Equine clinical reproduction and academic research; facilitating collaboration and co-ordination for real benefit to the breeder via a Colloquium for Equine Reproduction

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Introduction

The horse industry in Great Britain contributes to 0.5 % of total consumer spending and is estimated to generate £3.4 billion expenditure each year. In 1997, British Thoroughbred bloodstock exports were estimated to be worth £160 million (DEFRA 2004) and undoubtedly horse breeding is integral to the industry. The value of Sport Horse breeding further adds to these data. However, mares can exhibit poor fertility rates and the report of 43 % of Thoroughbred mares failing to produce a live foal in 2005 has changed little over the past 50 years (BHB 2007).

Despite the importance and size of the horse breeding industry within the United Kingdom (UK) and consistently poor fertility rates, academic

researchers working to improve clinical reproduction and enhance breeding efficiency are few. Since the closure of the Equine Fertility Unit in 2007, no dedicated group specialising in reproduction research exists. Rather, a small number of isolated individuals operating within larger university departments and veterinarians who carefully collect breeding records and disseminate their data through peer-reviewed journals are all that remain. Connecting the grass roots breeder to the low distribution of these specialists across the UK is challenging. A platform is required to facilitate a unified understanding of current research and the dissemination of ideas within and between groups of breeders, veterinarians and academics. Therefore, the Colloquium on Equine Reproduction, a one-day Workshop hosted by Aberystwyth University, was devised and held on 30th October 2010.

The aim of the Colloquium was to create a centre of gravity for a diverse range of mammalian/equine reproduction academics, horse breeders (from both from large and small commercial stud farms), veterinarians and students to create a synergistic network of contacts and collaborators and to facilitate structured discussion. Prominent scientists within the field were invited to present an overview of their current research, and in light of these talks, delegates were invited to contribute their opinions regarding the value of such research to them, and to make suggestions for potential study directions of real benefit to their breeding operations. Each presentation was followed by a question and answer session and a designated Discussion Forum towards the end of the programme enabled delegate input for indentifying issues of importance to them.

The aim of this Report is to summarise the research that was presented at this unique Forum and to highlight key issues identified and potential mechanisms to address them.

Early pregnancy research: translating novel human reproduction advances to equine science

Professor Eytan R. Barnea (a human obstetrics and gynaecology specialist, currently director and senior consultant at CAMcare and Clinical Associate Professor at Robert Wood Johnson Medical School, Camden, New Jersey) presented the body of his research investigating Preimplantation Factor (PIF), a novel peptide secreted by viable embryos, low or absent in non-viable ones(Barnea 2004; Stamatkin *et al.* 2009b). Preimplantation Factor is found throughout mammalian species (human, cow, mouse and pig). It was demonstrated that the embryo secretes PIF to promote maternal tolerance without resorting to deleterious immune suppression (Barnea 2007). Locally (*in situ)* PIF promotes embryo receptivity by the endometrium in preparation for implantation by modulating local immunity increasing adhesion molecules and controlling apoptosis (Paidas *et al.* 2010). Also, PIF promotes trophoblast invasion thereby facilitating further embryo implantation and pregnancy progress (Duzyj *et al.* 2010). PIF detection by monoclonal antibody based enzyme-linked immunosorbant assay (ELISA) demonstrated that increased PIF concrations in mouse and cow embryo cultures are associated with progress to the blastocyst stage (Stamatkin *et al.* 2009b; Stamatkin *et al.*

2009a). In women, PIF detection in embryo culture media highly correlates with good embryo quality and importantly, live birth. In contrast, PIF negative embryos mostly do not implant following single embryo transfer (ET). In pregnant women serum PIF chemiluminescent ELISA accurately detects early viable pregnancy and correlates with good pregnancy outcome. In bovine pregnancy at < 20 days after breeding, PIF detection is associated with 95 % viable calving rate while ultrasound based diagnosis at day 28 and beyond only correlates with 84 % (Ramu *et al.*). Despite this expanding body of knowledge regarding PIF function and utilisation in other species, the potential applications to equine clinical practice have not yet been explored.

