked at the Reproduction Centre Keros in Belgium and since 2012 has been 'back home' in his capacity as Professor Equine Reproduction at the University of Gent. He also introduced me to veterinarian Katrien Smits who has guided me through the practical aspects of ICSI. Dr. Smits was in charge of the first ICSI foal, born in the Benelux in 2009. Presently she is employed as a postgraduate researcher at the University of Gent where she is fine-tuning the ICSI technique and looking for new possibilities, such as freezing of egg cells and genetic analysis of ICSI embryos.

The technique is usually referred to as ICSI but actually, we are dealing with two different techniques: OPU (Ovum Pick Up) and ICSI (Intra Cytoplasmic Sperm Injection), but it's okay to call it ICSI. The OPU technique

involves the retrieval of egg cells from the mare's body. This is carried out on non-pregnant mares by inserting a probe into the vagina. The mare receives an epidural anaesthetic, a treatment which is not totally without risk. The probe, which consists of an ultrasound and a high-tech needle, is held against the vagina wall. This enables the ultrasound to produce an image of the ovary which itself is held in place via the rectum. Then you consecutively prick the needle through the vagina wall, via the abdominal cavity right through to the ovary and into the follicle. Next the follicles, which are basically just sacs filled with fluid and a tiny egg cell, are pierced through the vaginal wall and sucked empty with the needle. Sometimes an egg cell sticks against the wall and in a bid to enhance the chances of success the follicle is washed clean and the wall is scraped off the follicle. So next time when you're engaged in a discussion about the correct terminology, scraping or pricking, it's the same thing!

In an ideal situation the presented mare has around 15 to 20 immature egg cells in her ovary. Why immature egg cells? Because it's easier to manage egg cells in their immature state. Treatment of mature egg cells is a much more delicate affair and as a rule they need to be fertilised pretty quickly.

The more egg cells are ready the more chances of a pregnancy. According to Professor Daels this is one of the first advantages of ICSI. You can plan a visit to the OPU centre that suits your mare's routine. Before heading off to the OPU centre your own vet can give her a scan to make sure the trip is worth your while. When there aren't enough egg cells in position you can simply postpone the OPU until sufficient follicles have emerged. Now back to the egg cells because in the meantime they have been transported to the laboratory where they are retrieved from the flushing fluid and examined. The best egg cells are placed in an incubator where they are left to mature until they're ready to be fertilised. Even though fertilisation seems a straightforward process, it is in fact a highly complicated and delicate process.

What you do is take a few mature egg cells and a fraction of the amount of semen normally used for traditional stud services with fresh or frozen semen. Gather all these ingredients in a Petri dish with every egg cell floating in a separate drop of fluid.

Then you get to work with a micro manipulator, which is a combination of a microscope and two robotic arms. The microscope explains itself really, you can see very tiny things many times enlarged. We're talking now



When performing OPU by means of transvaginal ultrasound guided aspiration or from ovaries from deceased mares, one might collect 10-30 immature oocytes depending on the cycle of the mare.

about sizes of just a few micrometers, 1 micrometer is 0,000001m.

The robotic arms have a holding pipette in one hand and an injection pipette in the other. Both instruments are operated with a joystick while looking through the microscope. In addition to the movement of the instruments they both also have a suction- and blow function.

If you're not very dexterous with the console of the play station or still drop pallets of wood shavings from the loader then don't even think of it.

Now find the egg cell, manoeuvre your holding pipette alongside the cell and use suction to fixate the cell to the pipette. That stops the egg cell from rolling away when you want to inject it. You know, just visualise the story of the olive and the cocktail stick.

Then you look for a mobile sperm cell which is correctly shaped and active. You immediately curb that activity, or more like, damage the sperm cell's tail to stop it from swimming away all the time. Suck the sperm cell up into the injection pipette and go back to your egg cell.

Pierce the egg shell, puncture the egg cell membrane and inject the sperm cell into the egg cell. Mission accomplished, you have just fertilised an egg cell. Now go back to step 1 and fertilise all the remaining egg cells.

When all egg cells have been fertilised they must be placed in a different incubator with different climatic conditions. Don't make a mistake at this point! By damaging the tail of the sperm cell chemical substances are released that activate the next phases of the process. Human egg cells and those of other animal species are fairly transparent which makes it easy to monitor the cell division. Equine egg cells however, are dark which makes inspection a lot more difficult.



When aiming for the dominant follicle one collects only one mature oocyte.

Are you not the patient type? Then again, this is not your thing. Just wait!

