Czech University of Life Sciences Prague

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FAKULTA AGROBIOLOGIE, POTRAVINOVÝCH
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The 4th ČZU Prague hybrid seminar "Biotechnology in small ruminant reproduction: an international experience - 2025"

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The Book Of Abstracts

Ing. Filipp Georgijevič Savvulidi, Ph.D.

Ing. Martin Ptáček, Ph. D.

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Dear colleagues,

Dear students,

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 the 4th ČZU Prague hybrid seminar "Biotechnology in small ruminant reproduction: an international experience - 2025". We are delighted that many distinguished speakers responded favorably to our call for contributions! We thank all our invited speakers; we thank all the attendees of the seminar! We thank the Czech University of Life Sciences Prague, the Faculty of Agrobiology, Food, and Natural Resources, and the Department of Animal Science for providing the rooms and equipment for organizing the seminar. Furthermore, we would like to express thanks to the company Avantor, which again sponsored this event.

In conclusion, we do believe that our main aims (to give the younger generation of students and scientists an overview of the modern methods currently used in top-level laboratories around the world and to build even tight bonds between several laboratories working in a similar field) were successfully fulfilled, same as in previous years.

We thank you once again! We hope to see you attend the planned 5th CZU hybrid seminar!

Sincerely,

Filipp Georgijevič Savvulidi & **Martin Ptáček,**

Seminar Organizing Committee.

Direct link to the webpage of the the 4th ČZU Prague hybrid seminar "Biotechnology in small ruminant reproduction: an international experience - 2025":

Content

How to lift the low success rate of artificial insemination in sheep

Taylor Pini[1]

[1]School of Veterinary Science, The University of Queensland, Australia

Contact person: t.pini@uq.edu.au

Keywords: seminal plasma, proteome, glycome, immune response, cervix, frozen sperm, ram.

Artificial insemination (AI) in sheep offers significant genetic and biosecurity advantages, yet its widespread adoption is hindered by low pregnancy rates when using frozen semen via cervical insemination. This work explores the physiological and molecular barriers to successful AI and presents novel insights into the role of seminal plasma (SP) in overcoming these challenges. Proteomic analyses revealed that SP exposure alters the sperm proteome and glycome, enhancing mucus penetration and immune evasion without significantly affecting motility. Notably, binder of sperm proteins (BSPs), particularly BSP1 and BSP5, were shown to mitigate cryocapacitation effects and improve post-thaw sperm function. Additionally, SP modulates the female reproductive tract's immune response, including protection from neutrophil-mediated phagocytosis and altered cytokine signaling, particularly TGF-β expression. These findings suggest that SP components could be harnessed to enhance the viability and fertility of frozen sperm in cervical AI. However, the interplay between SP-induced sperm remodeling and the female immune environment remains incompletely understood. This work lays the foundation for translational strategies aimed at improving AI outcomes in sheep, with broader implications for reproductive technologies in other species.

Impact of heat stress on reproductive performance in Barbados Blackbelly rams

Nuradilla Mohamad-Fauzi[1,2,3], Mohd Shahmi Hakimi Mazlishah[1,2], Muhammad Syafiq Roslan[1,2], Noorhidayah Mamat[2], Mohd Fadzil Firdzaus Mohd Nor[3,4], Shamsul Azlin Ahmad Shamsuddin[1,2], Nor Azlina Abd. Aziz[1,5], Noor Hashim Hashida[1,2]

[1]Glami Lemi Biotechnology Research Centre Universiti Malaya, 71650 Jelebu, Negeri Sembilan, Malaysia.

[2]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.

[3]Institute for Advanced Studies, IAS Complex, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.

[4]Institute of Ocean and Earth Sciences, IAS Complex, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.

[5]Centre for Foundation Studies in Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.

Contact person: dilfauzi@um.edu.my

Keywords: temperature-humidity index, thermal tolerance, sheep, tropics, management system.

