

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

Physiology of reproduction in male and semen technology

Effects of environmental heat stress on ram's scrotal circumference and semen quality of the INRA180 sheep**Bouchra El Amiri¹, Anass Benmoula¹, Moussa El Fadili², András Horváth³, Otto Szenci³**¹Institut National de la Recherche Agronomique de Settat, Maroc, Morocco; ²INRA, Animal Production Department, Rabat- Maroc; ³Department of Obstetrics and Clinic for Production Animals, H-1078 Budapest, Hungary.**Keywords:** ram, heat stress, scrotal circumference, semen quality.

Due to its location between a temperate climate in the North to a tropical climate in the south, Morocco is a country suffering from extreme impact and sensitivity due to global climate changes. Recent scenario predicts a decrease in precipitations (11%) and an increase of temperatures (1.3°C) by 2050. In this context, it is well documented that ram's exposition to high environmental temperatures causes a seminal degeneration (Cárdenas-Gallegos, Archivos de Medicina Veterinaria, 47, 39-44, 2015) with intensity varying between breeds. Moroccan sheep raised in harsh conditions during summer suffer from such effects. However, there is a lack of studies regarding this issue. Thus, the present study aims to determine the effect of different environmental heat stress condition on the INRA180 ram semen quality. During summer period (July - October), 12 INRA180 rams 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. 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 whole day. The evaluation of fresh semen quality, mass motility (MM), individual motility (IM), concentration (C), volume (V) and scrotal circumference (SC) were recorded 15 days (at 10 am) before starting the experiment to determine the initial parameters. After 2 months of animal's exposure, the same measurements were recorded. During the experiment, the mean temperature was 27.30±1.48 and 31.55±3.4 in sub humid and semi-arid respectively. Statistical analysis were performed using SAS, ANOVA program (SAS Institute Inc., Cary, NC, USA). The results showed that the MM decreased significantly in the two groups (G1= 3.08±0.2; G2 = 2.43±0.4) compared to G0 (4.4±0.18). Individual motility decreased significantly in the two groups (G1=68.04±1.3%, G2= 32.33±1.75%) when compared to G0 (IM= 97.98±1.27%). For G2, C (2.04±0.68 x 10⁹ spz/ml) decreased significantly (P<0.05) and not for G1 (3±0.77 x 10⁹ spz/ml) (P > 0.05) when compared to G0 (C= 3.41±0.87 x 10⁹ spz/ml). However, the volume and SC were not affected by the heat stress exposure, whatever the ram group was (P > 0.05). The present study shows that the exposure of INRA180 rams to environmental heat stress causes a considerable decrease for MM, IM and C. These results are in agreement with those of previous studies under environmental as well as direct testis insulation heat stress (Soleilhavoup, Journal of Proteomics, 109, 245–260, 2014). The increase in temperature stress disturbs or fails thermoregulation and consequently increases the testicular temperature (Moule, Aust. J. Agr. Res. 1, 456, 1950). It will lead to local hypoxia and deleterious effects on the tissue inducing an alteration of the spermatogenesis process and reducing the quality of ram sperm (Marai, Ann. Arid Zone, 39, 449-460, 2008). To conclude, the exposure of INRA180 rams to thermal stress negatively affects the quality of the ram's sperm in semi-arid and sub-humid conditions.

Thanks for CNRST-Morocco and the NIH-Hungary and Mr Rahim and Mrs Nejjam.

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Physiology of reproduction in male and semen technology

Characterisation of the sire contribution to fertilisation failure and early embryo survival in cattle

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Keywords: bull fertility, superovulation, embryo ie.

Reproductive efficiency is a major driver of profitability in cattle production systems, particularly in seasonal systems where high 6-week in-calf rates are critical. The preponderance of research effort in the field of bovine reproductive physiology has focused on ways to improve cow fertility, while the contribution of the sire to poor herd fertility has received less attention. However, bull fertility is a major contributor to overall reproductive performance, particularly in herds where natural service is predominately used. Even in highly selected bulls in AI centres, where sperm quality is scrutinized, significant variation exists in field fertility. It is not clear where along the developmental axis such differences originate. The objective of this study was to determine whether subfertility in AI bulls is due to issues of sperm transport to the site of fertilisation (based on accessory sperm number), fertilisation failure or early embryo development.

