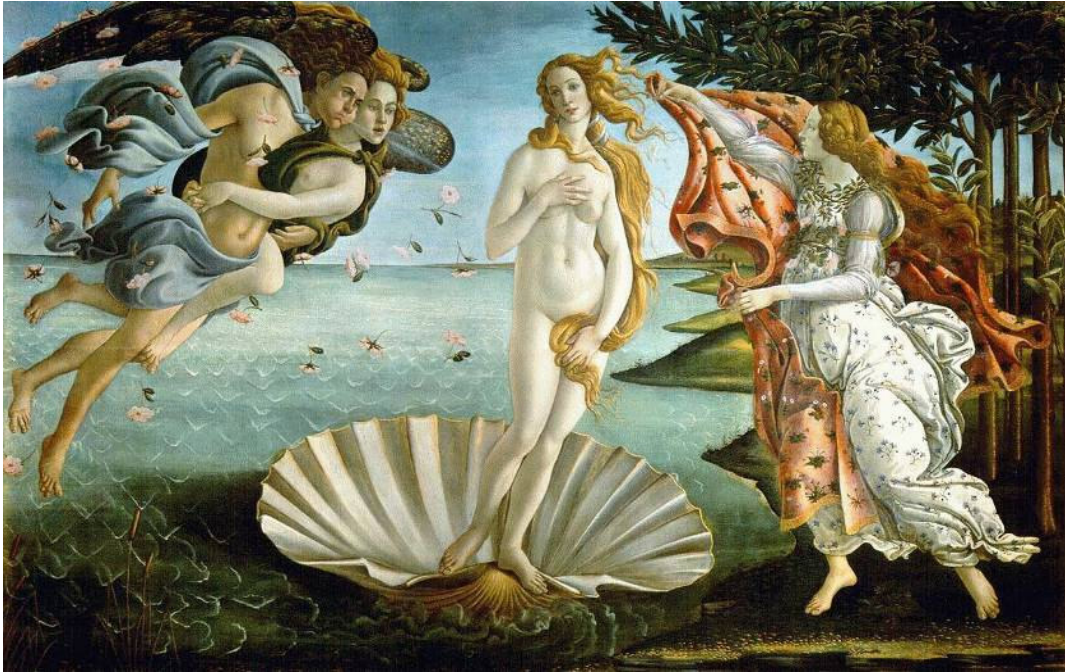


Equine Medicine and Reproduction



Breeding Confidence

Inaugural Lecture, April 14th 2008

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Faculty of Veterinary Medicine

Breeding Confidence

Inaugural Lecture

delivered with respect to acceptance of the office of Professor of Equine
Medicine and Reproduction at the Faculty of Veterinary Medicine, Utrecht
University on Monday 14th April 2008

by

Dr. Tom Arjun Edgar Stout

Right Honourable Rector Magnificus,
Professorial colleagues, lecturers and employees of the University,
Lady and gentlemen students,
Family, friends and colleagues from outside the University.

Esteemed listeners, Ladies and Gentlemen,

You have undoubtedly registered that the title of my inaugural lecture is ambiguous; it can be interpreted in two different ways. This is no accident. I deliberately chose this double-edged title because it neatly defines the multi-faceted nature of the chair to which I have been appointed; 'Equine Medicine and Reproduction'.