AIM

The consistently high levels of ambient temperature and humidity in the tropics are projected to worsen due to climate change, particularly with the increasing frequency of heat waves. Barbados Blackbelly (BB) is a prolific sheep breed known for their tropical adaptability, which potentiates them to be commercialised and improve sheep production in Malaysia, but the downstream effects of heat stress on reproductive performance have never been characterised in this breed within the context of the Malaysian tropical climate. Our studies sought to investigate the effect of short-term, acute heat stress on the reproductive performance of BB rams, as well as the impact of different management systems on the occurrence of longer-term heat stress in BB rams.

METHODS & RESULTS

In the first study, BB rams (n = 6) experienced moderate heat stress after 14 days of exposure to Temperature-Humidity Index (THI) of 89-94. In the second study, BB rams reared in the semi-intensive system for 8 weeks experienced significant higher THI than the rams kept indoors in an intensive system (n =12 per treatment group). In both studies, high THI

significantly decreased sperm motility and increased % abnormality, but did not affect sperm concentration, which could be attributed to oxidative stress damage. Oxidative stress markers malondialdehyde and hydrogen peroxide were elevated, but only significantly in the long-term study. Significant changes in cortisol and testosterone were observed in the rams exposed to short-term heat treatment, but there were no differences between rams in different management systems, indicating that BB rams can acclimate to long-term exposure of heat stress. In parallel to the hormone data, changes in sexual behavioral parameters observed were also not significant.

CONCLUSION

All in all, we show that heat stress had some negative influence on BB rams' reproductive performance, but the lack of severity suggests evidence of tropical adaptability in BB sheep. From our data, we can propose that the semi-intensive system can still be practiced to rear BB sheep in the tropics, provided that appropriate mitigation strategies are implemented to minimize the impact of heat stress. Findings from our studies also show that existing THI-based heat stress thresholds for ruminants are not suitable for BB sheep in Malaysia and that there is a need for thermal comfort indices to be developed for small ruminants in the tropics.

Acknowledgment: These studies were funded by the Ministry of Higher Education (MOHE) Malaysia under the Fundamental Research Grant Scheme (FRGS/1/2022/STG03/UM/02/7). The authors would like to thank the staff at the Glami Lemi Biotechnology Research Centre for their assistance during the experimental runs.

Interaction of female reproductive fluids with sperm in sheep

Carmen María Picazo Córdoba[1]

[1]Grupo SaBio IREC (CSIC-UCLM-JCCM). ETSIAMB. University of Castilla-La Mancha, Albacete, Spain

Contact person: carmenmaria.picazo@uclm.es

Keywords: extracellular vesicles, flow cytometry, reproductive fluids, sperm

AIM

EVs isolation and characterization in sheep through transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA) and flow cytometry evaluations to optimize sperm incubation media in in vitro fertilization (FIV).

METHODS

By the different techniques mentioned previously, is posible EVs characterization. NTA allows to analyze phenotype, concentration, and size distribution of EVs. The path of EVs movement is detected to measure the velocity of the particles, which is in correlation to their sizes. Transmission electron microscopy is used to determine the size, shape, and the structure of EVs. Various morphologies of EVs, ranging from spherical, to cup shape have been observed under TEM. However, the electron beam may have a detrimental effect on biological samples. Otherwise, flow cytometry allows to evaluate the size, shape and the presence of specific markers on EVs. Its principle is based on the excitation of fluorochromes present in the incubated sample, and their subsequent emission. Flow cytometers are equipped with lasers and can detect several stains simultaneously, allowing a multi-parametric approach.