Now it takes seven to eight days for the egg cell to develop into an embryo. Every time you open the lid of the incubator this may change the climate inside and that may very well affect your chances of success. At the end of this process you have two options. You can either freeze the embryos or implant them in a recipient mare. Research has shown that freezing has no influence on the survival rate of the embryo. Freezing the embryos enhances their mobility because they can then easily be transported around the world and it gives you complete control of what you want to do with the embryos.

These days, the OPU technique has been widely accepted and there are many locations in Europe where your mare's egg cells can be collected. Unfortunately, the technique for freezing egg cells is still not very advanced so you have to get to work with the egg cells straightaway. As said before, you can leave them to mature in the incubator and then fertilise them. But if your OPU centre doesn't carry out ICSI it is also possible to transport the egg cells at room temperature. The eggs are not affected by travel and can be placed in an incubator at one of the few existing ICSI centres across Europe. In fact, their condition remains so stable that no difference has been established between egg cells that first underwent transport or those that were left to mature without delay.

But if OPUs are relatively common, egg cells can easily be transported and you also possess good hand-eye coordination as well as patience, then it surely cannot be that difficult to be successful? Can it?

In one of the European laboratories they have

succeeded in performing ICSI on a small scale. Results were excellent, the clients were happy, not a cloud in sight. Until suddenly cell division came to a halt and fertilisation of egg cells failed altogether. The problem lasted a few days, then turned into weeks and then weeks turned into months. People frantically tried to figure out the cause of this singular phenomenon. In the end an expert was consulted who examined all fluids, soaps and disinfectants. He checked if the readings of the temperature on the display of the incubator matched the actual temperature inside. He examined the air as well as the laboratory itself and its surroundings. And this is how the expert came to the conclusion that a few months back new tarmac had been laid in the vicinity of the laboratory. The gases and substances that were released during this process had drifted into the laboratory and most literally had ruined the climate for successful fertilisation. That gives you an idea of how delicate ICSI is.

So before you decide to give ICSI a go in your bathroom make sure to remove all perfumes.

To get a clear idea of the success rate I was given the following data: from an Ovum Pick Up with 12 follicles on average 8 egg cells can be retrieved; from these about 5 continue to mature and can be fertilised, resulting in an average of 2 embryos that can be implanted or stored in nitrogen. For implantation a success rate of 65% is normal. So well over one out of two embryos actually becomes a foal.

But before anyone approaches me at a competition to give me the story about a neighbour who managed to get 3 embryos and ditto foals from just 1 OPU, let me remind you that these figures are averages. There are always exceptions to the rule, but it gives a fair idea of the chances you have when embarking on this adventure.

It also shows you can have control over OPU/ICSI. To make the most of your venture you must create the best possible starting position. In order to maximise your chances of success with OPU it's best practice to postpone taking action until the mare's scan reveals a sufficient number of follicles. You can do all this outside of the traditional breeding season and embryos can be defrosted and implanted any time you want. So in theory you could arrange to have all embryos implanted in April with all foals being born in March of the following year instead of having to get up many nights in spring and summer to help your mares with foaling. Weaning will also be a lot easier because all ICSI foals will be more or less the same age.

Because you can plan the moment of implanting it also gives you more scope to choose a recipient mare. A surrogate mare of your own or rather one from the centre that carries out the service? With standard embryo transfer you will be allocated a mare that is currently available and in the right phase of her cycle, which is not necessarily the mare you would prefer.

You can also choose any stallion you like and use him when the time suits you. There's no need to consider traditional fresh semen and allow for the competition calendar of the stallion of your choice. Is your mare in season, should she be covered now but the stallion is off-duty because he's out competing? Outdated practices with ICSI. Semen quality is poor or bad? One active little sperm cell is all it takes.

Say, you are planning to extend your OPU/ICSI and need to implant 20 embryos next spring. What you can do is divide them over several stations and that will give you a good indication of which centre comes up with the best results. This technique really means a shift in the centre of power with all its positive and negative consequences.

Okay, back to breeding and in particular to the recipient mares. One reason for the interview with Professor Daels was a lecture he gave at the University of Hasselt. In that lecture he came up with a number of noteworthy figures and statements I would like to share with you. For some this may be previously acquired knowledge, for others probably blasphemy.

Extensive research has been carried out in France which involved monitoring of over 50,000 horses throughout their lives, as foals, sport horses, stud stallions, broodmares... As it turns out, a mare's first-born performs below average. The norm is what you can expect from the combination of dam and sire, mare and stallion, both in terms of performance as well as physical development. Don't shoot the messenger, this is what the figures say and figures don't lie.

