AUSTRALIAN COLLEGE OF VETERINARY SCIENTIST 2010 ANNUAL CONFERENCE
COLLEGE SCIENCE WEEK
GOLD COAST INTERNATIONAL HOTEL
1-3 JULY 2010
EQUINE CHAPTER CONFERENCE
PROGRAM AND PROCEEDINGS

Scientific Meeting Equine Chapter Program Coordinator – John Chopin and Allan Gunn

Education Forum Coordinator – John Hyland

Proceedings Editor – John Chopin
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<td>Katie Lott</td>
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SPEAKERS BIOGRAPHIES

Angus Adkins graduated from the University of Melbourne in 1989. He worked in racing and stud practice at Flemington before moving to Murdoch University in Western Australia as a resident in equine medicine and surgery. Angus gained Membership of the Australian College of Veterinary Scientists in equine surgery in 1995 and Fellowship of the College in 2002, allowing him to be registered as specialist in all aspects of equine surgery.

John Aitken graduated with a PhD from the University of Cambridge in 1974. After postdoctoral fellowships with the World Health Organization in Geneva and the University of Bordeaux, he moved to the MRC Reproductive Biology Unit, University of Edinburgh, where he received an Honorary Professorship in the Faculty of Medicine and in 1995 was elected a Fellow of the Royal Society of Edinburgh. He is currently Director of ARC Centre of Excellence in Biotechnology and Development and Laureate Professor of Biological Sciences at the University of Newcastle. His research interests focus on the cell biology of germ cells, fertilization and early embryonic development.

Stephanie Ayres graduated from the University of Melbourne in December 2008 with an interest in small animal surgery. She completed a rotating internship in emergency and critical care, internal medicine and surgery at Pet Emergency and Queensland Veterinary Specialists in 2009, and has recently moved to the Pet Emergency and Specialist Centre in Melbourne to undertake an 18-month surgical internship.

The science career of Mark Baker began at Monash University, Melbourne Australia, majoring in both Biochemistry and Chemistry. Mark accepted a position with Dr. Alfons Lawen studying the biochemical mechanisms responsible for the mediation of apoptosis. He was awarded an Australian Postgraduate Award to embark on a PhD where he purified, identified and then cloned, for the first time ever, a transplasma membrane NADH-reductase, namely Porin 1. Mark was then invited to work with Prof. R. John Aitken at the University of Newcastle on the role of such systems in the generation reactive oxygen species by male germ cells. He managed to identify the enzymes responsible for NADH and NADPH-depant reduction of lucigenin, work that has now unraveled the seemingly highly controversial area. Mark has pioneered new areas of research into sperm proteomics. In particular, he has introduced Difference in 2D-gel Electrophoresis (DIGE) and label-free LC-MS/MS quantitation to determine proteins that become functionally modified in spermatozoa. This work has led to numerous publications and two international awards - a Postgraduate Trainee Merit Award and a Lalor International travel award which were presented at the 2005 American Andrology Society meeting in Seattle. Both awards were based on the outstanding contribution to reproductive science. The proteomic analysis is recognized worldwide invitations to speak at the Lourne proteomics conference, the prestigious Gordan conference, AOHUPO conference in Cairns and recently, to the 2009 HUPO mass spectrometry convention in Berlin.
Patrick Brogan has a Bachelor of Science 2000 with Psychology/Biology major, graduated Vet with honours from Sydney in 2005. He spent 6 Months in race track/small animal practice at Newcastle. Patrick then moved to Satur Veterinary Clinic (Scone NSW) in July ’06 until April ’08 then became a locum Veterinarian in mixed practice April ’08 until January ’09 in Perth. Patrick commenced Murdoch University January ’09 as inaugural resident in Veterinary Reproduction. He is with Murdoch University Equine Reproduction Services August ’09 which is soon to expand into Canine services.

Sophia Butler is a PhD candidate supervised by Professor Michael McGowan at the School of Veterinary Science, at the University of Queensland. She is completing her PhD in developing oestrous synchronisation protocols for fixed-time artificial insemination of Bos indicus heifers in northern Australia. Sophia completed a Bachelor of Science in Agriculture with first class Honours at the University of Sydney. Sophia has been involved in the stud stock industry from her family cattle property in the Hunter Valley in NSW, and moved to QLD to pursue her love for reproduction in the Beef industry.

Rebecca Butt graduated from Western College of Veterinary Medicine in 2002 and practiced in a rural, mixed practice in the central USA for two years. In 2006, Rebecca completed an internship in large animal veterinary medicine at the University of Guelph, in eastern Canada.

Joan Carrick graduated from the University of Queensland in 1983 with post-graduate honours in Equine Medicine. After some years working and studying in America Joan’s interest in intensive care and biomedical research lead her to the University of Tennessee Post-Graduate Medical School to undertake a Fellowship in Critical Care. Joan became a Diplomate of the American College of Veterinary Internal Medicine (Large Animals) in 1997 and then returned to Australia to work.

John Cavalieri graduated from the University of Melbourne with a BVSc in 1986. Following graduation he worked in rural veterinary practice and spent a short time in equine practice. He completed a PhD at James Cook University in 1996 on the topic of synchronisation of oestrus in Bos indicus cattle, and a residency and Board certification in Theriogenology in the USA in 1998. After returning to Australia he worked as a research fellow at the University of Melbourne until 2001 and then started a private practice focusing on veterinary reproduction in 2002. At the end of 2005 he started work at James Cook University assisting with the development and implementation of the new BVSc curriculum. He has been a registered specialist in veterinary reproduction since 2002 and obtained a graduate certificate in education in 2008.

Judy Cawdell-Smith (BVSc University of Sydney 1982; DipVetClinStud University of Sydney 1983) is a lecturer in Equine Science at The University of Queensland. Following graduation she undertook and internship and residency at the then Rural Veterinary Centre at Camden under Professor David Hutchins. After a period of running a horse transport business and study with her husband Terry, Judy returned to equine stud practice in the Hunter Valley. She then moved to the University of Queensland. She is currently completing her PhD in the role of the Processionary Caterpillar in Equine Amnionitis and Foetal
Loss. Her main areas of interest are pregnancy, more specifically foetal and placental development and mare infertility.

**John Chopin** graduated from The University of Queensland Veterinary School in 1990. After 18 months in general mixed practice, John completed a residency in equine medicine and surgery (UQ). John then completed a PhD with Dr Kerry Dowsett in equine frozen semen and the endocrinology of ovulation in the mare. A theriogenology residency followed, mentored by Dr David Pascoe and Dr Reg Pascoe at Oakey Veterinary Hospital. This included a teaching sabbatical at The UC Davis. From here John completed his Fellowship and joined Coolmore Australia in the Hunter Valley as an equine reproductive specialist.

**Iain Clarke** spent almost 30 years as a research scientist/fellow at Prince Henry’s Institute of Medical Research. He moved to Monash University in 2005 and became Chairman of Dept Physiology in 2007. His research has largely focussed on ways that the brain controls hormonal systems in the body and vice versa. He has published over 400 research papers. Professor Clarke uses large animal models to investigate numerous aspects of how brain systems control reproduction and metabolic function. Current projects are:-

- Analysis of control of food intake and energy expenditure in genetic models
- Endocrine profiling for predisposition to obesity
- Novel methods of controlling energy expenditure in muscle
- Role of kisspeptin in reproductive function
- Gonadotropin inhibitory hormone (GnIH) control of reproduction and feeding
- Melanocortin control of metabolism and reproduction

**Jessica Crisp** grew up on a beef cattle property near Wandoan in South West Queensland. She attended the University of Queensland St Lucia campus and studied a Bachelor of Agricultural Science specialising in Animal Science (Hons). After a year as a Technical Officer at Katherine for the Northern Territory Department of Primary Industries, Fisheries and Mines, she started her PhD also at the University of Queensland in the School of Veterinary Science. Jessica’s PhD is looking at novel early life indicators of *Bos indicus* bull fertility; she is currently in her third year.

**Simon de Graaf** is a Lecturer in the Faculty of Veterinary Science at The University of Sydney of basic reproductive biology and clinical techniques in assisted reproduction. He manages an active research program supervising undergraduate and postgraduate research students in the development of sperm sexing technology in domestic and wildlife species, the role of seminal plasma in sperm function and the interaction between sperm and the female reproductive tract. He has published a book chapter on reproduction in sheep, over 20 refereed articles in international journals and presented research
findings at numerous conferences, several of which were invited national and international conference papers. Simon consults to the Australian artificial breeding industry, including providing instructional courses for standardisation of semen assessment, processing and freezing for semen processing centres.

Laura Fennell was an intern and reproductive associate at Goulburn Valley Equine Hospital before moving to the University of Melbourne, Werribee to undertake a residency. She is sitting fellowship examinations in Equine Medicine in 2010 and is currently working with the Flemington Equine Clinic.

Erica Gee is Massey veterinary graduate who worked in large animal practice before returning to university to complete a PhD (Copper nutrition in pasture-fed pregnant mares, and the effects on developmental orthopaedic disease in their foals). From 2006-2008 Erica completed a residency in equine reproduction at Colorado State University, and in 2008 became a Diplomate of the American College of Theriogenologists. Erica is currently employed as a senior lecturer at the Institute of Veterinary, Animal and Biomedical Sciences, Massey University.

Zamira Gibb graduated from the Bachelor of Animal and Veterinary Bioscience with first class honours in 2006. In 2007 Zamira was awarded the Rural Industries Research and Development Corporation Postgraduate Research Scholarship to undertake a PhD focussed on improving the efficiency of flow cytometrically sex-sorting stallion spermatozoa. Zamira is in the final stages of completing her thesis and will be submitting in September 2010.

Allan Gunn, from Barn Veterinary Services, Muswellbrook, NSW, has practiced veterinary medicine in the UK, Zimbabwe, China and Australia, with the last 10 years as an equine stud veterinarian. He went on to achieve MACVSc qualifications in horse medicine and reproduction and became a Diplomate of the American College of Theriogenologists in 2008.

Dave Hanlon graduated from Murdoch University in 1992 and then completed a 3yr residency and Masters Degree in large animal reproduction at Massey University. In 1996 he joined Matamata Veterinary Services where he provides specialised equine reproduction services. In 2001 he became a Registered Specialist in equine reproduction after become board-certified with the American College of Theriogenologists. In 2002 he was invited to lecture in equine reproduction at Cornell University, New York and was the resident veterinarian at Cornell's Equine Research Park for the northern hemisphere breeding season. Dave is an adjunct Senior Lecturer in Equine Reproduction at Massey University and is currently completing a PhD. Current areas of research that Dave is involved in are: identifying the causes of reproductive wastage in thoroughbred mares, development of a new intravaginal progesterone-releasing device for oestrus control in mares and using the mare to develop a model for human infertility.
**Carl Hockey** graduated as a Veterinarian from The University of Queensland in 2002. He then took up a position as an intern at The University of Queensland’s Dayboro Veterinary Surgery, where he was involved in practising and teaching mixed animal medicine and surgery with a focus on dairy cattle. Whilst at the Dayboro Veterinary surgery, he began a part time Masters Research project on methods of selecting cows for AI, which upon receiving a scholarship from Dairy Australia he upgraded to a full time PhD in 2005. Since this time Carl has been conducting studies into improving methods of selecting cows for AI including assessing the performance of activity meters in dairy cattle under paddock management systems, and the effects of interval from AI to ovulation on conception rates. Carl is currently in the process of submitting his PhD thesis and is looking forward to the next opportunity that this will lead to.

**Fiona Hollinshead** graduated from The University of Sydney, Australia in 1996 with a Bachelor of Veterinary Science (Honours) and in 2004 with a PhD entitled, “Flow cytometric sorting of ram spermatozoa: Production of offspring of a pre-determined sex using in vivo and in vitro fertilisation.” From 2004 to 2006 she was a clinical resident in the Theriogenology (Small and Large animals) department at Cornell University, New York, USA and in 2006 became a Diplomate of the American College of Theriogenologists and in 2007 a Registered Specialist in Small Animal Veterinary Reproduction with the Australasian Veterinary Boards Council. Fiona currently runs a small animal reproduction referral centre located in New Zealand’s Waikato Region, Matamata (“Glenbred”) and is a consultant and Adjunct Senior Lecturer in The Faculty of Veterinary Science, The University of Sydney.

Following graduation from the University of Sydney in 2004, **Matt Izzo** worked in a mixed dairy practice on the south coast of NSW. In 2007 joined the University of Sydney as a resident in ruminant health and production. Currently completing a Masters degree looking at neonatal diarrhoea in the Australian dairy industry. Obtained membership to the ACVSc in dairy medicine in 2009. Currently undertaking the only fellowship program in dairy medicine and production in Australia under the guidance of Dr John House and Ian Lean. Recently, undertook an externship at the University of Wisconsin-Madison with some of the leading individual and population based cattle veterinarians in the world. Currently, the secretary of the cattle chapter; a member of multiple international organisations such as the AVA, ACV, NMC and AABP; accredited countdown advisor and a member of the NSW dairy pathways technical advisory committee.

**Haja Kadarmideen** is a Principal Research Scientist at CSIRO Townsville and Adjunct Professor at the University of New England, Armidale. Prior to joining CSIRO (in 2006), he was a chair and tenure-track professor of statistical and quantitative genetics at the Swiss Federal Institute of Technology (ETH) Zurich, Switzerland for over 5 years. He has veterinary medicine and science degrees from India (BVSc and MVSc) where he practiced large animal veterinary medicine for about 3 years. In 1994, he joined a PhD program in animal genetics / genomics at the University of Guelph, Canada. To date, he has
accumulated over 16 years of research and teaching experience and expertise in quantitative genetics and genomics, animal breeding, bioinformatics and computational biology in 6 countries (Canada, The Netherlands, UK, USA, Switzerland and Australia). Over his scientific career, he developed and used methods to estimate genetic parameters in animal pedigrees, detect genes/QTLs/eQTLs and use them in breeding programs, analyse high throughput genetic markers (SNP) and microarray gene expression data to detect causal genes and biomarkers for production, reproduction and disease traits in farm animals. In CSIRO, he leads a systems genetics group and various research projects that apply systems genetics approaches to the prediction of animal performance (in muscle growth, reproduction and parasite resistance) and genotype-environmental-managerial interactions in cattle grazing.

Natali Krekeker graduated from veterinary school in Berlin, Germany in 2001. Completed a doctorate degree (Dr. med. vet.) at the same school on osteochondrosis in warmblood foals and their dams. Undertook a residency in Theriogenology (animal reproduction) at Cornell University, USA, from 2002-2004 and got board certification of the American College of Theriogenologists. Worked as a Clinical Instructor in Theriogenology at Cornell University. Currently trying to finish up a PhD program in reproductive pathology with Dr Patrick Wright at The University of Melbourne.

Olga Lawlor graduated in 2003 from the University of Limerick with a BSc in Equine Science. Immediately she started work at Airlie Stud both hands on with the horses and running their equine laboratory. After 18 months she took a year out to travel and following this accepted a position at Scarvagh House Stud. She set up and managed an equine laboratory to service both the farms needs and that of surrounding equine practitioners. She spent several years working between the northern hemisphere breeding season with Scarvagh House Stud and the southern hemisphere breeding season, as a scientific officer in the laboratory at Scone Equine Hospital. She returned to the northern hemisphere in January 2010 after the southern hemisphere season and is currently writing an MPhil with Aberystwyth University on equine endometrial cytology and bacteriology. Olga has worked in the thoroughbred industry on three continents and has a love of equines of all disciplines.

Katie Lott graduated from University of Queensland in 2005. Completed a rotating internship at Queensland Veterinary Specialists and Pet Emergency in 2006 and a medicine internship at QVS earlier this year.

Tias Muurlink is a specialist surgeon and Head of Equine Clinical Services at the Equine Centre of the University of Melbourne. Prior to coming to the Equine Centre 5 years ago, Tias spent seventeen years were in private practice which included large amounts of equine stud work and surgery.

Scott Norman has worked in clinical practice in Queensland, including as a Registrar at the Pastoral Veterinary Centre, Goondiwindi and undertook his residency training in Theriogenology at the University of Florida in the late 1980’s. At the age of 26 he became the youngest veterinarian to be admitted as a Diplomate of the American College of Theriogenologists. Scott completed a PhD in the field of cattle reproduction and has produced 17 peer-reviewed publications with current research interests including the synchronisation of oestrus in mares and cattle, remote sensing of oestrus and parturition, the effects of nutrition on foetal growth and development, the role of kisspeptins in reproduction, the treatment of endometritis in mares, and methods of improving post-thaw viability of frozen semen. Scott is an active bovine and equine practitioner and is a registered specialist in veterinary reproduction. He joined the School of Animal and Veterinary Sciences at Charles Sturt University after a successful teaching career in the veterinary program at the University of Queensland which was acknowledged in 2006 by him being awarded the AVA teaching excellence award. Scott is a strong supporter of the AVA and is an active member of the Australian Cattle Veterinarians, Australian Reproduction Veterinarians and the Equine Veterinarians Australia.

Jim Rodger A Fellow of the Aust. College of Veterinary Surgeons, he is a registered specialist in Animal Reproduction (Equine), has a Post Graduate in Equine Medicine and has his own practice - Rodger and Associates in Jerry’s Plains, NSW.

Martin Sillence graduated with BSc(Hons) in pharmacology The University of Leeds, 1981, and PhD Animal Physiology and Nutrition, 1985. Two years as a postdoctoral fellow in the Department of Dairy and Animal Science, The Pennsylvania State University. From 1987, 9 years with CSIRO in the Division now known as Animal Industries in tropical beef cattle research, followed by 11 years at Charles Sturt University as Professor of Animal Science where he helped to establish an equine research program focussed on nutrition and reproductive research. Was involved in establishing vet science and led the establishment of the animal science degree programs at CSU before joining QUT as Assistant Dean (research) for the faculty of Science and Technology - a $30 million pa research enterprise with over 500 PhD students. Currently involved in 3 equine research projects with collaborators, concerning equine therapeutics, metabolic syndrome and laminitis.

Trained in Bacteriology and Immunology at the University of Glasgow Tony Smithyman is the founder of Cellabs Pty Ltd, one of Australia’s oldest biotech companies, which specializes in the development of diagnostic tests for tropical and infectious diseases. In recent years he has turned his attention to the rapidly escalating problem of antibiotic resistance and in 2005 formed Special Phage Services, a research
company aimed at the development of phage therapy products for the treatment of bacterial infection. Ongoing projects cover not only human and veterinary pathogens but also plant, aquaculture, and environmental targets.

After graduating from the University of Melbourne in 2009, Clint Yudelman took a slight detour in his career to partake in the reality cooking show My Kitchen Rules. In a balancing act between final year exams and dinner parties for the show, Clint managed to complete his veterinary degree and ended up coming second in the competition. Putting away the chef’s knife and slinging a stethoscope around his neck, Clint then jumped straight back into work where he is now employed as a rotating intern in EMCC, Internal Medicine and Surgery at Pet Emergency Room/Queensland Vet specialists in Brisbane.
This report is a retrospective analysis of artificial inseminations in bitches performed at Applecross Veterinary Hospital since 2007 comparing surgical insemination (SAI) with transcervical insemination (TCI) using fresh, chilled or frozen semen.

All surgical inseminations were performed once under general anaesthesia between 48 and 96 hours after ovulation as determined by serial serum progesterone concentrations. All TCIs were performed in conscious, standing bitches without the use of chemical restraint between 24 and 96 hours after ovulation as determined by serial serum progesterone concentrations and using a rigid fibre optic endoscope (Wilson, 1993).

Domestically and imported frozen semen that had been frozen by a variety of methods was used. Sperm numbers were estimated by manual counting using a Makler® counting chamber and recorded as the total number of live motile sperm used for insemination as counted immediately prior to insemination.

Pups born were the total number of full term pups born or delivered by Caesarean section.

410 pure bred bitches from 39 breeds were inseminated (327 SAI vs 73 TCI). No greyhounds were included in this study due to their apparent increase in fertility over the general pure bred dog population and likelihood of biasing the data (Pretzer et al, 2006).

Results are shown in Table 1.

Multiple paired t-tests yielded significantly fewer pups born per litter when comparing frozen semen TCI to SAI (P<0.001) but no other differences when comparing methods or semen type (P>0.05). Significantly more sperm were used in all fresh and chilled inseminations compared to either method using frozen semen (P<0.001).

The results are comparable with those published when allowing for number of inseminations, number of sperm per insemination and total number of sperm used per bitch (Linde-Forsberg et al 1989, 1989a, Thomassen et al 2006).

Reduced fertility was attributed to low sperm numbers, single inseminations and previously recognised failure to conceive problems within individual bitches. Pyometra developed in three SAI and two TCI cases post-insemination.
Table 1.

<table>
<thead>
<tr>
<th>Semen Type</th>
<th>Method</th>
<th>Bitches Inseminated N</th>
<th>Sperm Numbers x 10^6 (±SD)</th>
<th>Number Whelped N (%)</th>
<th>Pups born N (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>Surgical AI</td>
<td>138</td>
<td>319 (± 274)</td>
<td>80 (58.0)</td>
<td>5.2 (± 3.3)</td>
</tr>
<tr>
<td></td>
<td>TCI</td>
<td>52</td>
<td>641 (± 408)</td>
<td>36 (69.2)</td>
<td>4.3 (± 2.5)</td>
</tr>
<tr>
<td>Chilled</td>
<td>Surgical AI</td>
<td>18</td>
<td>414 (± 208)</td>
<td>15 (83.3)</td>
<td>4.4 (± 2.8)</td>
</tr>
<tr>
<td></td>
<td>TCI</td>
<td>8</td>
<td>542 (± 238)</td>
<td>4 (50.0)</td>
<td>6.5 (± 3.5)</td>
</tr>
<tr>
<td>Frozen</td>
<td>Surgical AI</td>
<td>181</td>
<td>111 (± 88)</td>
<td>107 (59.1)</td>
<td>5.0 (± 3.3)</td>
</tr>
<tr>
<td></td>
<td>TCI</td>
<td>13</td>
<td>104 (± 49)</td>
<td>6 (46.1)</td>
<td>2.8 (± 1.9)</td>
</tr>
</tbody>
</table>

References
Gestational Diabetes Mellitus (GDM) is a very rare form of Diabetes Mellitus (DM) which occurs during pregnancy. It is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Currently there is much literature published about human GDM but very little is known or published about canine GDM. This is a review of the current literature.

Pregnancy is a diabetogenic condition. Insulin resistance is a normal feature of pregnancy. Yet, despite the profound insulin resistance in late canine pregnancy, GDM is very uncommon in bitches when compared to other species such as humans. There are only 15 reported cases of GDM in the bitch. This is due to the young breeding age of bitches compared to the normal age of onset for DM in dogs and a lack of screening tests as performed with women.

The hormones of pregnancy implicated in GDM are progesterone and progesterone mediated growth hormone. They interfere with the action of insulin, causing a post-receptor deficit and effectively, type II DM. The net result is both an increase in insulin resistance and a decrease in insulin sensitivity. Despite the profound insulin resistance during late canine pregnancy, more so than in the non-pregnant dioestrus bitch, GDM still remains very uncommon. The significant foetal drain of maternal blood glucose is likely to explain this.

According to the present information on GDM, multiparous bitches develop GDM, usually in their third pregnancy. First onset is in late gestation. All reported cases of canine GDM have shown the typical clinical signs of DM during pregnancy leading to diagnosis. This is in contrast the human GDM where detection is made during pre-clinical stages of the disease via screening tests. The clinical signs of GDM are the same as DM with the classical quartet of polyphagia, polydipsia, polyuria and weight loss. Bitches treated by termination of pregnancy at time of diagnosis are less likely to develop permanent DM compared to bitches that go to term with insulin therapy.

GDM in the bitch is associated with a high neonatal morbidity and mortality rate. The neonatal mortality rate is almost double from bitches with GDM compared to healthy bitches. Eighty percent of the bitches that go to term experience dystocia from foetal macrosomia. Further complications may occur and the neonate has an increased risk of developing DM later in its life.


OBJECTIVE: To evaluate the characteristics of a population of bitches undergoing Caesarean section and report the associated aetiological diagnoses.

STUDY DESIGN: Retrospective case series of 510 cases of Caesarean section performed on 453 bitches between 1 July 1999 and 31 June 2009.

MATERIALS AND METHODS: Medical records were reviewed for information related to signalment, reproductive history, gestation, clinical features, ultrasonographic findings and treatment in bitches undergoing Caesarean section. The litter size and puppy mortality rates were reviewed. An aetiological diagnosis was sought for each case based on reported findings. The descriptive nature of the study dictated that statistical analysis was rarely required. Chi-square and Fisher’s exact tests were used and two-tailed p-values < 0.05 were considered significant.

RESULTS: Median age was 4 years with an interquartile range (IQR) of 2 to 5 years. Brachycephalic, dolichocephalic and giant breeds were overrepresented, whereas mesocephalic and small breeds were underrepresented compared with a control population consisting of all Australian-registered dogs. The mean interval from insemination or mating to Caesarean was 60.0 days (IQR, 59 to 61). In most cases (73.1%), the owner presented their bitch because of a perceived abnormality in the progression of labour.

Non-zero foetal heart rates were detected in 96.6% of cases in which ultrasound was performed successfully. In most of these cases (77.5%), the lowest recorded heart rate was 180 or less.

Forty-one bitches (9.1%) had anatomical vaginal abnormalities, the most common of which were stricture, stenosis or scarring of the vagina or vestibule and vaginal or vulvar hypoplasia. Eighty-one (17.9%) had uterine abnormalities, the most common of which were rupture, adhesions, ischaemia and torsion.