RESULTS

The knowledge of EVs from different female reproductive fluids has allowed to see the influence of them over sperm physiology. In fact, uterosomes promote sperm capacitation, what is possible because their transfer of SPAM1 protein to sperm, which reorganizes the sperm membrane releasing cholesterol, that promotes capacitation. Oviductosomes contain the ATPase PMCA4, a calcium pump that promotes sperm motility by regulating intracellular pH and calcium providing the sperm with the calcium levels necessary for hyperactivation. This key phenomenon of capacitation allows the sperm cell to free itself from the isthmus sperm reservoir during its transit through the oviduct. Finally, folliculosomes have a role in follicular growth and oocyte maturation.

CONCLUSION

Further research should be done on the components of gamete maintenance media to establish a clear composition, determining compounds that can compensate for the disadvantages of ESS use. Then, the use of EVs could be an option, since although there is still no established isolation method that allows high yield and high purity, they are physiologically present in reproductive fluids. Finally, nowadays a new generation of flow cytometers with detection limits suitable for EVs samples is facilitating their accurate characterization.

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Advances in ram sperm storage

Luca Palazzese[1], Martina Lo Sterzo[1], Margherita Moncada[1], Domenico Iuso[1], Marta Czernik[1], Pasqualino Loi[1]

[1]Department of Veterinary Medicine, University of Teramo, Italy.

Contact person: Luca Palazzese, email: lpalazzese@unite.it

Keywords: Freeze-dry, ICSI, Ram, Spermatozoa, DNA fragmentation.

AIM

The most commonly used method for sperm preservation worldwide remains cryopreservation with liquid nitrogen (LN). Frozen semen doses can retain viability for decades and, upon thawing, remain capable of successful fertilization and offspring production. However, the substantial environmental impact of LN production has driven interest in alternative techniques, including the freeze-drying of spermatozoa.

METHODS

The study utilized semen collected from fertile Sardinian breed rams with a verified excellent health status. Both frozen semen (via slow freezing) and freeze-dried samples were employed. Details of the freeze-drying and freezing protocols are available in Moncada et al. (2025), Theriogenology, doi: 10.1016/j.theriogenology.2025.117390. In vitro fertilization was conducted through Intracytoplasmic Sperm Injection (ICSI), and the resulting embryos were cultured in vitro. The spermatozoa underwent assessments for membrane integrity, DNA fragmentation levels, and lipid composition.

RESULTS

While freeze-drying of spermatozoa offers a promising alternative to cryopreservation, current findings indicate that rehydrated spermatozoa remain immotile. Despite this, in small mammals such as mice and rats, these immotile spermatozoa have successfully produced offspring via ICSI.

In contrast, freeze-dried ram semen is unable to activate oocytes independently, necessitating chemical activation for embryo development to progress to the blastocyst stage. Advances in freeze-drying techniques have eliminated the need for liquid nitrogen, resulting in significant improvements in embryonic development, with blastocyst formation rates reaching 13%.

Furthermore, alternative approaches such as spin-drying have shown potential in preserving sheep spermatozoa in an anhydrous state. These spermatozoa have retained the capacity to generate pre-implantation stage embryos after two years of storage at room temperature.

CONCLUSION

Freeze-drying of spermatozoa is emerging as a feasible alternative to liquid nitrogen storage. This approach warrants greater research focus, given the limited studies conducted so far, which have predominantly centered on small mammal models.

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Artificial Insemination of Small Ruminants in Türkiye: Current Status, Techniques, and Outcomes

Mustafa Bodu[1]

[1]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Selcuk University, Konya, Türkiye

Contact person: mbodu@selcuk.edu.tr

Keywords: artificial insemination, sheep production, semen quality, estrus synchronization genetic improvement

Türkiye ranks among the leading countries in sheep production, with a population of over 43 million. Artificial insemination is widely applied to improve reproductive performance and genetic quality in small ruminants. Vaginal, transcervical, and laparoscopic techniques are commonly used, with laparoscopic insemination providing the highest success rates by delivering semen directly into the uterine horns. The outcome is influenced by several factors, including semen quality, processing methods, ewe condition, environmental factors, and technician experience. Estrus synchronization protocols, particularly those involving progesterone-releasing devices and hormonal support, play a key role in optimizing insemination timing and success when using frozen semen. Türkiye's diverse sheep breeds offer significant opportunities for targeted genetic improvement. Future advancements such as antioxidant supplementation, protective additives during cryopreservation, and the use of precision livestock technologies are expected to support better fertility outcomes. Standardized procedures and trained personnel will be essential to achieve consistent and efficient results in the field.

