



Scientists at work at the ViaGen laboratory, Wisconsin USA. For a single successful clone foal, a large number of embryos are produced, making cloning a labour intensive process.  
Photo credit: ViaGen

# Horse Cloning

## earning itself a controversial profile

ARTICLE/PHOTOS: DR ALEONA SWEGEN, BVSC, VETERINARIAN, JERRY'S PLAINS VETERINARY CLINIC

CLONING seems to have earned itself a controversial profile through a somewhat shaky portrayal in science fiction and occasionally the media. The truth is that most, if not all leaps of science in the past have been met with much contention. This is neither surprising nor objectionable, as it's undeniably important to poke and prod, scrutinise and examine every idea before we accept - or reject - it as part of our everyday lives. However, the key factor here is to be well informed, and to try to understand exactly what we are dealing with and what impact it may have.

Otherwise, the debate becomes futile. Add to that the intrinsic human fear of the new and the uncertain, and we've hit a wall.

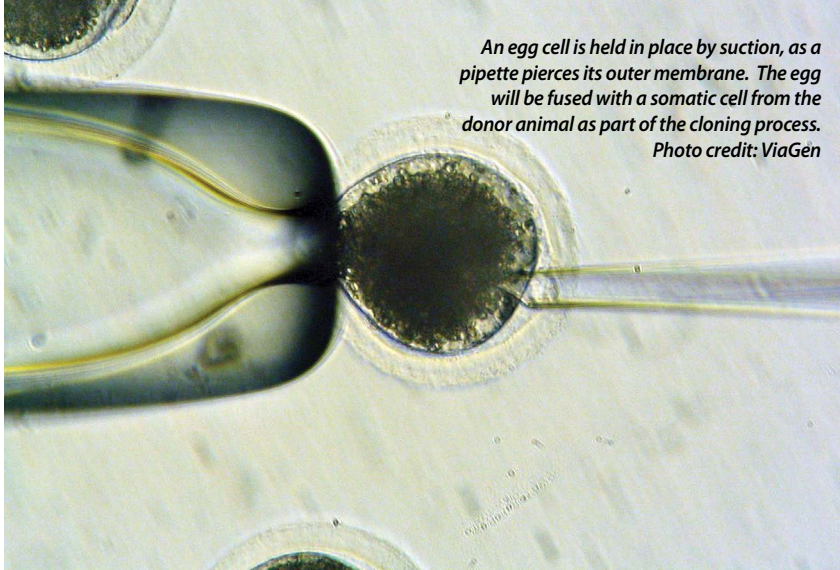
Immediate reactions to the concept of cloning often relate to the emotional or productive value of animals and people attempting to 'immortalise' them by cloning over and over again, with mass production of animals and loss of individual

identity as central concerns. In reality, this is neither a likely nor practical scenario as we come to understand that an animal's genetics are only a fraction of what constitutes an individual. There are many other factors and these will be discussed later on.

A horse was successfully cloned for the first time in 2003. Since then the procedure has gradually eased its way out of the purely scientific arena into the everyday, with numerous performance horses (in particular geldings) cloned in the USA and Europe.

For those willing, cloning is now well and truly, definitively, tangibly possible. There is no denying, however, that not all of the questions have been answered - both scientific and ethical.

This article is a brief rundown of the cloning concept and a handful of the associated quirks. I hope to answer more questions than I raise, but make no promises!



An egg cell is held in place by suction, as a pipette pierces its outer membrane. The egg will be fused with a somatic cell from the donor animal as part of the cloning process.  
Photo credit: ViaGen