The most common anaesthetic agents were alfaxalone and isoflurane. The Utrecht pattern was used in 418 cases (88.9%). Few anaesthetic or surgical complications were reported.

Overall, 8.4% of pups were dead on delivery; mortality rose to 11.5% at 2 hours post-surgery. Particularly small (one or two puppies) or large (eight or more puppies) litters were observed in 76 (14.9%) and 84 cases (16.5%) respectively. Mortality was increased in cases where the lowest recorded foetal heart rate was less than 100 or greater than 200 beats per minute.

The origin of dystocia was considered to be maternal in 63 cases (13.2%), foetal in 177 cases (37.0%) and related to litter size in 79 cases (16.5%). In 160 cases (33.0%), it was idiopathic.
Discussion: These results suggest that risk of requiring Caesarean section is reduced in small and mesocephalic breeds and increased in giant, brachycephalic and dolichocephalic breeds. The mean interval from insemination to Caesarean of 60.0 days is considerably shorter than some previous reports. Clients and veterinarians should be aware of the variability in normal gestation length to avoid incorrectly diagnosing prematurity or failure to commence labour. Vaginal and uterine abnormalities were more frequently associated with dystocia than previously reported. Factors found to be associated with an increased incidence of uterine abnormalities were large breed size, large litter size, old age, pluriparity and previous Caesarean section.

Fifty-four bitches that ultimately required Caesarean section presented prior to the detectable onset of labour with no clinical abnormalities detected by the owner. This may prompt clinicians to offer pre-partum check-ups to allow early detection of dystocia and hence early intervention if required. In 45.4% of all cases, second stage labour had not commenced at the time of presentation. In these cases, since stage I labour is poorly defined and sometimes lengthy, the clinician without access to ultrasonography may be forced to wait until 24 hours have elapsed or until second stage labour commences before being able to confidently diagnose dystocia and consider Caesarean section. This is a concern, as foetuses are at risk of hypoxia and death during this time. With foetal ultrasound, the detection of low heart rates gives an early indication of foetal distress and allows more timely intervention.

Alfaxalone and propofol both confer minimal risk to the bitch when used for induction of anaesthesia. Isoflurane is appropriate for maintenance. A review of the medical records revealed no subsequent visits indicating complications of surgery involving dehiscence of the uterine incision, suggesting that the Utrecht pattern is appropriate for closure of the hysterotomy. Studies conducted in recent times have reported overall low bitch mortality following Caesarean section but this is the first study to record a zero mortality rate.

It is hoped that these findings may direct clinicians in their recommendations to clients with pregnant bitches and aid in the formulation of an appropriate protocol for diagnosis and treatment of dystocia.

References
While dystocia occurs in only five to six percent of canine and feline pregnancies, dystocia is a true obstetrical emergency and clinicians must be familiar with the various treatment modalities. Options for treatment of dystocia include manipulative therapy, medical management in the form of ebolic therapy and surgical therapy (caesarian section). This presentation will focus on the use of oxytocin as an ebolic agent. Oxytocin is widely overused in situations it may be contraindicated. Clinicians should be aware of these potentially life-threatening contraindications and how to recognize them. Dose rates and treatment protocols for oxytocin are not well established due to a deficiency of organized studies into the viability of oxytocin as a treatment for small animal dystocia.

Oxytocin is widely used in small animal practice as first line of treatment for dystocia. Contraindications to its use include obstruction, systemic illness of the bitch or queen, fetal distress, a closed cervix and maternal exhaustion. The most significant contraindication is the presence of an obstruction. Inducing uterine contractions against an obstruction may result in uterine rupture and/or separation of placental sites. Premature separation of placentas results in fetal hypoxia, anoxia and eventually fetal death. Identifying the presence of contraindications is difficult for the clinician to establish, with readily available techniques, such as history taking, physical examination and to a certain extent radiography, being subjective measures that do not offer the entire clinical picture. Less readily available techniques, including ultrasonography and tocodynamometry, provide tangible information for the basis of veterinary intervention. Ultrasonography identifies fetal distress, a clear indication for cesarean section, where tocodynamometry is capable of identifying the presence of weak uterine contractions, the primary indication for oxytocin.

The indications for ebolic therapy, primarily uterine inertia, are poorly defined with no real data to demonstrate their validity. Drug dosages for oxytocin vary widely between texts, providing opportunity for misuse of a potentially dangerous drug. Two retrospective studies on canine and feline dystocia have demonstrated a seventy per cent failure rate for patients treated medically, with a caesarian section required to deliver remaining fetuses. In one prospective study, where twenty-seven dogs were diagnosed with primary uterine inertia due to low serum oxytocin levels, an average of fifty-nine per cent of the medically managed cases required surgical intervention. The limited success with oxytocin in dystocia, in combination with the numerous associated contraindications and lack of data demonstrating an oxytocin deficiency in the parturient bitch or queen, makes oxytocin a controversial choice for universal treatment of dystocia. There is no consistency in recommendations for an oxytocin regimen in dystocia, due to the lack of scientific research in its use. With no way of definitively eliminating the presence of the most serious contraindication, obstruction, use of oxytocin in dystocia carries an inherent risk to the well-being of the fetuses and bitch or queen.

The author believes that a caesarean is the preferred method of treatment for dystocia and that owners should be informed of their options, with oxytocin considered only when cost constraints prevent surgical therapy. Recommended regimen for oxytocin involves no more
than one dose before moving to surgical therapy, with a canine dose of 1-5 IU subcutaneously or intramuscularly and a feline dose of 1-3 IU subcutaneously or intramuscularly.


Table 1: Indications for Dystocia

1. Crying and biting at vulva
2. No signs of labour within 24-36 hours of temperature decrease < 37.7°C
3. Abnormal vaginal discharge: profuse haemorrhage, odorous mucopurulent discharge, green discharge (dog) or red-brown discharge (cat) without production of off-spring (indicating placental separation)
4. Prolonged gestation – if the bitch or queen has gone > 70-72 days since first breeding, > 68-70 days since last breeding, or > 58 days from the onset of diestrus
5. No fetus produced after 2-3 hours of onset of stage II (clear discharge, intermittent contractions)
6. Partially delivered fetus for > 10-15 minutes
7. Strong contractions > 30 minutes with no fetus
8. Signs of systemic illness, toxemia: depression, weakness, fever, vomiting, tremors etc
9. Weak or absent contractions, resulting in delay > 2 hours without production of offspring
10. Failure to deliver all fetuses within 18-24 hours (24-36 for cats)
11. Evidence of an obvious cause of dystocia, e.g. pelvic fracture or a fetus palpably stuck in the birth canal

Adapted from: 1, 3-5,10
NEW INSIGHTS INTO ENDOMETRITIS IN THE BITCH

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The University of Melbourne, 250 Princes Highway, Werribee, Vic 3030

Background
It was widely believed that endometritis did not exist in the canine species and that a bacterial infection would inevitably result in pyometra. Recent studies, however, showed that bacteria, including uropathogenic E. coli, could be isolated from infertile bitches without showing the hallmarks of pyometra (Fontaine et al., 2009). In this study the bitches with a history of at least two unsuccessful subsequent breedings and a prior full infertility work-up were included in the study. Positive uterine culture and cytology were considered diagnostic for endometritis. Treatment was initiated on the basis of an antibiogram and all treated bitches conceived in the next cycle. Interestingly, the mean age of these bitches was four years and therefore more than three years lower than reported in bitches affected by pyometra. This suggests that bitches might contain bacteria associated with subclinical endometritis for multiple years before some unknown factors allow bacterial proliferation and subsequent pyometra. Further evidence for an underlying role of the impaired immune defense in uterine disease was observed when experimentally infected bitches did not show the typical recurrence of pyometra in subsequent oestrous cycles (Tsumagari et al., 2005). This suggests that the bacteria, which were introduced in dioestrus, were sufficient to induce pyometra but the research dogs were able to overcome the disease in subsequent cycles.

Diagnosis and treatment
Diagnosis of endometritis is recommended in late dioestrus because no bacteria should be found in the healthy uterus during that time. Intrauterine samples for bacterial culture and cytology should be obtained by transcervical catheterization by endoscopy. Sterile saline (2 ml/10kg body weight) is flushed into the lumen and reaspirated. The recovered liquid is then used to perform a bacterial culture and cytology. Cytology is interpreted as positive if leukocytes and bacteria (± phagocytosis) is present. A diagnosis of bacterial endometritis is made in case of positive cytology in combination with heavy growth of bacteria. A diagnosis of none-bacterial endometritis is made in case of positive cytology and no bacterial growth. If bacterial endometritis is diagnosed an appropriate antimicrobial drug should be chosen on the basis of sensitivity and given for 2 weeks. If diagnosis is done in dioestrus luteolysis should be initiated (e.g. aglepristone or prostaglandinF2α) in order to eliminate the risk of pyometra in those bitches.

References:

NEW INSIGHTS INTO CANINE DYSTOCIA

FK HOLLINSHEAD BVSc (Hons), PhD, Diplomate ACT, Registered Specialist in Small Animal Reproduction

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Dystocia is defined as the inability to expel one or more foetuses through the birth canal without assistance. The reported incidence of dystocia in the bitch is between 5 and 16% (Linde Forsberg and Eneroth 2000; Bergstrom et al., 2006) although certain breeds, especially the brachycephalic type, have a much higher incidence (Linde Forsberg and Persson, 2007). The aim of the clinician when presented with a bitch in dystocia is to protect the bitch’s life, maximize the likelihood of neonatal survival and to maintain the fertility of genetically valuable bitches. Knowledge of the normal events of pregnancy and parturition as well as an understanding of the underlying causes of dystocia in the bitch is required so that diagnosis and appropriate treatment can be implemented in a timely manner, as timing of obstetrical aid significantly affects pup survivability.

Dystocia can be either foetal or maternal in origin, or a combination of both. Both causes of dystocia can be either anatomical or physiological in aetiology (Gaudet, 1985). The most common cause of dystocia in the bitch is primary uterine inertia which occurs in approximately 75% of dystocia cases (Darvelid and Linde Forsberg, 1994). Primary uterine inertia is the failure of the bitch to expel normal sized foetuses through an unobstructed birth canal due to inadequate uterine contractions (Gaudet, 1985). Complete primary uterine inertia is the failure of the initiation of labour at full term. Partial primary uterine inertia is when labour begins and one or two pups are delivered but there are insufficient myometrial contractions to complete the normal birth of all foetuses despite the presence of a patent birth canal (Gaudet, 1985; Linde-Forsberg and Persson, 2007). Secondary uterine inertia occurs when the birth canal is obstructed and normal uterine contractions cease due to fatigue. Foetal causes of dystocia include foetal death, malpresentation, malformations and foetal oversize.

Factors that pre-dispose a bitch to primary uterine inertia include “single pup syndrome” (the presence of only one pup and its failure to initiate parturition), an overstretched myometrium associated with very large litter sizes, nutritional imbalances (obesity or malnutrition), age (higher incidence in bitches > 4 years), parity (greater incidence in older primiparous bitches), and excessive human intervention.

Studies investigating the underlying pathogenesis of uterine inertia in the bitch are limited. In a recent study, we examined the relationship between ionised calcium (iCa), parathyroid hormone (PTH), oxytocin and the acid-base status of bitches selected from a colony of bitches with a history of an increased stillbirth rate (Hollinshead et al., 2010). The findings of this study indicated that the development of sub-clinical respiratory alkalosis caused by panting, anxiety and fear which is often associated with the onset of whelping resulted in decreased tissue sensitivity to PTH thus causing a transient decrease in blood iCa and subsequently myometrial contractions resulting in uterine inertia and an increased stillbirth incidence. This is very similar to the clinical model for the development of hypocalcaemia or “milk fever” in the dairy cow.
Similar to the dairy cow, manipulation of the cationic/anionic difference in the diets of pregnant bitches may reduce the incidence of primary uterine inertia.

Indications for veterinary assistance during whelping include general unwellness of the bitch, prolonged gestation length, presence of green vaginal discharge not followed by the delivery of a pup, greater than 2 h since the last pup was born, weak contractions without delivery of a pup for more than 4 h or the presence of strong uterine contractions for greater than 20-30 min without expulsion of a foetus.

Clinical examination of a bitch presented with dystocia should include the determination of any metabolic status, acid-base, blood glucose and electrolyte derangements, examination of the birth canal to determine if the underlying cause of the dystocia is obstructive or non obstructive and an abdominal ultrasound to assess foetal viability.

Dystocia can be treated either medically or surgically (caesarian section) depending on the underlying cause, condition of the bitch at presentation and foetal wellbeing. Medical treatment may include minor manipulation and digital extraction of an obstructed pup, or for the treatment of primary uterine inertia, the administration of intravenous oxytocin, calcium gluconate or a combination of both. Only 25 to 30% of primary uterine inertia cases respond successfully to medical treatment (Darvelid and Linde-Forsberg, 1994; Linde Forsberg and Persson, 2007). Non-responsive medical cases must be treated surgically. Bitches with dystocia caused by obstruction (foetal or maternal) or when foetal vitality is poor are immediate surgical candidates. In a Swedish study carried out by Bergstrom et al., 2006, 64% percent of bitches affected by dystocia were ultimately treated surgically.

References:


Canine pyometra is a prevalent uterine infection of mainly middle-aged bitches in dioestrus. Up to 24% of intact bitches develop a pyometra before ten years of age (Egenvall et al. 2004). Uropathogenic *E. coli* is the most commonly isolated pathogen (Fransson and Ragle 2003).

Originally cystic endometrial hyperplasia (CEH) and pyometra were defined as one disease entity. CEH was thought to arise first and therefore predispose the uterus to a secondary bacterial infection, which resulted in pyometra.

Currently, pyometra and cystic endometrial hyperplasia are considered two separate disease entities. Although, both diseases bear many similarities and can be found as subsequent events, the conditions have the potential to derive independently. Any stimulus in a progesterone-influenced uterus can lead to CEH and therefore the presence of CEH in pyometra could be the result of a uterine reaction to the bacterial infection.

The pathogenesis of pyometra is poorly understood. Many factors, such as the influence of age, ovarian hormones, breed, parity, and treatment with exogenous hormones have been shown to play a role in the development of the disease (Verstegen et al. 2008).

The mean age is reported to be between 7 to 8.5 years, although a range from 4 months to 18 years has been reported. A breed predisposition has been described. Pyometra is believed to be facilitated by the unique canine oestrous cycle, during which an oestrogen phase is followed by a relatively long progesterone dominated phase (dioestrus). The overwhelming proportion of affected bitches present within 8-12 weeks of their last heat. The length of the dioestrous phase in a non-pregnant bitch is not significantly shorter than in a pregnant bitch. This suggests the lack of a luteolytic mechanism in the dog. Leukocyte inhibition and decreased myometrial contractions, in the progesterone influenced uterus facilitate bacterial growth. The progesterone influence in dioestrus results in uterine stromal and glandular epithelial proliferation and increased glandular secretion. These effects are cumulative. Therefore, the risk of uterine disease increases with each estrous cycle.

Although there is no evidence of abnormal ovarian hormone concentrations in the pathogenesis of pyometra it has been shown that progesterone is necessary to initiate a CEH reaction and oestrogen potentiates the effect by upregulating the expression of progesterone receptors.

The role of uropathogenic *E. coli* in the pathogenesis of the disease has recently been investigated. They possess, among other uropathogenic virulence factors, so called adhesins, which facilitate the bacterial binding to an epithelial surface. We have shown that it was indeed the adhesin FimH which facilitates bacterial binding to the canine endometrium. Insertionally
disrupting fimH in a strain (P4), which was isolated from a bitch affected by pyometra, lead to significantly reduced binding to uterine epithelium. Furthermore, it could be demonstrated by fluorescent microscopy that the bacteria preferentially bound to the luminal surface of the uterus. In a different strain (P3) however, which carries three adhesins, insertional activation of one or two adhesin genes can be compensated for by the remaining ones. We demonstrated that even disruption of all three known adhesin genes in that strain still results in binding capacity of almost half of the wildtype’s. This suggests that pathogenic differences between E. coli strains could play a role in the infectious process.

The role of the bitch’s immune response has recently been emphasized by the diagnosis of endometritis in the bitch. It was widely believed that endometritis did not exist in the canine species and a bacterial infection would always result in pyometra. Latest studies, however, show that bacteria can be isolated from subfertile bitches that do not show the hallmarks of pyometra. Interestingly, the mean age of these bitches was lower than in those with pyometra. This suggests that bitches might contain bacteria in the form of subclinical endometritis for some time before unknown factors allow bacterial proliferation and subsequent pyometra.

We are currently comparing gene expression in relation to innate immunity in bitches between different stages of the oestrous cycle. We have already identified a 100-fold difference in the gene expression of certain beta-defensins (antimicrobial peptides) comparing dioestrus, oestrus and anoestrus uterine tissue.

Future research into the interactions of uropathogenic E. coli and the host’s immune response might help elucidate the pathogenesis further.

Clinical signs:

Vulvar discharge varies with patency of the cervix but often also present in closed-cervix pyometra. In open-cervix pyometra, bitches present with more copious amounts of vulvar discharge and are often less clinically ill. Bitches with closed-cervix pyometra are almost always systemically ill. Vomiting and depression are often presenting complaints along with fever, polyuria/ polydipsia, and abdominal distention mediated by septicaemia, bacteraemia and/or endotoxaemia.

Diagnosis:

- **Ultrasoundography**: thin-walled tubular uterus filled with hypoechoic fluid +/- flocculent material; wall can appear thick if severe CEH changes are present.
- **Laboratory findings**: White blood cell count is most commonly > 35,000 cells/μl (neutrophilia with left shift and toxic change). But normoleukocytosis and leukopenia are possible and do not necessarily exclude the disease; anaemia, elevated ALP and prerenal/ renal azotemia are common.

Prognosis:
The prognosis for survival is good with medical and surgical treatment if uterine rupture has not occurred. In regard to future reproductive performance it should be noted that the disease can reoccur and it is strongly recommended to breed the bitch in the next cycle and have her ultrasonographically examined for pregnancy 28 days after the LH surge; however, reoccurrence rate is dependent on age, parity and preexisting uterine pathology; most commonly it is reported to be ~20% within 12-14 months of diagnosis; it is possible that she will have a new infection at that stage and if that is the case ovariohysterectomy should be strongly recommended. It has been shown that dogs that do not respond well to medical treatment within 5 days have a very poor prognosis to retain their breeding potential and ovariohysterectomy should be elected; clinical improvement should be noted within 2 days and resolution of the disease should occur within 7 to 10 days.

Treatment:

● Surgical treatment:
Ovariohysterectomy is the treatment of choice in all non-breeding animals. Owners of breeding animals should also be informed about the likelihood of recurrence. Patients are often in poor condition for surgery and should be stabilized with intravenous fluids and antibiotics before the procedure.

● Medical treatment:
Medical treatment is a valid choice in breeding animals.

The rationale behind medical treatment is twofold:

● removal of progesterone allows cervical opening
● drainage of pus and elimination of bacteria through an open cervix aided by uterine contractions

1) Prostaglandin F2α (PGF2α): not registered/ approved for the use in dogs! Can be used off-label; main advantage is luteolytic effect and ecbolic effect; very inexpensive!

Especially in the case of closed-cervix pyometra it is paramount to start with low doses of PGF2α in order to minimize the ecbolic effect of the drug and reduce the risk of uterine rupture. Once luteolysis occurs and the cervix opens the dose can be increased and evacuation of the uterus is a desired effect of treatment.

● dinoprost: non-synthetic, inexpensive
dose:

10-15 μg/kg three times a day (TID) for 2 days subcutaneously (SC)

25 μg/kg TID for 2 days SC

50 μg/kg TID for 5 days SC

the dose can be adjusted to sensitivity of the bitch; some dogs are more sensitive and react with more side effects; others might need dose up to 100 μg/kg for 2 to 3 days.
side effects: side effects are dose dependent and diminish after several injections; they include: tachypnoea, vomiting, diarrhea, urination, anxiety; start about 20 min after treatment; walking the dog for 15 min after administration seems to alleviate the side effects; dog should be hospitalized for at least one hour after treatment to observe side effects.

- **cloprostenol**: synthetic PGF2α analogue, slightly more expensive but less side effects; less uterine contractions than with dinoprost; reported to be 100 % effective if given at a dose of 1 μg/kg once a day for 10 days

2) **Aglepristone**: progesterone-antagonist; competitively prevents progesterone binding to its receptor; more expensive than prostaglandin; no side effects; causes luteolysis but poor uterine contractions; works well in combination with prostaglandins in closed-cervix pyometra; aglepristone is given first and prostaglandin treatment can be started 36 to 48 hours later; cervix will open 26 hours (+/- 13 hours) after first aglepristone injection; dose: 10 mg/kg given twice 24 hours apart; can follow with an injection 8 days later.

3) **Antimicrobial therapy**: should be initiated immediately with a wide-spectrum antibiotic. Culture and sensitivity should be performed but therapy has to be started at time of diagnosis. Excellent results have been achieved with amoxicillin/clavulanic acid, cephalosporins and potentiated sulfonamides. If oral antibiotics are given care must be taken to give the drugs at a different time as the prostaglandin, which will often lead to vomiting. Antimicrobial therapy should be continued for at least 14 days after resolution of vulvar discharge.

**Follow-up care:**

- weekly blood cell counts should be performed to demonstrate decreasing neutrophilia (left shift should be no longer present).
- ultrasonography is recommended; visible reduction in lumen size after 5 to 7 days
- serum progesterone levels; although levels can be hard to interpret if aglepristone is used; if prostaglandins are used reduction within 48 hours

breeding management in next cycle and early pregnancy diagnosis by ultrasonography to exclude recurrence of the disease; note that treatment will often lead to shortened interoestrous interval; can be lengthened with mibolerone in order to allow for sufficient time for uterus to remodel; start one month after end of medical treatment and keep administering it for 2 to 3 months.

**References:**


This paper reviews existing and emerging genetic and genomic technologies to improve performance in farm animals with a focus on reproduction in dairy and beef cattle. It covers three inter-related areas and provides an outlook on impact of emerging technologies on reproductive biology and theriogenology. Conventional quantitative genetics and animal breeding strategies will continue to be an important basis on which to improve animal’s genetic merit for desirable reproductive characteristics in herds. The genetic and phenotypic trends in many cattle breeding countries show that substantial progress has been made in improving milk and meat production. However, the consequences of genetic selection for milk and meat production on reproduction (and health / disease resistance) are very unfavourable (Kadarmideen, Thompson and Simm 2000, Kadarmideen, Thompson, Coffey et al. 2003). Modern animal breeding programs are now aimed at arresting the decline in reproductive ability of animals by optimising selection for production and reproduction, simultaneously via Total Merit Selection Index (Kadarmideen and Simm 2002).

Advances in molecular genetics have led to identification of genes or genetic markers controlling variation in reproductive ability of animals. Many chromosomal regions (quantitative trait loci or QTLs) have been identified for various reproductive traits on 26 of 30 bovine chromosomes. These QTLs can be incorporated in predicting genetic merit as per the methods of Meuwissen, Hayes, Goddard (2001). Currently selection and breeding decisions based on this approach are practiced by many breed societies and genetic companies around the world. An alternative form of identifying genes affecting reproduction is candidate gene approach which exploits variation within genes known to affect reproduction (for instance, GnRH, PRL, BPL, STAT genes etc.). Recently completed bovine genome sequencing and assembly projects (The Bovine Genome Sequencing and Analysis Consortium 2009) have led to the paradigm shift in QTL and candidate gene approaches. Currently, the genome-wide association studies (GWAS) uses 50000 or more dense genetic markers called Single Nucleotide Polymorphisms (SNP) to accurately detect genes for reproduction; these SNP discoveries are used in predicting genome-wide merit of animals and conducting ‘genomic selection’. The genomic selection predicts the performance of an animal (at birth) given their genotypes at SNPs without ever recording a phenotypic observation. Hence it has huge impact on cost of traditional progeny testing schemes and length of time to make selection decisions (Schaefffer 2006).

Functional genomics and systems genetics (based on microarray gene expression profiling or MGEP) helps detect and annotate differentially expressed (DE) and co-expressed (CE) genes and construct underlying gene networks of reproduction. For instance, we have used these approaches to unravel the biology and genomics of sheep resistance to gastrointestinal
nematode (GIN) infections as well as sheep muscle growth and development (Kadarmideen, Watson-Haigh, Andronicos 2010). The same approaches can be applied for reproductive traits. For instance, animals with clearly distinct reproductive outcomes or characteristics could be used to identify DE and CE genes. GWAS and MGE are often practiced as independent approaches to study reproductive phenotype; the power to unravel the biology of reproduction is greatly increased if both MGE and GWAS are jointly conducted in genetical genomics framework. Recently, we have applied this integrated approach in identification of loci regulating expression GnRH and GnRH-receptor genes in inbred strains of mouse; this has implications for livestock species.

In the near future, there will be further revolution in genomics due to transition from Sanger sequencing (called first generation sequencing) to next generation sequencing or NGS (Metzker 2010); The NGS techniques are rapidly replacing microarrays due to inventions of a number of robust sequencing technology platforms and falling cost for generating sequence-based data. In addition to gene expression studies, NGS offers detection of cSNPs, novel and rare transcripts, novel protein isoforms, alternative splice sites, ncRNA, and allele specific expression in one single experiment. NGS will continue to rapidly open new applications in the biological sciences and scientists will have to adapt to these challenges. The scientists working in the areas of computational and systems biology, statistical genomics and bioinformatics, in particular, would need to keep up with these rapidly evolving new technologies.

