BOOK OF ABSTRACTS

2nd CZU hybrid seminar

ANIMAL REPRODUCTION, SPERM CRYOPRESERVATION AND ANALYSIS: AN INTERNATIONAL EXPERIENCE

3 – 5 MAY 2023 — PRAGUE — CZECHIA

Czech University of Life Sciences Prague

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BOOK OF ABSTRACTS
1st version
2nd CZU hybrid seminar
Animal reproduction, sperm cryopreservation and analysis: an international experience
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Welcome word

Ladies and gentlemen, Dear seminar participants,

We were very pleased to welcome you again, either online or in person, to the Czech University of Life Sciences Prague on the occasion of the second annual seminar dedicated to the topic of animal reproduction. We are delighted, that many distinguished speakers responded favourably to our call for contributions!

We thank the Czech University of Life Sciences Prague and the Faculty of Agrobiology, Food and Natural Resources for providing the rooms and equipment for organising the seminar. Similarly, we would like to express thanks to the company Avantor, which sponsored this event. We are very greatful for their contribution.

The organisation of this seminar was a joint idea of several colleagues from Animal Science Department at Czech University of Life Sciences Prague. The aim of the seminar is to give to the younger generation of students and scientists an overview about the modern methods currently used in top-level laboratories around the world, and to build even more tight bonds between several laboratories working in a similar field.

We believe that these goals have been met.

Jan Pytlík

On behalf of the Organising Committee

Programme

Wednesday 3 May 2023

Introduction

13:00 – 13:15 (CET) **Welcome word by Seminar Organising Committee**

13:15 – 13:30 **Welcome word by Sponsor**

Invited Speakers

13:30 – 14:00

Dr. Jonathan LaMarre
University of Guelph
Small RNAs in bull fertility

14:00 – 14:30

Dr. Szabolcs T. Nagy
Hungarian University of Agriculture and Life Sciences
Application of next generation cytomics in AI semen quality control

14:30 – 15:00

Dr. Felipe Martínez-Pastor
University of León
Trends on sperm processing for improving preservation and fertility

15:00 – 15:30

Dr. Agniezska Partyka
Wroclaw University of Environmental and Life Sciences
HSPs in livestock reproduction with a focus on the poultry sperm

15:30 – 16:00

MSc. Jakub Vozaf
Slovak University of Agriculture
Preserving genetic diversity: the state of sperm cryopreservation in Slovak national ram breeds

Thursday 4 May 2023

Invited Speakers

9:00 – 9:30 (CET) **Dr. Nurlan Malmakov**
Kazakh Research Institute of Animal and Fodder Production
Cryoconservation of ram semenRISPR/Cas9 and its actual and future application in the livestock industry

9:30 – 10:00 **Dr. Jane Morrell**
Swedish University of Agricultural Sciences
Alternatives to antibiotics in semen extenders for artificial insemination

10:00 – 10:30 **MSc. Aleksandar Cojkic and MSc. Pongreecha Malaluang**
Swedish University of Agricultural Sciences
Metagenomic identification of bull and stallion semen microbiome

10:30 – 11:00 **Dr. Sean Fair**
University of Limerick
Identification of potential biomarkers of bull fertility

11:00 – 11:30 **Dr. João Pedro Barbas**
National Institute for Agricultural and Veterinary Research
Artificial insemination of milk ewes by refrigerated sperm – Portuguese experience

11:30 – 12:00 **Dr. Allai Larbi**
Chouaib Doukkali University
How to success with AI in sheep and goat

12:00 – 12:30 **MSc. Natalia Szysiak and Dr. Urszula Kosior-Korzecka**
University of Life Sciences in Lublin
KISS-1/GPR54 mRNA expression in pituitary glands and the relationship between kiss-10 and luteinizing hormone secretion from pituitary cells of cyclicand pcos – affected sows

12:30 – 13:00 **Dr. Thorsteinn Olafsson**
Andrews University
Sheep diseases and AI