Holstein Friesian bulls (3 High, HF and 3 Low, LF, fertility) were selected from the national population of AI bulls based on adjusted fertility scores from at least 500 inseminations (n=840 bulls; HF: +4.34% and LF: -12.7%). Synchronized beef heifers (n=19, 3-4 per bull) were superovulated using follicle stimulating hormone. Heifers were blocked based on estimated number of follicles at the time of AI and inseminated with semen from HF or LF bulls. Following slaughter 7 days later, number of corpora lutea (CL) were counted and the uteri were flushed. Recovered structures (oocytes/embryos) were classified according to developmental stage and stained with DAPI to assess number of cells and accessory sperm.

Mean number of CL per superovulated donor was not different between groups (HF: 17.4 ± 8.2, LF: 17 ± 8.4). Overall recovery rate (total structures/total CL) was 52.6% (HF:49.6% v LF:55.3%; P>0.05). Mean number of embryos recovered per recipient was 8.6 ± 5.2 and 9.4 ± 5.5 for HF and LF, respectively (P>0.05). Overall fertilisation rate was 95.9% and was not different between groups. The percentage of morula (14.9 v 36.2%), blastocysts (40.5 v 23.1%) and expanded blastocysts (44.6 v 40.7%) was not different between HF and LF bulls. Mean embryo cell number was greater for HF (91.5±3.42) v LF (77.2±3.21) bulls (P<0.05). Overall, 17.0% (29/171) of structures had at least one accessory sperm. Number of accessory sperm was highly variable (range HF:0 to 45; LF:0 to 8; P<0.01). For those structures with accessory sperm, the mean number of sperm per structure was 12.7±3.66 v 2.9±0.75 (P<0.05) for HF and LF bulls, respectively.

In conclusion, while fertilisation rate did not differ between HF and LF bulls, the number of accessory sperm, a proxy for the number of sperm reaching the site of fertilisation, was lower in LF bulls, although highly variable. Despite differences in embryo cell number at Day 7, differences seen in field fertility between HF and LF bulls used in this study are unlikely to be due to differences in fertilisation rate or early embryo development. It is likely that differences occur later in development, perhaps associated with maternal recognition of pregnancy or implantation.

Funded by Science Foundation Ireland (16/IA/4474).

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Physiology of reproduction in male and semen technology

Bisphenol-A concentration affects ram and boar sperm motility

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Keywords: Bisphenol-A, spermatozoa, CASA.

There is growing concern about the effects of bisphenol-A (BPA) on fertility. There are reports on decreasing sperm fertility due to plastic quality, potentially associated with leaching (Porcine Health Manag 3:15, 2017). Therefore, we have tested the effect of BPA on boar and ram sperm motility. We used frozen ram semen doses from our cryobank (extended in TALP-HEPES post-thawing) and boar commercial doses (refrigerated) from AIM Ibérica, 3 males per species (triplicated by male). BPA (Merck, Darmstadt, Germany) was prepared in DMSO and added to sperm aliquots ($30 \times 10^6 \text{ ml}^{-1}$) at 500, 200, 100, and 10 μM , adding DMSO to the control. The tubes were incubated at 37 °C for 2 h. Videos of motile spermatozoa were acquired each 30 min (20- μm chamber, $\times 10$ negative phase, 200 fps) and processed with the OpenCASA software (PLoS Comput Biol 15:e1006691, 2019). Data were analyzed by linear mixed-effect models. Total motility decreased as the concentration of BPA increased, with different dynamics for each species. Ram spermatozoa showed significant effects of incubation time and the concentration (main effects, no interaction). However, only 500 μM caused a significant decrease. Progressive motility showed similar results, with $P < 0.05$ for 200 μM vs 10 and control. Boar data showed a significant interaction concentration \times time, with 0 and 10 μM behaving similarly and decreasing with time, whereas 10 to 500 μM abolished motility from time 0 (most samples below 10%), remaining similar for the rest of the experiment. Sperm velocities (VCL: curvilinear; VAP: average-path; VSL: straight-line) showed significant interactions for ram, overall being lower in 500 (all times) and 200 μM (decreasing with time). For boar, the dynamics for each variable varied, in general showing small changes with BPA and incubation. Linearity parameters (LIN: linearity; STR: straightness) were affected by BPA in ram ($P < 0.001$), decreasing with concentration but not as dramatically as for other variables, whereas WOB (wobble) was not affected. However, boar data showed interactions for LIN and STR ($P < 0.01$), increasing with time for 100 to 500 μM , and time and dose-dependent decreases for WOB ($P < 0.05$). The ALHmax (lateral head movement, maximum) showed a significant interaction in ram, with a decreasing trend for 100 and 200 μM , whereas 500 μM showed a lower value for the duration of the experiment. For boar, there was no interaction, with ALHmax decreasing with time and BPA concentration ($P < 0.01$). In conclusion, BPA affected motility in both kind of samples. However, only boar refrigerated spermatozoa were clearly affected at concentrations below 500 μM . These results are relevant for quality control, and help to explain dramatic prolificacy changes reported in boar doses involving faulty plastic material. Furthermore, these results highlight the usefulness of boar sperm as biosensors (Basic Clin Pharmacol Toxicol 123:3, 2018).