Last but not the least veterinarians will play a crucial role in generating good quality and quantity of reproduction related data in cattle using new herd and animal level monitoring and management devices (e.g. heat detection and hormonal activity meters, intra-uterine devices, ovarian ultra sound scans etc.). Such phenotyping will be a basis for applying any novel genetic/genomic technologies and hence to have an impact in cattle reproduction.

1. Conventional quantitative genetics and animal breeding strategies for improving reproductive performance in cattle

The key to genetically improve performance of animals is to keep accurate records of their performance for (genetic) evaluation and selection of best animals / culling worst animals. Hence, performance recording of cattle is crucial and are now conducted using state-of-the-art information technology tools and purpose built computer databases that are routinely implemented by national recording organisations and various breed societies. Veterinarians play an important role in assisting accurate and consistent reporting of disease, health or reproductive problems by farmers / producers to national recording organisations and various breed societies. The performance recording is standardized in most industrialized countries as per the guidelines of International Committee on Animal Recording (ICAR); this is more so for dairy cattle than beef cattle. Pedigree recording is also crucial for genetic evaluation as these methods predict future performance of animals based on performance of its relatives.

Quantitative genetics method is a well proven method to improve reproductive performance and productivity. Development of selection index principles in animal breeding programmes in
early 1940’s (Hazel and Lush 1943) followed by application of Best Linear Unbiased Prediction (BLUP) of animal’s genetic merit from early 1970’s (Henderson 1975) have had major impact in animal breeding and it continues to do so. These methods accurately quantify transmittable genetic merit of animals or breeding potential (estimated breeding values or EBVs; expected progeny differences or EPDs) for economically important traits and are expressed in real units of measurement (e.g. percent pregnancy or non-return rates). To assess the relative superiority of a bull or a cow, the animal’s EBV is compared with those reported by a breed average. With continuing advances in statistical and computing technology, quantitative geneticists successfully refined and modified BLUP methodology. This has led to accurate predictions of genetic merit of cattle and hence accurate selection of best animals for a range of desired traits simultaneously via Total Merit Index (TMI).

One of the traditional quantitative genetics approaches that does not require DNA genotyping but is aimed at detecting evidences of segregating major genes based only on phenotype and pedigree in BLUP framework is called complex segregation analysis [Janss et al. 1995]. It uses mixture (polygenetic and monogenetic) effect models and detects major genes, if present in the data. Stringent statistical tests are conducted for Mendelian transmission at the major gene to rule out environmental and polygenetic effects. These approaches are more often applied in detecting major genes for clinical diseases & reproductive and auto-immune disorders in biomedical sciences than agricultural sciences. Examples of such segregation analysis applied to large animal populations for the analysis of production and health traits include cattle [Ilahi and Kadarmideen 2004, Karacaören et al. 2006], pigs [Kadarmideen and Janss 2005 and 2007, Kadarmideen and Ilahi 2005] and poultry [Hagger et al. 2004].

There has been very high emphasis on milk and meat production in dairy and beef cattle production programs over many decades (understandably due to food security issues after the Second World War). The genetic and phenotypic trends in many cattle breeding countries show that substantial progress has been made in improving milk and meat production. However, the consequences of genetic selection for milk and meat production on reproduction (and health / disease resistance) were very unfavourable, as seen in antagonistic genetic correlations between production versus “functional” traits (Kadarmideen et al. 2000 and 2003). Evidences are provided from various developed countries to support this trend. Hence it is important to arrest the decline in reproductive ability of animals. Modern animal breeding programs have taken these issues seriously by optimising selection for production and reproduction, simultaneously (Kadarmideen and Simm 2002).

As mentioned earlier conventional genetic/breeding approaches to make genetic improvement in reproduction rely heavily on availability of good quality and quantity cattle reproduction data, which could only be achieved with good collaboration between veterinarians, artificial insemination companies or stud breeders and recording organisations (Kadarmideen 2003). Recording for cow fertility should address two essential components: first the cyclicity- how early the cow comes to heat after calving; and the second, the ability to conceive at the first mating or insemination and to maintain the pregnancy (Kadarmideen 2003). These two components would reflect most biological phenomenon underlying reproduction in cattle. Because heat observations are difficult to observe and record in field conditions, number of
days between calving and first mating or insemination and conception successes or non-return rates (NR) should be used in genetic evaluations. Until full-scale recording and reliability of mating and insemination records improves, some indirect fertility traits can be used (example, calving interval or body condition scores). There is definitely an economic sense to putting more emphasis on reproduction in breeding program because TMI that includes reproduction show 38% more profit than the TMI that does not include reproductive traits (Kadarmideen and Simm 2002).

2. Molecular genetics enhanced animal breeding strategies for improving reproductive performance in cattle

Identification of genes or genetic markers controlling variation in economically important quantitative traits, such as reproduction and complex diseases or disorders in animals has been practiced for over 20 years. The gene mapping or so called quantitative trait loci (QTL) mapping uses a few hundred multi-allelic microsatellite markers that are spread evenly and densely across the entire genome. QTL mapping is based on two steps: a) Creation of genetic linkage maps (in centiMorgen or cM units) based on recombination rates and/or distances using genetic markers. Analogy to linkage map is a large voluminous text book and markers are like page numbers on the book referring to a point in the genome and b) application of statistical modelling and analysis of genomic regions flanked by markers for their effect on range of traits (such as NR). For instance, investigating whether an animal carrying one form of a gene (allele) has more ability to be cycling regularly and have high NR than an animal carrying a different form of the gene (allele). These techniques have been extensively developed and applied in various livestock species for various traits and numerous QTLs have been found. For reproductive traits in cattle, there are several studies showing QTL or genomic regions that may contain genes affecting reproductive ability of cattle. For instance, Jemaa et al. (2008) found QTLs for NR traits on bovine chromosome 1 (68 - 98 cM), chromosome 2 (100-119 cM) and chromosome 3 (20-30 cM) and Holmberg et al. (2007) found NR QTLs on Chr 9 (25-30 cM). Hoglund et al (2009) lists several QTLs for various reproductive traits on all 26 chromosomes. Often, identification of causal genes is difficult but the genetic markers that are closely linked to or co-segregating with putative genes may be used to derive associations.

An alternative form of identifying genes affecting reproduction is candidate gene approaches. These approaches exploit variation within genes known to affect reproduction (for instance, GnRH, PRL, BPL, STAT genes etc.). The candidate gene approach looks at explaining variation in reproductive performance between animals because different animals carry different polymorphisms within a gene, each having different end result due to changes in protein and/or metabolites that produced by allelic variants. Some examples of detected candidate genes for reproductive traits include the study of Huang et al., (2009) for BPL, GHR PRLR genes, Kadarmideen et al., (2008) for PRL, Gpr54, FOS genes, Khatib et al. (2009) for STAT1 and STAT3 genes.

Whether it is a QTL based approach or candidate gene based approach, the identified gene information can be put into practical genetic improvement of reproduction. The incorporation of genetic marker in selection and breeding decisions of elite animals is called marker assisted selection. If marker information is incorporated in traditional BLUP EBVs then it is called Marker
**Assisted breeding value estimation.** These approaches and their impact on improving performance of animals are thoroughly discussed in Dekkers et al., (2004).

Bovine genome sequencing and assembly projects (The Bovine HapMap Consortium 2009 and The Bovine Genome Sequencing and Analysis Consortium and Elsik et al. 2009) have led to the end of low throughput gene mapping using microsatellites and a paradigm shift in QTL and candidate gene approaches. It is due to availability of high throughput genetic markers ranging from 50000 or more Single Nucleotide Polymorphisms (SNP). This number is expected to reach to 300,000 markers in the near future. These markers can be genotyped using high throughput Affymetrix or illumina GeneChips. The genome-wide association studies (GWAS) relate each one of those highly dense SNP markers with observed phenotypic traits (such as reproduction) in a “reference population” to identify SNP-QTLs across the genome that explains most genetic variation in phenotypes (Kruglyak 2008). Results of GWAS are then used in “Genomic Selection” of livestock in a “selection population” where estimated SNP effects are used predict the performance of an animal (at birth) given their genotypes at SNPs without ever recording a phenotypic observation, with an accuracy up to 70%. This dramatically changes traditional progeny testing schemes in cattle and other species because it eliminates the need to maintain and record performance of large number of animals from test bulls or sires for many years. In addition, GWAS still offers identification of major QTLs or candidate genes. This genome-wide genetic evaluation of animals is now practiced by livestock genetic evaluation industries and genomic companies (e.g. Pfizer Animal Genetics and Merial). This has become quickly adapted because the traditional genetic evaluation schemes suffer from the longer time span needed to prove genetic merit of animals and costs involved in progeny- or sib-testing schemes. In fact, Schaeffer (2004) claims, via empirical simulations, that the use of SNP GeneChip® arrays can cut down the cost of typical progeny testing in dairy cattle (and other animals) by astonishing 92% and the genetic improvement could be two-fold compared to normal conventional methods. However, it is important to address the lack of biological knowledge in these “gene discovery” or ‘genomic selection’ projects because of high number of false positives and biologically irrelevant SNPs. For instance, SNPs that are within the coding regions of genes which in turn are involved directly in producing proteins, metabolites or hormones affecting reproduction would need to be given higher weights than those that are peripherally involved. To this end, softwares such as FuncSNP (developed in our lab by Goodswen et al. 2010) that appropriately identifies functionally relevant SNPs for a given trait would be useful.

**3. Functional genomics and systems genetics enhanced animal breeding strategies for improving reproductive performance**

Microarray gene expression profiling (MGEP) has become popular in livestock species in the last decade that uses high-throughput transcriptomic arrays (e.g. Affymetrix or Agilent arrays) containing up to 30000 gene transcripts to reveal underlying gene (co)regulation in a set of biological conditions. Most transcriptomics experiments are focused on detection and annotation of differentially expressed (DE) and co-expressed (CE) genes and construction of gene networks (for review of transcriptomics, see Kadarmideen and Reverter 2007). For instance, we have used these approaches to unravel the biology and genomics of sheep resistance to gastrointestinal nematode (GIN) infections as well as sheep muscle growth and
development (Kadarmideen et al. 2010a, and 2010b). These methods or techniques are the same for reproductive traits, where animals with clearly distinct reproductive outcomes or characteristics could be used.

While the above mentioned GWAS and MGEP are often practiced as independent approaches to study a disease or reproductive phenotype, the power to unravel the biology of such traits is greatly increased if both MGEP and GWAS are jointly conducted in the same population to study genetics of -omics variation (genetic variation in transcriptome; Kadarmideen et al. 2006). Until the invention of high-throughput -omic techniques, it has not been possible to identify genetic variation in these omics traits. Genetical genomics is a novel method in integrative systems biology that helps us to investigate the inheritance of such regulatory loci, so called expression quantitative trait loci or eQTL. Recently, we have identified eQTLs regulating expression GnRH and GnRH-receptor genes in inbred strains of mouse that have implications for livestock species (Kadarmideen 2008)

4. New genomics approaches to improve reproductive performance

Hybridization-based approaches typically involve incubating fluorescently labelled cDNA with custom-made microarrays (e.g. Agilent arrays) or commercial high-density oligo microarrays (Affymetrix arrays as described in previous section); these methods have several limitations, which include:

a. reliance upon existing knowledge about genome sequence
b. high background levels owing to cross-hybridization
c. A limited dynamic range of detection owing to both background and saturation of signals
d. comparing expression levels across different experiments is often difficult and can require complicated normalization methods

The main factors that enabled transition from Sanger sequencing (called first generation sequencing) to next generation sequencing (NGS) are inventions of number of robust sequencing technology platforms and falling cost for generating sequence-based data. The fundamentals of NGS technologies are reviewed by Metzker (2009 and 2010). These NGS approaches are quantitative in that they directly determine the entire sequence thereby more accurately estimating RNA expression levels in cells or tissues than microarrays. We can directly compare results between experiments. In addition to gene expression studies, NGS offers detection of cSNPs, novel and rare transcripts, novel protein isoforms, alternative splice sites, ncRNA, and allele specific expression in one single experiment. Computationally, RNA-Seq has an advantage in that it can capture transcriptome dynamics across different tissues or conditions without sophisticated normalization of data sets. Hence, the evolution of ‘omic’ science has come full circle with a new focus on next generation genome sequencing. In nutshell, NGS will continue to rapidly open new applications in the biological sciences and scientists will have to adapt to these challenges.

The generation of high-throughput -omics data (proteomic, metabolomic, metagenomic, transcriptomic, genomic and nutrigenomic data) have resulted in data deluge containing information on genes (& their sequences), metabolites, proteins, cellular dynamics and...
organisms' responses to genetic and environmental perturbations. Systems biology, in practice, is a result of these new technologies that enables hugely comprehensive data at all levels of biological system to be collectively analysed using state-of-the art mathematical, computational biology and bioinformatic principles and tools. The principle of systems biology is that, in order to understand how an organism functions, (e.g. how human/animal grow, develop disease etc.,) we must study interactions at various levels both within and across biological systems but not in isolation. It is the vision of system biology to be ultimately able to link different processes within and between cells, tissues, organs, functions of the system and eventually up to the individual level traits to provide complete blueprint of functions. A branch of systems biology which focuses on integrating genetic factors (SNPs, QTLs etc) causing variation between individuals in intermediate -omic traits (whole genomic gene expression levels, metabolomic or proteomic levels etc) is called systems genetics. Livestock systems genetics will become increasingly important as more and more genes or QTLs affecting various intermediate traits are found in addition to SNPs or QTLs causing variation in complex polygenic traits measured on the animal itself such as reproduction traits. This ‘systems genetics’ would also include combining these data with information on signalling pathways and gene networks from other bioinformatic resources for a broader and deeper understanding of systems biology of complex traits. The most exciting development in genomics of reproduction will be to integrate NGS based transcriptomic and metabolomic investigations on reproduction with GWAS studies on reproduction.

References

PREDICTING OVULATION IN DAIRY CATTLE

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The University of Queensland, School of Veterinary Science

A series of studies were conducted to assess the performance of different methods of predicting ovulation in lactating dairy cattle and to determine if accurate ovulation prediction could improve conception rates to AI.

Two studies were conducted to assess the performance of a commercially available neck-mounted activity meter to detect cows about to ovulate in two paddock-based Holstein-Friesian dairy herds. The activity monitoring system recorded cow activity count in 2-hourly periods. Study I investigated the ability of the system to detect cow ovulatory periods in dairy herds managed in two different Australian environments and breeding systems using 5 activity alert algorithms. Herd 1 consisted of approximately 130 milking cows calving year-round in a sub-tropical environment and kept in a single dry lot paddock. Herd 2 consisted of approximately 400 milking cows calving seasonally in a temperate climate and fed pasture by rotation through multiple grazing paddocks. Ovulatory periods and non-ovulatory days were identified using milk progesterone monitoring alone or in combination with ovarian ultrasonography; using these “gold standards” 141 and 135 ovulatory periods were identified in 64 and 135 cows in Herds 1 and 2, respectively. Results of sensitivity, specificity and positive predictive value for each of the herds and algorithms are shown in Table 1.

Table 1. Activity meter performance for detecting ovulating cows in two different management systems using a range of activity alert algorithms.

<table>
<thead>
<tr>
<th>Measure of performance for detecting cows about to ovulate</th>
<th>Herd</th>
<th>Activity alert algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>W-DP</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>1</td>
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<td>90.4</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>1</td>
<td>98.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>98.2</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>predictive 1</td>
<td>73.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>75.8</td>
</tr>
</tbody>
</table>

Study II investigated the ability of the activity meter system to predict the timing of ovulations in paddock-based pasture-fed dairy cattle (Herd 2). The time of ovulation was estimated by
repeat trans-rectal ovarian ultrasonography at approximately 0, 12, 24 and 36 hours after artificial insemination (AI). The mean times (± SD) from onset and end of increased activity to ovulation were 33.4 ± 12.4 and 17.3 ± 12.8 hours, respectively (n=94). Fifty percent of cows (n=47) ovulated within the 8 hour period between 30 to 38 hours after the onset of increased activity, 76.6% (n=72) within the 16 hours between 24 to 40 hours, 85.1% (n=80) within the 24 hours between 18 and 42 hours and 90.4% (n=85) within the 32 hours from 19 to 51 hours after the onset of increased activity. Results from these studies show that in paddock-based dairy cows in two diverse management systems, this neck-mounted activity meter system detects high proportions of cows that are about to ovulate and provides a useful indication of when ovulation is likely to occur. However the specificities and positive predictive values using the algorithms assessed may be lower than desirable.

A third prospective observational study was conducted in two Australian dairy herds to assess the potential for improving pregnancy rates (proportions of inseminations that result in pregnancy) to artificial insemination (AI) if the time of ovulation could be predicted with more certainty. Herd 1 calved year-round and inseminations were performed during two periods each day. Herd 2 calved during autumn-winter and inseminations were performed only after the morning milking each day. In both herds, the AI to ovulation interval of enrolled cows was determined by trans-rectal ovarian ultrasonography approximately 0, 12, 24 and 36 hours after AI, and pregnancy was assessed by palpation per rectum 35-56 days after AI. Also, in Herd 1 vaginal electrical resistance (VER) measurements were taken at approximately 0, 12, 24, and 36 hours after AI, and in Herd 2 cows were fitted with neck mounted activity meters that monitored cow activity count in 2-hour periods. There was substantial variation in the intervals from AI to ovulation within and between herds (mean ± SD 21.2 ± 10.7, n=102; 14.7 ± 10.4, n=100 in herds 1 and 2, respectively). Pregnancy rates were higher for inseminations close to, but preceding, ovulation. Using combined herd data (n=202), the most frequent AI to ovulation interval did not correspond to the interval with the highest observed pregnancy rate (Table 2.). Thus it is possible pregnancy rates may be improved with methods that allow better insemination timing relative to ovulation.

In Herd 1, mean VER during the peri-ovulatory period varied with time from ovulation. Lowest values (mean ± sem, VER= 64.8±1.2, n=55) occurred approximately 18 hours before ovulation and were significantly lower than measurements approximately 6 hours before ovulation (67.4±1.0; n=73; p=0.003). Further work is required to determine if VER can be used to identify ovulation time and hence the optimal time to inseminate in individual animals. In Herd 2 a modest proportion of inseminations (26.9%) occurred between 24 and 40 hours after the onset of increased cow activity where the highest pregnancy rate (67.9%) was observed, whereas a significantly lower pregnancy rate (42.4%; risk ratio 0.6; 95% CI 0.4 to 0.9; p=0.036) was observed for inseminations between 8 and 24 hours after the onset of increased cow activity where the highest proportion of inseminations (56.7%) occurred. Thus cow activity monitoring may be useful to identify the optimal time to inseminate cows.
Table 2. Risk ratios for pregnancy following inseminations at different intervals from ovulation.

<table>
<thead>
<tr>
<th>Time of AI (hours from ovulation)</th>
<th>n</th>
<th>Pregnancy rate (%)</th>
<th>Adjusted risk ratio</th>
<th>(95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;32 to 48</td>
<td>21</td>
<td>14.3</td>
<td>0.3</td>
<td>(0.1-0.8)</td>
<td>0.058</td>
</tr>
<tr>
<td>&gt;16 to 32</td>
<td>101</td>
<td>28.7</td>
<td>0.6</td>
<td>(0.4-1.0)</td>
<td>0.039</td>
</tr>
<tr>
<td>&gt;0 to 16</td>
<td>63</td>
<td>50.8</td>
<td>Ref. group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After ovulation</td>
<td>5</td>
<td>20.0</td>
<td>0.3</td>
<td>(0.1-0.8)</td>
<td>0.041</td>
</tr>
</tbody>
</table>

n=number of inseminations; Risk ratio is adjusted for herd and days from previous calving to insemination; 95% CI = 95% confidence interval; Ref. group= reference group for adjusted risk ratio and p value.

Collectively these studies show that activity monitoring can detect a high proportion of ovulating cows and give a useful indication of time of ovulation. In addition the mismatch between AI to ovulation intervals with the greatest frequency of insemination and those with the highest pregnancy rates in the two herds studied suggests methods of predicting ovulation could lead to gains in conception rates in Australian dairy herds. Randomised controlled trials are now required to confer effects of varying intervals from AI to ovulation on pregnancy rates. The interval from onset of increased cow activity to time of AI was associated with pregnancy rates, suggesting that activity monitoring may be useful for identifying the optimal time to inseminate. Mean vaginal electrical resistance during the peri-ovulatory period varies with time from ovulation. Further work is also required to determine if vaginal electrical resistance predicts the time of ovulation with sufficient accuracy for use in commercial dairy herds.
CORRELATIONS BETWEEN HEIFER BREEDING OUTCOMES AND FIRST LACTATION REPRODUCTIVE AND PRODUCTION OUTCOMES.

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Introduction

In order to achieve good reproductive performance and improved herd profitability, it is essential that optimal rearing practices for replacement stock are built in to any operation’s protocols. On most Australian dairy farms, the breeding programmes designed for heifers are minimalistic and often involve solely bull breeding or one or two services by artificial insemination prior to bull mating. With increased consolidation of the dairy industry, herd size has continued to increase with in some cases a move away from pasture based operations to more intensive feeding operations. Some of these larger herds have begun to design more intensive heifer rearing programmes with continuous weight and height monitoring, hormonal synchronisation and multiple insemination breeding programmes.

The practice of performing multiple inseminations has led to the question as to how many times should a heifer be artificially inseminated prior to using a bull and are heifers that are difficult to get pregnant more likely to take a prolonged time to conceive again in their first lactation. In order to achieve optimum reproductive efficiency in year round calving herds, a cow needs to achieve a 13 month calving interval.

The aim of this study was to examine the records of a commercial dairy herd which performed multiple inseminations in nulliparous heifers to determine if the number of breedings required to achieve conception was associated with any differences in productivity, reproductive performance, and risk of culling during the first lactation.

Materials and Methods

Study Herd

The study herd was a two thousand milking cow freestall operation in western NSW. Heifers were moved into the breeding herd at approximately thirteen months once they had achieved a minimum liveweight of three hundred and seventy five kilograms. On entry to the breeding group heifers were administered prostaglandins to synchronise oestrous. Heifers returning to service are bred up to five times with artificial insemination after which they are run with a bull. Pregnancy is diagnosed by rectal palpation every 2 weeks for AI bred heifers and every 3 months for bull bred heifers. According to their conception dates heifers are moved onto a close up ration three weeks prior to calving.

All lactating cows were enrolled in a biweekly Presynch-OVSYNCH program, commencing at thirty five days in milk. All cows detected on heat through the use of tail paint and pedometers
were artificially inseminated by trained technicians. All eligible cows were pregnancy tested at greater than forty days and reconfirmed at one hundred and fifty days post breeding. Empty cows are enrolled into an OVSYNCH program. All records are entered into DC 305.

All cows were herd tested bi-monthly and this was used to calculate ME305.

**Statistical analysis**

For statistical analysis heifers were divided into two groups: heifers that conceived with less than five breedings (GP1) and heifers that conceived on their fifth or greater breeding (GP2).

Data was analysed using STATSDIRECT (StatsDirect Statistical Software, StatsDirect Ltd, Cheshire, UK). Survival analysis using cox regression was used to analyse the difference between the groups in the days to conception for the first lactation. Analysis of Variance (ANOVA) was used to compare the ME305 and proportion of heifers culled in the first lactation between the groups.

**Results**

After removing all heifers with incomplete records a total of 2476 heifers were analysed, with 1931 heifers in GP1 and 545 heifers in GP2. Statistical analysis showed that GP2 heifers had an increased days to conception, increased percentage of culling, increased failure to conceive and decreased ME305 in the first lactation when compared to GP1 heifers (p<0.05).

**Conclusion**

The results of this analysis indicate that nulliparous heifers that take more than 4 inseminations to conceive are more likely to take longer to conceive in the subsequent lactation, produce less milk, and are more likely to be culled from the herd during or at the conclusion of the first lactation than heifers that conceive with four or less inseminations. This data is useful for on-farm decision making regarding heifer management and culling decisions.
A large amount of variation in calf output exists for individual *Bos indicus* bulls in multiple-sire mating groups in northern Australian beef herds. Only 35-57% of this variation can be explained by the current physical, seminal and behavioural fertility traits measured prior to mating (Holroyd RG, Doogan VJ, De Faveri J et al. 2002). As seminal plasma proteins (SPPs) play a vital role in sperm development and function along the reproductive tract, including sperm maturation, storage, capacitation and motility (Killian GJ, Chapman DA, Rogowski LA 1993) they may be a contributing factor to this observed variation in calf output. Various statistical associations have been found between SPPs and fertility scores of *Bos taurus* sires (McCauley TC, Zhang H, Bellin ME et al. 2001) and significant relationships have also been found between SPPs and artificial insemination conception rates for Holstein bulls (Moura AA, Koc H, Chapman DA et al. 2006).

This study was conducted as a part of the current Cooperative Research Centre for Beef Genetic Technologies (Beef CRC) and investigates the role of SPPs in *Bos indicus* bull fertility. Seminal plasma samples from 56 two year old Brahman bulls with varying proportions of morphologically normal sperm (7-94%) were analysed to determine SPP protein profiles of electro-ejaculated bulls in northern Australia. The percentage of morphologically normal sperm in the ejaculate was analysed as part of a routine bull breeding soundness evaluation at the time the seminal plasma sample was collected. 258 proteins were separated using 2-dimensional (2D) SDS-PAGE techniques and the protein gels were analysed using PDQuest software.