Final Conclusion 13:00 – 13:30

Organising Committee

Anežka Málková

Kristýna Petričáková

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Presentations

Presentations can be found at the link or QR code below:

https://katedry.czu.cz/ksz/arsca

Artificial insemination of milk ewes by refrigerated semen-Portuguese experience

Barbas JP

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CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal

AIM

The objective was to present a summary of reproductive results after artificial insemination (AI) with refrigerated semen in two portuguese native milk breeds, namely Saloia and Serra da Estrela. Oestrous synchronization was done with vaginal sponges (FGA) and cervical artificial insemination was performed without previous estrous detection using refrigerated semen in the first 6 hours after semen collection. A summary of reproductive results are presented considering different extenders, periods of AI and after drugs and hormone treatments in Serra da Estrela, Saloia and Merino breeds. Also the effect of natural breeding after AI is presented and natural breeding distribution in Saloia breed.

MATERIAL AND METHODS

In this summary we have used 2 milk native breeds, namely Saloia and Serra da Estrela. In all ewes estrous synchronization was done using vaginal sponges (20 mg FGA). At sponge removal 500 UI eCG was injected (IM). Ejaculates were collected by artificial vagina being evaluated for individual motility and sperm concentration. Semen extender was usually skimmed milk. Artificial insemination (AI) was done by cervical route with refrigerated semen (10 ºC), 54 hours after sponge removal without previous estrous detection. Semen doses contained $300*10^{6}$ total sperm/dose. In producers AI are usually done in May, but we have considered other periods (September). In some trials, drugs and or hormones were used in AI protocosl with the objective of incresing fertility. In some experiments with Saloia breed we have joined rams 15 days after AI during a period of 45 days and evalute the corresponding fertility.

RESULTS AND CONCLUSION

In this section some results concerning fertility are presented in a variety of exploiting conditions. Usually Saloia breed are inseminated in May with 39 % fertility. We have experiments in Serra da Estrela using Oxytocin with 38,1%, without differences from control ewes. Semen deposition is a determinat factor which influences fertility due to ewe cervix conformation. In Saloia breed, ewes treated with PgE1, 6 hours before AI have improved fertility (30.4 to 60.7 %) with vaginal semen deposition and from 57,9 to 70.6 % with cervix semen deposition. In ewes inseminated with frozen semen by cervical route we have obtained unsatifactory results (24 %). However some improvements in fertility were detected in treated ewes, with vaginal deposition (0 to 5.8 %) and cervical depoistion (33 % to 45 %). In Saloia (n=420) breed inseminated in May with refrigerated we have average fertility of 48.5 % (Saloia), 53.5 % (Serra da Estrela) and 65 % (Merino).

CONCLUSION

Medium and variable fertility (39 -53 %) were obtained in milk native ewes inseminated in May with refrigerated semen considering several exploitations conditions. Fertility was influenced by breed, month (May vs September), extender composition, ram, place of semen deposition, drug treatment and exploitation conditions. But the the determinant factor was semen type (frozen refrigerated). We need to proceed our studies concerning all the factors that influence fertility and how we can improve it. Ram introduction in the subsequent oestrous (after AI) is suggested to improve reproductive parameters in our native breeds.

Keywords: ewes; cervical artificial insemination; refrigerated semen; fertility; natural breeding

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ACKNOWLEDGMENT: INIAV, Unit of Biotecnology and Genetic Resources and ACRO (Saloia breeders Association).

Metagenomic identification of bull semen microbiome

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AIM

The aims of these studies were (i) to determine the microbiota in raw semen from healthy bulls and to examine the relationships between the bacterial community and overall fertility (Study I); and (ii) to evaluate seasonal differences in bull semen microbiota from commercial frozen semen samples (Study II).

MATERIAL AND METHODS

In Study I, raw semen samples from 18 Estonian dairy bulls were used, while in Study II semen samples from 18 Swedish dairy bulls in three different season were evaluated (54 samples in total). For both studies, DNA extraction (Qiagen commercial DNA extraction kit) was performed at the Swedish University of Agricultural Sciences, followed by 16S rRNA amplification and sequencing (Illumina Inc) at SciLife Lab, Stockholm. The analysis of the 16S rRNA sequencing data was performed using Nextflow pipeline ampliseq v.1.1.2 for the first and v2.2.0 for the second study. The reproductive efficiency of the bulls was assessed by analyzing the non-return rates (NRRs) observed 90 days after the first artificial insemination (AI) procedure. Fertility was classified as high and low, with the threshold at 51%. Data analysis was performed using R statistical software (R Core Team, 2022; v 4.2.2).