## • The cloning process

By definition, cloning is the production of an identical fragment of DNA, or a genetically identical cell or organism, by non-sexual means. Plants and bacteria reproduce by making clones of their own cells. As far as animals go, there are two basic ways in which genetically identical individuals can come to exist. One is embryo splitting – this occurs in identical twins, and can also be done in a laboratory by splitting an embryo into two in its early life. Natural identical twins have not been observed in horses, but the ones split in the lab can be transferred to recipient mares as per normal embryo transfer, and will result in two foals carrying the same genetic material. The second way is nuclear transfer, and this is the method of “cloning” that we usually talk about. This process is quite simple in principle. To start from the basics, all of our genetic information is carried in our cells in the form of DNA (deoxyribonucleic acid). To clone a horse, we need the DNA of that horse, and an oocyte (an unfertilised egg) with its original DNA removed. The oocytes can come from any horse and can be matured in a lab. The nucleus containing the DNA is removed from the cell, and the oocyte without its genetic material becomes what’s called a ‘cytoplast’. In the meantime, ‘new’ genetic material is obtained from the horse to be cloned (i.e. the donor). The DNA can come from almost any cell, but usually a small skin biopsy is taken and the cells are cultured in the lab until the appropriate stage of growth. The cytoplast and a single donor cell are then fused together to form a ‘reconstructed oocyte’, and this continues to develop into an embryo after undergoing an activation process (reprogramming the cell to act as an embryo rather than a skin cell). After a few days of incubation the embryo is transferred to the uterus of a recipient mare (no different to standard embryo transfer) to be carried to term.

## • Is it really that simple?

So the theory is simple – egg from one horse, genes from another, fused together and we have an embryo, and subsequently a foal, genetically identical to our donor horse. But of course it’s easier said than done, with many stumbling blocks along the way. Some relate to the process itself, while some are unique problems that the equine species presents us with. All of these ensure that cloning a horse is a pretty inefficient and expensive process.

For example, the oocytes to be used need to be in a particular stage of development, and of high quality. Removing the DNA can be extremely difficult in horse oocytes, and the cell can be damaged in the process. This means hundreds of oocytes may be needed for one successful clone, and are not always readily available. Another hurdle has been the reprogramming part of the process. This is crucial as we must ‘convince’ the DNA of the embryo that it is no longer running a skin or muscle or bone cell, but is now the control centre of a future foal - so the genetic material remains unaltered but certain genes need to be switched



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Aleona is a veterinarian working for Rodger & Associates, at Jerry's Plains Veterinary Clinic in the Hunter Valley, NSW.

Having grown up with horses and coming from a family background of involvement in horse breeding and training, she is passionate about equine reproduction and has developed a keen interest in assisted reproductive technologies.

Aleona is now fulfilling her dream of working in a reproduction-focused practice in the horse capital of Australia, and hopes to continue study for specialist qualification in the future.



“Paris Texas”, USA’s first cloned foal, produced by scientists at Texas A&M University. The first successful cloning of a horse took place in Italy in 2003. Photo credit to: Media Resources, College of Veterinary Medicine, Texas A&M University



Five clones produced from a single donor cell line. There are notable differences in markings and appearance despite the foals being genetically identical.

on or off for embryo development to continue. It has taken years to develop a successful method for this, however for many of the embryos it is all too much change in too short a time period, again contributing to a fairly low efficiency.

### • Are clones identical to donors?

Yes and no. Clones are genetically identical to their donors, but generally will not be identical in appearance and behaviour. When DNA was first discovered, it was thought of as 'the secret of life', the tiny piece of coding that defined completely who we are as individuals. However, since then we've learnt that every individual is a balanced combination of genetic and environmental factors. We also now know that just having certain genes isn't all there is to it – there's also the potential for genes to be switched on or off (we call this epigenetics). Most of you will know the effects that different methods of training and handling can have on two genetically similar foals – such as siblings or offspring. Or perhaps the effects of nutrition, worming and exercise. These are just a few of the factors that will affect the way an animal utilises its genetic material, and can result in very different outcomes. The points mentioned are some of the more obvious – there are also factors relating to the foal's early development that play a role. These relate to embryo transfer, where things such as uterine blood supply, placentation, and condition of the mare carrying the foal will affect the characteristics that the foal attains as an adult years later. Note that these are applicable in any instance of embryo transfer, but in a conventional embryo transfer we often don't realise their significance because we are not comparing the foal born to an 'original' copy.

Thus the clone may ultimately be bigger or smaller, have different bone structure, be quieter or flightier, and so on. Remember, all these factors can work both ways, and are not necessarily negative!

One of the most striking differences between a clone and a donor - or indeed between any two genetically identical horses - is the variation in white markings. These are partly determined by a genetic signal but also by a fairly random migration of cells during foetal development, so the distribution and shape of the markings will vary quite noticeably. Markings aside, however, it has also been said that equine clones are in fact quite different in their individual look and identity – at least more so than other species cloned in the past. A note on performance: as the horse trainers out there will know, it takes more than a horse's talent to make a champion, no matter which sport we consider. The numerous factors above, and the all-important human factor of training, make it unlikely (though certainly not impossible) that a clone will achieve the same competition levels as the donor.