The first possible explanation for this could be the stiffness and size of the uterus which is still small with a first pregnancy. The bigger the uterus, the bigger the area that can be used to send nutrients and oxygen to the placenta with the embryo in it.

The 2nd and 3rd foals perform progressively better and then performance in relation to the norm stabilises/normalises.

Towards the end of a mare's life cycle the quality of her offspring starts to diminish again. Again, the explanation can be found in the same context. The elasticity of the

uterus diminishes and the uterine wall may for instance have sustained some scarring.

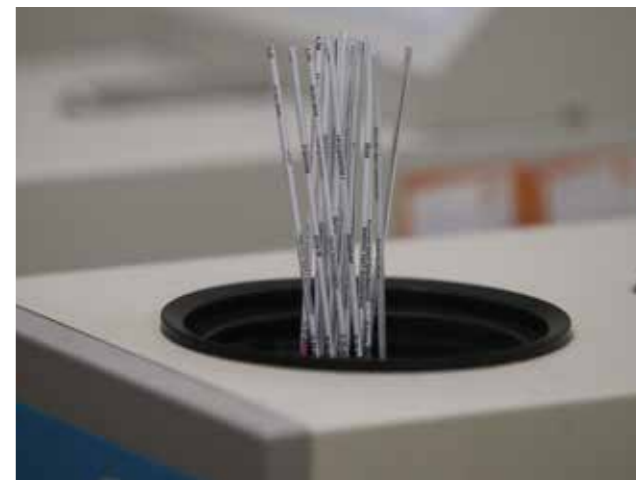
Amsterdam, 1944, the Hunger Winter. Men and women had to survive on the absolute minimum and due to wartime food shortages many inhabitants perished. The unborn babies in their mothers' bellies suffered with their mothers. To this day these people are diagnosed with afflictions like diabetes, cardiovascular diseases and disorders of lungs and kidneys. Serious deficiencies during the embryo stages produce lifelong consequences. So any deficiency your embryo incurs will also have lifelong consequences for him/her.

This, by the way, could well be the reason why many breeders have a sceptical attitude regarding ICSI. Criticasters often refer to the situation which involved cows, the first target group ICSI was used on.

First let me give you a short account of the history. Dairy cows give dairy calves, so far no surprises. But half of the dairy calves are bull calves which unfortunately have little economic value. No meat on the bones and no udder, so no milk. But the cows had to keep producing milk by giving birth to calves. So researchers went to the slaughterhouses where they retrieved the ovaries and egg cells from newly slaughtered beef cows. These egg cells were then fertilised and sold to farmers. The farmers, who previously used to buy semen to fertilise their cows, implanted the embryos of these small beef cattle into their dairy cows. This way the farmer had a pregnant cow which could produce milk after birthing, and a calf which, if it was a bull, would also generate some money. So along with the birth of calves we saw the birth of ICSI.

However, when fertilising the egg cells of cows with sperm cells other substances were added too. This caused the 'large cow syndrome' when calves were born with anomalies to organs and brains. For one, no other substances are added for equine ICSI and equally, so far no issues concerning new-born foals have been reported. Success rates in cows are higher however, which might lead to the conclusion that reproduction in cattle is less selective than in horses. In cattle, Nature doesn't phase out the 'production errors' so they are born with anomalies. This doesn't seem to be the case in horses. This isn't altogether new information, for instance conception in cows is known to be pretty straightforward, a piece of cake compared to the same procedures in horses.

But where were we? Yes, mares, their first-borns and



**Straws with embryos ready for deep-freezing.**

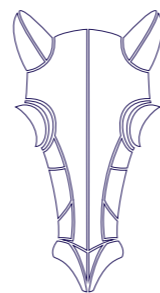


**Cesare Galli, founder of Avantea, is an authority on the subject of ICSI.**

recipient mares. If it is a first pregnancy for the surrogate mare her foal will perform below the norm for exactly the same reasons as with the biological dam. The same research also showed up that a foal born from a pregnancy when the dam had another foal at her side, performs better than the norm. But in these cases the explanation is more likely to be found in the overall health of a mare who year after year carries a foal. To make it even more complex: the research has revealed that offspring of 3-year-old mares generally perform better than the norm. Why is this? People who own a talented mare are keen to put her in foal so that they have one offspring from her before she is competed in the sport and possibly sold. And as a rule, talent performs better.