Acknowledgments to Topigs-Norsvin España (AIM Ibérica), C. Alquézar-Baeta and BIOFITER (OpenCASA developers), and L. Tejerina and L. Álvarez-Fernández.

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Physiology of reproduction in male and semen technology

***In vitro* fertilization as an assessment of cryopreserved boar semen**

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Keywords: porcine, oocyte, frozen semen.

Currently, artificial insemination in pigs uses fresh semen almost exclusively due to poor results using cryopreserved boar semen. The option to use frozen semen would be an advantage, *e.g.* due to long storage time and transport possibilities. The aim of this study was to investigate the usefulness of *in vitro* fertilization (IVF) as a fast method to assess the fertilizing ability of frozen semen from seemingly similar boars.

Semen from two Hampshire boars (A and B) cryopreserved using a slow cooling method, with similar post-thawing progressive motility as assessed by computer assisted sperm analysis, CASA (43% and 44% respectively) was compared. The semen was frozen in 500 μ l straws with lactose and egg yolk solution with the addition of 3% glycerol and thereafter kept in liquid nitrogen until use. After thawing, the semen underwent colloid centrifugation (PorciColl) to select the best spermatozoa. *In vitro* matured pig oocytes ($n = 1024$) derived from slaughterhouse material, were randomly divided into groups of 30 for fertilization by boar A or B. The time of fertilization was 4 h (short) or 24 hours (long) and the number of spermatozoa/ml for fertilization was either 0.6×10^6 (low) or 1.20×10^6 (high). The presumed zygotes were observed for cleavage rate, blastocyst production and number of spermatozoa attached to the zona pellucida on day 6 after fertilization (DAPI staining).

The low concentrations of spermatozoa as well as the very short incubation time resulted in very few or zero fertilized oocytes for both boars. This contributed to a very large variation in the data which limited the possibility to use robust statistical analyses. The results are therefore only presented descriptively as means \pm SD. Subjectively assessed sperm movement after thawing was similar between the boars (A: $28\% \pm 9$, and B: $33\% \pm 9$). Cleavage after low or short fertilization was similar between boar A and B ($12\% \pm 8$ and $16\% \pm 8$, respectively). Cleavage after high and long fertilization was similar between boar A and B ($56\% \pm 10$ and $59\% \pm 12$, respectively). Blastocyst development day 6 after low or short fertilization was lower in A than in B ($0.6\% \pm 1$ and $7\% \pm 5$, respectively). Blastocyst development day 6 after high and long fertilization was lower in A than in B ($3\% \pm 5$ and $14\% \pm 8$, respectively). Mean number of spermatozoa attached to the zona pellucida for boar A was lower than for boar B (0.9 ± 1.3 and 2.53 ± 3.4). In all parameters measured in this small study, boar B consistently had the best results even though both boars were subjected to colloid centrifugation to select the best spermatozoa. It is no surprise that there are boar differences in IVF-systems but the IVF-outcomes still may reflect fertility after *in vivo* insemination using frozen boar sperm. However, more studies are needed to confirm these results.

Funding was received from KSLA, Stiftelsen Nils Lagerlöfs fond (GFS2019-0068). INN and R. Jochems are gratefully acknowledged for allowing informative study visit prior to this study.

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Physiology of reproduction in male and semen technology

Application of complementary tests for the evaluation of chromatin structure in semen from AI boars

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Keywords: DNA fragmentation, spermatozoa, chromatin, SCSA.