Using a multiple regression model, eight of these proteins had a significant effect on the percent of morphological normal sperm and accounted for 88% of the total variation. Three of these proteins had a positive effect on the percentage of normal sperm while five had a negative effect.

A proportion of individual protein spots were cut from the protein gels and identified using peptide mass fingerprinting. Further confirmation of these results are currently being
conducted to correctly identify the above eight proteins and to validate the relationship between SPPs and percent morphologically normal sperm.

From these results, it appears likely that certain SPPs present in Bos indicus bulls are linked to fertility measures as observed in Bos taurus bull studies and may contribute to the variation in calf output recorded in breeding herds.

References


DEVELOPMENT OF OESTROUS SYNCHRONISATION PROTOCOLS TO ENABLE THE FIXED-TIME ARTIFICIAL INSEMINATION OF BOS INDICUS HEIFERS

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Development of oestrous synchronisation (OS) protocols to enable artificial insemination (AI) of large numbers of Brahman heifers will increase the adoption of artificial breeding in northern Australia. The use of AI in beef breeding herds is an efficient way of disseminating improved genetics. The use of fixed-time AI (FTAI) in large Bos indicus herds can eliminate the need for oestrus detection and permit 100% of a mob to be submitted to AI. The aim of this study was to evaluate the ovarian function in heifers treated with different doses of progesterone (P4) in intravaginal releasing devices, to provide information to develop best practice OS protocols for Brahman heifers in northern Australia. Brahman (n=30) and Brahman-cross (n=34; 3/4 to 7/8 Brahman) heifers weighing an average of 285kg (248-323kg) and 307kg (250-363kg) respectively, were allocated at random to one of 4 treatment groups: i) full dose Cue-Mate® (CM 1.56g P4; two P4 impregnated pods; Bioniche Animal Health Aust/Asia; n=17); ii) half dose Cue-Mate® (CM 0.78g P4; one P4 impregnated pod; n=15); iii) half dose Cue-Mate® + 300IU eCG i.m. at Cue-Mate® removal (CM 0.78g+G; Pregnecol; Bioniche Animal Health, Aust/Asia; n=14) and iv) double PGF2α group (2x PGF2α; cloprostenol; Estromil; Ilium Veterinary Products, Smithfield, Australia; n=18). Heifers in the Cue-Mate® treated groups received 1 mg ODB (oestradiol benzoate; Ciderol; 1mg/ml Genetics Australia, Bacchus Marsh, Victoria) at Cue-Mate® insertion (Day -10) and 24h after Cue-Mate® removal (Day -1) and 250µg PGF2α i.m at Cue-Mate® insertion (Day -10) and removal (Day -2). Heifers in the 2xPGF2α group received 500µg cloprosteno l on Day -16 and Day -2 intramuscularly (i.m.) Ovarian function was evaluated twice daily by transrectal ultrasonography on Day -1, Day 0 (expected oestrus) and Day 1, and once daily on Days -9 to -2 during Cue-Mate® insertion and on Days 4, 6, 8, 10, 12, 16 of the synchronised and return cycles. Concurrent blood samples were collected to determine plasma P4 concentrations. A treatment by breed x day interaction (P=0.002) was observed in the plasma P4 concentration of all heifers during intravaginal P4 treatment (Figure 1), with the Brahman heifers in the CM 1.56g treatment group having significantly higher (P<0.05) circulating mean P4 on Days -7 and -6 than the Brahman-cross heifers in the same treatment group and significantly higher (P<0.05) circulating P4 on Days -9, -8, -7 and -6 than heifers in the CM 0.78g and CM 0.78g+G groups combined and 2xPGF2α groups. Analysis of the slope of the mean plasma P4 concentration curve from Day 0 (day of ovulation) to Day 6 showed a significant day x treatment interaction (P<0.001; Figure 2). Although the mean diameter of the dominant follicle 54-56h post Cue-Mate® removal (time FTAI is recommended) was numerically lower for the CM 1.56g group (11.8±1.2mm) than the CM 0.78g (13.3±1.2mm), CM 0.78g+G (13.4±1.3mm) and 2xPGF2α (13.4±1.3mm) groups, no significant difference was observed (P>0.05). The proportion of heifers that ovulated between 53 and 77 hrs post Cue-Mate®
removal did not differ between the CM 1.56g (92.6%), CM 0.78g (85.2%) and CM 0.78g+G (75.3%), but was significantly lower in the 2xPGF$_{2\alpha}$ treatment group ($P<0.001$; 8.5%).

Analysis of individual heifer P$_4$ profiles revealed that 2/15, 1/15, 3/12 and 0/5 of heifers that ovulated in the CM 1.56g, CM 0.78g, CM 0.78g+G and 2xPGF$_{2\alpha}$ groups, respectively, failed to re-ovulate in the return cycle. Also, 1/15, 3/15, 1/12 and 0/5 of heifers that ovulated in the CM 1.56g, CM 0.78g, CM 0.78g+G and 2xPGF$_{2\alpha}$ groups, respectively, developed a CL which persisted into the return cycle. The use of lower dose P$_4$ containing intravaginal releasing devices in OS programs designed for FTAI improves the post-ovulatory P$_4$ rise in ovulating Bos indicus heifers which is likely to improve embryonic survival (Green et al., 2007; Carter et al., 2008; Clemente et al., 2009). This study has identified that a breed interaction may exist whereby Brahman heifers have a higher circulating P$_4$ than Brahman-cross heifers when treated with a standard P$_4$ (1.56g) intravaginal releasing device. This higher circulating P$_4$ may adversely affect development of the pre-ovulatory dominant follicle as reported by Carvalho et al. (2008) and observed in this study. Physiological evidence suggests that low-dose intravaginal releasing devices are recommended for best-practice OS protocols for FTAI of Brahman heifers in northern Australia.

References


KISSPEPTIN AND GONADOTROPIN INHIBITORY HORMONE: YING-YANG REGULATORS OF REPRODUCTION.

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Gonadotropin releasing hormone (GnRH) has been regarded as the primary driver of reproduction, since its discovery 40 years ago. This brain neuropeptide controls the synthesis and secretion of gonadotropins from the pituitary gland through its cognate receptor. Modulation of the secretion and action of GnRH is by various other brain systems as well as feedback action of sex steroids from the gonads. How various factors such as stress, nutrition, season and emotive states regulate/modulate GnRH function has been the subject of intense study, but the key elements that convey information to GnRH cells and/or gonadotropes have only recently been identified. These are kisspeptin and gonadotropin inhibitory hormone (GnIH), which are RF-amide peptides signalling through GPR54 and GPR147 respectively. Kisspeptin and GnIH cells are immediately distal to GnRH cells and GnIH cells also appear to play a hypophysiotropic role, modulating GnRH action at the level of the pituitary gland. Kisspeptin stimulates GnRH secretion and GnIH appears to inhibit secretion although there is no direct evidence for this. The neurons that produce these two potent RF-amide peptides mastermind reproductive function, responding to signals such as homeostatic hormones (e.g. leptin) and sex steroids. This is because the GnRH neurons do not possess the relevant sex steroid receptors or other receptors for factors that modulate reproduction. This allows convergence on GnRH cells of respective stimulatory and inhibitory factors. Based on our recent studies of pituitary gonadotropes, GnIH modulates GnRH function by convergence of signalling pathways. Thus, GnRH causes release of luteinising hormone by release of intracellular calcium and this secretory process is antagonised by GnIH. The stimulation of gonadotropin synthesis is effected by GnRH-stimulation of the ERK pathway and this is also antagonised by GnIH. A similar convergence of signalling pathways for GnIH and kisspeptin may also occur in GnRH cells, but relevant data are cursory at this stage.

The recent identification of kisspeptin and GnIH has significantly advanced our understanding of how the reproductive axis is regulated. Because these two peptides, like GnRH, act via GPCR, post-receptor signalling pathways are able to converge. Full appreciation of the extent to which kisspeptin and GnIH interact to regulate GnRH secretion and action should expand possibilities to modulate reproduction.
INTERACTION BETWEEN KISSPEPTIN AND GnRH NEURONS ACROSS THE EQUINE OESTROUS CYCLE – WHICH ONE IS IN CONTROL?

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Kisspeptin (KP) is a peptide product of the KiSS-1 gene. KP plays a key role in reproductive function including the regulation of gonadotrophin releasing hormone (GnRH) and luteinising hormone (LH) secretion in many species but little is known about its role in the mare. In this study, we examined the location of KP-producing neurons in the brain of the mare, their potential interactions with GnRH neurons, and temporal changes in their expression across the oestrous cycle. Mares (n=3/group) were euthanased at oestrus (just prior to ovulation), mid-dioestrus, and late dioestrus and the head was perfusion fixed with paraformaldehyde, and the hypothalamus collected. Coronal sections (40μm) were used for dual-labelling, immunostained for KP & GnRH as demonstrated in Figure 1.

Figure 1 - Example of GnRH neuron (green) - Kisspeptin fibre (red) interaction. Image taken from a Group 3 mare in the arcuate nucleus of the caudal MBH. The main image shows the xy-plane, to the right is the yz-plane, and the bottom is the xz-plane.

The majority of KP-immunoreactive (-ir) neurons were located in the arcuate nucleus/median eminence (especially mid and caudal regions), and periventricular nucleus. There was a trend (P=0.09) towards increasing numbers of KP-ir neurons across the cycle. GnRH-ir neurons, located primarily in the arcuate nucleus (especially mid arcuate), as well as the preoptic area, did not change in number across the cycle. Numerous interactions between KP and GnRH neurons were observed, primarily in the arcuate nucleus; KP fibres interacting with GnRH cell bodies, fibre-fibre interactions between KP and GnRH, and GnRH fibres interacting with KP cell bodies. Overall we found KP inputs to 32% of GnRH-ir cells, but the number of these...
interactions did not vary across the oestrous cycle. This study has confirmed the reciprocal innervation between KP & GnRH neurons in the mare. Although we did not detect variation in the degree across the oestrous cycle this may reflect the sample size issues inherent to equine research. Further research could lead to the development of improved control of the oestrous cycle and improved management strategies for the transition period.
TESTICULAR FUNCTION AND INSULIN-LIKE GROWTH FACTOR-1 RESPONSE TO GROWTH HORMONE TREATMENT IN SUB-FERTILE STALLIONS

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Introduction: Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) are thought to modulate testicular function (Roser 2008). However, the relative importance of these two hormones in stallion fertility has not been determined, nor has the efficacy of exogenous GH as a treatment for fertility problems. The aims of this study were to develop a reliable method for measuring seminal GH and IGF-1; and to determine the effects of equine somatotropin (eST) on semen parameters and GH/IGF-1 concentrations in blood serum and seminal fluid from two sub-fertile stallions.

Materials and methods:

Blood serum and seminal plasma GH concentrations were measured using a heterologous radioimmunoassay previously validated for use in equine serum (Noble 2001). Serum and seminal IGF-1 concentrations were measured using a two-site immunoradiometric assay (IRMA; DSL2800, Diagnostic Systems Laboratories, Texas, USA) also validated previously for use in equine serum (Noble 2001; Noble and Sillence 2001).

Two sub-fertile stallions were used to determine the effects of GH administration on fertility parameters. One stallion was a 19-year-old Standardbred (SB) with few spermatozoa and low progressive motility. The other was a 6-year-old Thoroughbred (TB) with testicular degeneration, characterised by poor motility despite a high percentage of normal, live spermatozoa. Both stallions were given 10 mg eST (BresaGen Ltd, Thebarton, SA) daily via intramuscular injection for either three months (SB) or two months (TB). At monthly intervals throughout the treatment period, blood and semen samples were obtained and stored at −20°C (blood serum) or −80°C (seminal plasma) until analysed. Samples were also obtained from both stallions for either two months (SB) or at seven months post-treatment (TB). A complete semen analysis (gel-free volume, colour, progressive motility, percent normal morphology,
live:dead ratio, sperm concentration and total sperm per ejaculate) was performed on the day of each semen collection.

**Results:** Immunoassays were validated for the measurement of GH and IGF-1 in seminal plasma. Both assays demonstrated good parallelism and recovery (89% to 109%). Intra-assay variation was minimal, except for the serum GH assay (15.5%), indicating that poor precision is a limitation of this assay. Unlike blood serum, the semen samples required processing by acid column chromatography prior to assaying for IGF-1 concentration to remove IGF-BP.

Semen parameters obtained from the SB did not alter throughout eST treatment. However, in the TB sperm concentration and total sperm per ejaculate decreased, whilst the percent live spermatozoa increased from 40 to 70% following eST treatment. These parameters returned to pre-treatment levels six months post-treatment.

Basal serum GH was undetectable (<0.42 ng/ml) for both stallions, and did not alter during treatment in the SB, but rose to 15 ng/ml during treatment in the TB. There was considerable variation between the SB and TB, respectively, in terms of basal seminal GH (100 ng/ml vs. 5 ng/ml) and IGF-1 (5 ng/ml vs. <2.5 ng/ml), as well as serum IGF-1 (300 ng/ml vs. 150 ng/ml). Seminal plasma GH, as well as seminal plasma and serum IGF-1, increased in both stallions during treatment (Figure 1).

**Conclusions:** Immunoassays suitable for the measurement of GH and IGF-1 in seminal plasma were validated. Both subfertile stallions responded hormonally to eST treatment, but neither showed a positive response in terms of semen quality
Figure 1: Hormone response of a subfertile Standardbred (SB; ●) and Thoroughbred (TB; ○) stallion to daily treatment with 10 mg eGH. Seminal GH (A) and insulin-like growth factor-1 (B), and serum insulin-like growth factor-1 (C). The treatment periods extended from June until September (SB), and from July until October (TB).

References:


Leptin was originally identified as a peripheral satiety signal made by fat cells and it was proposed that as fat accumulates leptin is secreted proportionally. Leptin then crosses the blood brain barrier and signals to the hypothalamus suppressing appetite and increasing energy expenditure as it rises (Zhang et al 1994). It is evident that if the leptin gene or its receptor is disrupted then morbid obesity occurs. However, obesity in humans is very rarely caused by a disruption of the leptin gene or its receptor, and in many species a good correlation exists between leptin concentrations in plasma with the degree of obesity. This early finding was unexpected and led to the leptin resistance hypothesis. Further studies have revealed that leptin actually appears to be almost ubiquitously expressed in many tissues. Consequently it remains unclear as to the role of leptin in the peripheral circulation.

There is increasing evidence that, in addition to its action on food intake and energy expenditure, leptin plays an important role in reproduction and development (Cervero et al., 2005). The ob/ob mouse is deficient in leptin and is obese and infertile. Fertility can be restored in both female and male ob/ob mice by the exogenous provision of leptin, which is characterized by an increase in basal LH and FSH (Mounzih et al., 1997). During the menstrual cycle, there are variations in serum leptin levels, with higher concentration reported in the preovulatory and midluteal phases and lower in the early follicular phase (Henson and Castracane, 2005). Indeed, the ob/ob mouse exhibits numerous reproductive abnormalities including unusually small ovarian weight in females and a small amount of interstitial tissue in males (Jones & Ainsworth-Harrison, 1957). Treatment of ob/ob mice with leptin results in a normalization of testicular and ovarian weight and function (Mounzih et al., 1997), thus suggesting that leptin may be required for normal growth and development of reproductive organs. The restoration of fertility to the female ob/ob mouse by the exogenous administration of leptin has demonstrated that leptin is essential for normal preimplantation and/or implantation processes (Malik et al., 2001). We and others have reported a beneficial effect of low physiological doses of leptin on early embryo development in culture and that higher doses impeded development. Leptin would also appear to be important during implantation and early pregnancy (Malik et al 2001; Henson and Castracane, 2005).

While it is clear that in the complete absence of leptin the reproductive systems does not function. The data from the above studies suggests that peripheral leptin may act as an inhibitor of both follicular development in the ovary and of early embryo development or implantation. It is interesting to speculate that leptin may have a permissive role when acting centrally and an inhibitory role in the reproductive tract, thus acting as a responder to over nutrition rather than under nutrition. Thus it would appear that reducing peripheral leptin
enhances reproduction in both mono and poly ovulatory species. To determine if this was also true in the horse we measured leptin in mares before and after joining to determine if there was any correlation between leptin concentrations and early pregnancy.

Using mares (n=30) from a stock horse stud served by a single stallion blood samples were taken at joining and at day 35 when eCG firsts starts to be secreted and days 60, 90 and 120. The mares in the experiment were pasture fed with no supplemental feed. The blood samples were assayed for leptin, glucose, NEFA and eCG.

Plasma leptin concentrations in those mares that became pregnant (n=25) and carried a foal to term were significantly lower (5.8 ± 0.8 ng/ml) at joining than those mares which did not become pregnant (8.2 ± 1.2 ng/ml; n=7). Leptin declined slightly over the rest of the sampling period of 120 days. Equine chorionic gonadotrophin was detectable in all pregnant mares at days 60-120 with the peak concentration varying from 15-over 100 iu/ml except for one mare which had no detectable eCG (despite being pregnant and giving birth to a live foal) and was excluded from the study. Glucose concentrations were also significantly different between those mares that became pregnant (5.2±0.25 mMol/l) and those that did not (4.5±0.35 mMol/l) at day 35. Glucose then declined in the pregnant group to be not significantly different from the non pregnant group for the rest of the sampling period. NEFA concentrations were not significantly different between the groups at any sampling period.

Although a much larger study is required with more frequent blood sampling to confirm these findings the data suggests that leptin in the lower physiological range may enhance the chance of reproductive success in mares, however the mechanism remains unclear. Leptin is likely to play an important role as a paracrine modulator of implantation and is expressed in the epithelial cells of the uterus and uterine tubes and there no evidence to show plasma leptin influences this local expression. Thus lower plasma leptin concentrations may be reflective of other important processes in pregnancy such as attenuation of the immune system (Matarese 2000) rather than being a direct causative effect on conception and implantation.

References


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ECG: IS IT NECESSARY FOR A SUCCESSFUL PREGNANCY?

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The recognition of a developing embryo by the maternal system is a critical stage in the reproductive process of mammals. There is considerable diversity in the mechanism utilized by various species. In humans, other primates and the horse the developing embryonic tissue secretes a hormone called chorionic gonadotrophin (hCG, eCG). This hormone is very similar to luteinizing hormone (LH) and binds to LH receptors in the corpus luteum and maintains the secretion of progesterone the hormone critical to the establishment and maintenance of pregnancy. In sheep and other ruminants the corpus luteum regresses at the end of the non pregnancy period in response to the rising concentrations of prostaglandin F\textsubscript{2a} (PGF\textsubscript{2a}) secreted by the endometrium in response to prolonged progesterone stimulation. The developing embryo in these species secretes the cytokine interferon tau which inhibits the secretion of PGF\textsubscript{2a} by the endometrium.

In primates the secretion of hCG occurs very early in pregnancy and is detectable within a week of conception. The synthesis of eCG within a pregnant mare does not occur until a considerable period of time has lapsed after conception. A unique feature of the equine embryo is the development of the chorionic girdle on the outer surface of the chorion between days 25 and 35 of gestation (Allen & Moor, 1972). The girdle is first noticed around day 25 as a series of shallow undulations in the chorion (Allen, 2000). By day 35, they have developed into elongated finger like villous ridges which adhere to the luminal surface of endometrium (Allen & Moor, 1972). Within 24 hours, the entire girdle peels off the foetal membrane and the now binucleate girdle cells start to invade the uterus (Allen et al, 1973). Over the next 3 to 4 days, these trophoblast cells penetrate down, through the endometrium’s epithelial cells to the basement membrane and glands before becoming sensile and enlarged, developing into endometrial cups (Allen & Moor, 1972). It is from these cups that the hormone, eCG, is secreted via lymph sinuses. Concentrations of eCG rise rapidly from day 38 to 40, reaching a peak ranging from 20-300 iu/ml around day 60 to 70 before declining steadily in parallel with the degeneration of the endometrial cups (Cole & Hart, 1930). However eCG like material has been found well beyond this period in urine and at term placenta (Roser et al 1989; McFarlane et al 1991).

Daels et al (1998) suggested that eCG may directly influence the primary CL to continue to secrete progesterone, a theory proposed initially by Squires & Ginther (1975). The idea that eCG is produced to maintain the primary CL for the first three months of gestation is further supported by Saint-Dizier et al (2003). They reported that the primary CL remained responsive to eCG throughout the period of gestation from around day 37 until day 130 when the foetoplacental unit became the source of hormones essential for the maintenance of pregnancy. Squires & Ginther (1975) reported that primary CL’s regress in size and secretory activity earlier in mares that were hysterectomised compared to pregnant mares. While
research seems to suggest that eCG may be necessary to help maintain the development and growth of the embryo by sustaining the primary CL, it has also been observed that some mares do not produce large quantities and, yet, are still capable of sustaining a full term pregnancy. This places in doubt much of the accepted literature that, without the presence of eCG in substantial concentrations, the survival of the primary CL is compromised. Could it be possible that other substances are being produced by these mares which are performing a similar task proposed of eCG? Could eCG in fact play a supportive role in the production of this/these other substances instead of a main role in the CL’s (primary or secondary) survival?

To examine the hypothesis that eCG may not be as important in the equine pregnancy as currently accepted we examined the eCG profile of 260 mares of various breeds we recruited from a commercial eCG production facility and from a stock horse stud. The mares were divided into 5 groups (0, 0≤5, 5≤10, 50≤100 and >100 iu/ml) based on the peak eCG concentrations and progesterone concentrations measured. Progesterone concentrations were not significantly different between those mares with eCG concentrations above 5iu/ml while those who had undetectable concentrations of eCG had significantly lower progesterone concentrations but were at least 70% of the other groups. Thus while eCG does appear to increase progesterone concentrations the effect while significant is marginal similar to the results observed by Daels et al (1998) where mares were given exogenous eCG. However importantly 4 mares had undetectable concentrations of eCG and one of these went on to deliver a healthy foal. The others had their pregnancy terminated at 90 days as part of the procedure at the commercial eCG production facility. This data strongly suggests eCG is unnecessary for a successful pregnancy and that perhaps this is the reason for the highly variable isoform composition between mares being reflective of an obsolete gene.

References


THE MARE AS A MODEL FOR HUMAN INFERTILITY

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Ovulation failure is reported in mares (Ginther, 1992), cows (Peter, 2004), bitches (Meyers-Wallen, 2007), camelids (Adams, 1991) and women (Qublan et al, 2006). In mares, the most commonly described anovulatory condition is the development of haemorrhagic anovulatory follicles (HAF) where the follicle fails to rupture and its cavity becomes filled with blood and fibrin strands (Ellenberger et al, 2008). The condition has a reported incidence of 5-20% of all oestrus cycles and is more common during the spring and autumn transition periods (Ginther et al, 2007; McCue and Squires, 2002).

Due to their size and the ease with which their reproductive tract can be imaged ultrasonographically, mares may offer a suitable comparative model for the study of a similar anovulatory condition in women known as luteinised unruptured follicle syndrome (LUF) (Ginther et al, 2008; Mihm and Evans, 2008). This syndrome is defined as a failure of ovulation and intra-follicular haemorrhage with luteinisation (Qublan et al, 2006) which is morphologically identical to HAF formation in the mare (Liukkonen et al, 1984; Zaidi et al, 1995). A model which reliably induces HAF formation in the mare may enable study of the LUF syndrome in women. Previous attempts to create a “HAF model” in mares have focused on exposing immature follicles to supraphysiological concentrations of LH through a combination of follicle ablation and prostaglandin treatment (Ginther et al, 2008). However, this model resulted in HAF formation in only 20% of follicular waves.

We have previously shown that the chorionic girdle can be successfully transplanted to ectopic sites such as the vulval submucosa in non-pregnant mares where the trophoblast cells differentiate (Adams and Antzcak, 2001). These transplanted trophoblast cells not only survived in the submucosa for a similar length of time as the endometrial cups in the uterus during pregnancy but they produced significant quantities of eCG that was detected in the serum of the recipient mares (de Mestre et al, 2008). eCG has LH-like activity in the mare and the protein structure of eCG is identical to that of eLH with a single gene encoding the β-subunits of both hormones (Saint-Dizier et al, 2004; Sherman et al, 1992) and we hypothesise that transplantation of chorionic girdle and associated eCG production in non-pregnant mares will result in HAF development. The aim of this study was to determine whether transplantation of equine chorionic girdle into non-pregnant recipients could act as a suitable model for HAF development in the mare.
Eight non-lactating mares of various breeds aged 3-7 years were used for this study and were maintained at the Baker Institute for Animal Health, Cornell University, USA. Transplantation of chorionic girdle was performed as previously described (Adams and Antczak, 2001). Chorionic girdles of day 34 embryos were obtained non-surgically and sectioned chorionic girdle strips were injected superficially into the vulval submucosa of four recipient mares. Preparation and injection of allantochorion was performed in the same way. Two control mares were transplanted with allantochorion and two control mares were transplanted with media only.

Mares were examined bi-weekly by transrectal ultrasound commencing 7 days prior to chorionic girdle transplantation. Ultrasound examination continued until 112 days after transplant. Blood samples were collected at the time of ultrasound examination and assayed for eCG and P4 concentrations.