Study on synchronization of estrus in Lacaune sheep during the ancestral season

Malinova Radka[1], Velkova Maria[1]

[1]Agricultural University – Plovdiv, Department of Animal Science, BG-4000-Plovdiv, Bulgaria

Contact person: radka.v.malinova@gmail.com

Keywords: Lacaune ewes, estrus synchronization, anestrous season, artificial insemination, prolificacy, reproductive performance.

AIM

The objective of the present study was to investigate the outcomes of estrus synchronization in Lacaune ewes during the anestrous season.

METHODS

A total of 318 ewes were treated with 60 mg Medroxiprogesteron acetate (MPA) intravaginal sponges for 14 days, followed by an injection of 250 IU of lyophilized serum gonadotropin hormone (PMSG) (Gonaser, HIPRA). The first artificial insemination was carried out 48 hours after sponge removal, followed by a second insemination approximately 5 hours after the first. Of these, 81 ewes underwent a natural breeding following a return to estrus. The synchronization procedure was carried out during the anestrous season, in April 2022. The animals were managed under an intensive, group-housing system. The farm has a dairy production orientation, with the ewes organized into groups and the reproductive management aimed entirely at ensuring year-round lactation.

RESULTS

The overall conception rate was 81.1%, with a biological prolificacy of 1.72 lambs per lambed ewe. Multiple pregnancies accounted for 64.5% of gestations. The abortion rate was 3.7%. Metod of mating significantly influenced fertility and prolificacy, while ewe age had no notable effect. The results demonstrate that the application of an appropriate hormonal protocol allows for highly effective breeding of Lacaune ewes during the anestrous season.

CONCLUSION

The biological prolificacy (number of lambs per lambed ewe) reached 1.72 ± 0.063, and 64.5% of all pregnancies were multiple, including 52% twin, 9.7% triplet, and 0.3% quadruplet lambings. The average number of lambs per ewe in the flock (prolificacy) was 1.34 ± 0.08.

Abortion rate was low (3.7%), suggesting effective pregnancy maintenance under the applied hormonal protocol.

These findings support the use of hormonal synchronization protocols as a reliable method for anestrus season breeding in Lacaune dairy ewes, facilitating year-round lambing and milk production planning in intensive sheep farming systems.

Advances in prediction of ram semen freezability

Lucie Langerová[1], Martin Ptáček[1], Filipp Georgievič Savvulidi[1]

[1]Department of Animal Science, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences (CULS) Prague, Prague, Czech Republic

Contact person: langerova@af.czu.cz

Keywords: freezability, sperm, ram, equilibration, cryopreservation

AIM

Like other livestock, rams have interindividual variability, which affects the results of the cryopreservation process. Based on the sperm cryotolerance, we can classify rams as good or bad freezers. To improve the efficiency of cryopreservation and insemination doses production is the effort to predict the freezeability of ram sperm cells. In our recent study, it was confirmed that conventional variables (motility, volume, concentration) of native ejaculate before freezing are not a strong predictor of freezability. The aim of subsequent study was to verify a defined procedure based on shortened equilibration during freezing, which could serve as a tool for predicting sperm freezability in rams of the Wallachian sheep breed.