Producing a sire is costly due to breeding and innovation expenses. Moreover, AI centres can incur in compensations if they fail to detect subfertile boars. Thus, the pig industry has an interest on procedures for early detection of these boars. Sperm DNA fragmentation (SDF) affects fertility, but it is only one of many parameters in sperm chromatin. We tested SDF together with abnormal histone retention and disulphide bridges between protamines, as a proposal for routinely testing boars entering production. We used ejaculates from 11 boars of known fertility in an AI centre (Topigs-Norsvin). Samples (two ejaculates per boar in different weeks) were analysed on the day of collection and after 11 days of storage at 17 °C. Flow cytometry analyses (in duplicates) performed SCSA (DNA fragmentation, %DFI, and chromatin immaturity, %HDS), determination of disulphide bridge levels (DSF) and % of spermatozoa with histone retention (HR). We compared the techniques (linear mixed-effects models, male and ejaculate as random factors), assessed correlations and clustered the boars according to the chromatin parameters (R statistical environment). Results are shown as mean±SD. SCSA showed a low incidence of chromatin alterations. Storage increased DNA damage (%DFI; 1.1%±1.2 to 3.2%±2.9), and decreased HR (22.3%±3.3 to 9.1%±6.6) and DSF (36.4±5.6 to 25.2±4.7), all P<0.001. %HDS was not affected (7.6%±3.2 to 8.7%±2.9, P>0.05). %DFI negatively correlated with HR (-0.54, P<0.001) and DSF (-0.32, P<0.05), other correlations not being significant. A hierarchical cluster analysis yielded 4 groups of boars for each technique. Two boars with high initial %DFI or %HDS clustered individually and the other were grouped in two clusters of 2 and 7 boars respectively. The average DBE (Direct Boar Effect, a relative index) was higher for the first cluster (0.37 vs 0.04). The first cluster grouped boars with lower chromatin alterations or less detrimental changes with refrigeration, whereas the second cluster grouped boars with worse chromatin status and lower storage resilience. We were able to assess a small number of boars estimating chromatin status and resilience to storage. SCSA and DSF could be more discriminant, but all the parameters showed some association with fertility variables. The scope of this study is limited by the sample size, but our results suggest that chromatin analysis have a good potential for being applied in the AI centers. However, equipment cost is an important issue for considering routine use, since these techniques require flow cytometry.

Supported by grant RTI2018-095183-B-I00 (MICINN, Spain) and "Lanzadera" project (FGULEM-FUESCYL). We thank Topigs-Norsvin España, AIM (León) and L. Álvarez, and B. de Arriba.

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Physiology of reproduction in male and semen technology

The Porcicoll colloid selects a population with improved chromatin status in fresh and refrigerated boar semen

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Keywords: porcicoll, spermatozoa, colloid, centrifugation, chromatin.

The pig industry undergoes increasing pressure for innovating while keeping costs low. Selecting an optimized sperm population could increase the useful life and efficiency of artificial insemination (AI) doses, while minimising problems due to low-quality ejaculates. The Porcicoll colloid allows easily selecting high-quality spermatozoa by single-layer centrifugation (SLC). Sperm DNA fragmentation reportedly decreases after selection, but few studies have explored other chromatin parameters. We aimed at testing if Porcicoll SLC was capable of modifying the chromatin structure in boar semen. Ejaculates from 10 boars (Topigs-Norsvin, León, Spain) were collected in 3 consecutive weeks. They were submitted to SLC (S1: 1 ml and S4: 4 ml Porcicoll) or analysed directly (C: control), on the day of collection and after 3 days of storage at 17 °C. Pellets resuspended in BTS were analysed by flow cytometry in duplicates by: SCSA (DNA fragmentation, %DFI; chromatin immaturity, %HDS), disulphide bridges levels (DSF, monobromobimane, mBBr) and histone retention (HR, CMA3). The effects of the SLC and processing day were tested by linear mixed-effects models (R software, results as mean±SEM). Treatment×day interactions were not significant, therefore both factors were analysed as main effects. %DFI was low in all cases and not affected by day. SLC decreased this important parameter, showing an effect removing spermatozoa with damaged DNA (C 0.83 vs S1 0.41, S4 0.36, SEM 0.05, P<0.001). The treatment had no effect on %HDS. DSF fell on day 3 respect to day 0 (P<0.001), not being affected by SLC. However, the medium-mBBr population (a measurement of free thiols) increased with SLC and decreased at day 3 (P<0.001) and high-mBBr decreased with SLC (P<0.001). HR as CMA3 mean fluorescence or moderate-CMA3 population decreased at day 3 (P<0.001), no effect of SLC. However, the high-CMA3 increased at day 3 (P<0.001) and decreased with S1 (P<0.001 vs C and P<0.05 vs S4). Porcicoll SLC modifies the chromatin status of boar semen, both fresh and refrigerated. Our study disclosed that changes not only affect DNA integrity but also other chromatin parameters rarely considered, nevertheless important. The Porcicoll variants S1 and S4 yielded similar effects. Therefore they could be used in different contexts according to practical requirements. Supported by grant RTI2018-095183-B-I00 (MICINN, Spain). We thank Topigs-Norsvin España, AIM (León), L. Tejerina and B. de Arriba.

