acetate (4 times 12h apart) [Barrier-Battut I.,et al. Theriogenology, 2001, **55**, 1679-95] and injection of a long acting deslorelin preparation [Ferris R.A. et al,. J. Equine Vet. Sci., 2012, **32**, 285-88] are commercially available and are widely used worldwide for the induction of ovulation in mares. A preliminary study has suggested that a single subcutaneous injection of a large dose (6 mg) of buserelin acetate in saline solution also induces ovulation [Levy I. & Duchamp G. Reprod. Domest. Anim., 2007, **42**, 550-54] Objectives were: To confirm that an intravenous injection of hCG (1500 IU) and a single subcutaneous injection of 6 mg buserelin acetate in saline solution have the same efficacy for the induction of ovulation and to evaluate the efficacy of a reduced dose of buserelin (3 mg) as compared to a 6 mg dose. Donor or recipient mares in oestrus having a growing follicle with a diameter of 35 mm were injected either subcutaneously with buserelin acetate (6mg) in saline solution (n=50) or intravenously with hCG (1500 IU) (n=41). The follicular growth was checked every 12 hours until ovulation. Donor and recipient mares in oestrus having a growing follicle with a diameter of 35 mm were treated by a subcutaneous injection of buserelin acetate in saline solution with a dose of respectively 6 mg (n=192) and 3 mg (n=341). There was no statistical difference in rates of ovulations occurring between 24 and 48h after the injection of buserelin 90% (45/50) *versus* hCG 78% (32/41). There was no statistical difference in rates of ovulations occurring between 24 and 48h after injection of 3 mg buserelin (79% - 269/341) and 6 mg buserelin (78% - 150/192). In the 2 groups, there were same rates of both multiple ovulations (16 *vs* 19 %) and early ovulations (before 24 hours after injection) (13 *vs* 15 %). Rate of early ovulations was lower during the beginning of breeding season 7% (before May 1) than later 18 % (after May 1). This study confirms previous results [Levy I. & Duchamp G. Reprod. Domest. Anim., 2007, **42**, 550-54] and clearly indicates that a single subcutaneous injection of 3 mg of buserelin in saline solution is effective to induce ovulation. Those results suggest that initial studies using GnRH or one of its agonists in saline solution conducted to an incorrect conclusion: the inability of a single injection of GnRH or an agonist in aqueous base to induce ovulation in estrous mares. The use of implants, repeated injections or preparations with long acting base of GnRH agonists does not seem necessary to induce the ovulation. Further studies should be conducted to test lower doses of buserelin administerd as a single injection as suggested by Lindholm *et al* [Lindholm A.R.G., et al. Anim. Reprod. Sci., 2010, 121, 68-70].

70
Is there a repeatability in the size of follicles at the time of ovulation within individuals in mares?

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In practice, the size of the ovulating follicle is often considered as an individual parameter of each mare, with a great repeatability between oestrus cycles. However the few published studies on this subject compared the size of preovulatory follicles checked during only 2 or 3 consecutive oestrus cycles of mares [Cuervo-Arango J et.al Theriogenology, 2008, 69, 681-687. Jacob JC et.al Reprod. Dom. Anim., 2009, 44, 92-99.] To evaluate in the same herd of mares where oestrus cycles and follicular growth were checked all along the year during successive years and consider individual, seasonal and meteorological influences on the size of the follicle the day before ovulation. A retrospective study was done based on the registration of follicular growth checked during 18 years in a herd of 21 donor mares used to produce experimental embryos. Only data obtained from 504 oestrus with spontaneous ovulations (5 to 36 oestrous cycles/mare) were analysed 1) to evaluate the influences of time of year, meteorological parameters (temperature, sunshine duration, rainfall) and number of ovulations on the diameter of preovulatory follicle on the day (24 hours) before the ovulation and 2) to assess the degree of repeatability of this diameter in individual mares. Statistical analyses were performed using non-parametric Mann and Whitney's U-test, student's test, ANOVA and Pearson's correlation test. Double preovulatory follicles being smaller than single ones (38.5 *vs* 40.1 mm), only cycles with single ovulation were kept for analyses. Standard deviations calculated for each mare from the diameter of preovulatory follicles the day before ovulation varied among the 21 mares from 2.68 to 6.11 mm. This individual standard deviation was lower than the standard deviation calculated for the total population (5.19 mm) for only 7 out of 21 mares. Ovulations in August occurred when preovulatory follicles were significantly smaller than in all other months of the year. The variability of the size of the preovulatory follicle the day before ovulation was higher in the beginning of the year and at the end of summer than during the breeding season. This size is negatively correlated to the mean temperature of the 5 previous days before ovulation. There was no relationship between this size and the other meteorological parameters. There was a tendency of individual repeatability of the size of preovulatory follicle the day before ovulation for only a third of the mares. These data suggest that knowledge of mare's history on the diameter preceding ovulation would not be as useful for estimating the optimal follicle diameter to breed the mare at the optimal time as previously published [1,2]. In those previous studies, only 2 or 3 consecutive cycles were checked for each mare, and season effect on the size of preovulatory follicle had probably played a greater role. This well documented effect of the season was again found in this study. This study showed, for the first time, a negative correlation between the temperature during the five last days of oestrus and the size of follicle on the day before its ovulation without any influence of other meteorological parameters.