Applications for PIF within equine stud medicine may include early pregnancy diagnosis, potentially at day 10 after ovulation, as opposed to conventional ultrasound detection at day 12 to 18. Within the Thoroughbred industry a prompt negative pregnancy diagnosis is essential to shorten time to repeat breeding, maintaining value of foals born earlier in the season and minimising their wastage. Due to its immune-modulating properties, PIF has potential as a therapeutic agent for inflammatory and immune disorders. Mating-induced endometritis (MIE) is ubiquitous in mares and when inflammation persists (persistent mating-induced endometritis; PMIE), clinical disease results and this occurs typically in around 15 % of animals (Zent *et al.* 1998). In Thoroughbred broodmares, PMIE is further exacerbated. Worldwide, the market for a prophylactic product to combat equine endometritis is estimated at $26 million (Bioniche Life Sciences Inc, 2004). Preimplantation Factor may offer a non-toxic therapeutic solution to prevent MIE progressing to PMIE as it

was shown to have antipathogenic properties, modulating immune receptors such as Toll-like Receptors, among others (Paidas *et al.* 2010). Embryo viability in culture may be assessed by measuring PIF concentrations from medium prior to transfer to a recipient mare and where PIF concentrations are low, the embryo may be treated with synthetic peptide in order to rescue it from demise; particularly pertinent to equine embryo transfer taking into account that mares are very difficult to super-ovulate. Monitoring serum PIF concentrations during pregnancy may indicate impending parturition or abortion, enabling best preparation of the breeder and veterinarian.

The presentation concluded with questions from the floor which stimulated lively debate. The presence of an Early Pregnancy Factor (EPF) has been previously demonstrated in both women and mares yet attempts to accurately measure EPF from the horse have been unsuccessful (Gidley-Baird *et al.* 1983; Marino *et al.* 2009). The question of how EPF and PIF are distinct from each other was raised and promptly resolved based upon knowledge that, unlike PIF, EPF is not pregnancy specific.

Some scepticism was issued in the ability of PIF to be an early biomarker and facilitator of successful pregnancy, considering it is recognised that placentation events are unique to the mare. However, for implantation to occur the embryo has to be viable despite the fact that there is a comparatively late implantation of the equid conceptus when chorionic girdle cells finally invade the endometrium at day 34 to 37 (Allen and Wilsher 2009). The effect of the glycoproteinaceous capsule present from day 7 after

ovulation that persists for a further 20 to 25 days (Betteridge 1989), which is simialr to the human decidua which also can develop without the presence of the embryo and can develop using oestrogen/progesterone. Furthermore, over the years several compounds have been proposed as pregnancy factors for equine pregnancy and none have been successfully utilised as a reliable diagnostic tool or indicator of viability.

In order to understand the true role and value of PIF secretion in equine pregnancy studies characterising serum concentrations of the mare during early pregnancy are highly appropriate. Overall, PIF may be a valuable serum biomarker for improving equine reproduction, an active subject of investigation within IBERS at Aberystwyth University. Also, studies to determine PIF expression in horse blastocysts are ongoing by Prof Barnea's research group. These experiments will provide the needed evidence to consider PIF of relevance and value for use to improve equine reproduction.

Placental structure and function in the mare

Professor Allen, head of the Paul Mellon Laboratory in Newmarket, UK, presented his work and that of his co-worker, Dr Sandra Wilsher, investigating placental structure and function in the mare. The development of the embryo, feotus and corresponding placental events unique to the mare were detailed chronologically and included; i) in-depth consideration of mechanisms that stimulate movement of the embryo from the oviduct into the uterus 6 days after ovulation, comparatively much later than equivalent events in the human; ii) the species-unique blastocyst capsule that surrounds the embryo and

gradually disentegrates from around day 20; iii) invasion of the chorionic girdle into the maternal endometrium at day 36, to form the equine Chorionic Gonadotrophin (eCG) -secreting endometrial cups which undergo necrosis from around day 90 to 100. Within this early period of pregnancy several, as yet unanswered questions were highlighted by the speaker as important avenues for further research.