Given my background in reproduction, the most obvious interpretation of the title '*Breeding confidence*' is confidence in the breeding industry. This relates directly to the current and future role of my group in fundamental and applied research into (in)fertility and assisted reproduction, primarily in the equine species. In other words, the development of a scientific basis for confidence in the Dutch breeding industry, including the prediction and monitoring of the fertility of breeding horses and the development of more successful and reliable techniques to achieve and maintain pregnancy in horses. The other possible interpretation of 'Breeding confidence' is the more general 'propagation of trust'. The Equine Department has only existed in its current form for 9 years, but even in that short period it has been through a number of difficult moments including a second reorganisation arising from financial necessity. With regard to the Chair of Medicine and Reproduction, the combination of the two disciplines under one head has stimulated considerable comment both within and outside the department. With this in mind, I would like to use this public platform to share my vision of the future of the combined discipline and the role of its professor. The disciplines are not going to be merged; but they will be brought closer together. It goes without saying that it is no longer realistic to expect the chair to be the highest authority over the full range of veterinary medicine under his charge. But it is his responsibility to ensure that the necessary expertise is in-house, in the form of the senior lecturers and lecturers under his command. The professor is, in short, primarily the manager of a group of specialists and emerging specialists. In situations where a new leader or manager is appointed who is not leading authority over the full breadth of his area of responsibility, one of the most important formative management tasks is the previously alluded to 'propagation of trust'. This applies not only to the propagation of trust within the group; it is just as important to ensure that outsiders have confidence that the group under its new leadership is heading in the right direction. Within the group, the main initial task is to establish and transmit a clear, broadly-supported, forward-looking strategy. From the group, the emphasis will also be on communication; giving a clear signal to the Dutch equine industry and the international veterinary community that the group is productively and innovatively pushing the boundaries of veterinary education, research and patient care.

With respect to the environment within which we operate, these are exciting times for the Dutch equine industry. Recent years have witnessed an enormous growth and development in the hobby and sport horse industries. The number of recreational horses and riders has increased significantly, Dutch horses and riders have been successful in equestrian sports, and foreign interest in Dutch bloodlines is growing rapidly. As a result, the equine industry is becoming an increasingly important part of the Dutch economy. Recent estimates suggest well over 400,000 horses in the Netherlands and a total turnover of around €2 billion; larger than the famous Dutch flower-bulb industry. This growth in the equine industry has not gone unnoticed by the national government and, as a result, the welfare and interests of both horse and rider are increasingly an integral part of policy for the countryside and agriculture.

The horse's place in society has also changed. The horse of today is increasingly either of significant financial value as an elite athlete or parent of elite athletes, or of inestimable value as a companion. The horse is thus much more than the agricultural animal of old. This

shift in the value and use of the horse has inevitably left its mark on equine veterinary medicine. Horses can now be registered as ‘not intended for human consumption’ and thereby qualify for a much wider assortment of medicinal products. On the other hand, rider, trainer and veterinarian need to be aware of the possible consequences of administering medicines to the equine athlete; horses can also be caught and punished for ‘doping’. The flourishing international market for horses, equine semen and equine embryos is also laced with possible consequences. The risk that exotic diseases, or at least diseases novel to the Netherlands, being imported has increased enormously; yet another challenge for all those involved with equine health.

Veterinary medicine as a whole is also changing. Just as in human medicine, enormous advances have been made during the last years and decades in the areas of knowledge, skills and equipment. Veterinary medicine is becoming increasingly complex and specialised, primarily because of the increasing demands and expectations of owners, and also because the expectations of the owners of small animals, horses and farm animals are becoming increasingly divergent. It will, therefore, come as no surprise that the omniscient vet evocatively and humorously described by James Herriot, someone who was able to treat ‘all creatures great and small’ with similar skill and with whom the general public most readily associate the word ‘veterinary’, may soon be the exception rather than the rule. Although Dutch vets are still trained to treat numerous species in the event of an emergency, it is no longer realistic to imagine that the necessary knowledge and technical expertise required to remain at the ‘cutting edge’ over the full breadth of veterinary medicine can be assimilated in one person. Neither is it realistic to expect that a student, in 6-7 years, acquire the necessary skills to satisfy the demands of clients in all the various branches of veterinary medicine from day 1 of his or her fledgling career. It is no coincidence that the number of dedicated, recognised or specialised equine veterinarians and referral clinics has increased during the last decade. In this light, the reorganisation of the faculty of veterinary medicine from a discipline based (Reproduction and Obstetrics, Internal Medicine, Surgery) to an animal type (small animals, farm animals, horses) entity was inevitable; as was the decision to compel students to graduate with a ‘study-direction’. Nevertheless, the cross-species, broad basic education is certainly not yet confined to history’s dustbin. On the contrary, I am convinced that it is precisely the comparative nature of this education that uniquely equips the veterinarian as practitioner, researcher or policy maker to be creative problem solver with the ability to think beyond the narrow confines of his or her own direct field of interest.