The influence of surrogate mares is negligible! Well, not entirely, but now I've got your interest piqued! As said before, if your surrogate mare gestates your embryo in what for her is a first-time pregnancy, then you know

# Sport Horse Insurance



Contact MS Amlin's Sport Horse insurance specialists, led by David Ashby, to get a quote for your tailor-made policy

Your bespoke policy can include All Risks of Mortality and Theft, Loss Of Use, Life-Saving Surgery, Foal Insurance, Prospective Foal Insurance, Broodmare Insurance, Stallion First Season Sub-Fertility, Stallion Permanent Disability and Transit/Operation only Insurance.



ASCA Z

Telephone: +44 (0)800 917 9712  
Email: [askequine@msamlin.com](mailto:askequine@msamlin.com) | [msamlin.com/equine](http://msamlin.com/equine)

MS Amlin Underwriting Services Limited is authorised and regulated by the Financial Conduct Authority.

**MS Amlin**



ICSI foal of Olympic mare Sapphire and World Champion Cumano.

that your foal will statistically perform below average. But it's not always fair or justified to look at surrogate mares with trepidation, especially not when considering character. Three examples:

In polo sports the horse's drive and willpower are decisive factors. The best horses therefore are not for sale because they are unique trump cards for the team. Most of these horses are mares because, as polo players claim, geldings don't exhibit the same drive as their female counterparts. Stallions are not used in polo sports because stallions and mares...yes, you get the drift. The successful mares are not used for 'traditional' breeding but instead have their embryos flushed. And that's not all. In Argentina they're having a ball using the cloning technique and the results speak for themselves. The mare Cuartetera of legendary polo player Adolfo Cambiaso was cloned six times. In the polo scene the mare is described as the Messi of polo sports and by cloning her Cambiaso had a string of horses to choose from to continue the game with a fresh and fitter horse but it never meant a change of character. With his six clones he won the prestigious Tortugas Open in Palermo. According to him it's an immense luxury to ride the 'same' horse and get the same commitment all of the time.

In Thoroughbred racing there's a thin line between anonymity and eternal fame. They usually speak in terms of stamina, a characteristic that should make the difference between victory and defeat, first place or a place in the mob. Anyone who's slightly familiar with this world surely has heard that a serious defeat can break a horse's character and end his career.

When the successful Thoroughbred mares have a foal at their sides they obviously need to be covered again. The Thoroughbred stallions, strictly used for traditional mating only, are often stationed in another country or even continent. So it's simply out of the question to expect these young foals to travel long distances on the truck, let alone fly them overseas. That's why there are nurseries in the vicinity of breeding studs. These are adoption centres where the foals are taken care of and raised by adoption dams. We don't expect breeders of Thoroughbreds, dealing with foals by sires with stud fees of up to €100,000.-, to take unpredictable risks and take chances their foals might miss out on inheriting the much sought-after character of their successful dams. So apparently, the character of the adoption dam has no influence on the character of the foal.

The final anecdote is by Professor Daels himself. It's generally known that some stud stallions put a strong stamp (good or bad) on their offspring's character. But those stallions weren't present at their foals' conception. Nor were they present at their births and they don't do maternity visits either. But still, the foals inherit part of their sires' character. It's a simple story that tickles our thoughts.

## ICSI or ET

One way or another you always end up with embryo transfer, the only difference being the phase preceding the actual transfer of the embryo. With ET the egg cell is fertilised inside the donor mare, with OPU/ICSI this is done in the laboratory. The last stage is implanting the embryo into the recipient mare.

Judging by the results, 1 OPU/ICSI session provides a better chance of an implanted embryo than ET does. ET procedures average a 35% chance of an embryo, with ICSI the chance of success averages over 60%. However, ICSI procedures come with a considerably higher price tag than ET. With ICSI you are more in control and have more scope, with ET you depend on the natural cycle. Don't forget, for years ET has been the answer to enable active sport horses to be used for breeding. ET also comes with fewer risks than ICSI, which requires an epidural anaesthetic and involves puncturing of the vaginal wall. This is why active sport horses are hardly ever subjected to OPU. It's beyond question that both techniques will significantly evolve over time and in its wake more ICSI centres will come onto the market. The result will be more supply and consequently lower prices which will help to make ICSI more attractive. Finally, one remarkable finding is that both ICSI and ET produce more colts than fillies. On average, ET has a 56% yield of colts and with ICSI the average goes up to 72%. Mother Nature sticks to 50/50. The choice is yours.