Physical or chemical embryo-endometrial interactions that induce an embryonic signal to initiate maternal recognition of the pregnancy state are not understood, unlike the situation in cattle, sheep and other ruminants where down-regulation of oxytocin receptors in the endometrium result in a decline in prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secretion which enables the corpus luteum to survive and therefore prevent a return to cyclicity. In the mare, previous hypotheses suggested embryo-derived oestrogens as the possible signalling mechanism. However, yet this proposal was rejected, notably through a series of well designed experiments undertaken at the Equine Fertility Unit (Wilsher and Allen, 2010). As such, the exact mechanism remains unknown.

The unique development of endometrial cups during early pregnancy in equids, requires an active invasion of chorionic girdle cells into the endometrium during days 36 to 38 of gestation. The mechanism which suddenly blocks the invasiveness of these cells around days 38 to 40 remains unknown. Necrosis and sloughing of the fetal endometrial cups into the

uterine lumen during days 100 to 140 of gestation includes both humeral and cell-mediated immune responses from the maternal immune system.

In the mare oestrogen receptors are expressed densely by both the endometrium and trophoblast, concurrent with an increase in oestrogen secretion by the secondary corpora lutea from around day 40 after ovulation, whereas progesterone receptors are initially absent in these tissues. This raises the question of whether oestrogen is of greater importance than progesterone in equine pregnancy?

Single layer Centrifugation

Dr Jane Morrell of the Swedish University of Agricultural Sciences (SLU) presented details of her work and that of her colleagues; designing, optimising and evaluating a semen processing technique termed Single Layer Centrifugation (SLC). Single Layer Centrifugation is a simplified variation of Density Gradient Centrifugation (DGC) which has been utilised to improve the quality of sperm preparations intended for AI (Morrell 2006). Dr Morrell believes SLC can sucessfully further faciltate improved sperm quality, applicable to a range of species, including equids (Morrell 2006).

Single Layer Centrifugation relies on the centrifugation of semen samples through a single layer of a glycidoxypropyltrimethoxysilane-coated silica colloid with a species-specific formulation (Androcoll-E). Through selection of sperm cells based on their density, SLC has the potential to select populations

of sperm that are progressively motile, morphologically normal, viable and with high chromatin integrity. The ability of SLC to improve these fertility-linked parameters has been demonstrated (Johannisson *et al.* 2009) and, although the sample size in this study was small (10 ejaculates from 3 stallions), the findings have been confirmed by another study (Morrell *et al.* 2009). Single Layer Centrifugation has also been shown to aid the removal of morphologically abnormal cells (Morrell *et al.* 2009), although not spermatozoa with detached heads (characteristic of blocked ampullae or sperm accumulation syndrome). Overall, the data presented at the Colloquium for Equine Reproduction tended to show that SLC significantly improves progressive motility, viability, morphology and chromatin integrity in processed samples. Anecdotal reports suggest the procedure may also hold promise for stallions with low fertility rates which may be due to poor morphology, viability or chromatin intregrity of their sperm. The SLC procedure can also be scaled-up to process whole ejaculates; something which cannot currently be achieved using DGC.