The development of 18 HAFs was recorded during 19 follicular waves (94.7%) in mares transplanted with chorionic girdle. The process of HAF development that we observed was typical of that previously described (Ginther et al, 2007; McCue and Squires, 2002; Ginther et al, 2008; Ellenberger et al, 2008; Cuervo-Arango and Newcombe, 2008). None of the control mares produced a HAF during the study. Transplantation of chorionic girdle resulted in elevated eCG concentrations for at least 77 days. This confirms what we have reported previously (de Mestre et al, 2008). In association with the formation of HAFs in our study, we observed a sustained elevation in serum progesterone concentrations in all mares.

In conclusion, we have shown that transplantation of chorionic girdle into the submucosa of the vulva in non-pregnant mares induces HAF formation. The mechanism of action is presumed to be via the exposure of follicles to high levels of eCG produced by the trophoblast cells of the chorionic girdle. This model appears to offer a potential method of studying the HAF syndrome in mares and possibly the LUF syndrome, a similar condition, in women.

References

THE RENAISSANCE OF OXYTETRACYCLINE – A TREATMENT FOR EQUINE ENDOMETRITIS?

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Despite their widespread use within the equine breeding industry, there is a lack of published data regarding the efficacy and pharmacokinetics, after intrauterine infusion, of many antibiotics. One antibiotic which is not commonly used for the treatment of endometritis in mares is oxytetracycline (OTC). This is despite its potential effectiveness for this condition based on bacterial susceptibility spectrums. The reason for the reluctance of veterinarians to use OTC seems to be due to minimal evidence suggesting OTC causes irritation and potential long-term damage to the endometrium (Bennett et al., 1980).

There is also a lack of data describing the pharmacokinetics of intrauterine administration of OTC in the mare. Thus, it is not known if intrauterine administration of OTC achieves adequate concentrations in the endometrium to be effective against target bacteria.

This study was designed to develop a model for investigating the pharmacokinetics and potential pathology of antibiotics in the mare after intrauterine infusion, using OTC as the test drug. The null hypotheses to be tested were that intrauterine infusion of 6 grams (g) of OTC does not result in endometrial uptake; intrauterine infusion of OTC does not reach the tissue minimum inhibitory concentration (MIC) of the common bacterial pathogens causing endometritis in the mare; intrauterine infusion of OTC does not result in systemic absorption; and intrauterine infusion of OTC does not result in endometrial pathology.

Eight mares acted as the treatment group and two as the control group, each mare also received pre-treatment biopsies, acting as self-controls. Six grams of oxytetracycline as the dihydrate was infused into the mares’ uterus for three consecutive days beginning on day 2 of oestrus. Endometrial biopsies were collected at eight, 24 and 48 hours following the final infusion. A final biopsy was collected during the oestrus following treatment. Endometrial and plasma OTC concentrations were determined by extraction followed by HPLC. Endometrial integrity was assessed using standard histological preparation and staining followed by categorisation using a modified Kenny grading system (Kenney and Doig, 1986; Schlafer, 2007).

With regard to the pharmacokinetics, results of this study demonstrate that OTC was present in endometrium above MIC for at least eight hours and there was negligible systemic absorption. The concentrations detected in the endometrium at both three and eight hours post-infusion were greater than the reported MIC of the four common bacterial pathogens causing endometritis in mares as shown in Figure 1.
With regard to effects on the endometrium, results of this study suggest that once daily infusion of 6 g of oxytetracycline as the dihydrate for 3 consecutive days can cause transient inflammation, but is unlikely to be detrimental to the endometrium of the mare in the longer term. However, studies involving larger numbers of mares and using post-treatment pregnancy rate as an end-point are warranted. Until these studies are performed, the conservative recommendation is that intrauterine oxytetracycline infusion be used only in specific cases where more routine treatments have failed and culture and sensitivity results suggest oxytetracycline may be a suitable antibiotic choice.

Reference List


USE OF UTERINE BACTERIOLOGY WITH CYTOLOGY AS PREDICTIVE FACTORS FOR CONCEPTION.

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Endometritis has been reported to be the third most common medical condition of horses (Card, 2005) and is an important cause of reduced fertility in mares (Hurtgen, 2006) in particular the most common cause of embryonic loss before day 35 in normally cycling mares (Knottenbelt, 2003). Previous reports in the literature on the diagnosis and treatment of endometritis in the mare are contradictory and opinions vary considerably (Liu and Troedsson, 2008). Uterine bacteriology with cytology has long been reported to be a useful diagnostic tool in identifying mares with endometritis (Wingfield Digby and Ricketts, 1982) and at risk of reduced 28 day pregnancy rates (Riddle et al., 2007), however, studies have failed to investigate the use of uterine bacteriology and cytology and their predictive value with regard to live foal rates, therefore, that is the aim of this study.

Material and Methods:

Guarded swabs for uterine endometrial cytology and bacteriology were simultaneously collected from 1614 mares on 17 stud farms in the Hunter Valley region of NSW by 5 veterinarians at Scone Equine Hospital during the 2008 breeding season. All mares, including maiden (181), foaling (1059), barren (306) and problem (67) had a swab taken for cytological and bacteriological analysis at the beginning of the season prior to covering. The 1614 mares were further broken down into age groups as follows: Group A (young 3-8 years), group B (middle aged 9-13 years), group C (old mares 14-18 years) and group D (oldest mares >18 years). The swabs were transported to the laboratory at Scone Equine Hospital on the same day as collection. Cytology was classified as non-inflammatory 0-5% neutrophils, mild inflammation 5-15% neutrophils, moderate inflammation 16-30% neutrophils and severe inflammation >30% neutrophils (Card, 2005). Bacterial growth was classified as no growth, mixed flora (2 or more organisms) or positive (for one bacterial isolate only). Swabs that had a cytological presence of >10% neutrophils and no bacterial growth were also put in cooked meat medium and cultured aerobically for 72 hours in order to identify possible anaerobic pathogens. Pregnancy outcome was classified as missed, slipped (in accordance with Australian stud book definitions) or live foal. Pearson’s $\chi^2$ analysis (Genstat) was used for statistical analysis throughout, a p value of <0.05 was considered significant.

Results:

Age group was significantly (p < 0.001) associated with pregnancy outcome, live foal rates varying between the young (76%), middle aged (74%), old (65%) and oldest mares (31%). There
was a significant (p< 0.001) association between reproductive status and pregnancy outcome, with foaling rates varying between 77% for maiden mares and 61% for problem mares. 1518 (94%) of the 1614 paired cytological and bacteriological samples were normal (< 5% neutrophils and no growth). These control mares had a live foal rate of 74%, 19% missed and 7% slipped. There was a significant association (p< 0.005) between positive bacterial growth and decreased pregnancy rates. Of these positive mares 56% had a live foal, 40% missed and 4% slipped. The major contributors to the positive category were beta haemolytic streptococci (62%) with a live foal rate of 71% and Escherichia coli (12%) with a live foal rate of 17%. There was no significant association between mixed bacterial flora and inflammation or live foal rates though they were lower (71%) compared to controls; 22% mares missed and 7% slipped. There was a significant (p< 0.05) association between inflammation and live foal rate. Mares that fell into the mild inflammatory category had a live foal rate of only 33%, while the overall percentage for mares with mild, moderate or severe inflammation having a live foal was 61%. There was a positive association (p< 0.05) between mares that had a positive swab, inflammatory cytology and pregnancy outcome, with poorer live foal rates for these mares (53%), with 41% of mares classified as missed and 6% as slipped.

Discussion:

Individually both uterine bacteriology and cytology are diagnostic in predicting pregnancy outcome, as mares with either a positive culture or inflammation on cytology had lower live foal rates than the control group. However, they are most prognostic when used together and mares with an inflammatory cytology and positive bacteriology were the least likely to produce a live foal (53%). Our data did not support the finding of Riddle et al., which stated that all mares with two or more organisms (mixed flora) isolated in the uterus had moderate or severe inflammation on cytological analysis. The type of pathogen isolated is important with a live foal rate of only 17% in mares with Escherichia coli isolated. Sampling techniques used in this study were viewed to be successful as only 2.7 % of swabs were hypocellular yielding inadequate epithelial cells to allow analysis. Mares with a mild inflammatory response had a disproportionately low number of live foals, it is thought that these mares may not have been viewed as high risk and therefore not treated as aggressively as mare with moderate and severe inflammation or may have developed post breeding endometritis. Analysis of the data is ongoing and further research is being undertaken to assess such things as the predictive value of the intermediary and last mare swab versus first mare swab in repeat breeders and the possibility of creating a predictive equation for live foal rates based on mare age, reproductive status, and uterine bacteriology with cytology. In conclusion, this study demonstrates that cytology and bacteriology are predictive of live foal rates.

References:


USE OF MARES PREVIOUS BREEDING RECORD TO PREDICT FERTILITY

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Introduction

Although the breeding efficiency of thoroughbred horses has improved during the previous 20 years, foaling rates of thoroughbred horses in Australia remain between 60% and 70%. Large numbers of brood mares are bought and sold each year. The value of each mare is primarily determined by the racing performance of the individual, her close relatives or her progeny. However, the mare’s fertility is a critical determinant of her capacity to generate income and deliver a return on the owner’s investment. There is no recently published data investigating the use of breeding history of mares to predict future foaling rates. This study was designed to determine whether previous breeding performance is able to predict fertility of thoroughbred brood mares.

Materials and Methods

The breeding records of 190 thoroughbred mares were retrieved from the Australian Stud Book. Mares that had uterine or clitoral samples submitted to SEH laboratory for bacterial culture during 2006 were randomly selected for inclusion in the study. Inclusion in the data set required the mare had a return submitted to the Stud Book for the 2009 foaling season (2008 breeding season) and more than 3 mare returns recorded from 2003 to 2007. The outcome of each breeding season from 2003 through 2007 was recorded as foaled, slipped or missed. Years the mare was not served were not included in the analysis. The live foaling rate, the proportion of years the mare missed certification as pregnant and the proportion of pregnancies slipped (aborted or delivered a dead foal) were calculated for each mare. Mares with a live foaling rate of greater than 50% were classified as “good” breeders (130) and with a live foaling rate of 50% or less were classified as “poor” breeders (60). The probability that a mare would produce a live foal in 2009 was determined for good and poor breeders. Mares that had slipped 50% or more of their pregnancies were classified as high risk to slip and mares that had missed 50% or more of the years served were classified as high risk to miss. The probability that a high risk to slip mare would slip in 2009 and that a high risk to miss mare would miss in 2009 were calculated.
Results

In 2009 there were 121 (64%) live foals delivered, 41 mares missed and 28 mares slipped from the 190 mares included in the analysis. This is similar to the rates reported for the Australian thoroughbred population for 2008 breeding season by the Australian Stud Book. The probability that a poor breeder would not produce a live foal in 2009 was higher than a good breeder not producing a live foal (p=.0009, RR 1.9, 95% CI 1.3 – 2.7). The probability that a poor breeder would slip the fetus tended to be higher than the probability that a good breeder would slip the fetus (p=0.07, RR 1.8, 95% CI 0.9 – 3.6). The probability that a poor breeder would miss pregnancy certification was higher than the probability that a good breeder would miss (p=0.002, RR 2.3 95%CI 1.3 – 3.9).

The probability that a mare would slip the fetus conceived in 2008 was higher for mares that had previously slipped 50% or more of their pregnancies compared with mares that had slipped less than 50% of their previous pregnancies (p=0.0045, RR 4.8 95% CI 2.7 – 8.5). The probability that a mare would miss in 2008 was higher in mares that had previously missed pregnancy certification 50% or more of the years served than in mares that had missed less than 50% of pregnancy certifications in previous years(p= 0.046, RR 2.1, 95% CI 1.1 – 4.0).

Discussion

As expected, the previous breeding history of a population of mares predicts future foaling rate for the population accurately. The foaling rate of mares with a good breeding history was over 70%, which is higher than the breed average, and the foaling rate of mares with a poor breeding history was 47% which is much lower that the breed average. A mare with a poor breeding history was almost twice as likely not to produce a foal in 2009 as a mare with a good breeding history. A mare that had missed more than 50% of previous breeding seasons was two times more likely to fail to miss in 2008 than mares that recorded pregnancy certification.
more than 50% of the time. In addition, a mare that had a history of aborting 50% or more of her pregnancies had a relative risk of slipping almost 5 times that of a mare with a history of slipping less than half her pregnancies.

These data show that mares can be classified based on breeding history and this classification indicated the probability of the mare delivering a live foal in the following year. Mares that have frequently aborted previous pregnancies are highly likely to abort again. Mares that have previously missed are likely to repeat the problem. Identification of problem mares before the breeding season would allow appropriate intervention to improve the probability of delivery of a live foal.
INCIDENCE OF PSEUDOMONAS AERUGINOSA & KLEBSIELLA PNEUMONIAE ISOLATION FROM CLITORAL SWAB SAMPLES: EFFECT ON PREGNANCY RATES AND ANTIBIOTIC SENSITIVITY PATTERN

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Introduction: Samples of clitoral bacterial flora are obtained from thoroughbred mares before natural service to detect the presence of the venereal transmitted bacteria, Klebsiella pneumoniae and Pseudomonas aeruginosa. The presence of these bacteria prevents breeding of the mare until the infection is cleared. This limits the number of opportunities a mare has to conceive during the breeding season and is likely to result in a lower pregnancy rate. Changing antibiotic resistance patterns of pathogenic bacteria are of great concern in both human and animal populations. Bacteria resistant to commonly used antibiotics can be difficult to eliminate, thus the amount of time infection is present is increased. This is of concern to the thoroughbred breeding industry, as a mare with a persistent clitoral infection may reach the end of the breeding season without the opportunity to conceive. This project investigated the incidence of detection of Klebsiella pneumoniae and Pseudomonas aeruginosa in clitoral samples of a thoroughbred mare population, described the antibiotic resistance patterns of the bacteria, and analysed the effect of the presence of Klebsiella pneumoniae or Pseudomonas aeruginosa in clitoral samples on the annual pregnancy rate.

Materials and Methods: The results of 11,208 clitoral samples obtained from thoroughbred mares during 2006, 2007 2008 and 2009 were reviewed. The samples were cultured on Oxoid Pseudomonas CFC and MacConkey No3 agar and incubated at 37°C for 36-48 hrs. Positive cultures of Klebsiella pneumonia were identified by Biomerieux 20EAPI. Presumptive identification of Pseudomonas aeruginosa was by appearance, pigmentation, positive oxidase test, and odour. Isolated organisms were tested for antibiotic sensitivity using a modified Kirby Bauer method: isolates were incubated on Oxoid Sensitestate plates for 18-24hr at 37°C. During 2007, 2008 and 2009, antibiotic disks were used for comparative sensitivities, with disks containing 30µg ceftiofur, 5µg enrofloxacin, 10µg gentamicin, 30µg neomycin, 75µg ticarcillin, and 30µg amikacin oxoid discs.

The breeding outcome of 253 mares with a positive (Klebsiella pneumoniae or Pseudomonas aeruginosa) clitoral sample and of 373 mares with a negative clitoral sample collected at the same time on the same farm were retrieved from the Australian Studbook website. Data was entered into a spreadsheet and transferred to a statistical program for analysis.

Results: Klebsiella pneumoniae or Pseudomonas aeruginosa was isolated from 375 (3.3%) clitoral samples collected from 253 mares during 2006, 2007 and 2008; of which 35.2% were Klebsiella pneumoniae and 64.8% were Pseudomonas aeruginosa. There was no difference in pregnancy rates between mares positive for Pseudomonas or Klebsiella. Mares with a positive clitoral sample were more likely to end the breeding season with a status of “missed” or “not served” than mares with negative clitoral samples. However, the rate of pregnancy for 373 mares with a negative clitoral sample was 69.2%, which was similar to the pregnancy rate for
the 253 positive mares (68.2%). The number of mares that tested positive each year did not significantly increase during the study period.

*Pseudomonas aeruginosa* was found to be resistant to more of the antibiotics tested than *Klebsiella pneumoniae* (Table 1). The resistance of both *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* to gentamicin is very low and does not change during the years investigated. *Pseudomonas aeruginosa* was more likely to be resistant to neomycin than *Klebsiella pneumoniae* \(p<0.0001, \text{RR} 1.9, 95\% \text{CI} 1.5 \text{ to } 2.5\). The resistance to neomycin was different over the years investigated due to changes in the resistance of *Pseudomonas aeruginosa* (2007: 84\%, 2008: 58\%, 2009: 86\%). *Pseudomonas aeruginosa* was more likely to be resistant to ceftiofur than *Klebsiella pneumoniae* \(p<0.0001, \text{RR} 5.7, 95\% \text{CI} 3.6 \text{ to } 9.3\), and the resistance patterns do not change over the years for either organism. *Pseudomonas aeruginosa* was more likely to be resistant to enrofloxacin than *Klebsiella pneumoniae* \(p<0.0001, \text{RR} 22.5, 95\% \text{CI} 7.4 \text{ to } 68.4\) and the resistance is different over the years due to changes in the resistance of *Pseudomonas aeruginosa* (2007: 82\%, 2008: 66\%, 2009: 94\%). Both organisms were predominantly sensitive to amikacin, however, *Pseudomonas aeruginosa* is less likely to be resistant to amikacin than *Klebsiella pneumonia* \(p=0.0115, \text{RR} 0.28, 95\% \text{CI} 0.10 \text{ to } 0.74\) and the resistance patterns do not change over the years for either organism. *Pseudomonas aeruginosa* is less likely to be resistant to ticarcillin than *Klebsiella pneumoniae* \(p<0.0001, \text{RR} 0.12, 95\% \text{CI} 0.08 \text{ to } 0.18\) and the resistance is different over the years due to increases in resistance of *Klebsiella pneumoniae* (2007: 46\%, 2008: 81\%, 2009: 89\%) and *Pseudomonas aeruginosa* (2007: 0\%, 2008: 10\%, 2009: 17\%).

**Table 1.** Average antibiotic resistance rates for clitoral *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates from 2006, 2007 and 2008.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>% Resistant Gentamicin</th>
<th>% Resistant Neomycin</th>
<th>% Resistant Ceftriaxone</th>
<th>% Resistant Enrofloxacin</th>
<th>% Resistant Amikacin</th>
<th>% Resistant Ticarcillin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>14.3</td>
<td>75.4</td>
<td>96.9</td>
<td>80.2</td>
<td>2.7</td>
<td>9.7</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>12.2</td>
<td>39.1</td>
<td>16.9</td>
<td>3.6</td>
<td>9.9</td>
<td>78.8</td>
</tr>
</tbody>
</table>

**Discussion:** One of the primary objectives of this project was to determine the difference in pregnancy rates between mares with positive clitoral swab results and those with negative results. *Klebsiella pneumoniae* is able to be sub-typed into a number of distinct groups, not all of which are pathogenic venereal bacterium. It was hypothesised that a clitoral swab positive for *Klebsiella pneumoniae* would result in a significantly lower pregnancy rate; hence utilization of sub-typing techniques would be important to improve pregnancy rates of these mares. There
was no difference in pregnancy rates between mares positive for *Klebsiella pneumoniae* and mares with negative clitoral swab results, therefore expensive sub-typing of isolates has no cost benefit.

The fact that a positive clitoral sample did not significantly affect pregnancy rates between the groups was unexpected. Mares with positive clitoral swab results were more likely to end the breeding season with a “missed” or “not served” status. However, decreased opportunities to breed in mares with positive clitoral swab results did not result in a lower pregnancy rate.

The organisms analysed in this study had unique resistance patterns, however both had a low incidence of resistance to gentamicin and amikacin. The only other antibiotic *Pseudomonas aeruginosa* was reliably sensitive to was ticarcillin; however, the incidence of resistance of *Pseudomonas aeruginosa* to this antibiotic increased during study period. Similarly, *Klebsiella pneumoniae* only had one other antibiotic it was consistently sensitive to; enrofloxacin, but its resistance rate did not change during the study period.
In comparison to other domestic species, the reproductive performance of horses is relatively low. Allen et al (2007) found that the foaling rate of Thoroughbred mares visiting stud farms in the UK during 2003 was 78.5%, a figure only marginally better than the foaling rate of a similar population of mares in 1983. Similar findings have been recorded in Australian Thoroughbred mares with some 10% of mares failing to carry a foal to term, and in any one year more than 20% of mares served failing to conceive (Bourke, 1995). This is in spite of significant technological advances in veterinary science such as ultrasonography, ovulation inducing drugs, treatment for endometritis etc. over that time period. Obviously reproductive wastage is a significant cause of financial loss to the Thoroughbred industry; no other livestock industry would accept a situation where 20% of its breeding herd failed to be productive each year. Given the value of the thoroughbred industry to the Australian and New Zealand economies ($1.4 billion per year in New Zealand from the IER Size and Scope study (2005)) it is surprising that little work has been done to determine the main factors that drive reproductive performance on Australian and New Zealand stud farms. Therefore we must assume (perhaps incorrectly) that the reproductive performance of mares in Australasia is similar to those overseas and that the causes of wastage are also similar. Given that horses in the southern hemisphere are managed differently (pasture-based, outdoors, larger farms etc.) to those in the northern hemisphere is likely that reproductive performance and the factors limiting its potential also differ. By knowing where these opportunities exist we can begin to focus on those areas of mare reproduction which are likely to enhance the production of viable foals and therefore improve the economic performance of the Thoroughbred breeding industry.

Previous studies investigating the reproductive performance of Thoroughbred broodmares have mostly used a univariate approach, whereby the outcome of interest (dependent variable), such as foaling rate, is singularly compared to an exposure variable (eg. mare age). This approach does not allow for the control of confounding variables (ie. exposure variables that are related to each other) such as stud farm and stallion for example.

The objectives of this study are to identify the main factors that determine the reproductive performance of Australasian Thoroughbred stud farms. Statistical techniques that control for confounding will be used, ie. multiple logistic regression for dichotomous outcomes such as pregnancy, and survival analysis for time-to-event outcomes such as time to conception after foaling. A prospective cohort study is currently underway involving 7 farms in the Waikato region of New Zealand and 2 farms in the Hunter Valley region of Australia. Data has been collected from these farms since the 2006 breeding season.

This presentation will outline some of the preliminary findings from this study.
References

Laparoscopic surgery is well suited to examining and performing procedures on the intra-abdominal equine reproductive tract. Advantages include superior access and visibility for many procedures, minimally invasive surgery giving lower morbidity and earlier return to work and many procedures can be performed standing (Walmsley 1999; Caron 2009). These impressive advantages have allowed surgeons to improve outcomes and also to offer a number of procedures which are not feasible by any other approach.

Initially reproductive laparoscopic surgery was mainly confined to the examination and biopsy of intra-abdominal organs, ovariectomy and cryptorchidectomy. As confidence in the techniques developed further indications were added including testes sparing inguinal hernia repairs, assessment and treatment of bleeding post castration, and tumour and mass removal. More recently application of PGE to the oviducts and imbrication of the mesometrium have been used in attempts to improve the fertility in selected mares.

Ovariectomy and granulosa thecal cell tumour removal are now commonly performed laparoscopically. Dissection and haemostasis is frequently achieved by the use of an electrosurgical device, the Ligasure® (Hand, Rakestraw et al. 2002; Hubert, Burba et al. 2006). Diseased ovaries up to 30 cm in diameter have been successfully removed by this technique (Lloyd, Walmsley et al. 2007). This device uses low voltage combined with high current and pressure to achieve haemostasis and can safely cauterise vessels up to 7mm diameter (Hand, Rakestraw et al. 2002; Hubert, Burba et al. 2006). Other means of haemostasis and dissection have been used and include various monopolar and bipolar cautery methods; the harmonic scalpel (Dusterdieck, Pleasant et al. 2003); stapling devices (Van Hoogmoed and Galuppo 2005); ligatures for example Endoloop® (Boure, Marcoux et al. 1997); and polyamide ties (Cokelaere, Martens et al. 2005). Cystic structures on the ovary can be aspirated and the use of endoscopic specimen bags assist in allowing large ovaries to be removed through relatively small flank incisions. Commonly laparoscopic ovariectomy is performed standing under sedation and local anaesthesia, which aids in reducing costs and morbidity.

Cryptorchidectomy may be performed with the horse standing or under general anaesthetic. Laparoscopic surgery allows excellent visualisation of the regions from the inguinal area to the kidney and allows the surgeon to diagnose with confidence the presence or absence of testes (Kelmer, Kramer et al. 2006). Testes from an inguinal location can usually be retracted into the abdomen. There has been some interest in techniques of ligation of the mesorchium and leaving inguinal or normally descended testes in situ (Rijkenhuizen and Grinwis 1999). This has not proved universally successful in causing complete testicular necrosis. Studies done to investigate this have demonstrated there is some collateral circulation to parts of the testes (Voermans, Rijkenhuizen et al. 2006). Again, haemostasis and transaction of the mesorchium in
castration techniques may be achieved by electrocautery (eg. Ligasure®) or using ligatures (Fischer and Vachon 1998; Hanrath and Rodgerson 2002; Varasano, Catini et al. 2008).

Laparoscopic surgery has proven useful in the diagnosis of post castration bleeding. The excellent visualisation in the dorsal abdomen in the standing horse can allow effective location and control of bleeding in these compromised horses without the need for general anaesthesia (Rijkenhuizen 2002). Inguinal hernia repair in entire males can be challenging if the surgeon’s goal is to spare the testes. There have been a number of laparoscopic techniques developed which may improve the prognosis in these cases (Marien 2001; Rossignol, Perrin et al. 2007; Caron 2008).