### • Are cloned horses normal?

As far as we know – yes. Those that make it through the rough-and-tumble of in vitro incubation, gestation and what may be a tough few first days of neonatal life, generally go on to live normal, healthy lives. In other species, there have been a few conditions specifically associated with cloned offspring – for example 'large calf syndrome' in cattle – which have affected their survival and long term health. In horses however, no such conditions have been observed to date. The vast majority of cloned foals thus far have survived to adulthood and are no different to any other horse. A few have been affected by pneumonia, septicaemia, perinatal asphyxia syndrome (dummy foals) and angular forelimb deformities in the first few days of life – but of course these can and do affect all foals, not just clones. Whether they are more common in cloned foals is hard to say because of the statistically small number of them born so far. And even if the numbers were conclusive, we would need to then determine whether the cause is the cloning process itself, the in vitro stage of development, or factors relating to embryo transfer (embryo handling, placentation, nutrition, etc). Remember that these foals spend the first few days of their existence being incubated in a dish, and these may not be the ideal conditions for development, despite our best efforts and continual improvement!

Something that calls for further investigation is mitochondrial DNA, and its effect on the health of cloned horses. Mitochondria are tiny organelles found inside cells which process energy and control many of the cell's functions. When a cytoplasm and donor cell are combined, the mitochondria of the oocyte remain in the cell and therefore in the developing embryo. Mitochondria also carry a very small amount of genetic material – mitochondrial DNA (mtDNA). So the cloned animal will inherit the mitochondrial DNA of the 'random' animal that supplied the oocyte. At this point in time, we really don't know whether this has any consequence at all on the health and appearance of a clone. In humans, mtDNA has been implicated in a number of health disorders (Parkinson's disease is one example), but these are unique to each species and we are not aware of any inheritable mitochondrial disorders in horses. Thus we assume that there is some potential for mtDNA to affect the health and performance of cloned animals, both in positive and negative ways. So far there has been no cause for concern with cloned horses, however the exact effects and their significance are yet to be investigated.

As far as reproductive health is concerned, we know that clones are able to reproduce normally, and, importantly, pass on the same genes to their offspring as the donor animal would. Although the clone itself may not be phenotypically (in appearance) identical to the donor, the genetic material is exactly the same and therefore offspring of clone and donor are equivalent (this is a crucial point and highlights the main practical asset of the cloning procedure). The epigenetic status of the DNA is reset when gametes are formed, so these changes do not affect the progeny.

The only way a clone's foal can be distinguished from a donor's foal is by the differences in mitochondrial DNA. However this only applies to the foals of female clones, as the mitochondrial DNA is only passed down in the oocyte and not in sperm cells (which lack mitochondria-containing cytoplasm). Therefore foals produced by a stallion clone are genetically indistinguishable from the donor's foals.

### • The future of cloning

Why should we or shouldn't we clone horses? Which horses should we clone? Is it worth the hassle and expense? Is it really useful, or

just "fun"? Is cloning ever going to become as common as embryo transfer, or artificial insemination with frozen semen?

These are all intriguing questions and everyone out there will have their own answers. There are numerous perspectives from which to look at the issue. Breeders, competitors, veterinarians and geneticists work tirelessly on selective breeding, trying to constantly raise the bar and improve on current bloodstock. Strictly speaking, a cloned animal is not an improvement on current genetics but simply a duplication, and therefore not progressive. That is, if we think of the 'genetic pool' as encompassing all the horses that exist on the planet. In practice, however, cloning can present an opportunity to provide genetic improvement in isolated gene pools, similar to AI with frozen semen. The greatest value of the procedure lies not in producing 'copies' of a particular animal – as we now know clones are all but the same – but rather, in being able to pass on the valuable genes of that animal to the next generation. For instance: in areas where a stallion would not otherwise be accessible, or in cases where a stallion or mare has become infertile due to accident, disease or castration. The latter is a frequent scenario as many high performing horses are geldings, and cloning is a method of 'salvaging' the valuable genetics that would otherwise be lost from the gene pool. An animal that dies unexpectedly before reproducing may also have a second chance to pass on its qualities to the next generation with the help of nuclear transfer.