Assuming that future studies reveal SLC treatment of equine semen produces improvements in fertility rates that correlate with the observed improvements in sperm quality, the technique has valuable commercial potential as a tool for processing subfertile stallions' ejaculates, and potentially for improving the cryosurvival of cells by selecting more viable (and potentially cryo-resistant) populations of spermatozoa. Unfortunately the sample sizes in many of the trials of SLC preclude generating statistically significant pregnancy-rate data to unequivocally demonstrate the positive effect of SLC on fertility. Future

large-scale trials to establish the fertility of SLC-processed semen are warranted to assist in determining how useful this technique could be and to what extent conception rates may be improved. Sourcing sufficient numbers of stallions and mares to test the resultant fertility rates of the processed sperm, whilst adhering to rigorous, standardised experimental design is highly challenging and the authors expressed their wish to collaborate with industry representatives willing to help further evaluate SLC in practice.

The need for scientific research to support the breeding industry

Dr John Newcombe, a practicing veterinarian, stud medicine specialist and author of over 50 peer-reviewed articals written from observations made from his clinical records, outlined the most pertinent research questions that would be of greatest benefit to the grass-roots breeder and veterinarian.

A ubiquitous problem exists in maintaining the quality of frozen semen post-thaw (in terms of progressive motility and sperm velocity) especially in light of the industry standard that assumes, simply by convention, that 35 % progressively motile sperm is acceptable. The 35 % progressive motility value was contested as being adequate, particularly as resulting pregnancy rates in practice from these inseminates, are as low as 40 % at first insemination. An agreed minimum standard was proposed by the speaker, below which semen should be sold as ‘not of marketable value’. To determine this standard a multicentre field trial, using a large population of stallions was recommended whereby the post-thaw semen characteristics are rigoursly evaluated and correlated with ultimate pregnancy rates.

The difficulty in obtaining chilled semen at weekends and Bank Holidays was raised, which requires much estimation on the part of the practitioner/stud manager to predict the day of ovulation so that chilled semen arrives within 24, or even 12 hours before ovulation. Studies leading to improvements in chilled semen storage techniques would be a major step forward to enable sperm viability of up to 48 hours, preferably at temperatures of 15 ^{o}C, which is far more convenient than chilling to 5 ^{o}C. However, temperatures of 5 ^{o}C are favourable to maintain sperm motility, fertility and minimse bacterial growth (Aurich 2008). One study provided a protocol and proposed a chemically defined diluent (INRA 96) for succesfully storing semen at 15 ^{o}C (Batellier *et al.* 1998), although these findings contrasted those of more recent work (Price *et al.* 2008).

Comment [dn1]: Conjecture on my part, is this really a big inconvenience? I can't remember now what John's reasoning for preferring chilling semen to 5 C

The determinates of fresh semen viability within the uterine environment following AI is not well understand and may be multifactoral. Dr Newcombe has observed in practice that semen can survive up to 7 days in the mare with successful pregnancy outcome, while other inseminates exhibit longevity of less than 12 hours (despite accurate determination of ovulation). Factors hypothesised by the speaker as determinants of longevity that require investigation include: the effect of utilising sperm concentrations in the order of billions, representative of natural covering, as opposed to millions as is common for AI; the importance of progressively motile sperm and percentage dead must be better understood and the existence of 'cohorts' of sperm at various stages of maturation within the inseminate relative to the timing of AI.

Mating-induced endometritis (MIE) is ubiquitous in mares and persistent mating-induced endometritis (PMIE) whereby inflammatory uterine fluid accumulation causes subfertility, occurs in at least 15 % of animals (Zent *et al.* 1998). The current post-insemination standard treatment comprises of adminsitering ecobolic agents such as oxytocin to stimulate uterine myometrial contractions to expel the persistent fluid, yet as a practictioner, Dr Newcombe described this practice often necessitated repeating in some mares. The development of a longer acting or more poweful ecbolic agent was highlighted. Alternatively, the applictions for PIF, as outlined earlier by Prof Barnea, may translate well to equine practice as a potential anti-inflammatory agent that may be administered as a semen extender. Furthermore, the underlying innate immune response to insemination remains poorly understood. It is well established that different inseminates elicit a differential neutrophil response (Kotilainen *et al.* 1994). However, when the inseminate type is fixed, the greatest variable observed in practice (using neutrophil cell counts from the uterine lumen and time to their resolution as a marker of inflammation) and indeed *in vitro* (using $PGF_{2\alpha}$ secretion as an inflammatory marker; (Nash *et al.* 2008; Nash *et al.* 2010) is between individual animals; the mechanisms contributing to this variation is unknown.