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Physiology of reproduction in male and semen technology

Extended equilibration time and glutathione increase viability and chromatin compaction in post-thawed bull semen

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Keywords: semen, chromatin, cattle.

Holding bull semen overnight before freezing is often used for samples that cannot be frozen on the day. However, there are contradictory reports regarding the effect on sperm quality. Also, supplementation of antioxidants to the semen extender could reduce the detrimental impact of reactive oxygen species (ROS). Therefore, this study aimed to assess the effects of extending the equilibration time to 24 h and the addition of glutathione in the post-thawing quality of bull semen. Semen from 8 Holstein and 4 Asturiana de los Valles bulls (an autochthonous breed in Asturias, Northern Spain) was collected by artificial vagina (3 ejaculates per bull) and frozen (BioXCell, IMV) after 4 (4E) or 24-h equilibration (24E) at 5 °C and with reduced glutathione (2 mM GSH, G2) or without GSH (G0). The cryopreserved doses were assessed immediately post-thawing (37 °C 30 s) and after a 5-h incubation at 37 °C (as a stress test). We analysed sperm viability, acrosomal status and apoptosis (YO-PRO-1/propidium iodide/PNA Alexa 647), and chromatin status (SCSA, acridine orange staining) by flow cytometry. Data were analysed by linear mixed-effects models in the R statistical environment (results as mean±SEM of %; no significant interactions detected). E24 improved viability (62.3%±1.0 vs 57.4%±1.2, P<0.001), and apoptotic ratio (9.7%±1.1 vs 12.4%±1.5, P<0.001) post-thawing, but reduced viability (40.2%±1.4 vs 45.7%±1.2, P<0.001) and acrosomal status (45.5±1.3 vs 52.0±1.1, P<0.001) after the incubation. G2 slightly improved viability (60.8±1.2 vs 59.0±1.8, P=0.013) post-thawing, and acrosomal status overall (63.4%±0.9 vs 62.1%±0.9, P=0.041). DNA fragmentation (%DFI) was not affected (1.9%±0.8 overall). Chromatin immaturity (%HDS) post-thawing was smaller in E24 (4.2%±0.4 vs 5.4%±0.3, P=0.005) and G2 (4.5%±0.2 vs 5.2%±0.3, P=0.038), with no significant differences after the incubation. Extending the equilibration time up to 24 h could be advantageous for work planning in breeding centres. E24 could even improve sperm quality in some cases. However, post-incubation results suggest the presence of sublethal damage not evident post-thawing. G2 yielded small effects, but it could be considered for freezing sensitive samples. Future work as insemination trials could elucidate if the lower resilience of samples submitted to E24 represents a threat. Nevertheless, differences were small and therefore increasing the equilibration time could still be considered if deemed convenient. In any case, individual bulls on high genetic value should be tested, since the sensitivity of the spermatozoa to extension times could vary among males.

Supported by grant RZP2017-00008-00-00. We thank the breeder associations ASCOL and ASEAVA and INDEGSAL technicians Beatriz de Arriba and Lucía Tejerina.

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Functional characterisation of sperm from high and low fertility bulls

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Keywords: fertility, bull, sperm physiology.

