BOOK OF ABSTRACTS

CZU hybrid seminar

ANIMAL REPRODUCTION, SPERM CRYOPRESERVATION AND ANALYSIS: AN INTERNATIONAL EXPERIENCE

3 MAY 2022 — PRAGUE — CZECHIA

BOOK OF ABSTRACTS
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CZU hybrid seminar
Animal reproduction, sperm cryopreservation and analysis: an international experience
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Welcome word

Ladies and gentlemen, Dear seminar participants,

It is our great pleasure to welcome you either in person or through online environment. We are delighted, that many notable speakers answered our call for contribution favourably. Some of them even made a trip to our campus, which is located in Prague, one of the most beautiful cities in the world.

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 thank the Czech University of Life Sciences Prague and the Faculty of Agrobiology, Food and Natural Resources for providing the rooms and equipment for organizing the event and all the sponsors who supported our event. We would like to also express thanks to the company Avantor, which sponsored this event. We are very greatful for their contribution.

We hope that you will enjoy all lectures with fruitful discussions.

Jan Pytlík

On behalf of the Organising Committee

Programme

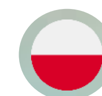
8:20 – 8:25 **Welcome word by Seminar Organising Committee**
Ing. Jan Pytlík

8:25 – 9:00 **Ing Martin Ptáček, Ph.D.**
Ing. Kristýna Petričáková
Ing. Filipp Georgijevič Savvulidi, Ph.D.
Czech University of Life Sciences Prague
Conversation programs of native small ruminants and poultry breeds in Czechia

Invited Speakers

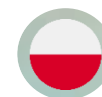
9:00 – 9:45 **Prof. Szabolcs Tamás Nagy, Ph.D.**
Hungarian University of Agriculture and Life Sciences
"Flow spermmetry" – the past, present and future of flow cytometry in sperm physiology

9:45 – 10:30 **Prof. Agniezska Partyka, Ph.D.**
Wroclaw University of Environmental and Life Sciences
Advances in cryopreservation of poultry semen



10:30 – 11:15 **Prof. Joaquin Gadea DVM, Ph.D.**
University of Murcia
New techniques in sperm assessment

11:15 – 12:00 **Mgr. Sylwia Judycka, Ph.D.**
Institute of Animal Reproduction and Food Research
Standardization of cryopreservation protocols and practical use of cryopreserved salmonid fish sperm



Lunch 12:00 – 13:00

Invited Speakers

13:00 – 13:45 **RNDr. Karina Savvulidi Vargová, Ph.D.**
Charles University
CRISPR/Cas9 and its actual and future application in the livestock industry

13:45 – 14:30 **Prof. João Pedro Barbas, Ph.D.**
National Institute for Agricultural and Veterinary Research
Cryopreservation of semen from small ruminants

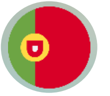
14:30 – 15:15 Fernando Juan Peña Vega DVM, Ph.D.

University of Extremadura

Multiparametric flow cytometry: a potent tool for the study sperm biology

15:15 – 16:00 Prof. João Carlos Caetano Simões, Ph.D.

University of Trás-os-Montes and Alto Douro



Seasonality in males and females of small ruminants and ovulation synchronization

Final Conclusion 16:00 – 16:05

Organising Committee

Martina Jánošíková

Kristýna Petričáková

Martin Ptáček

Jan Pytlík

Filipp Georgijevič Savvulidi

Support and sponsorship

Presentations

Cryopreservation of semen from small ruminants: an overview

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AIM

Cryopreservation is an important technique for the maintenance of germplasm long term viability. However, the cooling process includes fast structural changes in spermatozoa (SPZ), which causes cell damage such as the reduction of membrane fluidity and modification of lipid and protein organization. There are several factors that influence frozen semen quality. In our laboratory, we have focused on the production of an extender for semen of small ruminants that could reduce deleterious effects of cryopreservation on sperm viability while keeping good fertilizing capacity.

MATERIAL AND METHODS

In our research several ejaculates from sheep and buck males from Portuguese native breeds were used. Several factors that may affect sperm quality parameters in fresh and frozen semen, namely breed, individual variation, season of semen collection, extender composition and protocol of semen cryopreservation, were evaluated. Morevoer, in thawed semen individual motility, sperm vitality and abnormalities were evaluated. In our andrology laboratory we have formulated an extender for small ruminant semen cryopreservation named EZN-Extender which contains buffers, carbohydrates, salts, egg yolk and antibiotics. Semen was collected by using an artificial vagina, and quantitative and qualitative parameters were immediately evaluated in fresh semen. Our EZN semen extender have different formulations for bucks or rams. In bucks, seminal plasma must be removed by centrifugation before dilution. Afterwards semen was diluted to 800 (bucks)and 1200 (rams) $*10^6$ sperm cells/mL, distributed into Cassou Straws (0.25 mL) and equilibrated during 4 hours till 4ºC; finally, the straws were frozen in nitrogen vapours during 20 minutes, prior to immersion in NL2.