RESULTS

In Study I, 107 bacterial genera were identified in 18 raw bull semen samples; in Study II 388 genera were identified in the 54 commercial bull semen samples in three seasons; winter, spring and summer (234, 211, and 233 genera, respectively). In Study I, there was also considerable variation between individual bulls both in the total number of identified bacterial genera (ranging from 12 to 89) and in the number of the Top 20 genera present (ranging from 5 to 20). However, two genera, *W5053* and *Lawsonella*, were enriched in the low fertility group (Study I). The Top 20 genera differed between studies; six genera were present in both sets of bulls (*Campylobacter, Cutibacterium, Fusobacterium, Histophilus, Porphyromonas* and *Staphylococcus*). In Study II, the number of bacterial genera with > 1% of relative abundance differed between the seasons, with the most bacterial genera being identified in spring (n=17) followed by summer (n=14) and winter (n=13). The genera identified in all seasons (n=9) were *Acidocella, Bacillus, Burkholderia, Campylobacter, Geobacillus, Histophilus, Lactobacillus, Pseudomonas* and *Streptococcus*, while the *Prevotella* was the only genera identified in just one season (summer).

CONCLUSION

There were differences in the number of bacterial genera and top 20 most abundant genera between bulls at the same location and between countries. Furthermore, the number of genera present in different seasons varied. Differences in the microbial communities of healthy bulls have been documented and may be associated with fertility potential.

Keywords: 16s rRNA sequencing, fertility, season

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ACKNOWLEDGMENT: We thank the personnel at the Kehta cattle breeding station, Animal Breeders' Association of Estonia and Viking Genetics, Scara Sweden, for providing the bull semen samples. This study was funded by KSLA, Stockholm (grant number to AK) and by FORMAS, Stockholm (2017-00957, to JMM). The authors acknowledge support from the National Genomics Infrastructure in Stockholm funded by Science for Life Laboratory, the Knut and Alice Wallenberg Foundation and the Swedish Research Council, and SNIC/Uppsala Multidisciplinary Center for Advanced Computational Science for assistance with massively parallel sequencing and access to the UPPMAX computational infrastructure. We thank SLU Bioinformatics Infrastructure (SLUBI) for the management and processing of the sequencing data. Support from NBIS (National Bioinformatics Infrastructure Sweden) is gratefully acknowledged. This project was supported by the UMBLA platform at SLU.

Identification of potential biomarkers of bull fertility

Fair S

Laboratory of Animal Reproduction, Department of Biological Sciences, Biomaterials Research Cluster, Bernal Institute, Faculty of Science and Engineering, University of Limerick, Limerick, Ireland

AIM

Despite passing all quality control checks at animal breeding centres, bulls with apparently normal semen quality can yield unacceptably low field fertility rates. The hypothesis of this *in vivo* study was that this variation in fertility could be at least in part due to molecular differences in sperm-uterine interactions. The aim of this study was to profile the transcriptome and perform histological and immunohistochemical analysis of the bovine endometrium in response to sperm from high fertility (HF) and low fertility (LF) bulls.

MATERIAL AND METHODS

Uterine biopsies and uterine explants were collected post-mortem from nulliparous heifers 12 h after a fixed time AI to a synchronised oestrus with frozen-thawed semen from 5 HF (fertility rate 4.01% ± 0.25, mean±s.e.m.) and 5 LF (fertility rate -11.29% ± 1.11, mean±s.e.m.) bulls (mean of population was zero). Semen from each bull was used to inseminate two heifers (10 heifers per treatment). Uterine biopsies were also collected from control (CTRL) heifers (n=8) which were not inseminated. High quality RNA extracted from uterine biopsies was analysed by RNA-seq and differential gene expression assessed. Uterine biopsies were collected for polymorphoric neutrophil number (PMN) quantification using histology and for immunohistochemistry analysis.