Tumour and mass removal or biopsy can be performed laparoscopically. There have been reports of uterine tumours resected (Muurlink, Walmsley et al. 2008) and of ovariohysterectomy (Gablehouse, Cary et al. 2009) in standing mares with the aid of laparoscopy. Recently the application of PGE to the oviducts using laparoscopic guidance has been reported. Fourteen of 15 mares with a history of chronic infertility became pregnant in the original report of this technique (Allen, Wilsher et al. 2006). Another technique recently described uses laparoscopic techniques to imbricate the mesometrium in older mares which have uteri which are sub-optimally positioned to allow natural drainage. The resulting uteropexy elevates the uterus and may result in improved fertility (Brink, Schumacher et al. 2010). These techniques add a new dimension to the management of sub fertile mares; however, more data is needed to fully assess the value of these procedures.

References

LAPAROSCOPIC APPLICATION OF PGE\textsubscript{2} TO RE-ESTABLISH OVIDUCAL PATENCY IN INFERTILE MARES

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Temporary blockage of the oviduct is a recognised cause of infertility in the absence of identifiable pathology of either the structure or function of the reproductive tract in the mare (Allen, Wilsher, Morris et al 2006). The presumptive diagnosis of this condition is made when a mare consistently fails to conceive despite being inseminated at the appropriate stage of oestrus and follicular development, by a stallion of known high fertility, and when the mare has show normal function of the cervix, uterus and ovaries, and has no evidence of infective endometritis or chronic degenerative endometritis that might interfere with conception. Oviducal blockage prevents the passage of the oocyte or zygote by acting as physical obstruction (Liu, Lantz, Schlafke et al 1991). A number of studies have identified the presence of obstructive masses, although the likely origin of the mass is speculative (Tsutsumi, Suzuki, Takeda et al 1979). Previous studies had identified increased fertility rates after physical flushing of the oviduct or following the application of PGE onto the oviduct (Zent, Liu and Spirito 1993. Allen, Wilsher, Morris et al 2006). The role of embryo-derived PGE\textsubscript{2} has been well demonstrated in the active transport of an embryo through the oviduct (Weber, Freeman, Vanderwall et al 1991).

Ten thoroughbred mares with a tentative diagnosis of oviducal blockage were treated by laparoscopic application of PGE\textsubscript{2} onto the oviduct 4 days following ovulation after natural service. The surgery was undertaken using the technique described by Allen et al. Briefly, the mares were sedated in a crush and both flanks were clipped and surgically prepared. A 10 mm laparoscope was inserted from the left flank and 0.2mg of a PGE\textsubscript{2} gel was applied to the entire length of oviduct. The procedure was then repeated from the right flank. The mares tolerated the procedure well and there were no surgical or post-operative complications encountered. The mares were allowed paddock exercise the day following surgery. The results are shown below. An 80% 15 day pregnancy rate was achieved, although one mare lost her pregnancy between an early and mid-ultrasound pregnancy examination. Of the ten mares, 30% were pregnant on the same cycle, 30% on the second cycle and 20% on the third cycle following surgery.
<table>
<thead>
<tr>
<th>Mare</th>
<th>Age</th>
<th>Years barren</th>
<th>Pregnancy post-treatment</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>4</td>
<td>Pregnant 3rd cycle</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>2</td>
<td>Pregnant 2nd cycle</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>2</td>
<td>Pregnant 1st cycle</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>2</td>
<td>Pregnant 2nd cycle</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>3</td>
<td>Failed to conceive</td>
<td>Served on two cycles</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>2</td>
<td>Pregnant on 1st cycle</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>2</td>
<td>Pregnant on 2nd cycle</td>
<td>Lost pregnancy between 18 and 30 d</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>2</td>
<td>Pregnant on 3rd cycle</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>1</td>
<td>Pregnant on 1st cycle</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>2</td>
<td>Failed to conceive</td>
<td>Served on three cycles</td>
</tr>
</tbody>
</table>

References:


OESTRUS SUPPRESSION IN THE MARE

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b Veterinary Large Animal Teaching Unit, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

Oestrous behaviour may account for variations in performance and temperament of ridden mares. Suppression of cyclicity may be advantageous for mares with oestrous cycle-related behaviour and performance problems, and less commonly for mares that show signs of pain or colic during oestrus. Commonly used hormonal methods of oestrus suppression in the mare include synthetic progestins such as daily oral supplementation with altrenogest (Regumate®), which can be time consuming and expensive.

When exogenous oxytocin is administered in the mid-luteal phase (starting before day 10 post-ovulation) it may result in prolonged dioestrus, and thus may be a useful method to suppress oestrus in cycling mares. The optimal route, dose rate and dose intervals of oxytocin administration to induce prolonged dioestrus have not been determined, nor has the duration of oxytocin-induced prolonged dioestrus been described (Goff et al 1987, Stout et al 1996, Vanderwall et al 2007).

We hypothesised that oxytocin given once daily (10 IU either intravenously or intramuscularly) from day 7 to 14 post-ovulation would be effective at suppressing oestrus in mares for up to 70 days.

The effects of oxytocin on follicular activity and oestrous behaviour were evaluated. Eighteen cycling mares were assigned to one of three treatment groups on the day of ovulation, starting in mid-November. All mares were treated once daily on days 7 to 14 post-ovulation. Mares in the OXY-IM group (n = 6) were injected intramuscularly (IM) with 10IU oxytocin, mares in the OXY-IV group (n=6) were injected intravenously (IV) with 10IU oxytocin, and mares in the control group with injected with 1ml of saline intravenously. Mares were teased by a pony stallion 3 times weekly for 9 weeks and categorised as displaying oestrous, dioestrous or neutral behaviour. Transrectal ultrasound examinations were performed three times weekly, or daily when a 35 mm follicle was identified until ovulation. Assessors of mare teasing behaviour and follicular activity were blinded to mare treatment group.

Results of teasing behaviour are shown in Figure 1. For all 18 mares there was agreement between teasing behaviour and ultrasonic ovarian activity: mares displaying dioestrus in response to the stallion had ultrasonic evidence of at least one corpus luteum. In the control group three mares cycled regularly; another mare ovulated once after the trial started, and then displayed prolonged dioestrus. The other 2 mares in the control group displayed prolonged dioestrus throughout the trial. In the OXY-IV group 5 mares displayed prolonged
dioestrus, while the others cycled regularly. In the OXY-IM group 5 mares cycled regularly and one mare displayed prolonged dioestrus.

Figure 1. Teasing behaviour of individual mares grouped by treatment (Oxy IM = 10IU oxytocin administered intramuscularly daily from day 7 to 14 post-ovulation; OXY IV = 10IU oxytocin administered intravenously daily from day 7 to 14 post-ovulation; Saline = 1ml saline administered intravenously daily from day 7 to 14 post-ovulation. Stippled bars indicate when mares showed oestrous behaviour, grey bars indicate when mares displayed dioestrous behaviour in response to a stallion.

The results of this study are inconclusive, as 3 out of 6 control mares displayed prolonged dioestrus instead of cycling regularly as expected. Most mares ovulate at approximately 21 day intervals during the physiologic breeding season. The incidence of prolonged dioestrus/ovulation failure in normal mares during the physiologic breeding season is up to 8.2% (McCue and Squires, 2002). The specific causes of prolonged dioestrus/ovulation failure have not been identified, but may be more common in older mares and obese mares. Factors associated with the high incidence of prolonged dioestrus in our control mares require further investigation. We can only speculate that 10IU intravenous oxytocin given daily from day 7 to day 14 post-ovulation may prolong dioestrus, while intramuscular oxytocin does not.
References


REPRODUCTIVE EFFICIENCY OF THOROUGHBRED AND STANDARDBRED HORSES IN NORTH-EAST VICTORIA

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Goulburn Valley Equine Hospital, Congupna, Victoria, Australia

Objective: To evaluate the reproductive efficiency of horse farms in north-east Victoria and identify aspects of management to be targeted for improving reproductive efficiency.

Design: Retrospective study.

Procedure: Records from seven Thoroughbred (TB) and four Standardbred (STB) studs in north-east Victoria from 1990 to 2001 were reviewed; 8813 cycles in 4455 mares were analysed. TB mares were inseminated by natural mating, whereas most STB mares (89%) were artificially inseminated.

Results: The overall early pregnancy rate per cycle was 68.8% for TB mares and for STB mares, 68.3%. Multiple pregnancies per cycle were more frequent in TB (8.3%) than in STB (4.6%) mares (P < 0.001). Early embryonic death occurred in 7.1% of TB and 7.5% of STB pregnancies. TB mares had fewer inseminations per cycle (1.03) than STB mares (1.43) (P < 0.001). There was a significantly lower proportion of barren reproductive status within the TB than the STB mares. Pregnancy rate per cycle among stallions ranged from 48% to 79%.

Conclusions: On-farm pregnancy rates in both breeds were higher than previously reported and likely reflect improvements in reproductive management. The disparity between breeds in the inseminations per cycle and proportion of barren mares exposed the differing structures of the two industries, and presents a target for improving the reproductive efficiency in STBs. The difference between breeds in the multiple pregnancy rate per cycle likely reflects the higher ovulation rate of TB mares. The variability in pregnancy rate per cycle between the 22 stallions was associated with differences in individual inherent fertility and the quality of stallion management.
Table 1. Effect of number of inseminations per cycle on the pregnancy rate in all cycles

<table>
<thead>
<tr>
<th>No. of inseminations</th>
<th>Thoroughbred</th>
<th>Standardbred</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cycles</td>
<td>Pregnancy rate (%)</td>
</tr>
<tr>
<td>1</td>
<td>5919</td>
<td>69.0</td>
</tr>
<tr>
<td>2</td>
<td>195</td>
<td>63.6</td>
</tr>
<tr>
<td>≥ 3</td>
<td>9</td>
<td>44.4</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.08</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Values within the same column with different superscripts differ significantly, χ² test, Bonferroni P<0.016.

Table 2. Effect of age on reproductive parameters for Thoroughbred and Standardbred mares

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Thoroughbred</th>
<th>Standardbred</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early pregnancy rate 1st cycle</td>
<td>Embryonic death (13–45 days)</td>
</tr>
<tr>
<td>2–8</td>
<td>73.7%&lt;sup&gt;a&lt;/sup&gt; (1451/1968)</td>
<td>5.1%&lt;sup&gt;a&lt;/sup&gt; (99/1944)</td>
</tr>
<tr>
<td>9–13</td>
<td>72.4%&lt;sup&gt;a&lt;/sup&gt; (1047/1446)</td>
<td>7.9%&lt;sup&gt;b&lt;/sup&gt; (114/1438)</td>
</tr>
<tr>
<td>14–18</td>
<td>63.3%&lt;sup&gt;b&lt;/sup&gt; (431/681)</td>
<td>10.0%&lt;sup&gt;b&lt;/sup&gt; (65/650)</td>
</tr>
<tr>
<td>&gt;18</td>
<td>53.7%&lt;sup&gt;b&lt;/sup&gt; (73/136)</td>
<td>11.8%&lt;sup&gt;b&lt;/sup&gt; (14/119)</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Values within the same column with different superscripts differ significantly, χ² test, Bonferroni P<0.008.
Table 3. Effect of reproductive status on the pregnancy rate for Thoroughbred and Standardbred mares

<table>
<thead>
<tr>
<th>Reproductive status</th>
<th>Thoroughbred</th>
<th>Standardbred</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnancy rate</td>
<td>Pregnancy rate</td>
</tr>
<tr>
<td></td>
<td>1st cycle</td>
<td>All cycles</td>
</tr>
<tr>
<td>Maiden</td>
<td>72.8%</td>
<td>72.2%(^a)</td>
</tr>
<tr>
<td></td>
<td>(385/529)</td>
<td>(532/737)</td>
</tr>
<tr>
<td>Barren</td>
<td>70.9%</td>
<td>66.6%(^b)</td>
</tr>
<tr>
<td></td>
<td>(732/1033)</td>
<td>(1053/1581)</td>
</tr>
<tr>
<td>Lactating</td>
<td>70.5%</td>
<td>69.1%(^a,b)</td>
</tr>
<tr>
<td></td>
<td>(1670/2368)</td>
<td>(2277/3296)</td>
</tr>
<tr>
<td>Slipped</td>
<td>60.9%</td>
<td>51.3%(^b)</td>
</tr>
<tr>
<td></td>
<td>(14/23)</td>
<td>(20/39)</td>
</tr>
<tr>
<td>P value</td>
<td>0.54</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*Excludes 470 cycles (345 mare seasons) that were of unknown reproductive status for Thoroughbreds and 156 cycles (103 mare seasons) for Standardbreds.

\(^a,b\)Values within the same column with different superscripts differ significantly, \( \chi^2 \) test, Bonferroni \( P<0.008 \).

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NOCARDIOFORM PLACENTITIS FROM AUSTRALIA WITH IMPLICATIONS FOR EAFL AND MRLS

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Introduction

Foetoplacental infection is diagnosed in about a third of abortions submitted in Kentucky, USA. Nocardioform actinomycetes organisms were seen as an important cause of chronic placentitis in a survey from 1986 to 1991 (Giles et al., 1993; Hong et al., 1993). The pathological lesions of the “nocardioform-like placentitis” are characterised by variable amounts of thick, mucoid, viscous exudate dotted with white granular structures and focal placentitis of the chorion, in particular the base of the horns or the body (Hong et al., 1993; Bryant, 2008). The diagnosis of nocardioform placentitis in Kentucky peaked in 1998 and 1999 foaling season with 94 and 144 diagnosed cases respectively, subsequently there have been only 93 diagnosed cases between 2001 to 2008 foaling seasons (Bryant, 2008).

Gram positive bacteria belonging to the order Actinomycetales are commonly referred to as actinomycetes and have been isolated from pathologically affected placentitis since the condition was first diagnosed in 1986. The most common actinomycete isolate from affected placentas is the newly recognised actinomycete Crossiella equi (Donahue et al., 2002). A variety of other actinomycetes isolated from nocardioform placentitis cases have been isolated worldwide including a variety of Amycolatopsis sp. from Kentucky and South Africa including Amycolatopsis kentuckyensis, Amycolatopsis leonstonensis, and Amycolatopsis pretoriensis (Labeleda et al., 2003). Two potentially emerging equine abortigenic actinomycetes and causes of placentitis have recently been described; Mycobacterium spp. and Cellulosimicrobium cellulans (Bolin et al., 2004; Bryant, 2008) the latter formerly known as Oerskovia xanthineolytica has been isolated previously in Australia from an aborted equine foetus (Thomas and Gibson, 1982).

The geography of this form of placentitis has been limited to Kentucky (USA), Northern Italy (Cattoli et al., 2004), Florida (Christensen et al., 2006) and South Africa. Interestingly the temporal emergence of this disease in Kentucky preceded the emergency of another new reproductive disease in the mare. Nocardioform placentitis reached its peak in 1999 (Donahue et al., 2002), two season prior to the outbreak of Mare Reproductive Loss Syndrome (MRLS) in Kentucky (Sebastian et al. 2008).

Equine Amnionitis and Foetal Loss (EAFL) was a major cause of abortion in the Hunter Valley, Australia during the 2004 season. The condition is similar in many respects to MRLS. There are similarities in case definition and pathology (Perkins, 2005), however the prominent bacteria isolated in each of these conditions differs. In EAFL cases environmental coryneform/actinomycetes predominate compared to alpha-haemolytic streptococci in MRLS.
cases (Todhunter 2009, Cohen 2003). Pathology such as the fibrinous pericarditis and uveitis found in MRLS do not occur with EAFL (Todhunter et al., 2009). In both MRLS and EAFL there is a caterpillar causal hypothesis, with MRLS been associated with exposure of pregnant mares to the Eastern Tent Caterpillar (Malacosoma americanum) (Webb et al., 2004) and EAFL associated with exposure to the Processionary Caterpillar (Ochrogaster lunifer) (Cawdell-Smith et al., 2009).

This case series of nocardioform placentitis has some similar pathology to EAFL and throws light on the possible mechanisms of pathogenesis of nocardioform placentitis.

**Materials and Methods**

A total of 513 equine placentas were examined during the 2008 (341) and 2009 (172) breeding seasons on one farm in the Hunter Valley, NSW, Australia. During the two seasons six placentas were diagnosed as having nocardioform placentitis (5 in 2008 and 1 in 2009). Gross description and photographs were taken along with samples for bacteriology and histology. Bacteriological samples were taken using transport media, Gram stained and cultured and then identification by partial sequencing of 16s rRNA gene of the isolated bacteria (Muscatello and Browning, 2004). The partial sequence was then compared to those in the GenBank database using BLASTN to establish an accurate identification to the species or genus level. Histological samples were placed in buffered formal saline and then processed with H&E stain.

**Results**

The age of mares varied from 8 to 18 years of age and parity from primiparous to 13 foals. Clinical examination of the foals did not reveal obvious clinical abnormalities other than some evidence of emaciation and slightly immature coat in some of the foals. The longest time to stand was 80 minutes and the longest time to suck was 2 hours 20 minutes in those foals that were not muzzled due to neonatal isoerythrolysis risk. Foal birthweight ranged from 45 to 58.5 kilograms. Gestational age varied from 329 days to 343 days. Placental weight varied from 5 to 8 kilograms.

Of the 6 diseased placentas, 2 had lesions restricted to the chorion at the base of the non-pregnant horn, 2 had lesions at the base of the pregnant horn, 1 placenta had lesions on the distal half of the pregnant horn and another lesion in the caudal body, 1 placenta had lesions on the tip of the non-pregnant horn. Lesions were avillous with thick, mucoid, brown exudate with occasional white granulomatous masses (2-3mm in size). In addition to the lesions particular to nocardioform placentitis, 2 of the placentas had evidence of funisitis and amnionitis, pathological lesions consistent with EAFL.

From the diseased placentas, 3 recovered *Amycolatopsis* spp. (2 in 2008 and 1 in 2009) whilst each of remaining placentas recovered pure or heavy growth of the following bacteria; *Cellulosimicrobium cellulans, Microbacterium oxydans* and *Cellulomonas flavigena*.  

95
Discussion

The pathology of these placentitis cases is noteworthy because although nocardioform placentitis has been recognised in Australia (A. Begg, personal communication) it has not been formally published. The pathology of two of these placentas showed lesions that are found in EAFL and raise the question of the possibility that the 2 diseases were concurrent in these two cases or that nocardioform placentitis might be another pathological expression of EAFL. The bacteria isolated also provide a link to both EAFL and nocardioform placentitis in the USA. The finding of similar environmental actinomycetes in these cases to that isolated from EAFL cases might strengthen the argument that nocardioform placentitis and EAFL may be different pathological expressions of a shared underlying reproductive pathogenesis. The shared genera and species identification of bacteria isolated in these nocardioform placentitis cases with that seen in Kentucky, makes these findings relevant to nocardioform placentitis cases in the USA and also potentially MRLS.

EAFL and MRLS share some common pathology and possible pathogenesis mechanics with each associated with exposure hairy caterpillars. If nocardioform placentitis present in this case series was associated with caterpillar exposure it may explain the specific geographic distribution of nocardioform placentitis. Nocardioform placentitis is seen mainly in Kentucky, Italy, and South Africa and now in Australia. It has not been reported in other geographical locations and does not occur in the Newmarket area of England (K. Whitwell pers comm. 2008) an area of intense horse breeding activity that actively screens a lot of equine placentas. If the geography of nocardioform placentitis and EAFL/MRLS are similar then it is possible that nocardioform placentitis might be an extension of EAFL/MRLS caused by a shared environmental factor which may be the hairy caterpillars.

Conclusion

This is the first formal reporting of nocardioform placentitis in Australia. There is shared pathology and bacteriological findings with EAFL a similar disease to MRLS. These links raise the possibility that nocardioform placentitis is a variant of EAFL (or MRLS) and maybe caused through exposure to a similar environmental factor.

Acknowledgements

Simon Arnot, Anna Blishen and Coolmore Australia for their assistance and support with the production of this work.

References


ULTRASOUND FINDINGS IN MARES WITH PRECOCIOUS SIGNS OF IMMINENT PARTURITION – USE AS PREDICTORS OF FOAL SURVIVAL

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Introduction Placentitis is a significant contributor to the delivery of premature, septic or dysmature foals that require expensive intensive care. Ultrasound examination is used to monitor mares with placentitis. However, the use of the published normal ultrasound parameters to determine foal survival has not been tested. This study was designed to determine the sensitivity and specificity of ultrasonographic variables for pregnancy outcome in mares with placentitis.

Materials and Methods The records of 212 mares that presented with premature lactation and/or vaginal discharge from 2005 to 2009 were examined. Combined utero-placental thickness (CUPT), placental separation and folding, fetal heart rate, activity, and aortic diameter, and fluid echogenicity, the treatment regime and pregnancy outcome was recorded. A total ultrasound score was derived by addition of the incidence of abnormal ultrasonographic variables detected during each examination. In addition, a trans-rectal ultrasound score was derived by the addition of the incidence of an abnormal CUPT, placental separation, fluid echogenicity and fetal activity detected by trans-rectal ultrasonography. Pregnancy outcome was recorded as good if a live healthy foal was delivered and poor if the foal was aborted, stillborn or required intensive care to survive. Data were entered into a spreadsheet program and analyzed using a statistics program (JMP 7, SAS Institute Inc, Cary NC, USA). Contingency analysis and Fishers Exact Test were used.

Results Mares that presented with premature lactation and premature lactation were more likely to have a poor outcome than mares that presented vaginal discharge alone (p = 0.02, RR 1.7, 95% CI 1.0–2.8) or mares that presented with premature lactation alone (p<0.0001, RR 3.0, 95 % CI 1.9-4.7). Mares that presented with vaginal discharge alone tended to be more likely to have poor outcome than mares that presented with premature lactation alone (p=0.056, RR 1.8, 95% CI 1.0-3.2).
Except for placental folding detected during trans-rectal ultrasonography, there was an increased probability of a poor pregnancy outcome when each individual ultrasound variable was abnormal. (Table 1) The relative risk with 95% confidence interval (CI), sensitivity and specificity of a normal ultrasound variable to predict a good pregnancy outcome are presented below.

<table>
<thead>
<tr>
<th></th>
<th>Trans- rectal Ultrasonography</th>
<th>Trans-abdominal Ultrasonography</th>
<th>Fluid Echo Genicity</th>
<th>Fetal Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>199</td>
<td>126</td>
<td>126</td>
<td>126</td>
</tr>
<tr>
<td>p value</td>
<td>0.03</td>
<td>0.002</td>
<td>0.002</td>
<td>0.006</td>
</tr>
<tr>
<td>Relative Risk</td>
<td>1.6</td>
<td>2.1</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>95% CI of RR</td>
<td>1.0-2.5</td>
<td>1.2-3.6</td>
<td>1.4-3.2</td>
<td>1.4-3.7</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>.68</td>
<td>.67</td>
<td>.33</td>
<td>.44</td>
</tr>
<tr>
<td>Specificity</td>
<td>.48</td>
<td>.62</td>
<td>.89</td>
<td>.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.85</td>
</tr>
</tbody>
</table>

Table 1. The abnormal ultrasound variables associated with poor pregnancy outcome.

The outcome of the pregnancy was significantly associated with total ultrasonography score (p<0.0001) and trans-rectal ultrasonography score (p<0.0001). The proportion of poor outcomes doubles between a score of 3 and 4, therefore the outcome of mares with a score of 3 or less was compared with the outcome of mares with a score of 4 or more (Figure 2). The probability of a poor pregnancy outcome was increased when the total ultrasound score was > 3 (p<0.0001, RR 4.1, 95% CI 2.4-7.1) or the rectal score was >1 (p<0.0001, RR 3.4, 95% CI 1.8 – 6.3)
Table 2. The sensitivity and specificity of a total ultrasound score > 3 and trans-rectal ultrasound score >1 for a poor pregnancy outcome

<table>
<thead>
<tr>
<th></th>
<th>Total Ultrasonography Score &gt;3</th>
<th>Trans-rectal Ultrasonography Score &gt;1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>.66</td>
<td>.74</td>
</tr>
<tr>
<td>Specificity</td>
<td>.88</td>
<td>.71</td>
</tr>
</tbody>
</table>

Discussion Despite intensive treatment, more than half the mares that presented with combined premature lactation and vaginal discharge failed to deliver live healthy foals. This was in contrast to mares that presented with premature lactation (81%) or vaginal discharge (67%) alone.

When examined individually, the presence of an abnormal ultrasound variable indicated a greater chance of a poor pregnancy outcome. When an individual ultrasound variable was abnormal, the relative risk of delivery of a dead or compromised foal was between 1.5 and 2.3 compared with when the ultrasound variable was normal. A single abnormal ultrasound variable detected between 33 and 68% of the pregnancies that ultimately delivered a dead or compromised foal.

Although the ultrasound scores indicated higher relative risks of a poor outcome, utilisation of either total or rectal ultrasound score did not improve the accuracy of the ultrasound examination for predicting the outcome of the pregnancy. The total ultrasound score was no more effective at detecting the pregnancies that delivered a poor outcome (sensitivity) than the CUPT measured either during trans-rectal or trans-abdominal ultrasonography. In addition the total ultrasound score was similarly effective at detecting the pregnancies that would deliver a healthy foal (specificity) as separation or folding of the placenta detected during trans-abdominal examination.
CAUSES OF ABORTION FROM 2005 TO 2009 IN THE HUNTER VALLEY, NSW

REBECCA BUTT, JOAN CARRICK.

Scone Equine Hospital, Liverpool St, Scone.