METHODS

Four rams of the Wallachian sheep breed were used for this experiment. Ejaculate was collected at weekly intervals for a total of 7 collection days. An artificial vagina for small ruminants, pre-heated to 38–39 °C, was used for collection. The collected ejaculate was analyzed (semen volume, concentration, mass motility) and diluted (OptiXcell diluent). The filled straws were divided into 2 groups. Both groups were equilibrated together in a cooling box, at a temperature of +5 °C. The first group was the experimental. It was equilibrated for 15 min. Then it was frozen in a freezer with liquid nitrogen. After cryopreservation, these experimental variants were stored in a container with liquid nitrogen and then thawed in a water bath at 39 °C for 30 s. The second group (the control group) was equilibrated for the standard time of 120 minutes. Subsequent cryopreservation was carried out in the same way as the first experimental group. After cryopreservation, the control group was stored in a container with liquid nitrogen for 4 weeks. Thawed samples were evaluated using CASA and a flow cytometer.

RESULTS

A significant predictive potential of sperm parameters after 120 min. equilibration based on the assessment at 15 min. equilibration was observed primarily in indicators evaluated by flow cytometry. All prediction models were significant. The predictive value ranged from 77.5% to 61.7%. The predictive model for assessing sperm viability after thawing had a very strong

predictive power based on the coefficient of determination 78%. The correlation coefficient showed a medium-strong positive correlation between the indicators. In indicators evaluated by CASA the predictive value ranged from 26% to 52%. The predictive character of the variables was significant only for total and progressive motility, with weak positive correlation between the indicators.

CONCLUSION

The prediction of sperm freezability in rams based on shortened equilibration time represents an effective tool, which is fast and more economically efficient than used proteomic or more sophisticated methods.

Acknowledgment:

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Extender Supplements for the Improvement of Goat Sperm Freezability

Abbas Farshad[1]

[1]Veterinary Clinic for Reproductive Medicine and Neonatology; Justus-Liebig-University of Giessen, Germany.

Contact person: abbas.farshad@vetmed.uni-giessen.de

Keywords: phospholipase A, glycoprotein lipase, glycerin, DMSO, lecithin, mitochondrial antioxidants, trehalose, L-carnitine, antifreeze proteins, enzymatic antioxidants.

Cryopreservation of goat sperm is a crucial advancement in modern animal breeding, enabling long-term storage, improved reproductive management, genetic diversity conservation, and effective disease control through international germplasm exchange. Despite its benefits, the process of freezing and thawing places considerable stress on sperm cells, often leading to reduced motility, membrane and DNA damage, and a significant decline in fertilization potential, with sperm loss reaching up to 50%. This challenge is further intensified in goats due to species-specific seminal plasma enzymes such as phospholipase A and glycoprotein lipase. These enzymes degrade common extender components like egg yolk and milk, generating cytotoxic byproducts that compromise sperm viability. To counteract these issues, two main strategies are employed: refining freezing protocols—adjusting temperature descent rates (slow, medium, or fast) - and incorporating protective agents into extenders. These protective agents include permeating substances like glycerin, DMSO, lecithin, and mitochondrial antioxidants, as well as non-permeating compounds such as trehalose, L-carnitine, antifreeze proteins (AFPs), and enzymatic antioxidants (e.g., superoxide dismutase and catalase). Additionally, the integration of mitochondria-targeted antioxidants has shown potential in mitigating oxidative damage at the cellular level. Given the unique physiological attributes of goat sperm, tailored approaches are essential to improving cryopreservation outcomes. Future research should prioritize refining extender formulations, gaining deeper insights into sperm-membrane interactions, and exploring novel bioactive compounds that enhance post-thaw viability and fertility, thereby optimizing reproductive success in goat breeding

Semen collection techniques and welfare in small ruminants

Rodolfo Ungerfeld[1]

[1]Facultad de Veterinaria, Universidad de la República, Uruguay

Contact person: rungerfeld@gmail.com

Keywords: semen collection, electroejaculation, stress, pain, welfare, small ruminants

AIM

To summarize recent information on semen collection techniques, welfare problems generated by electroejaculation in ruminants, and treatments/alternative techniques that can reduce these problems and enhance semen collection results.