Traditionally, bovine artificial insemination (AI) centres have relied solely upon microscopy-based assessments, such as motility and morphology, as post-thaw measures of semen quality. However, amongst bulls deemed as acceptable based on these criteria, there is still considerable variation in fertility. The aim of this study was to investigate a range of sperm functional parameters in bulls of varying fertility to identify reliable discriminatory biomarkers. Holstein Friesian bulls classified as having either high (HF) or low (LF) fertility (n = 10 per phenotype) were used and had adjusted pregnancy rates of +4.1±0.15% or -7.6±1.50% (with a mean of 0%), respectively. For each bull, frozen-thawed spermatozoa were washed (300 x g, 5 min) and then incubated in modified TALP media and assessed at 0, 3 and 6 h (3 ejaculates assessed separately per bull). Motility and kinematic parameters were assessed using computer assisted sperm analysis (CASA) whilst nigrosin-eosin smears were prepared for morphology assessments at 0 h only (200 spermatozoa assessed per slide). For flow cytometric assessments, spermatozoa were incubated with two different staining combinations, 1) Alexa 647-PNA, merocyanine 540 and DAPI for acrosome integrity, lipid packing and viability and 2) MitoSOX red and Sytox Green for superoxide production and viability. Spermatozoa were stained with each combination for 15 or 20 min, respectively, at each time point prior to analysis. Potential differences between the fertility phenotypes were statistically analysed for each functional assessment using linear mixed model regression (REML; R version 3.4.1). Pairwise comparisons were determined using the predictmeans function with a Tukey adjustment. There were no differences between HF and LF bulls in relation to motility, kinematic parameters, morphology or superoxide production in viable cells, irrespective of incubation time (P>0.05). However, the percentage of viable cells with an intact acrosome as well as with high membrane lipid packing was greater overall in HF bulls (HF: 42.6±3.21% vs LF: 31.2±5.53%, P<0.05). Based on these findings, the tentative markers for fertility relate to cell viability and acrosome integrity. Since the flow cytometric assessment of viability is based on the integrity of the plasma membrane, further research is required to identify factors contributing to structural defects in the membrane and acrosome in bulls with low fertility.

Funded by Science Foundation Ireland (16/IA/4474).

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Physiology of reproduction in male and semen technology

Comparison of the uterine inflammatory response to sperm from high and low field fertility bulls**Eimear M. Donnellan¹, Kieran G. Meade², Sean Fair¹**

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Keywords: AI, fertility, inflammatory response.

Despite stringent quality control checks in animal breeding centres, some bulls with apparently normal semen quality yield unacceptably low pregnancy rates. We hypothesised that this was due, at least in part, to a differential uterine immunological response to sperm from high and low fertility bulls. The aims of this study therefore were to (i) optimise an *in vitro* protocol to assess the endometrial inflammatory response to frozen-thawed bovine sperm (ii) establish if sperm from high and low fertility bulls elicit a differential uterine inflammatory response and (iii) identify if the source of the uterine inflammatory response from frozen-thawed sperm is of sperm or seminal plasma origin. The experimental model used was heifer follicular phase uterine explants (8 mm biopsy punch) stimulated with washed frozen-thawed sperm *in vitro* (3-5 replicates per experiment). Experiment 1 investigated three sperm concentrations (5, 10 or 15 x10⁶sperm/ml) and three incubation time points (1, 3 and 6 hours). Experiment 2 assessed sperm from 3 high (fertility rate of + 4.27% ± 0.35, mean ± s.e.m.) and 3 low fertility (-12.2% ± 1.81, mean ± s.e.m) bulls (average fertility = 0%) where fertility was based on an animal adjusted model (AAM) which is based on calving rate while adjusting for a wide range of factors. Experiment 3 included explant co-incubation with pooled caudal epididymal sperm (CES) from mature bulls of unknown fertility with and without seminal plasma. Each experiment included a control explant (no sperm added). In all experiments, a panel of inflammatory mediators namely, *IL1A*, *IL1B*, *IL6*, *TNFA* and *CXCL8* were analysed by qPCR. Quantification of IL1-β and IL-8 in explant supernatants for experiments 2 and 3 were analysed by ELISA. For statistical analysis, repeated measures ANOVA was performed for experiment 1 and one-way ANOVA for experiments 2 and 3. In experiment 1, there was no effect of sperm concentration (P>0.05) but there was an effect of time for *IL6*, *IL1B* and *IL1A* with maximum expression at 6h (P<0.05). There was no sperm concentration by time interaction. There was no difference in the inflammatory response at the gene or protein level between high and low fertility bulls (Experiment 2) but a significant up-regulation of *ILB*, *TNFA* and *IL1A* gene expression from frozen-thawed sperm (irrespective of bull fertility status) was detected compared to the control. An up-regulation of IL-1β and IL-8 protein concentrations compared to the control (P<0.05) was also detected. There was no difference between the uterine inflammatory response of CES or CES and seminal plasma and no up-regulation of cytokine expression or protein concentration compared to the control (P>0.05). Overall, this study demonstrated an up-regulation of inflammation in the uterus in response to frozen-thawed sperm *in vitro* but CES or CES with seminal plasma do not mirror these effects indicating inflammation could be coming from another component of the frozen-thawed semen. Based on this *in vitro* model there does not appear to be any difference in the uterine immunological response of sperm from high and low fertility bulls.