The need for a reliable diagnostic tool for determining pregnancy before day 11 after ovulation was also suggested. The purpose would be to hasten the confirmation of a negative pregnancy diagnosis whereby the mare can be quickly returned to oestrus by exogenous manipulation of the oestrous cycle.

Moreover, pregnancy rates observed by Dr Newcombe are up to 70 % as revealed by ultrasound diagnosis on day 11 or later but fertilisation rates may be much higher, indicating a significant proportion of early embryonic loss. A definative diagnosis before day 11 would help uncover the timing of embryo demise and would be a useful step forward in understanding the underlying causative factors. It is worth considering again the real possibilities that PIF may potentially offer in this regard, as outlined by Prof Barnea.

Finally, factors affecting endometrial cyst occurence are not well understood: although anecdoctal evidence suggests that cysts increase with mare age and height, there appears to be no established correlation with mare parity or susceptibility to PMIE and cyst presence or absence, number, size or effect on fertility. To date this topic receives very little research attention.

Public Forum

A public discussion forum was devised and chaired by Dr Elizabeth Lane (University College Dublin), to allow opportunity for delegates to comment upon the presentations, identify issues pertinent to their breeding operations where resolution would improve fertility, discuss potential steps for addressing such issues and consequently build new networks and collaborations between the wide variety of particpiants. By way of introduction, Sabrina Barnwell, a veterinary surgeon specialising in stud medicine in Co. Limerick, Ireland, outlined typical issues that inhibited her day to day clinical reproduction practice. For example the problem of inseminating older, maiden mares with closed cervix was outlined. Thereafter, delegates were divided into specific

fields of interest for focussed, small-group discussion. Groups were defined as: pre-pregnancy; early pregnancy; late pregnancy and parturition; mare reproductive tract disease; stallion related factors; new technology requirements. Following a 30 min discussion period a representative from each group presented the main issues and any proposed solutions to the delegation and these points were collated and documented by a note-taker, to enable later dissimination through the present report. It was interesting to note that as well as physiological aspects for improving reproductive efficiency, several groups identified improvements to documentation, administration and maintenance of standards as being critical to enhance industry integrity and success. Key topics discussed within each interest group are detailed.

Pre-pregnancy and reproductive tract diseases groups

The pre-pregnancy group stated that whole animal factors affecting mare fertility require more indepth understanding. For example, the impact of the plane of nutrition upon the mare has recieved little research attention other than in Standardbreds and Quarter Horses (Cavinder *et al.* 2007; Thorson *et al.* 2010; Vecchi *et al.* 2010). The importance of acurately correlating pregnancy rate with body condition score (BCS) of the mare prior to covering was stressed. Furthermore, a recommendation was made by the group discussing mare reproductive tract disease which links well to understanding generalised mare health status and illustrates the pertinence of this issue within the breeding fraternity; mares sent to stud are often not accompanied with detailed documentation that states previous breeding history, reproductive and health status, which may assist the stud in succesfully

managing an individuals clinical programme to facilitate positive pregnancy outcome. The suggested solution was to design and promote the use of a standardised mare breeding record for use by veterinarians, whereby essential information, such as BCS, outcomes of previous coverings, double or single ovulations, extent of uterine fluid accumulation etc., can be documented. The form may travel with the mare, along with her passport, to stud where the attending manager/veterinarian can utilise the information to inform tailored and best practice. Of course, such a record could not be enforced industry-wide, however, should a record by devised, it may be disseminated to breeders and veterinarians attending the Colloquium for Equine Reproduction. Those willing to trial its use over the ensuing breeding season could be invited to a future Colloquium to present their experience of using the form. The intention would be that by reporting on the advantages (and identifying the disadvantages for improvement) in a public forum, the practice would be promoted and adopted by an increasing population of breeders and veterinarians. Indeed, Anne Brown (Gadbrook Stud, Peterborough, UK) offered that her veterinarian has such a form; Dr Nash will investigating gaining permission to acquire, adapt and circulate the record form in due course through a website specifically implemented as a result of the Colloquium for use of the delegates. A further advantage to instigating a standardised mare breeding record would be the provision of vast data available for collative research.