Of course there are downsides to a radical reorganisation. For example, the loss of a huge amount of knowledge and experience within an extremely short period of time, not to mention the fact that while the total staff numbers decrease, the workload doesn’t. However, there are also plus points to a reorganisation, and in our case these included the rebuilding of the clinic. Although the building process *per se* resulted in a number of years of disturbance, the ‘state-of-the-art’ facilities that we now have at our disposal were well worth the inconvenience. As a department, we are proud of our new facilities. At the same time, we are well aware that we now need to justify the investment by ensuring that Utrecht becomes recognised as a force and pioneer in equine medicine, in at least a number of carefully selected ‘focus areas’. I deliberately use the phrase ‘become recognised’ even though Utrecht has long been one of the big names in veterinary medicine because the competition from international veterinary schools and both national and international horse practices has increased dramatically; recognition as a ‘force to be reckoned with’ needs to be earned anew. The same applies to research; we have fantastic facilities, we have chosen to focus on a

narrow range of topics and we have updated our organisational structure to facilitate collaboration between clinicians and fundamental researchers. But here the challenge is even more daunting. To justify and preserve the basic central funding, an equivalent amount of money needs to be acquired from other sources (such as competitive scientific grants or industrial partners) and the research needs to be published in scientific journals with a suitably high '*impact factor*'. And all this needs to be realised without losing the feel for the veterinary field or deviating from the veterinary mission of the faculty.

What is my group actually going to do? I will start with what we have to offer to the 'top end' of the horse breeding industry. Quite rightly the breeding industry is dedicating increasing attention to genetic progress. To hasten this process, assisted reproductive techniques are being utilised on an increasing scale; in particular, embryo transfer and insemination with 'low-doses' of cryopreserved stallion sperm have seen a massive surge in interest. This intensive use of the genetic cream of the sport horse population has meant that any deficiencies in fertility have been more rapidly and determinedly unearthed. This can be a disaster for an owner who has invested time and money in a potential breeding animal. On the other hand, in the longer term it could be even more of a disaster for the studbooks if, with the help of expert veterinary attention and advanced reproductive techniques, we carry on breeding from animals with poor innate fertility, particularly if they transmit these deficiencies to their offspring. This is why it is important to examine the causes of subfertility; only then will we be able to determine whether the problem is 'fixable' and whether the fix is unlikely to have any detrimental effects in the longer term. The two topics that we aim to concentrate on in the short to mid-term are 'markers' for semen quality in the stallion and early embryonic loss in the mare.

Nowadays, and with the aid of artificial insemination, we expect the most popular breeding stallions to impregnate hundreds of mares per year. Whether this is realistic is either left to fate or estimated on the basis of a pre-breeding semen evaluation. Sometimes the semen quality of a stallion is so poor that it is immediately obvious that the goals need to be readjusted. However, it is also quite common for a stallion to satisfy the requirements for normal semen quality and still turn out to be poorly fertile or infertile. This is frustrating for everyone involved, not least because they have assumed that everything was OK. For the sake of comparison: in human medicine, the approach to male-factor infertility is currently quite straightforward; *in vitro* fertilisation via intracytoplasmic sperm injection. For a sub-fertile bull or boar the solution is also pretty straight-forward; slaughter. In the case of a valuable stallion, the decision is somewhat more complicated. If the animal represents an unusual bloodline or is worth vast amounts of money, an owner is unlikely to give up just like that. On the other hand, we need to ask ourselves whether it is responsible to produce offspring using modern reproductive techniques if it transpires that the underlying cause of sub-fertility is heritable. Before we are in any shape to give a sensible answer to this hypothetical question, however, there is an awful lot to learn. Suffice to say that the current routine semen evaluation does not provide sufficient information to make an accurate prediction of expected fertility, or to reveal a subtle defect resulting in infertility. On the other hand, during the last decade a number of parameters have been described, and related tests developed, that can give a much clearer picture of the fertilizing capacity of single spermatozoa (and by extension, the donor stallion). Unfortunately, these tests have yet to be validated and implemented in practice. The routine use of such tests would certainly make it easier to determine the cause of reduced fertility and to subsequently give advice about how to manage the problem. In the fullness of time, it will almost certainly become possible to prospectively examine a stallion, or semen preserved using various techniques, for likely fertility, and make much more accurate

predictions of the number of sperm required for an insemination dose that offers an optimal chance of pregnancy. It should be obvious that we are referring to the translation of a number of years of preparatory research to the practice situation. To enable this, we are going to need the collaboration and support of the end-users, the breeding industry, up to and including financial support. Optimising these tests for use in the field is only likely to succeed if the tests can be performed on the semen of popular stallions of varying fertility levels, and the results coupled directly with the hard fertility data.

