

**ABSTRACTS: 36TH ANNUAL MEETING OF THE ASSOCIATION OF EMBRYO TECHNOLOGY IN EUROPE (AETE)**

TAI, FTET and AI

**Cervical transcriptomic profiling of high and low fertility sheep breeds****Laura Abril-Parreno<sup>1,2</sup>, Paul Cormican<sup>2</sup>, Anette Krogenæs<sup>3</sup>, Xavier Druart<sup>4</sup>, Kieran Meade<sup>2</sup>, Sean Fair<sup>1</sup>**

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**Keywords:** RNAseq, cervix, ovine.

In sheep, cervical artificial insemination (AI) involves depositing semen at the cervical opening, as it is not possible to traverse the sheep cervix due to its complex anatomy. However, this method yields low pregnancy rates of less than 30% worldwide when frozen-thawed semen is used. The only exception to this is in Norway, where vaginal (shot-in-the-dark) insemination with frozen-thawed semen to a natural oestrus is performed by farmers and yields pregnancy rates of approximately 70%. Research in Ireland has demonstrated this is due to the ewe breed, since sperm can traverse the cervix in greater numbers in some ewe breeds (Belclare) than in other breeds (Suffolk). However, the molecular mechanisms underlying differences in sperm transport through the cervix and its secretions remain unknown. The aim of this study was to profile the transcriptome of the ovine cervix in four ewe breeds with known differences in pregnancy rates following cervical AI using frozen-thawed semen. These were Belclare and Suffolk in Ireland (high and low fertility, respectively) as well as Fur and Norwegian White Sheep (NWS) in Norway (both with high fertility compared to the Irish ewe breeds). Cervical post mortem tissue samples were collected from the four ewe breeds (all the ewes were parity 3-5) at the follicular and luteal phases of the oestrus cycle (n=8-10 ewes per breed at the follicular phase of a natural and synchronised cycle and at the luteal phase of a synchronised cycle). Following euthanasia, the ovaries were assessed for the presence of an active corpus luteum (luteal phase = Day 9) or dominant follicles (follicular phase = 12 h post detection of standing oestrus, Day 0). The reproductive tracts were then longitudinally opened and two sections were taken from the mid region of the cervix. High-quality RNA extracted from the cervical tissue samples was analysed by RNA-seq and differential gene expression was assessed. We identified 7232, 7716 and 510 differentially expressed genes (DEGs) in NWS, Fur and Belclare ewes (respectively) compared to the Suffolk breed (reference level) at the follicular phase of the oestrus cycle. At the luteal phase, 1661, 4984 and 2087 genes were differentially expressed in NWS, Fur and Belclare, respectively (FDR < 0.01). Gene ontology analysis identified enriched pathways for transmembrane transport (*CA5A*, *PLN*, *MT-COX1*), inflammatory response (*KLKB1*, *MLKL*, *TSPAN2*) and cervical remodelling (*TGFB111*, *NEXN*, *TAGLN*, *TPM1*, *COL9A2*, *TES*). Although, there was a considerable overlap in the differential expressed genes between ewe breeds, with 262 and 202 genes in common for the four ewe breeds at the follicular and luteal phases, respectively (P < 0.05). In conclusion, this study has shown that there are breed- and phase-specific differences in cervical gene expression between ewe breeds known to differ in sperm transport across the cervix. This novel study provides the first transcriptional analysis of cervical tissue in four economically important European ewe breeds and aids our understanding on why frozen-thawed sperm can traverse the cervix in some ewe breeds but not in others.

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**Effects of environmental heat stress on ram's seminal plasma oxidative stress and proteome in INRA180 sheep****Bouchra El Amiri<sup>1</sup>, Anass Benmoula<sup>1</sup>, Lilla Turiak<sup>2</sup>, Fanni Bugyi<sup>2</sup>, László Drahos<sup>2</sup>, Moussa El Fadili<sup>3</sup>, András Horváth<sup>4</sup>, Otto Szenci<sup>4</sup>**

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**Keywords:** heat stress, seminal plasma, oxydative stress, proteome, ram.