RESULTS AND CONCLUSION

In rams and bucks of native Portuguese breeds, individual motility, live and normal sperm were always lower values in thawed ($p<0.05$) semen. In fresh semen (Ram Saloia Breed) we

have determined seasonal and individual variation upon quantitative parameters but without differences in thawed semen. In bucks (fresh semen) we have detected a breed effect upon volume and concentration with no differences in thawed semen. We have compared some extenders for ram semen cryopreservation and showed that our EZN S-EXT (egg yolk based) was more consistent around the year in cryopreserving SPZ than commercial egg yolk-free based extenders and still seems to be the most appropriate S-EXT to be used in Merino sheep (native breed).

Globally, in small ruminants the cervical AI with frozen semen, had low (sheep) and medium (goat) fertility rates. Mean fertility after AI of Saloia and Merino breeds (native breeds) ranged from 17.5 to 30.6% comparing to 35.8 % in Serrana Goats and 44.6 % in Saanen goats. Sperm cryopreservation contributes to the expansion of reproductive techniques, such as artificial insemination, IVF and creation of germplasm banks. Presently, a lower fertility is generally accepted as a consequence of cryopreservation, so our efforts have also been directed to improve sperm viability and fertility.

Keywords: Sperm quality, Cryopreservation, Ram, Buck

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New techniques in sperm assessment

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AIM

Sperm assessment tries to evaluate the quantity and the quality of the spermatozoa present in the ejaculate with the objective of make a diagnosis of the potential fertilizing capacity. In animal production, the prediction of sperm fertilizing ability has great economic importance for breeding herds when artificial insemination is used, since it leads to the selection of only those males with optimal reproductive performance. In the human fertility clinic, the accuracy of sperm assessment is predictive of the potential fertilizing capacity of the sperm and facilitates the selection of the most appropriate treatment.

To date, sperm assessment has primarily relied on microscopic evaluation of seminal samples and biochemical assays and the use of computer aided sperm analysis (CASA) for measuring motility and morphometric sperm parameters is common nowadays.

The evolution of sperm assessment methods has paralleled technological developments, and recently, advances in microscopy offer the possibility to use new equipment to evaluate sperm parameters. In this manuscript, we review modern technologies that are emerging for sperm evaluation and that will very likely transform how sperm assessment is performed.

MATERIAL AND METHODS

From 2 D to 4D sperm evaluation

With the evaluation of the sperm morphology, two methodologies are used frequently, fixed and stained semen samples to obtain a 2D image and contrast phase microscope is used for the evaluation of unstained samples in wet samples. 3D sperm morphology can be determined optically by staining samples using a confocal microscope by scanning the sample at different depth. The invention of lasers has provided coherent light sources facilitating the control of light in general. One of the first techniques to use these new sources of light was holography, also known as interferometric phase microscopy (IPM). With the combination of interferometer measurements of the cells and a microfluidic platform, the automatic selection of human spermatozoa with normal morphology have been recently reported. Moreover, through addition of microfluidics to IPM, it is possible to estimate morphometry in vivo as well as the volume of motile spermatozoa

Whereas the previous techniques are suitable for the study of sperm morphology at the microscale, atomic force microscopy (AFM) is an alternative tool to evaluate nanoscale parameters.

For spermatozoa tracking, several studies in the last years have focused on the classic technique of “in-line spherical wave holography. This technology therefore represents a new

era in the tracking and evaluation of the 3D movement of sperm which will provide new insight into the biological relevance of this movement.

Spermatozoa and seminal plasma composition. Raman Spectroscopy

Raman Spectroscopy is a very specific technique that allows the biochemical analysis of cellular components and fluids. This technique uses a laser light source to irradiate a sample, and generates a Raman scattered light, which is detected as a Raman spectrum using a CCD camera. damaged and intact sperm cells can be distinguished by characteristic Raman bands and mitochondrial function can be monitored and recently differences in DNA, protein and lipid composition of bull spermatozoa and human spermatozoa. Seminal plasma composition has been also evaluated by Raman

RESULTS AND CONCLUSION

Emerging technologies for sperm evaluation are now in develo`ment and that will very likely transform how sperm assessment is performed.