With regard to the generation of new scientific information, in the coming years we intend, in collaboration with the Department of Biochemistry, Cell Biology and Histology to concentrate on the processes involved in the maturation of a sperm during its journey from the testicle to the place of fertilisation in the oviduct of the mare. When a sperm leaves the testicle, it is neither capable of independent motility nor of binding to or fusing with an oocyte. Only during a complicated, and poorly understood, two week maturation process in the epididymis does a sperm develop these characteristics vital to fertilizing capacity. Precisely because any disturbance to this maturation process can result in infertility, we want to understand how it is regulated. This research will concentrate on the role of defined proteins and vesicles originating from the epididymis or accessory sex glands and present in seminal plasma (including 'epididymosomes' and 'prostasomes'. Similar vesicles have also been described in man, and while it is assumed that they play a critical role in either the fertilization process or in inhibiting the female immune system to prevent premature destruction of the sperm, their precise role has yet to be proven. In this sense, the stallion will be used as an animal model to elucidate the function of the vesicles.

The other major topic of equine reproduction research will be early embryonic death (EED), and the contributions to this phenomenon of embryo quality and embryo-maternal communication. EED is an important source of financial loss to the breeding industry; approximately 10% of all pregnancies detected on day 14 after ovulation have disappeared by day 42. The main causes of EED in the mare are not known and neither, therefore, is it clear if or how EED can be prevented. There is however evidence that EED is more common in aged mares or after the use of assisted reproductive techniques such as IVF and oocyte or embryo cryopreservation. In women, chromosomal aberrations are known to be the most common cause of embryonic death, with the incidence also increasing with maternal age and the use of IVF. Recent research by our group suggests that chromosomal abnormalities are also likely to be a major cause of EED in the horse. Moreover, it appears that abnormalities already present, or acquired (e.g. during *in vitro* culture or cryopreservation), by the oocyte contribute to reduced embryo quality. One of these abnormalities is a reduction in the number of functional mitochondria. Nevertheless, further studies are required to determine the influence, type and causes of chromosomal abnormalities in EED in horses, including the predisposing factors and the cellular mechanisms by which these factors exert their detrimental influence on chromosome division and further cell division and differentiation. In this respect, we will initially be reliant on experience and expertise available in the field of human reproductive medicine. Subsequently, we expect the mare to become an extremely suitable model from which to generate information on the cellular causes of chromosomal non-disjunction and in which to develop better techniques for examining embryo quality.

This is not to suggest that EED results exclusively from embryonic abnormalities. The uterine environment and the exchange of biochemical signals between embryo and dam also play crucial roles in the establishment of pregnancy, for example by initiating the endocrinological changes responsible for ensuring that the mare remains in the progesterone

dominated gravid state. How the early horse embryo signals its presence within the uterus to initiate the necessary physiological changes is still a mystery, although there is an increasing body of information with respect to molecules and receptors that might be involved. Moreover, the completion of the equine genome project and the development of increasingly powerful molecular biological techniques mean that there is any increasing range of approaches at our disposal to finally answer the question: how does the mare know that she is pregnant?

The other half of my group, Internal Medicine, will also concentrate on two clinically relevant topics in the coming years: the '*equine athlete*' and '*emerging diseases*'. The decision to focus on the equine athlete was easy, given that horses are primarily bred for their athletic prowess and given that the discipline already has considerable expertise and know-how in this area of science (including two staff members who have recently received their PhDs). This research will attempt to determine characteristics of both the healthy athlete in full training and 'overtraining', and to examine their physiological or pathophysiological relevance. Newly developed markers and diagnostic tests will be translated as rapidly as possible to a multidisciplinary (i.e. together with orthopaedic surgery) clinical setting to improve our capacity to determine the reasons for, and design appropriate treatments for, poor performance. It is probable that the future of equine exercise physiology, just as for the elite human athlete, will involve a more proactive approach with the accent on athletic capacity and recovery during training or after competition as part of a structured training programme aimed at achieving and maintaining peak performance. To achieve this aim, our fundamental understanding of muscle and exercise physiology in horses first needs to be extended, and techniques developed to monitor the primary performance influencing parameters.