RESULTS

In the HF treatment relative to CTRL heifers there were 346 genes significantly differentially expressed in the endometrium with just 1 gene differentially expressed in the LF treatment relative to CTRL heifers. Comparing the HF and LF treatments directly there were 40 significantly DEGs (FDR<0.01). Transcriptomic analysis shows a predominant role for the inflammatory marker Interleukin-1 which was further confirmed by immunohistochemistry. Gene ontology analysis identified enriched pathways for cell adhesion, sperm cell movement, cell renewal as well as the inflammatory response. Further characterisation of the inflammatory response by the quantification of PMNs in the endometrium showed a significant effect of sperm, however, there was no difference in PMN numbers between HF and LF groups.

CONCLUSION

In conclusion, this study shows a distinct inflammatory response to sperm in the endometrium and a divergent transcriptomic response in the female reproductive tract to semen from high and low field fertility bulls, which could have important consequences for the outcome of AI.

Keywords: sperm, endometrium, inflammatory response, interleukin-1

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ACKNOWLEDGMENT: This research was funded by Science Foundation Ireland under the Investigators Programme (16/IA/4474).

KISS-1/GPR54 mRNA expression in pituitary glands and the relationship between KiSS-10 and luteinizing hormone secretion from pituitary cells of cyclic and PCOS – affected sows

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AIM

The aim of study was to compare the effect of kisspeptin-10 (KiSS-10) on *in vitro* luteinizing hormone secretion (LH) by pituitary cells of cyclic sows (n=10) and sows with follicular cysts (n=12). In addition, the concentration of KiSS-10 in the blood plasma and pituitary *kiss-1/gpr54* mRNA expression of both groups of animals were determined.

MATERIAL AND METHODS

The blood and the pituitary glands were obtained from the Polish Landrace sows. The animals were divided post-mortem into two groups based on the macroscopic estimation of the ovaries. The first group consisted of cyclic females, in the follicular phase of the estrous cycle, with numerous ovarian follicles with a diameter of 6-10 mm. The sows assigned to the second group were affected by polycystic ovarian syndrome (PCOS) (numerous bilateral ovarian cysts with no signs of luteinisation, with a diameter of 15-60 mm). Pituitary cells were cultured in McCoy 5A medium without hormones (the negative control), with GnRH ($4x10^{-9}$ M; the positive control), with KiSS-10 (10^{-11} – 10^{-7} M) or with KiSS-10 (10^{-11}–10^{-7}M) and GnRH ($4x10^{-9}$ M). After 2, 6, 12, 18, 24 and 30 hours of the experiment, the media for LH analysis were collected and the proliferation index (PI) of the control cells and those treated with KiSS-10 (10^{-11} – 10^{-7} M) or both GnRH ($4x10^{-9}$ M) and KiSS-10 (10^{-11} – 10^{-7} M) was determined. KiSS-10 in the blood plasma and LH in the culture medium were determined by ELISA assays using species-specific antibodies. The expression of *kiss-1* and *gpr54* mRNA was analysed using Real-Time PCR.

CONCLUSION

The obtained results show that the effect of KiSS-10 on pituitary cells of cyclic sows and sows with follicular cysts depends on the KiSS-10 concentration used. In the 10^{-9}-10^{-7} M concentration range, Kiss-10 exerted a stimulatory effect on LH secretion in both groups of sows, with the highest secretion observed at a concentration of 10^{-8} M. Despite of high positive correlation between the concentration of KiSS-10 and LH secretion both in cultures without GnRH and with GnRH, the level of KiSS-10-stimulated LH secretion was significantly

lower in cells coming from PCOS-affected sows than from cyclic sows. KiSS-10 concentration in blood plasma was higher in samples collected from cyclic sows compared to samples from PCOS-affected sows. Pituitary *kiss-1 mRNA* expression was lower and *gpr54* mRNA expression was higher in sows with follicular cysts than in cyclic sows.