The testis temperature must range from 2 to 8°C below body temperature to ensure successful spermatogenesis and disruption of this condition causes varied degrees of damages to the gonadal parenchyma and sperm production. Thus, exposure of animals to high temperatures potentially affects their reproductive efficiency. Seminal plasma (SP) is a component within the semen having a great role in maintaining sperm motility in many species. Previous studies focus on heat stress scrotal insulation on semen quality but there is a lack of studies based on environmental heat stress on oxidative stress status and seminal plasma proteome. In Morocco, animals are suffering from such effects mainly during summer when animals are grazing far away from their sheepfold. Thus, the present work aimed to study the effects of heat stress on rams's seminal plasma proteome and oxidative stress in INRA180 sheep. From mid Jun to the end of September 2019, semen samples were collected by artificial vagina (AV) from 12 INRA180 rams and then centrifuged to obtain seminal plasma (SP). The animals were randomly assigned to 3 groups. The control group (G0) was housed under sub humid conditions and was exposed to the sun during the grazing time from 7 to 11 am and from 3 to 6 pm. In the remaining time, animals were kept in a ventilated shed. The experimental groups were housed under sub humid (G1) and semi-arid (G2) conditions and were exposed to the sun during the whole day. From 15th to 30th of June, SP were collected once a week and used as a control. During the two months (July-August) of heat exposure no sample was collected. Then during the whole September, the samples were collected once a week. Total proteins, SOD activity and the level of GSH were evaluated in seminal plasma. Seminal proteins were analyzed by mass spectrometry. Statistical analysis to estimate the animal group effect were performed using SAS, ANOVA program (SAS Institute Inc., Cary, NC, USA). To compare the estimated means, the Dunnett test were used. After heat exposure, the total proteins (mg/ml) were lower in G1 ( $24.24 \pm 0.35$ ) than in G0 ( $25.49 \pm 0.02$ ) and G2 ( $26.84 \pm 0.06$ ). The SOD (U/mg prot) activity was significantly ( $P < 0.05$ ) higher in G0 ( $78.39 \pm 0.55$ ) than G1 ( $76.33 \pm 0.62$ ) and G2 ( $73.22 \pm 0.55$ ). The level of GSH (mg/dL) is highly affected by the heat stress exposure. The lowest value was recorded in G0 ( $18.68 \pm 0.13$ ) and G1 ( $18.83 \pm 0.14$ ) while the highest level was obtained in G2 ( $19.64 \pm 0.16$ ). The preliminary results of the proteomics analysis showed that 444 proteins were identified, and their appearance depended on the experimental group. Label free protein quantification showed that heat shock protein alpha was higher in G1 (4.7 times) and G2 (5.5 times) than in G0. Some proteins (Serpin domain-containing protein for instance) were only present in G0. Others were present in G1 and G2 while they were absent in G0. To conclude, the results in this study suggest that the environmental heat stress affects the oxidative stress indicator levels and the proteome and might be associated with semen quality.

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**Increasing the genetic potential in a nucleus of Romanian Buffaloes, by artificial insemination with sexed semen, after stimulation with OvSynch protocol - A Case Report****Stefan Ciornei, Dan Drugociu, Petru Rosca, Liliana Ciornei**

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**Keywords:** indigenous Romanian Buffalo (IRB), artificial insemination (AI), sexed semen, genomically tested bulls.

Although artificial insemination (AI) may mean a significant improvement in buffalo genetic improvement, its practical application has been difficult due to the low visibility of estrus and its poor human detection, the variable duration of estrus and the difficulty of predicting the time of ovulation. More recently, the development of protocols for synchronizing ovulation and planned AI in buffaloes has been used to overcome these constraints and to be able to use AI on a large scale. Genetic improvement is a dynamic process that must evolve over time, supporting and responding to the needs of breeders, the market and the local context.

As the number of buffaloes in Romania is decreasing in recent years, the application of current reproductive biotechnologies to these breeds is limited. However, AI is used occasionally in private small farms. In Italy, the country consecrated with tradition, in the production of milk buffaloes, AI has the most extensive use, but a large part of farms still using mount bull.

The farm "Terra di Buffala" has buffaloes of the Mediterranean breed, the Indigenous Romanian Buffalo (IRB) variety, and through this study we wanted to form a nucleus of females with high genetic potential. Thus, by implementing AI, an attempt was made to obtain female fetuses using sexed and genomically tested semen. The experiment was performed on a number of 20 multiparous buffaloes at over 60 days postpartum and lactation. Two batches of 10 were created for A.I. separated by two bulls, Oro and Aton, with 2 million female sperm straws. (S.C. Genomix, importer of the National Association of Young Buffalo Breeders from Italy). The females were at the beginning of the breeding season, February - March. The groups were compiled after a thorough gynecological and general examination, and subsequently the OvSynch therapeutic protocol (Gn-RH, PGF, Gn-RH) was started. According to the protocol, the females received on day zero and nine, 0.01mg buserelin acetate (Receptal®, MDS-Intervet, Holland) and PGF received on the fifth day, cloprostenol 500 µg IM (Estrumate®, MDS, Holland). The average body score was 3, the females had a completely involved and healthy uterus. The ovaries showed no signs of pathology, and their average size was 2 cm. Also, no corpus luteum was present at the beginning of the protocol. To prevent waste, they were AI only buffaloes that were at least interested in the bull, and that had a dominant follicle (DF) on the ovary (at least .9 mm). The AI method was tactile recto cervical, females were inseminated once at 18 hours after the second Gn-RH injection. The ovarian response was good, due to the selection of females, bull stimulation and the beginning of the breeding season. Therefore, 75% buffaloes (15/20) were diagnosed in estrus and inseminated. The conception rate was 60% (9/15), being diagnosed pregnant at 45 days (transrectal ultrasound white Honda HS-1600V®, Japan; 7.5 MHz). By categories of bulls the percentages were 50% in Oro (4/8) and 70% the Aton (5/7). 9 calves were born, the sex ratio was 88.8%, only one male was produced by Aton. We state that the goal of increasing the genetic potential of IRB by using AI with sexed sperm becomes achievable.