The '*emerging diseases*' are a threat for which the Dutch and European horse industries are going to have to be alert in the coming years. The climate is changing and as a direct consequence the diversity of vectors able to spread exotic horse diseases is expanding. These include various types of ticks, mosquitoes and biting flies. The EU and national government are already developing policies to prevent or control potential outbreaks. This includes stimulating the development of reliable diagnostic tests and techniques to fight transmission (e.g. vaccination). The '*Blue tongue*' epidemic of the last two years is an object lesson in how a previously unrecorded disease can spread like wildfire and, in the worst case, become endemic; all because the vectors are present year round. African Horse Sickness (AHS) belongs to the same virus family as blue tongue, and is spread by the same vectors. If AHS ever makes it to the Netherlands, the chance is high that it will spread rapidly, and because AHS is fatal in the majority of affected horses, this needs to be avoided at all costs. In collaboration with the Veterinary Faculty in Pretoria, South Africa experiments are currently being initiated to generate essential basic information about the time course of AHS viraemia and to improve available diagnostic tests. The ultimate aim will be to assist in the development of a better and safer vaccine. Because it is also known that viraemic bulls shed blue tongue virus into their semen, we will also address the question of whether this occurs with AHS. If it is the case, it has obvious implications for the international transport of stallion semen from AHS affected areas. These studies will use donkeys infected with virulent AHS virus (donkeys do not die of infection) or stallions vaccinated with a live virus vaccine after their arrival in an AHS area.

Besides AHS there are other *vector-borne* diseases that can dramatically affect the welfare of the individual horse, the export status of a country or even the health of the human population (e.g. West Nile virus). A number of these diseases are already present within either

Europe or even the Netherlands. However, given that their prevalence is not known, it seems sensible to initiate a national survey to establish the incidence and distribution of the diseases and their vectors. We intend to initiate such a survey in collaboration with the 'Utrecht Centre for Tick-Borne Diseases' and the 'Veterinary Medical Diagnostic Centre', both sub-departments of the veterinary faculty.

Horses can also suffer from the effects of biting insects without transmission of any disease; this is for example the problem in the midge hypersensitivity known colloquially as 'sweet itch'. In recent years, the discipline of medicine and partners at Utrecht and Wageningen Universities have conducted studies to determine why some horses are more sensitive for midge bites than others, how the related skin disease arises and whether it is possible to develop tests for midge hypersensitivity to enable preventative measures to be taken sooner rather than later; this research will continue.

I would like now to make a small digression from the 'horse' to describe the last area of research in which my group participates. As a legacy from my predecessor, Professor Ben Colenbrander, and as an element of the 'memoranda of understanding' with Pretoria University in South Africa and various veterinary faculties in Thailand, including Kasetsart and Chiang Mai, we have also become engaged in research into biodiversity and the reproductive biology of wild and endangered species. Experience with assisted reproduction and cryobanking is of course a useful start in these fields. Even more useful is the fact that experience of certain reproductive interventions in horses, is useful for predicting likely effects in elephants; for example, equine experience has proven critical in the development of contraceptive vaccines for elephants. In collaboration with Pretoria we are therefore currently examining the efficacy, applicability and safety of contraceptive vaccines in elephants. As you will probably be aware, in a number of south African countries the elephant can no longer be considered 'endangered'; indeed, the number of elephants in some reserves has increased to the point where they are destroying the vegetation and threatening biodiversity. Contraception is seen as a long term alternative to culling family groups. However, before we can conclude that contraception is preferable to shooting, we need to know for sure that the vaccine is effective and has no unexpected negative consequences for either the health of vaccinated animals or the family or social bonds that are so crucial to elephant society. In Asia the problem is reversed, there are too few elephants left in the wild because their habitat has been destroyed by man. And neither has breeding within the domestic population been easy; sub-fertility appears to be a common issue in elephant bulls and there are fears that the rates of inbreeding are rising rapidly because only a small number of bulls are used for breeding. To establish the extent of the problems, a European Union subsidised project was recently initiated together with institutes in London, Thailand and Sri Lanka to examine aspects of genetic diversity and reproductive biology in Asian elephants. Our wildlife connection was given a recent extra boost via an agreement with the Glasgow based Institute for Breeding Rare and Endangered African Mammals (IBREAM) to base its Global Scientific Project Coordinator in Utrecht.