Keywords: kisspeptins, follicular cysts, neuroendocrinology

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Small RNAs in Bull Fertility

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BACKGROUND AND AIMS

Reproductive efficiency is a critical parameter in the animal agricultural sector which is essential for successful animal production systems. Bull fertility is a key component of bovine reproductive efficiency that can be challenging to accurately assess early in the reproductive lifespan using standard metrics such as sperm morphology and motility. Our group and several others have recently begun to explore the utility of small RNAs in sperm and semen as biomarkers of bull fertility. In the present studies, we aimed to characterize the small RNA populations in sperm samples from bulls with subtle but significant differences in sire conception rate (SCR) in order to explore their utility as fertility biomarkers.

MATERIALS AND METHODS

Small RNA sequencing libraries were prepared from sperm samples obtained from Holstein bulls with SCRs of +2.1 +/- 0.7 (High Fertility – 6 bulls) and -2.1+/- 1.1 (Low Fertility – 6 bulls). After Illumina sequencing we utilized bioinformatic pipelines to characterize the levels of microRNAs and piRNAs in the sequencing data as well as specific features of the different RNA targets of these pathways and, for piRNAs, their sites of origin in the genome.

RESULTS AND DISCUSSION

The most abundant miRNAs in both fertility groups were the same; miRs -34b-3p; -100-5p; -191-5p; -30d-4p; -21-5p as were specific patterns of isomiRs present for each of these. No significant differentially expressed sequences with read counts higher than 200 were observed. 10 distinct pairs of miRNAs were positively correlated with each other in bulls with higher fertility and negatively correlated with each other in comparatively less fertile individuals. 8 additional miRNA pairs demonstrated the opposite trend. Pathway analysis (GO, KEGG) of predicted targets of the most abundant RNAs revealed potential roles of miRNAs present in bull sperm in the regulation of specific genes that impact spermatogenesis and embryo development. The piRNA populations also did not differ between fertility conditions when examined by principal component and differential expression analysis, suggesting that a high degree of conservation in the piRNA system is likely necessary for the production of viable sperm. Both fertility conditions demonstrated evidence of “ping-pong” activity – a secondary biogenesis pathway associated with active transposable element targeting and suppression. piRNAs from both groups were approximately the same length (29-30 nucleotide peak), with similar expression levels from clusters encoded on specific chromosomes. PiRNAs were predicted to target known transposable element families including LINEs, SINEs, and LTRs. Limited transposable element expression is known to be essential for spermatogenesis, thus epigenetic regulation of this pathway is likely to influence sperm quality and fertilizing capacity although this was not reflected by differences between the groups studied.

CONCLUSIONS

This work has characterized the miRNAs and piRNAs in the sperm of Holstein bulls and identified several subtle molecular differences between fertility conditions. Correlations involving specific miRNAs were identified that may reflect specific interactions in the control of gene expression related to bull fertility but are likely to require more extensive studies to establish their utility as biomarkers.

Keywords: sperm, miRNA, piRNA, biomarker

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ACKNOWLEDGMENT: Funded by NSERC (Canada) and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). Semen samples provided by SEMEX Canada.

Seminal Microbiome of Healthy Stallions in European Countries

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AIM

The bacterial microbiome impacts the system they live in or on. The seminal microbiota has substantial importance for the soundness of mares bred by the stallions, for retention of semen quality during semen storage for artificial insemination, and more targeted antibiotic use in semen extenders. Traditionally, bacterial identification was culture-based, which may mask the existence of some bacteria. Recently, investigations on the presence of microbiota in healthy stallions using a non-culture-based approach, 16S rRNA sequencing, displayed some bacteria that had yet to be determined in culture. The study aimed to define the bacterial diversity of semen from healthy stallions in three European countries using 16S sequencing.

MATERIAL AND METHODS

Bacterial DNA was extracted from ejaculates from thirty-seven stallions in Germany, Portugal, and Sweden. The DNA was amplified by PCR (Pro 341F and Pro 805R primers) and sent to SciLife Lab (Stockholm, Sweden) for 16S rRNA sequencing with Illumina platform. Data were interpreted using R software (version 3.3.1). Bacteria were specified at the phylum and genus levels. The difference ($p < 0.05$) between bacterial diversity (Shannon Index) and richness (observed ASVs); but there was no difference in species evenness (Pielou, $p>0.05$).