An important non-commercial use for cloning is in the preservation of endangered groups of horses – such as the wild Przewalski's horse and less well known Sorraia horses of the Iberian region. These animals feature small population numbers and narrow gene pools. Cloning offers a means of gene banking to prevent permanent loss of precious genetic material. Gene banks have been established for numerous endangered species and cloning may be resorted to in dire circumstances where the species approaches extinction. The practice is controversial, too, as some believe the capacity to clone endangered or even extinct species may cause us to be less compelled to address the original problems, such as habitat destruction and poaching. The issue is not a straight forward one, but one can easily see how such a technology could be called upon to aid conservation.

In the context of advanced reproductive technologies, it is hard to see why cloning should be viewed any differently than frozen semen AI, embryo transfer or in vitro embryo production. On the other hand, we can't deny that there are valid ethical concerns over the procedure becoming commonplace, and we need to be realistic.

Will cloning ever become very common? Is every child's favourite pony going to be duplicated at the blink of an eye? I doubt it. The most obvious limitations are cost and efficiency. The success rate is low, facilities and expertise available in only a handful of labs around the world, and last I checked it cost more than \$100,000 to produce a single clone – although continued work is likely to improve efficiency and decrease cost in future. Even so, with regard to the notion of 'mass production' of cloned animals, I cannot imagine a reason for anyone to excessively clone one particular animal. Especially a horse, an animal traditionally valued as an individual rather than a unit in a production system. This would have no commercial or emotional benefit, neither to the individual nor the industry as a whole.

Besides, by the time a clone has grown to competition or breeding age, it is likely that its genetics are no longer as 'valuable' as they were at the time of cloning, because, hopefully, the current generation is an improvement on the previous one. In the end, though, any horse

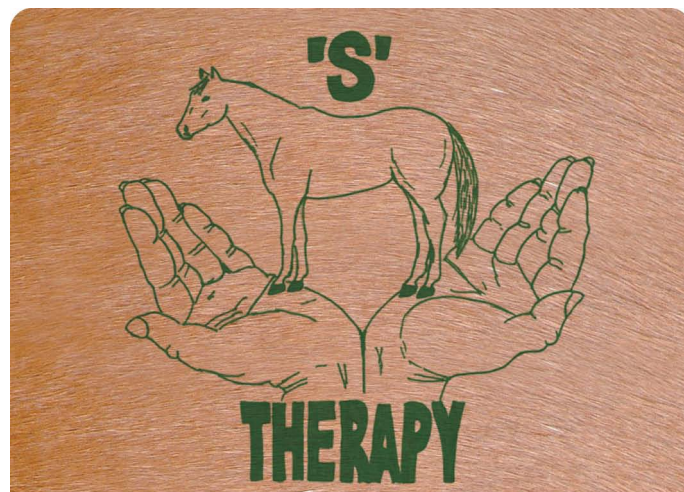
can be cloned where the owner so desires and the facilities and funds are available.

Of course, misuse and manipulation are a concern with any breeding technology. Identity disputes should not be a problem with proper management and considering the significant variation in the appearance of equine clones. Unfair competition is also considered an unlikely complication as performance depends on far more than just genes, as discussed above.

An arguably less concerning issue relating to cloning is the reluctance of some breed societies to register cloned animals. I can't really comment on the cause of this but am not aware of any science based reason to validate the policies. However this certainly does not apply to all organisations, and is likely to change in the future as the technology becomes more widely accepted. Generally this is not a problem for competition animals as clone status won't prevent them (or their progeny) from participating in events – e.g. cutting, dressage, showjumping.

From my perspective as a veterinarian, cloning is another valuable tool in our toolbox of assisted reproductive techniques – but one whose aim is preservation rather than progress. Health and welfare of the horse are paramount, and if I felt these were threatened in any way by such technology, no matter how great a scientific milestone, I would not be writing this article. The greatest benefit of cloning technology lies in acting as an 'insurance policy' against the loss of animal genetics that we cherish, for whatever reason – be it performance, conservation, or emotional value.

*Remember this is a vast and complex topic. This article is by no means a complete summary and I encourage everyone to seek out the widely available literature on the subject for further reading!* 📖



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