METHODS & RESULTS

Several studies summarizing the main welfare problems associated with the use of electroejaculation were presented, including reports of increased cortisol, body temperature, glycemia, biochemical and hematological changes, and behavioral responses in domestic and wild small ruminants. Alternatives include the use of anesthesia/sedation/analgesic treatments, with sedation and analgesia appearing as being probably the best options. The development of alternative techniques to collect semen, as the Transrectal ultrasound-guided massage of the accessory glands (TUMASG), was evaluated and compared to electroejaculation in conscious and anesthetized animals. In general, in conscious animals, welfare problems are reduced, and semen quality is enhanced. The use of different hormones that stimulate the motility of the reproductive tract to facilitate the ejaculation process was also tested, with the carbetocin (a long-acting oxytocin analogue) providing the best results.

CONCLUSION

Overall, there are numerous alternatives that reduce pain and stress, as well as others that improve semen quality, which can be incorporated into the routine without apparent disadvantages.

Acknowledgment: Juan Carlos Orihuela, Aline Freitas-de-Melo, Julia Giriboni, Julián Santiago-Moreno, Silvia Abril-Sánchez

Biomarkers of Sperm Quality in Livestock: G2P & AI2

Peter Sutovsky[1,3], Filip Tirpak[1], Lauren E. Hamilton[1], Jacob Rissman[1,2], Robert D. Schnabel[1,2], Tasrin Sultana[1], Miriam Sutovsky[1]

[1] Division of Animal Sciences, University of Missouri, Columbia MO, USA

[2] Genetics Area Program, University of Missouri, Columbia MO, USA

[3] Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia MO, USA

Contact person: sutovskyp@missouri.edu

Keywords: sperm quality, genome to phenome, male fertility, infertility

AIM

Tackling the complexity of cattle artificail insemination (AI), we employ a genome-to-phenome (G2P) approach to identify deleterius mutations affecting male fertility. Our goal is to identify biomarkers of paternally derived factors affecting reproductive outcomes. We focus on rare (less than 1% population frequency), deleterious homozygous mutations associated with genes controling gmale gonadal function and sperm output/quality.

METHODS

Based on extensive insemination and pregnancy records, along with genome sequencing, we have identified 85 subfertile bulls carrying alternative gene variants related to sperm quality and preimplantation embryo development. We employ immunofluorescence microscopy, Western blotting, image-based flow cytometry, and gene expression analyses to study the localization and functional significance of the candidate gene products.

RESULTS

Our research focuses particularly on spermatozoa exhibiting a nuclear vacuole (NV) phenotype at a frequence of >20% spermatozoa in an ejaculate, within a sub-cohort of five NV bulls that share mutations in fertility-associated genes—MSL3, PIR, PEG3, EP400, and TDRD9. In addition to a possible role in the NV pathology, these genes are involved in chromatin and DNA dynamics and are developmentally regulated during spermatogenesis and preimplantation embryo development. Deleterious mutations or altered expression of proteins contribute to stump tail phenotype due to axoneme and periaxonemal structure disruption. In this subproject, we focus on products of genes involved in sperm tail development, such as POC1, CEP152, KIAA0586, MICAL1, and. Using our high-throughput phenotyping pipeline, which includes epifluorescence microscopy, image-based flow cytometry (IBFC), and proteomic analyses, we

identified differential protein immunolocalization and abundance compared to spermatozoa from wild type (for said gene mutations) sires. We also confirmed the nuclear localization of candidate proteins in wild-type bull spermatids. These findings are being translated to automated, label-free semen analysis by integrating machine learning with advanced IBFC techniques—essentially, putting the AI into AI, a concept we refer to as AI².

CONCLUSIONS

The results of this study will deepen our understanding of spermatogenesis, link idiopathic infertility and pregnancy failure to inherited paternal factors, and elevate the efficiency of livestock breeding strategies while opening new horizons for the identification and treatment of human infertility.

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