RESULTS

The bacterial composition in semen from stallions in the three countries was inferred with 1,908 identifiable amplicon sequence variants (ASVs). The three most often seen phyla in the three countries were identically *Bacteriodetes*, *Firmicutes*, and *Actinobacteria*, covering about 80% to 95% relative ASV abundance. The three most typically noticed genera were about 40% relative ASV abundance in Germany, and approximately 80% to 95% in Portugal and Sweden, respectively. Bacterial diversity differed significantly among the countries (Shannon Index, $p = 0.017$). Germany had the highest diversity, while Sweden had the lowest. There were significant differences in ASVs between Germany and Portugal and also between Germany and Sweden. However, ASVs between Portugal and Sweden were similar.

CONCLUSION

Taxonomic classification of ASVs at phylum level was comparable in stallion semen from the three European countries but varied considerably at the genus level. Germany had the most remarkable microbial diversity. The bacterial composition in stallion semen from Portugal and Sweden were additionally comparable to those from stallions in Germany. These results

emphasize the necessity for a thorough understanding of the seminal microbiota in precise dimensions to provide targeted antibiotic use in semen extenders.

Keywords: microorganism, bacterial DNA, 16S sequencing

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Cryoconservation of Ram Semen and Artificial Insemination: Kazakhstani Experience

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AIM

Artificial insemination (AI) of ewes with fresh and frozen semen is an efficient technology for rational long-term use of genetically valuable sire-rams in the sheep genetic improvement programs. Research aim was a development of new and improvement of existing methods and approaches in this area to increase their efficiency and applicability in the sheep industry of Kazakhstan.

MATERIAL AND METHODS

Ewes in natural heat were identified and drafted with the aid of the teaser rams with prepuce covered by aprons made from sackcloth (50 x 50 cm). Teaser rams joined ewe flock at a ratio of 80–100 ewes per ram daily for one hour from 6 a.m. to 7 a.m. or from 6 p.m. to 7 p.m. Ram semen was collected with the aid of an artificial vagina. Semen quality was assessed with the aid of a phase-contrast microscope Olympus CH-30. Dilution, chilling, cryopreservation and thawing of semen, cervical and laparoscopic inseminations of ewes were performed according to methods described by Evans and Maxwell (1987) with some modifications.

RESULTS AND CONCLUSION

In general people who would like to freeze semen buy and use small or large computerized programmable freezers. Price for freezers starts from $5000. No problem if budget is strict. We developed a method for freezing of semen packed in the 0.25 ml straws in the 20-liter Dewar flask. Of cause, quality of a semen frozen in the Dewar flask will be slightly lower than one frozen in the programmable freezer, but it will produce acceptable fertility results.

Diluent for freezing ram semen consists of several main components: sugar, buffer, egg yolk, glycerol, antibiotic. We improved buffering capacity of the freezing diluent (Salamon and Maxwell, 1987) by replacement of tris biological buffer with bis-tris one which have useful pH range 7-9.2 and 5.8-7.2 respectively. During chilling in the fridge pH of semen diluted with tris dropped from 6.97 to 6.36, whereas pH of semen diluted with bis-tris dropped from 6.76 to 6.53 ($P<0.05$).

Ram semen is able to survive even if it was frozen two times in the liquid nitrogen. Double freezing of semen and freezing of semen in the glycerol-free diluent could be used for fast comparison of different freezing diluents or regimens.

When run cervical or laparoscopic AI in the field conditions we recommend use of:

1) 50-ml transparent plastic culture flask filled with warm water as a microscope's warm stage for accurate assessment of sperm motility;

2) metal thermos with 0.5 liter capacity for moisturizing the vaginal mirror with warm physiologic solution;

3) portable LED-flashlight, attached to the body of the AI-gun with electric insulating tape or scotch for quick finding of the cervix and accurate insertion of the AI-gun's tip into cervix entrance;

4) portable LED-flashlight attached to the laparoscope with rubber tube (13-mm inner diameter 5-cm long) as a replacement for an expensive light source ($2000).