71
Effect of asynchronous embryo transfer on the transcriptome of early equine conceptus membranes

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In commercial equine embryo transfer (ET), it is common to use recipient mares that ovulate after the donor mare (negative asynchrony). Whereas in other domestic species severe embryo-uterine asynchrony is incompatible with embryo survival, the equine embryo can tolerate up to 5 days of negative asynchrony, albeit at the expense of retarded development and an increased incidence of early embryonic death. How the embryo can adapt to an out-of-synchrony uterine environment is unknown, and neither is it clear whether development is simply delayed or whether it is otherwise compromised. The aim of this study was to examine the effect of an asynchronous uterine environment on the transcriptome of the bilaminar trophectoderm of early equine conceptuses.

Day 8 embryos were transferred to recipient mares that either ovulated on the same day as (synchronous; n=8), or 5 days after (asynchronous; n=8) the donor mare. Conceptuses were recovered 6 or 11 days after ET (day 14 or 19 of conceptus age; n=4 per group), and bilaminar trophectoderm was isolated and snap frozen. After thawing and RNA library preparation (Illumina TruSeq kit), RNA sequencing was performed using an Illumina NextSeq 500 platform. Generated reads were mapped to the equine genome (EquCab2.0) using TopHat2, the number of reads per gene was calculated using QuasR qCount, and statistical analysis was performed using DESeq2. On day 6 after ET, 472 genes were differentially expressed in the bilaminar trophectoderm of conceptuses recovered from a synchronous versus an asynchronous uterus (fold change >1.5-fold, FDR 1%); approximately half of the genes were up-regulated in the synchronous environment. By day 11 after ET, the number of differentially expressed genes had increased to 3191, of which 1751 were up-regulated in conceptuses from synchronous ET. Clearly, many of the differentially regulated genes will relate to differences in developmental stage. Indeed, conceptuses recovered from an asynchronous uterus resembled day 11 conceptuses at ET+6, and day 16 at ET +11. Nevertheless, when the transcriptome of ET+6 synchronous and ET+11 asynchronous conceptuses were compared, there were also 3263 differentially expressed genes, of which 1867 were up-regulated in the asynchronous group. Over-represented functional gene clusters included growth factors, nutrient transporters, adhesion and extra-cellular matrix molecules, and angiogenesis regulating factors. In conclusion, asynchronous ET leads to a rapid delay in conceptus development and alters gene expression in the bilaminar trophectoderm. As the conceptus develops, many extra-embryonic membrane genes are up or down-regulated to ensure adequate histotroph uptake and communication with the endometrium. While asynchrony between conceptus and endometrium will result in a mismatch, it appears that the conceptus can alter its gene expression and delay development until the uterine environment is favourable. The identity of genes differently regulated after asynchronous ET should help identify developmental changes driven by intrinsic programing from those triggered by uterine signals, thereby improving understanding of embryo development and embryo-uterine communication during early pregnancy.

Key Words: embryo transfer; uterine asynchrony; conceptus development

Acknowledgments

This work was supported by the EU FP7 project grant; EpiHealthNet, FP7-PEOPLE-2012-ITN -317146.

72
Oocytes from aged mares show reduced expression of mRNA for key spindle assembly checkpoint components

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In man and rodents, advanced maternal age is associated with a marked increase in embryonic aneuploidy, as a result of errors in chromosome segregation during meiosis. This can either lead to sub-fertility, primarily as a result of an increased incidence of pregnancy loss, or to the generation of chromosomally abnormal offspring. During meiosis, the Spindle Assembly Checkpoint (SAC) mechanism ensures correct alignment of chromosomes on the spindle before they are separated during anaphase [Musacchio ANat Rev Mol Cell Biol. 2007May;8(5):379-93].

An age-related reduction in SAC component expression in oocytes from aged females may predispose to mis-segregation events [Jones KT Development. 2013 Sep;140(18):3719-30].
Although little data is available on the incidence of aneuploidy in equine embryos, chromosomal abnormalities could contribute to the increased incidence of early pregnancy loss in older mares. The purpose of this study was to examine whether maternal age affects the expression of SAC components and their regulators in equine oocytes. Cumulus oocytes complexes (COCs) recovered from slaughtered mares were divided into groups depending on the age of the mare (young,< 15 years; old, ≥15 years) and cumulus morphology (compact or expanded) at recovery, and matured in vitro for 24h. After maturation, oocytes were denuded and sub-divided into those that reached MII and those that didn't (non-MII). RNA was extracted and cDNA synthesized from pools of 10 oocytes (n=4 per group). mRNA expression for SAC components and regulators (MAD2, BUB1, BUB3, BUB1B, TTK, NDC80, SPC25, AURKB, AURKC) was evaluated using RT-qPCR, with PGK1 and SRP14 as housekeeping genes. The effects of maternal age, cumulus morphology and success of maturation on mRNA expression for SAC components and regulators was analyzed using multiple regression. Gene expression for MAD2, BUB1, BUB3, BUB1B, NDC80, SPC25 and AURKB was similar in oocytes from young and old mares. Expression of TTK and AURKC was significantly reduced in oocytes from old compared to younger mares, irrespective of maturation stage or initial cumulus morphology. In mice, TTK1 is required for the proper timing of prometaphase, and is essential for SAC control, chromosome alignment and AURKC localization during meiosis I; its absence severely impairs chromosome segregation [Hached Ket al. Development. 2011 Jun;138(11):2261-71] .We therefore speculate that reduced expression of TTK and AURKC in oocytes from aged mares predisposes to aneuploidy and early embryonic loss.