Funded by Science Foundation Ireland (16/IA/4474) and the Irish Research Council (GOIPG/2017/1884).

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

Physiology of reproduction in male and semen technology

Photo-stimulation with red-led light of Duroc pig seminal doses was not effective to improve fertility of Iberian sows

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Keywords: porcine AI, spermatozoa, reproductive outcomes.

Different authors have pointed out the sensibility of the sperm cells from different species to be exposed to light (Shahar et al., Hum Reprod 26: 2274, 2011). In pigs, it has been reported an increase in the farrowing rate and litter size when the seminal doses for artificial insemination were photo-stimulated by red-led light (Yeste et al., Scientific Reports 6: 22569, 2016; Blanco Prieto et al., Rep Dom Anim 54: 1145, 2019). The former studies were developed with commercial breeds, mainly based in Large White and Landrace breeds. The reproductive parameters and outcomes from the Iberian breed pigs are different from the commercial breeds (Gonzalez-Anover et al., Theriogenology 75: 34, 2011). The aim of this study was to evaluate the possible effect of illumination of seminal doses with red-led light from Duroc boars and Iberian females in their specific production system. Semen samples were obtained from 33 fertile Duroc boars. Semen AI-doses were prepared from ejaculates that fulfilled the standard of sperm quality thresholds, using MR-A extender and stored at 16°C up to 48 hours before application. Photo stimulation of the AI seminal doses were carried out through a commercial system (Maxipig, IUL SA, Barcelona, Spain) that illuminated the samples with red led using the program of 10 min of light, followed by 10 min of darkness and finally 10 min additionally of light. The fertility study was conducted on 2 commercial farms at Murcia (Spain) using multiparous Iberian sows (farm A n=824; B n= 2137), that was randomly assigned to Led (L) or Control (C) groups. Post cervical insemination took place on 0 and 24 hours after the diagnosis of oestrus with seminal doses from the same ejaculate and same treatment. Categorical parameters as pregnancy and farrowing rate were analysed by Chi square test, continuous parameters as parity, pregnancy length and litter size were analysed by ANOVA. No differences were found between L and C groups in both farms ($p>0.05$) for parity, pregnancy rate, duration of pregnancy, farrowing rate and litter size (total, alive and died born piglets). Farrowing rates in farm A were 88.8% (n=383) for control and 89.6% (n=441, $p=0.67$) for led group. In farm B were C:90.5% (n=1035) and L: 90.1% (n=1102, $p=0.48$). In farm A total born piglets were 8.69 ± 0.11 for C and 8.71 ± 0.11 for L ($p=0.87$). In farm B 8.72 ± 0.7 for C and 8.70 ± 0.06 ($p=0.82$) for L. Under the productive conditions of Iberian breed the photo-stimulation with red-led light of Duroc pig seminal doses was not effective to improve fertility of Iberian sows. According to the data reported by Blanco Prieto et al., 2019, and analysed by Chi square test the increase in farrowing rate after photo stimulation was significant ($p<0.05$) only in 6 from 31 farms evaluated. Specific characteristic of the Duroc spermatozoa, Iberian sows or the productive conditions of these farms could be related to the inefficiency of the photo stimulation system. The influence of these factors must be analysed in further studies.

Supported by Fundación Séneca 20040/GERM/16 and UMU-CEFUSA agreement 33636.