Optimal time for laparoscopic insemination with frozen semen is 18-20 hours post detection of ewes in natural heat.

Keywords: cryoconservation, ram semen, cervical insemination, laparoscopic insemination

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Alternatives to antibiotics in semen extenders used for artificial insemination

Morrell JM

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The One Health concept deals with the interactions between humans, animals and the environment. These three areas are intimately associated; factors affecting one area unavoidably have an impact on the other two. Nowhere is this more apparent than in antimicrobial resistance (AMR), which is being driven by the use of antimicrobials in medicine and veterinary medicine. Antimicrobial agents are used to treat bacterial infections, for example in the production of safe animal-source products for human consumption. Good antibiotic stewardship is advocated; antibiotics should be administered for therapeutic purposes after determining sensitivity to the specific agent. However, one non-therapeutic use of antibiotics is in artificial insemination, where antibiotics are added to semen extenders to inhibit bacteria in the semen. Artificial insemination is the method of choice for breeding livestock in many countries. The addition of antibiotics to semen doses is mandated in regulations covering international trade in semen and embryos. Despite strict hygiene precautions during semen collection, bacteria are present in the semen and can cause a deterioration in sperm quality during storage or, in the worst case, disease in inseminated females. However, inseminated females, environmental bacteria and personnel handling the animals are exposed to these antibiotics. Several cases of resistance to antibiotics have been reported in bacteria found in semen; therefore, their continued use is not sustainable. Since semen insemination doses are much larger in pigs than in other animals, the potential risk from this material is correspondingly large. Possible alternative to antibiotics in boar semen extenders include low temperature storage, which was previously not possible for boar sperm doses, freezing semen, adding naturally-occurring substances that have antimicrobial activity in semen, and physically separating spermatozoa from the bacteria in semen, e.g. by centrifuging the semen through a colloid [1]. The results of some pilot experiments with these methods will be discussed in detail.

References

Morrell JM, Malaluang P, Cojkic A, Hansson I. Chapter in The Global Antimicrobial Resistance Epidemic – Innovative Approaches and Cutting-Edge Solutions, ISBN 978-1-80356-042-7. 2022

Keywords: antimicrobial resistance, One Health, boar semen, colloid centrifugation, cold storage, cryopreservation

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AI on sheep in Iceland; AI helps to defend against sheep diseases

Thorsteinn Olafsson

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AIM

A historical overview of the importation of sheep to Iceland and the diseases that accompanied the importation. How to use AI to prevent the spread of diseases and breed against diseases.

MATERIAL AND METHODS

Description of how Iceland is divided into sheep disease prevention zones. How fresh semen is used in sheep breeding instead of moving live sheep between regions.

RESULTS

Five to six percent of ewes are inseminated with fresh semen every year from two AI centres. Approximately 65% of the ewes are lambing and get 1.85 lambs on average.

CONCLUSION

The use of fresh semen is a good way to prevent the spread of sheep diseases.

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Heat Shock Proteins (HSPs) in livestock reproduction with a focus on the poultry sperm

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Heat shock proteins (HSPs) are known as one of the major molecular chaperone proteins in eukaryotic cells. They are commonly divided according to their molecular weight into the HSP100 (HSPH), HSP90 (HSPC), HSP70 (HSPA), HSP60 (HSPD), HSP40 and HSP27 (HSPB) families. HSPs are presented in the cytosol and different cellular components including mitochondria, endoplasmic reticulum, and nucleus. They have been known for first time as stress-related proteins, but strong evidence now refers to its protective role even under non-pathological and non-stressful conditions. HSPs participate in the regulation of essential cell functions, such as protein translocation, refolding, assembly and the recognition, prevention of protein aggregation, renaturation of misfolded proteins, degradation of unstable proteins, etc. Several studies have shown the pivotal role of HSPs as functional and structural candidates required to fulfill the males reproductive functions. Indeed, in males, HSPs are expressed throughout the testicular germ cells, Sertoli cells and along of the male reproductive tracts. They were also identified in the surface of sperm cell membrane of bull, boar, mouse, rat, stallion, dog and human.