Introduction: The recognition of EAFL (Equine Amnionitis Funisitis Loss syndrome) in 2004 significantly changed the protocols for fetal post mortems. The purpose of this retrospective was to investigate the causes of abortion in Hunter Valley Thoroughbreds from 2005 through to 2009 and review the gross and histological findings.

Materials and Methods: The records of 369 abortions submitted to Scone Veterinary Hospital from 2005-2009 were reviewed. Cases included all abortions, stillbirths and perinatal deaths occurring within six hours of birth. Gestational age, body condition, predation, autolysis, membranes missing, chorionitis, location of chorionitis, allantoitis, amnionitis, funisitis, fetal infection, cord length, placental mineralization, hypovillus regions and microorganism identification were recorded in a spreadsheet. Each case was assigned a single diagnostic category. Diagnostic categories were Placentitis, EAFL, Equine Herpes virus (EHV-1) infection, Fetal Infection of Unknown Origin, Umbilical Cord Torsion, Perinatal Death, Vascular Occlusion not due to cord torsion, and Miscellaneous Causes. No diagnosis was reached in 87 (23.6%) cases; 63 were due to insufficient fetal tissue or membranes, and 12 were due to advanced autolysis. These 75 records were removed from analysis. Data were entered into a spreadsheet and analyzed using a statistics program (JMP 7, SAS Institute Inc, Cary NC, USA). Continuous data was assessed for normal distribution. Logistic regression and Wilcoxin Ranks Sum Test was used to assess between continuous variables and diagnostic category and Contingency analysis and Fishers Exact Test were used to assess the relationship between categorical variables and diagnostic category. Significance was determined to be p<0.05.

Results: Diagnosis was achieved in 282 of 294 (95.9%) cases where adequate tissue was submitted for examination.

Placentitis There was no association of placentitis with year or gestational age. Umbilical cord length was inversely associated with development of placentitis. Median cord length of cases with a diagnosis of placentitis was 60 cm (IQR 45 – 70 cm) which was shorter (65 cm, IQR 51 – 81 cm ) than cases that did not have a diagnosis of placentitis (p=0.05). A diagnosis of placentitis increased the probability that fetal body condition was poor (p<0.001, RR 2.1 95% CI 1.5 – 3.0), the fetus was fresh (p=0.019, RR 1.4 95% CI 1.0 to 1.8), infection was detected in the fetus (p=0.0001, RR 2.4 95% CI 1.8 to 3.2), there were hypovillus regions, (p=0.03 RR 1.7 95% CI 1.1 to 2.7) and a microorganism was detected in the fetus (p<0.0001, RR 2.0 95% CI 1.5 to 2.8). Organisms were isolated from fetuses in 57% of placentitis cases.

EAFL A decrease in the incidence of EAFL was observed in 2009. There was no association of a diagnosis of EAFL with gestational age. The median cord length of cases with EAFL was 58 cm (IQR 45 to 67 cm) which was shorter than the umbilical cord length of cases without EAFL (65 cm IQR 50 to 80, p=0.009). Fetal body condition, autolysis, and the presence of hypovillus regions were not associated with a diagnosis of EAFL. A diagnosis of EAFL increased the
probability of fetal infection (p<0.0001, RR 3.0 95% CI 2.3 to 3.9) and that bacteria were isolated from the fetus (p<0.0001, RR 2.2 95% CI 1.7 to 3.0) but reduced the probability of mineralization of the placenta (p=0.03, RR 0.6 95% CI 0.4 to 1.0).

**EHV-1** There was no difference in the incidence of EHV-1 amongst the years. The median gestational age of fetuses with EHV-1 was 305 days (IQR 283 to 325 days) which was higher than that of fetuses that were not infected with EHV-1 (277 days, IQR 225 to 305 days, p=0.0008) The probability of EHV-1 infection was greater when the fetus was in normal body condition (p= 0.0003), when there was no autolysis (p=0.0003), no fetal infection (p=0.0002), no hypovillus regions (p=0.03), no placental mineralization (p=0.001) and no microorganisms were detected (p<0.0001). The relative risk of EHV-1 infection when there was a fresh fetus compared to when the fetus was autolysed was 5.6 (95% CI 1.9 to 16.2). The relative risk of EHV-1 when no fetal infection was detected compared with when fetal infection was present was 13.7 (95% CI 1.9-100.9). There was no association of umbilical cord length and EHV-1 infection.

**Fetal Infection of Unknown Origin** No association existed between fetal infection of unknown origin with gestational age, body condition, autolysis, hypovillus regions, or mineralization of the placenta. The length of the umbilical cord in cases of fetal infection of unknown origin was 52 cm (IQR 45 to 60 cm) which was shorter than the length of the umbilical cord in cases where infection source was known or where bacteria were not isolated (63cm, IQR 50 to 80cm).

**Umbilical Cord Torsion** Median gestational age of abortion due to umbilical cord torsion was shorter (243 days, IQR 223 to 264 days) than the gestational age of abortions not caused by umbilical cord torsion (284 days, IQR 236 to 312 days). The length of umbilical cords was longer (83 cm, IQR 72 to 93 cm) when the abortion was due to umbilical cord torsion than when the abortion was not due to cord torsion (60 cm, IQR 45 to 70 cm). The probability that umbilical cord torsion would be the cause of an abortion increased as the length of the umbilical cord increased (p<0.0001, Unit Odds Ratio 1.1). The area under the receiver operator curve was 0.84 and the ROC table indicated the “cut off” umbilical cord length for a diagnosis of cord torsion is 71 cm. The relative risk of umbilical cord torsion when the umbilical cord length is greater than 71 cm compared with 71 cm or less is 6.6 (95% CI 3.6 to 12.3).

**Vascular occlusion not due to umbilical cord torsion** There was no association with this group with year, gestational age, umbilical cord length, body condition, autolysis, hypovillus regions or detection of a microorganism.

**Perinatal death** Perinatal death was more likely when the body condition was normal (p=0.009, RR 1.4, 95% CI 1.2 to 1.6), there was no placental mineralization (p=0.03 RR 1.6 95% CI 1.2 to 1.9) and when a microorganism was not isolated (p=0.03, RR 1.5 95% CI 1.2 to 1.9) There was no association of perinatal death with year or with the length of the umbilical cord.
Table 1 Causes of Abortions submitted to the Scone Veterinary Hospital from 2005 to 2009

<table>
<thead>
<tr>
<th>Year</th>
<th>Infectious Causes</th>
<th>Non-infectious Causes</th>
<th>No Diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placentitis</td>
<td>EAFL</td>
<td>EHV-1</td>
<td>Fetal Infection unknown origin</td>
</tr>
<tr>
<td>2005</td>
<td>10</td>
<td>17</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>17.86%</td>
<td>30.36%</td>
<td>3.57%</td>
<td>17.86%</td>
</tr>
<tr>
<td>2006</td>
<td>14</td>
<td>11</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>26.92%</td>
<td>21.15%</td>
<td>5.77%</td>
<td>17.31%</td>
</tr>
<tr>
<td>2007</td>
<td>18</td>
<td>15</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>26.09%</td>
<td>21.74%</td>
<td>15.94%</td>
<td>0.00%</td>
</tr>
<tr>
<td>2008</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>23.81%</td>
<td>19.05%</td>
<td>4.76%</td>
<td>9.52%</td>
</tr>
<tr>
<td>2009</td>
<td>19</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>35.19%</td>
<td>9.26%</td>
<td>7.41%</td>
<td>9.26%</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>60</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>25.85%</td>
<td>20.41%</td>
<td>7.82%</td>
<td>10.20%</td>
</tr>
</tbody>
</table>

Discussion Placentitis was the single most prevalent cause of abortion. EAFL and umbilical cord torsion are important causes of fetal loss. Umbilical cord length greater than 71cm can predict cord torsion with 84% accuracy. Abortions due to non-viral infections were associated with shorter umbilical cords. EHV-1 infection is unlikely if there is fetal autolysis or poor body condition. Diagnosis was achieved in 96% of cases where sample submission was adequate.
CORRELATION BETWEEN INFLAMMATION IN THE PLACENTA AND NEONATAL HEALTH

JOAN CARRICK
Scone Equine Hospital, Liverpool St, Scone

Introduction
Although placental pathology is a critical determinant of neonatal foal health, more than half the foals that required veterinary treatment during the first 48 hr of life on a thoroughbred farm were delivered by mares with normal placentas (Keane S, 2009). Gross placental pathology may not represent acute placental dysfunction. This project was designed to investigate whether histopathological changes consistent with the presence of inflammation of the placenta would be better correlated with neonatal foal health.

Materials and Methods.
The placentas from 24 foals were examined. There were 12 placentas from foals that were admitted to the SHE NICU at less than 48 hours of age and 12 placentas from healthy foals. Five of the 12 placentas from healthy foals had gross placental pathology. Seven of the placentas from critically ill foals had gross placental pathology. Large avillous regions of the placenta, placental thickening, or the presence thick tenacious discharge was defined as gross placental pathology. Sections of the placenta at the cervical pole and in the body close to the umbilical insertion were obtained for histopathological examination by a Specialist Pathologist who was unaware of the gross pathological assessment or the health of the foal. Histopathological changes were recorded as
Chronic = chronic chorionitis, allantoitis or fibrosis,
Active = active chorionitis or allantoitis (acute or chronic),
Acute = acute chorionitis or allantoitis.

Results
There was no difference in the incidence of chronic or active histopathological change between critically ill or healthy foals. The placentas from critically ill foals were more likely to have acute histopathological changes than the placentas from healthy foals (p=0.047). None of the placentas from healthy foals had histopathological changes that were described as acute.

Placental Histopathology and Neonatal Foal Health

None of the placentas from healthy foals had histopathological changes that were described as acute.
Placentas that had gross pathology were more likely to have histopathology (p=0.05 RR 2.6 95% CI 0.9-7.7). Three were 3 placentas with no gross pathology that had histopathology, tow produced healthy foals and the changes were described as chronic or chronic active. Only one of the five placentas with no gross pathology that produced a critically ill foal had histopathology recorded. The histopathology of this placenta was described as acute and active inflammation.

**Discussion & Conclusions**

The gross and histopathological pathology of the placentas was well correlated. Histopathological examination of the placentas detected some changes that were not apparent during gross examination however these changes were not closely associated with the health of the neonatal foal. Acute inflammation of the placenta was a significant factor in the delivery of a foal that required intensive care to survive.

**References**


**Acknowledgments**

Dr Angela Begg for histopathological assessment of the samples
Mitotic division in the testis as elsewhere in the body produces “normal” diploid cells, which in the horse contain 32 pairs (i.e. total 64) of chromosomes. However at the initiation of spermiogenesis a meiotic cell division gives rise to haploid cells containing only 32 chromosomes with no pairing. While contained within the tubules of the testis they remain isolated.

Should, however some disruption occur, as in a traumatic incident and these haploid cells come into contact with the immunogenic cells of the body, they will be recognized as “not normal” and an immune response will be initiated. This can result in the male animal mounting an immune response to his own spermatozoa or their cellular haploid precursors.

The outcome of this is that if before or during ejaculation the spermatozoa are exposed to the antibodies being produced the spermatozoa will become inactivated or killed. This can result in infertility or subfertility in the male.

The condition is well recognized in humans where a low but constant cause of male infertility is quoted as being associated with antisperm antibody production. (Rumke, 1959), (Mumford, 1958)

In horses the condition has been suspected and described in infertility investigations but it has been difficult to demonstrate. (Papa et al., 1990), (Teuscher et al., 1994), (Zhang et al., 1990),

In this paper clinical cases suspected of having antisperm antibodies will be discussed and also the various attempts at demonstrating this as a cause of infertility. A new technique will be introduced as a means of demonstrating the problem using a technique adapted from the human field using antibody impregnated immunobeads (Clarke et al. 1985).

References:


CAUSES OF MALE INFERTILITY

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Male infertility is the single largest defined cause of human infertility affecting approximately one in 20 men of reproductive age (McLachlan and de Kretser, 2001). A majority of men in this situation generate sufficient numbers of spermatozoa to fertilize the oocyte, however in these patients, the gametes have lost their capacity for fertilization. Thus a fundamental concept in male infertility research is that this condition has little to do with the number of spermatozoa in the ejaculate but everything to do with their quality. Diagnostically, we do place significant emphasis on the concentration of spermatozoa in a semen sample and classify men as subfertile (oligozoospermic) when that number drops below a threshold of around 20 million spermatozoa per ml. However this is not because conception is an obstacle race featuring high rates of sperm attrition. In normal men who have had their spermatogenesis suppressed through the administration of steroids, conception has been found to occur with sperm counts as low as 3 million per ml (Wallace et al., 1992). When we perform a conventional semen analysis, we are using criteria such as sperm count, morphology and motility to give us indirect information about the underlying quality of the spermatogenic process that generated the spermatozoa. When spermatogenesis is impaired, sperm quality declines.

As for the cause of the defective sperm function seen in the patient population, our current hypothesis is that the fertilizing capacity of these cells is curtailed as a result of oxidative stress (Aitken and Clarkson, 1987). Spermatozoa are particularly susceptible to this form of stress for a variety of reasons. Firstly, they are professional generators of reactive oxygen species (ROS), which appear to emanate largely from the mitochondria although additional plasma membrane sources are possible (Aitken et al., 2003; Koppers et al., 2008). Secondly, these cells are poorly endowed with the enzymes that protect most cell types from oxidative stress (superoxide dismutase; glutathione peroxidase, catalase etc) because of the limited volume and restricted distribution of their cytoplasm. Thirdly, these cells possess abundant targets for oxidative attack in the form of polyunsaturated fatty acids and DNA.

The link between oxidative stress and male infertility was first established in studies conducted more than 20 years ago demonstrating that the spermatozoa of infertile men generate significantly higher levels of ROS than normal fertile donors (Aitken and Clarkson, 1987; Alvarez et al., 1987). In subsequent studies, the excessive generation of ROS had been correlated with lipid peroxidation, oxidative DNA damage and disruption of such fundamental attributes of sperm function as motility and sperm-oocyte fusion (Aitken and Krausz, 2001). The clinical significance of oxidative stress in the germ line was ultimately established in prospective studies in which the generation of ROS by spermatozoa was found to correlate with the incidence of spontaneous pregnancy during a prolonged 4-year follow-up of patients experiencing idiopathic infertility (Aitken et al., 2001). The final piece of evidence suggesting that oxidative stress is causally involved in the etiology of male infertility centres on the ameliorating effect of
antioxidant therapy. Although few studies in this area would be described as definitive, the
general thrust of the antioxidant studies that have been conducted is that such treatment
significantly rescues sperm function and increases pregnancy rates (Ross et al., 2010).

Given this wealth of data suggesting a role for oxidative stress in the etiology of male infertility,
it is now important that we understand the origins of this stress so that preventative measures
can be taken. In this context, we have proposed a two step hypothesis. In Step 1 the testes
come under oxidative stress as a result of a wide variety of conditions including diabetes,
varicocele, testicular torsion, life style factors such as smoking, exposure to environmental or
occupational toxicants, infection and electromagnetic radiation (Aitken and Roman, 2010). This
stress interferes with the spermiogenesis, a sensitive stage of spermatogenesis when
haploid round spermatids differentiate into spermatozoa. This process is entirely dependent on
the regulated translation of pre-existing mRNA species and therefore vulnerable to the known
ability of ROS to interfere with protein translation (Ling and Söll, 2010). Such impaired
spermiogenesis results in the generation of defective spermatozoa with poorly remodelled
chromatin that are vulnerable to oxidative attack. In Step 2, these vulnerable cells become
attacked by ROS. The latter could arise from a number of extrinsic sources such as infiltrating
leukocytes or redox cycling xenobiotics. However, we postulate that the most common source
is intrinsic, in the form of the spermatoza’s own mitochondria. These organelles are thought
to generate ROS as these vulnerable defective cells succumb to an apoptosis-like process
featuring in addition to mitochondrial ROS generation, motility loss, caspase activation and
phosphatidylserine exteriorization. Normally such programmed senescence would take place in
the female reproductive tract in order to facilitate the phagocytic removal of dead and
moribund spermatozoa. However, in cases of male infertility this process is activated
prematurely within the male tract, resulting in a loss a fertilizing potential, the induction of
extensive DNA damage and sub-clinical levels of leukocytic infiltration. Since such defective
cells are frequently employed in assisted reproduction cycles, there is now considerable
interest in developing strategies to overcome such oxidative damage to the male germ line that
may have application in other species where male infertility is a problem.

References
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the genesis of reactive oxygen species by human spermatozoa. Journal of Reproduction and
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Alvarez JG, Touchstone JC, Blasco L, Storey BT. (1987) Spontaneous lipid peroxidation and
production of hydrogen peroxide and superoxide in human spermatozoa. Journal of Andrology 8:338
There is considerable application of chilled and frozen semen globally in equine breeding programs and its use will continue to expand. However, currently there is mixed success with equine artificial insemination which in part is associated with an individual stallion’s inherent fertility and sperm response to the preservation process. The fact that horses are not selected on the basis of their reproductive performance will continue to drive the development of clinical techniques that attempt to identify the mechanisms and treat the causes of subfertility.

There is a burgeoning interest in the implications of DNA-damaged spermatozoa on fertility (Alvarez, Ollero, Larson-Cook et al., 2004; Baumber, Ball, Linfor et al., 2003; Fleming, Ilad, Griffin et al., 2008; Lin, Kuo-Kuang Lee, Li et al., 2008; Linfor and Meyers, 2002; Love, 2005; Love, Brinsko, Rigby et al., 2005; Morrell, Johannisson, Dalin et al., 2008; Neild, Gadella, Agüero et al., 2005). Historically, breeding soundness assessments in the stallion appear to succeed in ruling out those stallions with very clear reasons for subfertility but traditional assessments of sperm viability do not account fully for male factor infertility (López-Fernández, Crespo, Arroyo et al., 2007; Neild, Gadella, Agüero et al., 2005). By assessing parameters of sperm motility there is an indirect assessment of mitochondrial function and its ability to propel the sperm forward, with little regard to the evaluation of the integrity of the DNA (chromatin structure) or the membrane surrounding the sperm head (Love, Thompson, Brinsko et al., 2003). Spermatozoa with excellent chromatin structure but without motility or with other significant abnormalities will obviously be infertile; however, this logic cannot be implied to the situation in reverse (Giwercman, Richthoff, Hjøllund et al., 2003). Spermatozoa with defective chromatin structure (DNA aberrations) can still exhibit normal motility with the ability to fertilise oocytes. Reproductive failure then becomes evident at the onset of embryonic DNA expression and development (Larson-Cook, Brannian, Hansen et al., 2003; Silva and Gadella, 2006) which becomes increasingly relevant in the assisted reproduction technologies of ICSI and IVF (Fleming, Ilad, Griffin et al., 2008; Lin, Kuo-Kuang Lee, Li et al., 2008). Therefore, through the development and inclusion of motility independent variables in assessing and preparing stallion ejaculates we can expect to improve the reproductive performance of those individuals clinically exhibiting subfertility (Love, 2005).

It has been demonstrated that ejaculates from fertile stallions displayed significantly lower levels of abnormal chromatin than those from infertile stallions (Linfor and Meyers, 2002); and when combined with sperm morphology, chromatin integrity can be useful in predicting an ejaculate’s fertilising ability (Morrell, Johannisson, Dalin et al., 2008). Spermatozoa are vulnerable to oxidative stress, and defective spermatozoa and activated leucocytes in an ejaculate generate reactive oxygen species in copious quantities (Ainsworth, Nixon and Aitken, 2005; Baumber, Ball, Linfor et al., 2003). Proper condensation of DNA in spermatozoa appears to make them more stable and inherently less vulnerable to oxidative damage (Love, 2005; Love, Brinsko, Rigby et al., 2005; Silva and Gadella, 2006). However, the process of
cryopreservation and semen processing involving the physical shearing forces of centrifugation of unselected sperm populations promotes substantial DNA degradation and the generation of reactive oxygen species in spermatozoa (Aitken and Clarkson, 1988; Baumber, Ball, Linfor et al., 2003). Therefore, there has been considerable drive in the development of techniques that actively selects populations of spermatozoa with minimal morphological abnormalities or, chromatin defects, and maximal motility characteristics. Preparation techniques such as direct swim up, adherence methods, density gradients separation and membrane filtration have been applied to select populations of spermatozoa with minimal potential to generate reactive oxygen species thus improving storage capability and maximising fertility by reducing the non-compensable defects in an inseminate.

Recently a membrane based electrophoretic filtration system, known as the Cell Sorter-10 (CS-10) which preferentially separates spermatozoa with low levels of DNA damage has been developed. Separation of cells is based on their size and charge; cells with minimal DNA damage exhibit a greater negative charge and are drawn across the membrane toward the cathode, whereas larger cells (leucocytes, macrocephalic sperm) are not drawn across or are unable to cross the membrane (Ainsworth, Nixon and Aitken, 2005). Human trials indicate that it is a highly effective separation system, requiring a fraction of the skill and time of density gradient centrifugation for separating spermatozoa for assisted conception (Ainsworth, Nixon and Aitken, 2005; Fleming, Ilad, Griffin et al., 2008). Application of this system on samples of stallion semen has not yet been performed. Our initial clinical trials of the CS-10 unit in attempting to separate stallion sperm will follow the guidelines set out in previous work (Ainsworth, Nixon and Aitken, 2005; Fleming, Ilad, Griffin et al., 2008). We plan to assess the effectiveness of the separation system with parameters of motility, membrane function (hypo-osmotic swell test) and acrosome and chromatin integrity through the application fluorescent markers and flow cytometry (FITC-PNA, Ethidium Homodimer, Acridine Orange) in accordance with techniques detailed in previous work (Cheng, Fazeli, Voorhout et al., 1996; Love and Kenney, 1998; Love, Thompson, Brinkso et al., 2002). Preliminary trials with thawed semen have succeeded in the separation of sperm; however, currently it appears the application of an electric charge is rendering spermatozoa immotile. In the absence of charge motile sperm are able to cross the membrane with relative ease.

References


INTERACTION OF SPERMATOZOA WITH THE FEMALE REPRODUCTIVE TRACT

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There are a host of methods used to assess the functional integrity of spermatozoa in an attempt to predict their subsequent fertility. Traditional parameters such as motility, viability and morphology are now combined with more complicated assessments such as acrosome integrity, capacitation status, and the sperm chromatin structure assay to provide a better understanding of sperm physiology and an improved prediction of fertility to be obtained post-insemination (Gillan, Kroetsch, Maxwell et al. 2008). However, these analyses fail to explain the poor fertility of spermatozoa in circumstances where no obvious problems with sperm function or female reproductive physiology exist. A possible explanation lies in the discovery that sperm maturation, function and fertility are governed by a complex interaction between the sperm cells and the female reproductive tract (Fazeli and Pewsey 2008; Rath, Schuberth, Coy et al. 2008). This so called ‘maternal communication’ with spermatozoa must be taken into consideration if the issue of fertility is to be fully understood.

Spermatozoa interact with the female tract in a variety of ways during transit from the site of semen deposition in the anterior vagina, cervix or uterus to the site of fertilisation in the ampulla of the oviduct. Taking a vaginal depositor such as the sheep as an example, spermatozoa must first traverse the physical barrier of the cervix before entrance into the uterus, which is only achieved by a select portion of the initial ejaculate or inseminate. Within the uterus, spermatozoa interact with uterine epithelial cells as well as polymorphonuclear neutrophilic granulocytes and leucocytes (particularly in uterine depositors such as the pig and horse; Rath, Schuberth, Coy et al. 2008), before establishing a reservoir at the utero-tubal junction (UTJ; Druart, Cognie, Baril et al. 2009; Rodriguez-Martinez, Nicander, Viring et al. 1990; Taylor, Rath, Zerbe et al. 2008). This site appears to serve as a second selective barrier for spermatozoa as the concentration of sperm cells is significantly lower in the isthmus of the oviduct when compared to the UTJ (Druart, Cognie, Baril et al. 2009; Hunter, Nichol Crabtree 1980). Once through the UTJ, uncapacitated sperm cells bind with oviducal epithelial cells (OECs) of the isthmus to form the final sperm reservoir (Hunter 1981; Suarez 1998). Following capacitation, spermatozoa disengage from the OEC (Fazeli, Duncan, Watson et al. 1999; Lefebvre and Suarez 1996; Revah, Gadella, Flesch et al. 2000) and make their way to the ampulla. Here, a spermatozoon may fertilise the oocyte after binding with the zona pellucida and undergoing the acrosome reaction (Evans 2003). While there is some species variation in this chain of events, this is a reasonably accurate description of the physical interaction between sperm and the female reproductive tract in all species studied. These physical events have been relatively well elucidated, but the molecular basis for their regulation – the precise nature of the pathways of maternal communication with spermatozoa – remains poorly understood. There are no reported pathways in the vagina, cervix and uterus, but their presence in the oviduct has been established and the molecular basis of the interaction
relatively well studied. It is known that specific sperm surface proteins are required for sperm to be able to pass the UTJ (Cho, Bunch, Faure et al. 1998), that binding to OECs is mediated by sperm lectin and OEC carbohydrate residues, and that their subsequent release is controlled by glycosidases in the oviduct epithelium (Rath, Schuberth, Coy et al. 2008). Controversially, it is now apparent that this maternal communication is not unidirectional, that is to say that signalling occurs from maternal sources to sperm cells as well as from spermatozoa to the cells of the reproductive tract (Fazeli and Pewsey 2008). The presence of spermatozoa within the oviduct has been shown to elicit sperm-specific changes to the production of mRNA transcripts (Fazeli, Affara, Hubank et al. 2004), secretion of proteins (Georgiou, Snijders, Sostaric et al. 2007; Georgiou, Sostaric, Wong et al. 2005) and prostaglandins (Kodithuwakku, Miyamoto Wijayagunawardane 2007) by the oviduct. These findings have considerable implications for fertility as it is evident that spermatozoa are able to influence the oviducal microenvironment in which sperm transport, sperm binding and release, capacitation, transport of oocytes, fertilization and early cleavage stage embryonic development occur (Fazeli and Pewsey 2008). The oocyte and embryo are also capable of similar control over the microenvironment in which they reside (Fazeli and Pewsey 2008). Clearly, successful fertilisation and subsequent establishment of a pregnancy is the result of a complex ‘interactome’ of signalling pathways with any disturbances causing potential adverse effects on fertility.