The pre-pregnancy group identified a need for better understanding of follicle development and duration of dominance correlated to the quality of the

resulting oocyte and embryo. Dr Newcombe commented that he is undertaking steps towards this type of study from his own breeding records.

There was strong opinion from the group discussing mare reproductive tract disease, especially from veterinarians, that one of the greatest challenges to achieving satisfactory pregnancy rates is inseminating older maiden mares, who's cervices fail to adequately relax. Prof Robert Gilbert (Cornell University, USA) suggested that prostaglandin E_2 (PGE_2) topical cream may provide a practical solution to hasten and promote cervical relaxation, as well as acepromazine (ACP) or administration of oestrogens as alternatives. In order to take this forward, several veterinarians expressed a willingness to trial this approach over the coming breeding season and report back at an upcoming Colloquium for Equine Reproduction. Dr Nash will attempt to co-ordinate trialling of PGE_2 topical cream through veterinary surgeons via the proposed Colloquim website.

Eary pregnancy

Two prominent issues for research were proposed by those considering early pregnancy. Firstly, a methodology for increasing the recovery rate of embryos 5 days after ovulation for the purposes of freezing would be advantages for embryo transfer. In light of the fact that an embryo normally descends into the uterine lumen by day 6 after ovulation, one strategy suggested included speeding embryo development to hasten the passage of the embryo when thereafter it can collected by lavage.

Comment [dn2]: Why? I can't remember specifics but assume that later embryo's are more fragile so earlier embryo's better survival, but of course they don't enter uterine lumen until day 6.

Comment [dn3]: Is this really practical/likely even with research?

Second, the usefulness of measuring progesterone at day 24 to 27 after ovulation, or even regularly up until day 32 (either a single or multiple test, as determined by research) necessitates evaluation, because such a test would be highly advantageous for judging viability of the embryo before endometrial cup formation. The point was made that progesterone levels vary greatly (between mares or within-I can't remember?) although serum concentrations tend to be more consistent. On the other hand, determination of PIF, as outlined by Prof Barnea in his presentation, may offer an alternative gauge of embryo/foetus viability throughout pregnancy.

Late pregnancy and parturition

The discussion within this interest group focussed upon the significant benefit to the breeder of acurately predicting parturition. The group acknowledged that electrocardiographic monitoring of the foetal heart beat as an indicator of stress is currently being developed, yet the application of this technique as an indicator of imminent parturition in the undisturbed pregnancy appears to be limited (Nagel *et al.* 2010). Again, the application of measuring PIF, which is hypothesised by Prof Barnea to decline with impending parturition, is yet another possibility worth pursuing with further study.

Stallion related factors

The group considering male factors, as well as identifying specific topics of research need, also deliberated wider issues that inhibit research, namely funding and animal availability. Several bold, yet vital proposals were made. The establishment of a centralised breeding station that, although run

commercially, would provide a research facility was strongly supported. The maintenance of the facility could be partly funded by individual research groups and breeders contributing a small annual fee, whereby they would gain access to the amenities and be prioritised to trial products/methodolgies/standardised documentation once deemed appropriate by the station. The success of the facility would be dependant upon gaining Home Office premises approval and would require key staff with personal licences. Nonetheless, a centralised station whereby combined resources exist (for example, laboratory analysis expertise) and where animals, their environment and maintenance are standardised, could only be of substantial benefit to conducting robust, internationally acclaimed and co-ordinated research with real benefit to the veterinarian and breeder. The group went further to suggest a centralised organisation/body/working group to represent British horse breeders. The role of such a body would include lobbying MP's, creating economic impact reports, promoting changes of legislation (e.g. semen standardisation minimum requirements under trade description acts?) and generally enhancing unity within the industry, for example, between the British Equestrian Federation, the British Horse Society, DEFRA etc.