In this review, we will present the newest information about the role of HSP in male reproductive functions and our experience in that field.

Our study was aimed to determine the presence of HSP 60, 70 and 90 in freshly ejaculated chicken spermatozoa and the effect of cryopreservation on the expression of HSPs in cells. Pooled semen from 15 Greenlegged Partridge roosters was used in the study. Each pool was divided into three aliquots and diluted with EK extender supplemented with 5 mM N-acetyl-L-cysteine (NAC), 200 U/mL superoxide dismutase (SOD) and without antioxidants (control). After dilution, the samples were subjected to cryopreservation using the 'pellet' method and dimethylacetamide as a cryoprotectant. Sperm proteins were extracted from fresh and cryopreserved spermatozoa and subjected to Western blot analysis for HSP detection using a monoclonal anti-HSP 60, 70 and 90 antibody. A prominent 60 kDa protein band of the Hsp60 protein was detected in protein extracts from fresh and cryopreserved spermatozoa, while no Hsp70 and 90 staining was observed in chicken gametes. There was no evidence of heat shock proteins with mass of 60 kDa, 70 kDa, and 90 kDa in the seminal plasma analyzed of all samples. The relative expression level in frozen thawed control and with NAC semen was significantly higher than in fresh and cryopreserved with SOD semen ($P<0.01$). The results of this study suggest that Hsp60 level in chicken spermatozoa gradually increases after the freezing-thawing process. The addition of SOD to chicken semen before freezing does not

change the level of expression of the Hsp60 protein compared to fresh semen, which may indicate an important role of the antioxidant in reducing changes related to cryopreservation.

Keywords: heat shock proteins, semen, chicken, spermatozoa

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Application of next generation cytomics in AI semen quality control

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SPERM CHROMATIN STATUS

The gold standard in sperm chromatin analysis is the so-called Sperm Chromatin Structure Assay™ that reveals the rate of DNA fragmentation as well as abnormal chromain condensation. In our previous studies on bull sperm samples with known fertility (NRR and CR percentages), however, the test showed weak predictive value. We applied flow cytometric barcoding and fingerprinting techniques in R environment in order to reduce the effect of user experience. Fingerprinting increased the predictive value of SCSA (ROC analysis, AUC value was 63.9% compared to 55% with conventional data analysis), however, non-metric multidimensional scaling indicated an overlap between bulls with acceptable and poor fertility both with barcoding and fingerprinting.

SPERM HEAD MORPHOLOGY

Our previous studies revealed that sperm morphology evaluated with light microscopy has a high predictive value in fertility prediction. A quick, more precise and automated alternative would highly beneficial both for the basic and applied research and the breeding industry as well. Previously, pulse shape analysis via slit-scan flow cytometry was successfully applied to evaluate sperm morphology of several animal species. However, recent models of bench top flow cytometers are not capable of carrying out such pulse shape analyses. We tested the so-called PulSA approach, a pulse shape analysis originally developed to measure particle sizes and intracellular trafficking. Different ratios of mixed bull and stallion sperm samples modelling different percentages of abnormal sperm heads were analyzed with the PulSA approach and non-metric multidimensional scaling showed that these mixed samples clearly separated from samples containing 100% bull or stallion spermatozoa.

SPERM DNA CONTENT ANALYSIS TO IDENTIFY TRANSLOCATION CARRIERS

Breeding males are routinely screened for chromosome abnormalities, translocations and carrier animals should be excluded from breeding. Classic tests are carried out lymphocytes isolated from blood. In case of imported sperm samples, however, it is difficult to do such tests. We successfully applied histogram modelling and histogram profile analysis to identify known translocation carrier bulls based on their sperm DNA content.

CONCLUSION

Sperm DNA analysis with novel flow cytometric data analysis approaches can extend our arsenal of sperm quality control tools.

Keywords: domestic animals, sperm quality, DNA, automated data analysis

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