Knowledge of these interactions has helped shed light on fertility problems with otherwise normal spermatozoa, which have been described as highly functional by traditional means of assessment. One such example is ram spermatozoa liquid stored for 24 hours prior to cervical artificial insemination. Despite excellent motility, the fertility of ram spermatozoa stored using this technique appears to be compromised unless delivered by intrauterine insemination (Maxwell and Salamon 1993). Direct observation of the patterns of sperm migration within the reproductive tract of the ewe by fibred confocal fluorescence microscopy has since demonstrated that liquid storage causes a diminished capacity for migration of the cervix and a subsequent decrease in the number of spermatozoa at the UTJ and within the oviduct (Druart, Cognie, Baril et al. 2009). The molecular basis for this altered interaction of liquid-stored spermatozoa with the cervix of the ewe has not been established. A further example is the diminished fertility of sex-sorted bull spermatozoa in samples with seemingly acceptable levels of motility, viability and acrosome integrity (Underwood, Bathgate, Maxwell et al. 2010). Using the sheep as a model, recent results suggest that sex-sorted spermatozoa alter the expression of genes within the oviduct and by inference the microenvironment of fertilisation and early embryonic development (Beilby, de Graaf, Grupen et al. unpublished observations). While this does not appear to affect the in vivo fertility of sex-sorted ram sperm (de Graaf, Evans, Maxwell et al. 2007) it may have implications for the poor fertility obtained in cattle (Schenk, Cran, Everett et al. 2009), pigs (Bathgate, Grossfeld, Susetio et al. 2008) and horses (Clulow, Buss, Sieme et al. 2008).

Clearly, further work is required to ascertain the full extent of the interaction between sperm and the female reproductive tract. As further details of molecular pathways and sperm-tract signalling come to light it will be essential to investigate the precise effects of assisted reproductive technologies such as sex-sorting and sperm cryopreservation on the maternal communication interactome and any subsequent relationship to fertility.
References


Understanding the molecular basis of sperm-egg interaction is one of the most outstanding, pivotal questions in biology, with major ramifications. By resolving this interaction, not only would we be able to determine the process of “species specificity” (i.e. gametes only recognise each other from the same species), but it would also help in resolving two key areas of fertility regulation, namely pest animal control some cases of male infertility. The latter affects one in every twenty Australian men (1) with one of the most common attributes of defective sperm function being the inability of the spermatozoa to bind to the Oocyte (2).

Sperm-egg binding is mediated by specific glycoproteins that comprise the zona pellucida (ZP), a clear acellular shell that is secreted around the oocyte during folliculogenesis, and not only provides physically protection for the egg but also regulates sperm-egg interaction. The ZP comprises 3-4 major glycoproteins (ZP1-4) depending on the species, and it is generally believed that the primary sperm-binding characteristics of this structure are defined by ZP3 and ZP4. In contrast to our understanding of sperm-binding sites on the egg, the proteins that allow the spermatozoa to bind these glycoproteins have never been definitively resolved.

Recently, we have deleted a gene found in the initial regions of the male reproductive tract, the epididymis, known as RNASE10. The epididymis is a 32 meter long (in human) tube that connects the rete-testis to the vas deferens. As sperm transit through the lumen of the epididymis, epithelial cells secrete a complex proteaceous fluid that serves to modify the spermatozoa in such a way that they gain the competence for fertilization. As spermatozoa pass through the initial regions of the epididymis, they specifically gain the ability to recognize the ZP.

Amazingly, RNASE10 deficient mice are infertile. Although normal sperm cell counts, motility and acrosome reactions can be found, these spermatozoa fail to recognize and bind to the surface of the ZP. The function of RNASE10 is unknown. Although it has similarity to pancreatic RNASE, the protein itself does not possess RNASE activity. Although RNASE10 is secreted by the epididymis, the effect this protein has on sperm modification is also unknown. By using a proteomic approach, we have identified 30 significant peptides differences comparing wild-type with RNASE10 deficient spermatozoa. Some peptides possess extremely high molecular mass and charge, together with the signature low mass sugar-ions, demonstrating that these peptides are glycosylated – an important contributor to sperm-egg recognition. As such, they are likely candidates for proteins that play a critical role in the process of sperm-egg recognition. To date, we have identified a number of transplasma-membrane proteins that appear to be present in the wild type sperm, but absent in the RNASE10 knockouts. One protein of interest, found to be down-regulated in the knockout sample was mannosidase-O-transferase. This enzyme is involved in the regulation of target proteins through addition of mannose residues to specific sites. Interestingly, others have shown that the involvement of mannose is an important element of sperm-egg binding (3). Using this approach, we are now
in an extremely strong position to resolve once and for all how the epididymis confers on spermatozoa the competence for sperm-egg recognition.

References
IS FLOW CYTOMETRIC SEX-SORTING A REALITY FOR THE HORSE INDUSTRY?

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In 2000, the first viable pregnancies using sex-sorted stallion spermatozoa were obtained (Buchanan, Seidel, McCue et al., 2000). Fertility trials have shown that the fertility of fresh or liquid stored sex-sorted stallion spermatozoa approaches 95% of the fertility of non-sorted spermatozoa (Lindsey, Schenk, Graham et al., 2002). These achievements are overshadowed by the restricted time frame in which to perform inseminations in mares. This problem could be overcome by the ability to successfully cryopreserve the spermatozoa post-sorting; an additional step which, using current methods significantly compromises fertility, with reported pregnancy rates below 13% (Lindsey, Schenk, Graham et al., 2002; Clulow, Buss, Sieme et al., 2008). Due to significant advances being made in ruminant research, sex-sorted, cryopreserved bull spermatozoa has been commercially available for several years with 90% of the fertility of non-sorted spermatozoa. In addition, the fertility of sex-sorted, cryopreserved spermatozoa from sheep (de Graaf, Evans, Maxwell et al., 2007) and buffalo (Lu, Zhang, Lu et al., 2010) has been found to exceed that of non-sorted spermatozoa. It would appear that the process of sorting alone does not significantly decrease chromatin stability of stallion spermatozoa (Bochenek, Herjan, Okulski et al., 2005), but when sorted spermatozoa are cryopreserved, post-thaw longevity and DNA integrity are significantly compromised (Gibb, Morris, Maxwell et al., unpublished data).

Both the quality of spermatozoa post-sorting and the sorting efficiency must be improved before sex-sorted stallion spermatozoa can be made widely available on a commercial basis. The speed at which stallion spermatozoa is sorted (1000-3000 cells/s) is significantly lower than that of other species (3000-5000 cells/s). This may be due to; the smaller sperm head size making stallion spermatozoa more difficult to orient for fluorescence detection, and the use of an opaque staining diluent reducing the resolution between X- and Y- chromosome bearing populations. In addition, high insemination doses are needed in mares and as such, 1-6 hours are required to sort sufficient numbers of stallion spermatozoa for a single low-dose insemination (10-25 × 10⁶), compared to 5-30 minutes required to sort a single AI dose of 1-5 × 10⁶ for cattle.

The inability to synchronise oestrus in mares presents another hurdle as the time of ovulation cannot be reliably predicted. Fixed-time inseminations 36 hours after administration of an ovulation-inducing agent have resulted in pregnancy rates exceeding 70% using fresh or liquid stored sex-sorted spermatozoa (Lindsey, Varner, Seidel et al., 2005). However, the timing of ovulation is highly variable between mares (Barbacini, Zavaglia, Gulden et al., 2000) and given that the post-thaw longevity of sex-sorted, cryopreserved stallion spermatozoa is significantly reduced compared with non-sorted spermatozoa (Gibb, Morris, Maxwell et al., unpublished data), such fixed-time insemination regimes may fail to place viable spermatozoa in the tract close to the event of ovulation.
Recent modifications to the sex-sorting and cryopreservation protocols for stallion spermatozoa have yielded several improvements (Gibb, Morris, Maxwell et al., 2009; Gibb, Morris, Maxwell et al., 2010; Gibb, Grupen, Morris et al., 2008). The formulation of an optically clear staining diluent has resulted in more than a two-fold increase in sex-sorting efficiency compared with the traditional skim milk based diluent, significantly increasing the proportion of spermatozoa which can potentially be sorted (Gibb, Morris, Maxwell et al., 2009).

Future research must be aimed at reducing the deleterious effects of sorting upon stallion spermatozoa to permit successful cryopreservation post-sorting. With the recent advances made, this technology is on the brink of being commercially viable for equine breeding programs.

References


Artificial insemination (AI) is now common practice in the horse breeding industry and is accepted by the majority of breed societies. Semen can be used for AI as fresh, fresh-chilled or frozen. While frozen semen gives the greatest flexibility of transport and long-term storage, the conception rates associated with frozen semen are much more variable and considerably lower than those for fresh and fresh-chilled semen. The limitation of fresh-chilled semen is the relatively short period that the sperm remains viable and the need to maintain the sample at 5°C. The objective of this study was the development of a semen extender that can prolong the viability of sperm and that achieves this when the sample is stored at ambient temperature thus allowing more flexibility for transport while maintaining conception rates close to those for fresh semen. Specifically, the project investigated a bovine extender designed for the storage of semen at ambient temperature to determine if it would prolong the viability of stallion sperm when maintained at ambient temperatures.

Semen was collected from three stallions, assessed and extended with four different semen extenders, three commercially available extenders and the test extender. The extended samples were stored at both 17°C and 5°C. The semen was evaluated at the time of collection and then daily for seven days by assessment of the sperm for % motile, rate of motility, % live, % intact acrosome and chromatin structure. The test extender maintained motility above an acceptable level of 45% at 5°C for up to 96 hrs and at 17°C for up to 120 hrs. This was significantly longer than the commercial extenders (P<0.05). The test extender also maintained the rate of motility above the acceptable level of 2 for longer than the commercial extenders. The %live was not significantly different between the extenders nor was the %DNA denaturation. The % acrosome intact sperm was significantly less (P<0.05) for the test extender at both 5ºC and 17ºC, however, at 5ºC it remained above the threshold level until 144 hrs but at 17 ºC it was below the acceptable level by 24 hrs.

The test extender has been shown to have potential to prolong the viability of stallion semen for 96 hrs when stored at 5ºC. This is 48 hrs longer than the currently recommended time for fresh-chilled semen. This extender may be an option for use at 5ºC if adjustments are made to the total number of spermatozoa that are shipped as an insemination dose. This would compensate for the greater number of sperm that undergo the acrosome reaction in this extender. Further research is needed to confirm the fertilizing capacity of sperm extended with the test extender. Additional studies to identify the cause of the high % acrosome reaction of the sperm when stored with the test extender at ambient temperatures could lead to changes to the extender that might improve results and provide an extender that prolongs the viability of fresh semen at ambient temperatures.

This research was supported by the RIRDC Horse Program
THE PREVALENCE OF NEUTROPHILS IN DISMOUNT SEMEN SAMPLES OF THOROUGHBRED STALLIONS

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Brookes Veterinary Services, Scone, NSW.

Introduction
Semen dismount samples are routinely monitored for the presence of spermatozoa in order to confirm ejaculation has occurred in stallions during breeding barn management for natural mating, particularly in thoroughbreds (Umphenour et al 1992, Conboy 2007). Due to the relative ease of obtaining the sample, it is tempting to use various features of these dismount samples to assess the ejaculate for fertility prediction (Haag 1959, Gravance et al 1997, Love et al 2000)
The presence of poly-morphonuclear leukocytes (PMN’s/neutrophils) in the dismount sample is mentioned by Blanchard 2007, and were found to significantly decrease the odds of pregnancy in a multivariate model (Blanchard et al 2010). Amongst the parameters assessed were dismount sample sperm concentration, progressive motility and neutrophil score. However, no prevalence of neutrophils was mentioned.

There are anecdotal and documented reports (Conboy 2007) that assessment of PMN’s from a dismount sample of a stallion may be indicative of an inflammatory or infectious process in the reproductive tract of that stallion. Inference of stallion exposure to an infectious agent was mentioned by Conboy 2007, when dismount samples contain neutrophils. They suggested that the neutrophils are most likely from a mare with an inflamed, and possibly infected, reproductive tract, with which the stallion has mated.

To the authors’ knowledge, there are no reported findings on the prevalence of neutrophils in the dismount samples of thoroughbred stallions.

The aim of this study was to determine the prevalence of PMN’s in dismount samples from a group of stallions with no reported or suspected inflammatory conditions of the urogenital tract on a Thoroughbred breeding farm in Eastern Australia.

Materials and Methods
Sample Collection:
Dismount semen samples from seven stallions were collected Monday through Friday from 10 September to 7 December 2009 on a commercial Thoroughbred stud farm in Eastern Australia.

Fluid from the tip of the penis and urethra was collected into a 5ml plastic tube, labelled with the stallion’s initials, time and date of service.
The sample tubes were processed within 24 hours of sample collection. After agitating the sample, a 25 microlitre drop was placed near the base of a clean, labelled microscope slide. The fluid was smeared across the slide which was air dried, and stained with Diff Quik (R) stain (Schumacher and Moll 2010).

Cell Assessment:

The length of the slide was scanned initially at 100 times magnification (Olympus CH30RF200 microscope). The slide was then more critically evaluated at 1000 times magnification with an oil immersion objective in the reverse direction. Average numbers of PMN’s were counted in each field and the sample was scored according to Table 1.

<table>
<thead>
<tr>
<th>PMN Score Category</th>
<th>Poly-morphonuclear Leukocytes (PMN’s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;1 per 5 HPF (Minimum three cells per slide)</td>
</tr>
<tr>
<td>2</td>
<td>1 per 5 HPF -  &lt; 1 per 3 HPF</td>
</tr>
<tr>
<td>3</td>
<td>&gt;1 per 3 HPF- &lt;1 per HPF</td>
</tr>
<tr>
<td>4</td>
<td>1-2 per HPF</td>
</tr>
<tr>
<td>5</td>
<td>&gt;2 per HPF</td>
</tr>
</tbody>
</table>

Table 1: Sample PMN score according to numbers of PMN’s in Stallion Semen Dismount Sample High Power Fields (HPF)

Results
Four hundred and eighteen samples with complete data were collected. Counts were made of the number of samples within each scoring category. The results are shown in table 2. The prevalence of PMN’s in samples was calculated for scores using subtractive totals for counts for each score. The results are shown in table 3.

The sampling strategy (convenience) did not significantly over or under represent any of the stallions (chi-square test, p>0.5). The number of samples containing PMN’s were not significantly different between each stallion (chi-square test, p>0.1).

Data for the rate of serves by each stallion over time was not available. The samples appeared to be spread evenly over the entire time frame. Samples with PMN’s were also evenly distributed over time, and appeared to be of similar proportion to number of samples collected each week (see figure 1).

Other cells were detected, but not necessarily recorded. These included medusa cells (epididymal ciliated cells), and non stratified squamous epithelial cells such as parabasal-like and intermediate-like cells. Occasional mononuclear inflammatory cells were also noted.
<table>
<thead>
<tr>
<th>PMN Score Category</th>
<th>Number of Samples</th>
<th>Percent of Total (n=418)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>10.0</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>2.1</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>2.9</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>2.4</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Table 2: Number and Percent of Stallion Semen Dismount Samples in Each PMN Score Category

<table>
<thead>
<tr>
<th>PMN Score Category</th>
<th>Subtractive Total of Samples</th>
<th>Prevalence of PMN’s (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1</td>
<td>84</td>
<td>0.20 (0.16,0.24)</td>
</tr>
<tr>
<td>≥2</td>
<td>42</td>
<td>0.10 (0.07,0.13)</td>
</tr>
<tr>
<td>≥3</td>
<td>31</td>
<td>0.07 (0.05, 0.09)</td>
</tr>
<tr>
<td>≥4</td>
<td>22</td>
<td>0.05 (0.03,0.07)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.02 (0.01,0.03)</td>
</tr>
</tbody>
</table>

Table 3: Prevalence of PMN’s in Stallion Semen Dismount Samples by Subtractive Totals for PMN Score Categories.
Figure 1: Graph Showing the Weekly Number of Stallion Semen Dismount Samples Collected and the Number of Samples with PMN’s.

Discussion

To the authors knowledge, the only other report of PMN’s in stallion dismount samples is the decrease in the odds of pregnancy associated with the presence of neutrophils in a multivariate model (Blanchard et al, 2010). There do not appear to be reports of the prevalence of PMN’s in stallion dismount samples.

Conboy 2007 considered that the presence of PMN’s may be an indication of exposure of the stallion to an infectious agent, and Blanchard 2007 stated that PMN’s in dismount samples may be a reason for subfertility in a stallion.

This study shows that the presence of PMN’s in dismount samples occurs in an apparently normal population of mares and stallions in a commercial Thoroughbred breeding operation in Eastern Australia. Part of the stud farm acceptance of a brood mare for breeding to these stallions was a bacteriological negative uterine or cervical swab from that oestrus cycle.

According to this data, 20% of dismount samples have PMN’s present, and 7% have an easily recognisable number of PMN’s (Score 3 or more: more than one PMN per three HPF’s) present in a smear of the sample from apparently normal matings.

The PMN’s could originate from a wide source: the urogenital tract of mare and/or stallion, including stallion accessory glands. Barr bodies have been detected within PMN's from dismount samples (T. Little, personal communication 2010), indicating that at least some of the neutrophils are from the mare. Further work is required to elucidate this. The significance of the finding of PMN’s in dismount samples in relation to fertility requires further examination.
Conclusion
According to our results, approximately 20% of dismount samples from Thoroughbred stallions contain PMN's. To the authors’ knowledge, this is the first report of the prevalence of neutrophils in the dismount sample of stallions. Further work is required to determine the origin of the PMN’s, and the significance of their presence.

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Stud owner, management and staff for facilitating the study.

References
THE USE OF SERUM CREATININE TO PREDICT NEONATAL FOAL HEALTH

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Introduction
Placental function is an important determinant of neonatal foal health. Serum creatinine concentration in neonatal foals has been suggested to be an indicator of placental function and foetal stress (Palmer, J. 2006). A recent study showed that elevated serum creatinine concentration in newborn foals was positively associated with placental pathology and an increased likelihood of the foal requiring veterinary treatment (Keane, SE et al, 2009). This project investigated the value of serum creatinine obtained 12-36 hr after birth as a predictive test for neonatal foal health.

Materials and Methods
Serum samples were obtained from 447 foals between 12 and 24 hours of age. The samples were obtained from foals at two large thoroughbred farms with resident veterinarians and from blood submitted to two veterinary practices in the Hunter Valley, NSW during the 2009 foaling season. Serum creatinine concentration was determined using a Vitros 350 blood biochemistry analyser (Ortho Clinical Diagnostics, Rochester, NY). Foal health during the first 48 hrs of life was recorded. A foal was classified as systemically healthy if it required no veterinary intervention or if the veterinary attention was for a non-infectious orthopaedic disease, or a localised ophthalmic problem such as entropion. All other problems were classified as systemic illness and included perinatal asphyxia syndrome, sepsis, colic, diarrhoea, peritonitis, pneumonia and pyrexia with lethargy.

The results were recorded in a spreadsheet (Excel, Microsoft Computer Software) and descriptive analysis performed. The data was randomly divided into two populations; a model development population (230 foals) and a test population (219 foals), and transferred to a statistics program for statistical analysis (JMP 7, SAS Institute Inc, Cary, NC, USA). The relationships between serum creatinine and foal health was analyzed in the model development population using logistic regression, a receiver operator curve (ROC) was generated and the ROC table used to select an optimal “cutoff” level for serum creatinine. The accuracy of the “cutoff” to predict foal health in the test population was determined by Fishers Exact Test and the positive predictive value, negative predictive value, sensitivity and specificity was derived from the contingency table.

Results
Thirty two (32) (7.1%) foals were classified as systemically ill. In the model development population (230 foals) the probability that a foal would be healthy decreased as the creatinine level increased (p=0.0001, Unit odds ratio 0.99). The area under the
ROC was 0.72 and the ROC table determined the optimal “cutoff” for serum creatinine concentration to determine foal health was 194 umol/L.

The probability that a foal in the test population would develop systemic illness was higher when the serum creatinine was greater than 194 umol/L (p=0.048, RR 2.5 95% CI 0.99 to 6.1). The sensitivity of a serum creatinine >194 umol/L to predict the development of systemic disease was 0.47 and specificity was 0.75. The positive predictive value of a serum creatinine >194 umol/L to predict the development of systemic disease in a foal in the test population was 0.14 and the negative predictive value was 0.94.

**Discussion**

Apparently healthy neonatal foals with a serum creatinine> 194umol/L are at an increased relative risk of developing systemic illness during the first days of life. The sensitivity of the test is less than 0.47, indicating that a serum creatinine of > 194 umol/L detects almost half the foals that develop systemic illness during the first few days of life. In the test population a serum creatinine of > 194 umol/L predicted a far higher number of compromised foals than actually occurred. Only 14 % of the foals predicted to be at risk actually developed systemic illness. Hypercreatininaemia not associated with renal has been described in neonatal foals admitted to referral hospitals (Chaney et al 2010). The elevated serum creatinine subsequently decreased rapidly within 72 hours of birth. These foals had a very high incidence of neonatal encephalopathy. Serum creatinine concentration of >194 umol/L should therefore prompt further diagnostic work such as a complete physical examination and haematology as well as monitoring of serum creatinine over subsequent days.

The test very accurately predicted healthy foals; 94% of the foals that the test predicted to be normal, remained healthy. Further research to define other critical factors of neonatal foal health may improve the capacity to predict development of systemic illness in apparently healthy newborn foals.

**References**


**Acknowledgements**

The authors thank Dr Ristan Greer of the School of Veterinary Science, University of Queensland, Gatton, QLD, for advice about the statistical analysis.

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In recent years increasing concerns about the development of antibiotic resistance and a very thin pipeline of new antibiotics have prompted a search for alternative means of treating bacterial infections. One such alternative that is being explored, or rather re-explored, is that of bacteriophage therapy. Bacteriophages (phages) are a family of naturally occurring viruses, extremely widespread in nature, which bind specifically to, and destroy, a particular bacterial target, leaving other bacterial species (e.g. the normal flora) intact. This unique specificity conveys an obvious therapeutic advantage in situations where the maintenance of the normal flora is vital to the recovery of the animal. Furthermore, because of their unique mechanism of action, phages are equally effective against both antibiotic-resistant and sensitive bacteria.

The use of phages as a means of treating bacterial infections has had a long, and at times, controversial history (Smithyman and Morales, 2007). Phage therapy was first successfully used to treat dysentery patients in 1919, and enjoyed a vogue for some 20 years, before disappearing from Western science, shortly after the advent of antibiotics in 1941. However a series of classical studies, starting in 1982, have reconfirmed the therapeutic and prophylactic efficacy of bacteriophage preparations in a variety of settings (Barrow, 2001; Brussow, 2005; Higgins et al., 2005; Loc-Carrillo et al., 2005; Sulakvelidze and Barrow, 2005). In the veterinary field, the elegant experiments of Smith and colleagues (Smith and Huggins, 1982; Smith et al., 1987) demonstrated the efficacy of phage therapy on mice, calves, pigs and lambs suffering from scours caused by natural bovine enteropathogenic E. coli strains. The results showed that administering phage from 1 to 8 hours after infection could prevent diarrhoea, and even when phage application was delayed until the onset of symptoms mortality rates were significantly reduced. Moreover phage treatment conferred protection on subsequent litters in the same facility.

The treatment of infections in horses caused by bacteria such as Streptococcus equi, Rhodococcus equi and Pseudomonas aeruginosa are frequently complicated by side effects associated with antibiotic use. Of particular concern is the development of antibiotic-associated diarrhoea or colitis and hyperthermia, which may be fatal. This can occur with antibiotics given by any route including by injection. Other negative effects include secondary infections and allergic reactions, usually with hives and breathing problems. Long-term side effects in horses include damage to the liver and kidneys as well as the development of antibiotic resistance. Furthermore, even when bacteria are sensitive to antibiotics, delivery of effective levels of antibiotic to abscesses via the oral route can be difficult. Other problems may be associated with decontamination of equipment used for breeding. Pseudomonas sp. in particular, are generally very resistant to common disinfectants which may actually enhance pathogen colonization by constantly reducing the normal flora (Hurtgen, 2006).
We have recently investigated the *in vitro* activity of bacteriophage preparations ("cocktails") developed in our laboratories for the prevention and treatment of infections caused by animal (and human) isolates of *Pseudomonas aeruginosa*. A preliminary study showed that the cocktails were highly effective against *Pseudomonas aeruginosa* isolates responsible for causing infectious endometritis in mares. Though preliminary, this and other recent studies suggest that bacteriophage therapy could become an important tool for the equine industry by providing a safe, environmentally friendly, and effective means of controlling problematic bacteria.

**REFERENCES**