I have almost reached the end of my lecture, and some of you have undoubtedly noticed that I have dedicated very little time specifically to teaching or patient care. That does not mean that I consider them less important. On the contrary, the large busy clinic is the reason that I came to Utrecht, and the reason why I stayed. Patient care is our main area of contact with the real world, and the problems that arise in veterinary practice are, and should remain, both the inspiration and the ultimate goals of our research. Also in patient care, I am a great fan of innovation and will absolutely not settle for the 'tried and trusted'. Alongside

treating the patients entrusted to our care, we have an important part to play in the training of the next generation of vets and specialists. Disseminating knowledge and teaching skills, clinical acumen and self confidence to enthusiastic, students or would-be specialists with a thirst for knowledge is a special part of our daily business. I admit to being thoroughly old fashioned in that I consider it just as important that students develop a feeling of responsibility, the ability to make decisions and the realisation that we have duties and not only rights, as well as learning facts or techniques. Patient care and the related teaching is also our primary point of contact with our colleagues in practice. In this respect, the development of expensively equipped specialist equine clinics means that the exchange is increasingly two-way; we can learn from each other and collaborate in useful retrospective and prospective applied research projects. Moreover, just as the practices now have a structural role in student education, we should look at establishing a role in the training of veterinary specialist via an exchange of trainee specialists and staff.

I would like to end with a few words of thanks. Today is a special moment in my scientific career; a point at which I have arrived thanks to the help, advice and support of many; too many to name them all. Nevertheless, I would like to say a few words about the cadre of 'wise men' who have been instrumental in my development as a clinician, teacher and scientist and who by some bizarre coincidence all retire this year. Professor Twink Allen introduced me to equine reproduction and science, and I was privileged to be able to learn from him how science should be conducted, written and presented. It is a great shame and a scandal that the Thoroughbred Breeders Association of Great Britain thought it fitting to use the moment of Professor Allen's retirement to end the existence of his brain child, the 'Equine Fertility Unit'. The EFU has done a huge amount for the breeding industry and I am convinced that, in the fullness of time, the industry will regret its decision to shut the Unit with such obscene haste. In Utrecht, I came under the mentorship of Professors Ben Colenbrander and Bert van der Weijden. Professor Colenbrander is well known as a networker and facilitator *par excellence*; he pushed me, but above all allowed me the room to develop as a research leader. From Professor van der Weijden, and other colleagues from the old 'Obstetrics' department, I was able to learn skills, knowledge and a love of obstetrics and teaching obstetrics; and patience. The last would-be pensioner I would like to acknowledge is Andries Zandee; it has been a pleasure to work alongside such a committed, enthusiastic and encyclopaedic fan of Dutch horses. In short, all fanatics for whom it was much more than just a job.

I would not want to forget my family. I am delighted that my parents were able to join us. My interest in medicine and love for animals is down to them, and they also gave me the freedom and support to follow my chosen career path. The same is true of my wife Susanna, my favourite companion but due to my recent work commitments as good as a single mother to our two young children; I couldn't manage without your support based on understanding of the horse world and science. And it is with regret that I note the absence of her parents today due to health problems.

I have saved the last word for my colleagues, the people that I work with on a daily basis in the clinical disciplines of equine reproduction and medicine, the research group 'Biology of Reproductive Cells', the teaching committee for 'Reproduction', the University Equine Clinic and, last but by no means least, the Departmental management board. I hope that I have made it clear that I at least have confidence in a healthy and fruitful future for the Chair and those with which it collaborates.

Esteemed audience; thank you for your attention.

I have spoken