Keywords: male fertility, andrology, sperm motility

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Standardization of cryopreservation protocols and practical use of cryopreserved salmonid fish sperm

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AIM

Cryopreservation is the most promising technology for preserving biological specimens for many years. Cryopreservation enables long-term conservation of biological material (cells, tissues, embryos and organisms) at low temperatures, ensuring its viability after thawing. Sperm cells were the first mammalian cells which were successfully frozen. The cryopreservation of bull semen introduced in the 60s had a huge impact on the dairy bull industry, by achieving impressive breeding progress by using the sperm of a small number of breeders with outstanding genetic traits. This success was not repeated for other domestic animals. Today, cryopreserved semen is commonly used in cattle breeding and in assisting human reproduction. It is also successfully applied in horse and pet breeding. Unfortunately, so far it has not been possible to introduce cryopreservation into the practice of fish farming. A current problem in fish cryopreservation is the lack of procedure standardization what limits the commercialization of frozen fish semen in aquaculture.

RESULTS AND CONCLUSION

The presented research focuses on the development and improvement of cryopreservation procedures of salmonids semen (rainbow trout, brown trout, sea trout, brook trout, Atlantic salmon, Arctic char, grayling, sex-reversed females rainbow and brook trout) and percids (European perch and pikeperch) useful for the creation of sperm banks. The creation of cryopreserved sperm banks is an effective strategy for protecting the biodiversity of local fish populations, as well as provides great potential for fish breeding programs since sperm banks secures material from the most valuable breeding individuals and offers its use for further selection work. As a result of the presented research, an innovative, standardized (in terms of constant sperm concentrations in a straw and the final concentration of cryoprotectants) methodology of cryopreservation of fish semen using a simple glucose-methanol extender was developed. Standardization of sperm concentration in a straw has two fundamental advantages: 1) the conditions of cryopreservation technology are aligned in terms of this parameter, and 2) the fertilization procedure is simplified due to a constant and defined number of sperm in the straw. Determining the optimal concentrations of glucose and methanol in the extender turned out to be particularly important, because even small changes in the concentration of these cryoprotectants resulted in a significant reduction in sperm motility after thawing. It has been shown that due to species-specific differences in cryopreservation protocols, it is necessary to optimize cryopreservation conditions for each of the fish species studied.

The developed standardized procedure for cryopreservation of salmonid fish semen after its implementation into hatchery practice should significantly improve the work of the hatchery. The implementation of modern technologies will primarily result in organizational and economic benefits, as the number of fish required to maintain high genetic variability in the hatchery can be reduced due to the genetic material deposited in the sperm bank. In addition, in the event of an epidemic situation on the farm (fish diseases), or in the case when the females are already mature, while the males are not yet, the cryopreserved semen can be successfully used for fertilization.

Keywords: freezing/thawing, sperm concentration, cryoprotectants.

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"Flow spermmetry " the past, present and future of flow cytometry in sperm physiology studies

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THE PAST

Application of flow cytometry as an analytical tool in sperm studies has a several decades long history. Although the first studies focused on quantitative sperm DNA analysis – leading to the development of routine sperm sexing, a technology that revolutionized the dairy industry – later other opportunities were tested and the status of the plasma membrane, acrosome, mitochondria, etc. were studied either as single assays or multicolor applications. Flow cytometry and spermatozoa are the ideal match: cells are already in suspension, i.e. there is no need for tissue dissociation; the tests are quick and robust, statistically precise (but not necessarily accurate); on the other hand, there are some limitations, like no visual detection, and important sperm characteristics like motility or morphology cannot be tested directly. The lack of visual control has led to a lot of scientific effort to solve the „invisible sperm" problem, i.e. positive fluorescent labelling of every sperm cell.

THE PRESENT

The development of flow cytometers now allows to use multiple laser lines and several fluorescent colors can be evaluated simultaneously which has widened the opportunities of organelle-specific, multicolor sperm studies in the research environment. On the other hand, smaller and more and more user-friendly instruments have found their place in the routine semen quality control in Artificial Insemination Centers. Pre-set applications and ready-to-use kits are available for the end users. Small, bench-top sorters allow the separation and further analysis of different subpopulations.

THE FUTURE

The huge development of analytical instruments was not followed by a similar development of the analytical softwares – data analysis is mainly still based on user-dependent region drawing and subsequent gating. In the last years, however, automated data analysis options have emerged (in R environment, for ex.) in hematology, microbiology and other fields, and the application of these analytical approaches will be more and more frequent in sperm studies. At the same time, some almost-forgotten cytometric techniques, like pulse profile analysis for sperm morphology may re-appear. „Next-gen" flow cytometry practically has no limits...

The lecture summarizes a personal journey of 20+ years in the field of flow spermmetry, including totally unrelated events like blues jams, walks on seabed, target shooting and some heated lab activities...