In terms of areas of specific research need relating to the stallion, several key ideas were offered. Developing a male genetic marker of fertility would be offer a gold standard for the future of informed, refined and responsible breeding. Although this level of knowledge is likely a long way from fruition, several positive steps to enhance our understanding of stallion fertility can be instigated meantime. Understanding the nutritional factors affecting stallion

fertility has recieved very little attention, although studies currently being undertaken at Writtle College are a positive step to address gaps in the knowledge. A handful of studies have trialled feeding omega-3 fatty acids to prevent to improve semen quality (Brinsko *et al.* 2005). Beyond National Research Council (NRC) recommendations, few data exist to determine optimal daily rations for stallions with direct correlation to parameters of fertility, such as sperm concentration, progressive motility, membrane and chromatin integrity, or freezability. Predicting freezability and fertility of frozen semen and maintaining motility of chilled sperm were also raised by this group as parameters that require further study. Once the parameters that determine fertility of fresh, chilled and frozen semen have been established, minimum standards may be devised and universally agreed, as highlighted by Dr Newcombe in his earlier presentation, and critically must be coupled with accurate documentation. Mrs Caroline Sussex described a frustrating experience when receiving frozen semen from overseas which, on arrival and subsequent laboratory examination, was deemed useless. Although the fresh semen had been examined at the source as being of suitable quality before freezing, no subsequent examination post-thaw was performed by the suppliers and no documentation accompanied the inseminate to attest to its quality. Mrs Sussex suggested that legal documentation which states that frozen semen from an individual stallion has succesfully resulted in a pregnancy and a post-thaw semen analysis would be great progress.

New technology requirements

The primary focus of discussion within this group regarded the measurement of immune status of mares, especially in response to breeding and resultant MIE or PMIE. As such, this point links to the observation made by Dr Newcombe during his presentation, that there is great variability of uterine responses to insemination between mares and the factors that determine such variation remain unknown. The group suggested measuring lymphocte to neutrophil ratio as one potential candidate for susceptibility. The oxygen saturation of red blood cells was also considered.

Comment [dn4]: Was this in context of this point though? I can't remember?

Interestingly, this group also re-iterated comments made by other groups, highlighting their importance, both regarding correlating BCS at cover the potential effects upon pregnancy rates and the need for improving our ability to effectively freeze embryos intended for transfer.

Comment [dn5]: Expand on these if poss?

Conclusion

The Colloquium for Equine Reproduction achieved its aim in providing a platform for current research communication and an opportunity for the opinions of representatives from all corners of the equine breeding industry to be made. A feedback questionnaire completed by delegates verified that the event was met positively, meeting a strong requirement to provide the industry and academia with a common forum for the benefit of all. As a direct result of the Colloquium the present report was devised to summarise and disseminate the outcomes of the day to delegates and interested parties. In addition, to maintain the networks that were forged during the day and to aid progression of the ideas and strategies proposed, a website will be implemented for

delegates as a central point of contact. It is envisaged the website will not only publicise and promote the Colloquium *per se*, but communicate the identified gaps in the knowledge, standardised documentation requirements of the industry and potential strategies to tackle them. With members permission, the website will also enable contacts to be maintained between delegates, providing a vehicle through which studies can be postulated, designed, constructively criticised and hopefully undertaken using the wide availability of resources held by various member individuals through collaboration. In light of its success, every effort will be made to hold a future Colloquium for Equine Reproduction, evolving into an annual meeting.

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