Keywords: domestic animals, sperm quality, cytology, automated analysis

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Advances in cryopreservation of poultry semen

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The semen cryopreservation is a key biotechnological strategy used for the conservation and protection of the genetic background, which decreases in some species and conserves animal genetic biodiversity. Moreover, it is a crucial and until recently the most effective method of creating a genetic reserve of birds ex situ. Species differences in sperm susceptibility to cryopreservation and a decrease in the quality of thawed semen create the need to develop biotechnology of reproduction in birds and to conduct research on changes occurring in the gametes during this process.

Cryopreservation of semen causes irreversible damage to sperm cell membranes, which decreases sperm quality after freezing-thawing. During this process, sperm are exposed simultaneously to damage caused by thermal shock, freezing, and thawing, which consequently causes loss of sperm motility, osmotic changes in the cell membrane, and reorganization of its lipids and proteins.

Some of these limitations are a consequence of the structure of avian sperm cells. Poultry spermatozoa, mostly those of the Galliformes, have a filiform morphology with sperm heads being relatively narrower and longer, resulting in a smaller cell volume than those of mammalian gametes. The avian sperm membrane contains more polyunsaturated fatty acids (PUFAs) than sperm of mammals and have a lesser protein content, a lower cholesterol/phospholipid ratio and greater overall fluidity at physiological temperatures.

For many years, the best protocol for freezing semen of various species has been searched for. However, there is a question: Do we have the ideal protocol for the cryopreservation of avian semen? It seems that not! There are several factors that still bothered our heads such as: Which packing system is better for use? What cryoprotectant use to obtain satisfied results after insemination? Therefore, we are looking for new strategies to improve the outcome of semen cryopreservation.

In this review, we will present current knowledge and the newest effective strategies that exploit cryoprotectants and antioxidants used in assisted poultry reproduction techniques. This research area is still being developed, and therefore some aspects are not well recognized as they are in mammals.

Although antioxidant supplementation of semen extenders has been used for many years, the supplements are now more likely to be natural substances and small particle antioxidants. Furthermore, the combination of antioxidants with nanotechnology, is starting to be suggested more widely. Encapsulation of some substances that are weakly water soluble into nanostructured lipid carriers and liposomes is a promising options in active substances. Could this perhaps be the future method for the use of different antioxidants in avian sperm preservation?

Keywords: chicken semen, semen cryopreservation, cryoprotectants, antioxidants

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Comparison of commercial poultry extenders modified for cryopreservation procedure in genetic resource program of Czech golden spotted hen

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AIM

Czech Golden Spotted Hen (CZH) is the only one original national Czech hen breed, classified among genetic resources and protected under national rescue program. The sperm cryopreservation procedures of this breed are necessary to create a sufficient gene reservoir as there were less than 260 CZH both males and females in this program in 2020.

The aim of the study was to compare commercial fresh semen extenders supplemented by defined cryoprotective agent as to suggest important tool for cryopreservation procedure of original Czech hen breed.

MATERIAL AND METHODS

Ejaculates were obtained from 4 adult roosters (*Gallus gallus domesticus*) collected by dorso-abdominal massage technique twice a week. Semen samples passing initial limits were pooled and diluted in commercially available extenders with cryoprotective agent: Poultry media®, Raptac®, and NeXcell® supplemented by 9% N-Methylacetamide. Diluted semen was frozen in 0.25 ml plastic straws exposure to liquid nitrogen vapours (5 cm above) for 10 min and subsequently plunged into liquid nitrogen for storage. Sperm samples were thawed in a water bath tempered at 5°C for 100 s. Sperm parameters of viability (VIA; expressed by the plasma membrane integrity, and acrosome status) and sperm motility (MOT), were assessed using flow cytometry and mobile computer-assisted sperm analyser – mCASA (iSperm®) before and after cryopreservation procedure.

RESULTS AND CONCLUSION

Poultry media® (VIA = 51,11%, MOT = 23,58%) and Raptac® (VIA = 52,04%, MOT = 23,13%) with cryoprotective supplement showed comparable results with highest cryoprotective efficiency. Contrary, significantly lower VIA and MOT parameters were detected for CZH roster spermatozoa froze in NeXcell® extender in comparison with Poultry media® and Raptac® as well. Our study identified appropriate commercial fresh semen extenders modified for cryopreservation as to suggest important tool for cryopreservation procedure of original Czech hen breed. It will be, however, necessary to verify our promising *in vitro* results using artificial insemination technique in future.

Keywords: gene reserve, cryoprotectant, rooster, flow cytometry, sperm evaluation

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Perspective of cryopreservation program for original Sheep and Goat population in the Czech Republic

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AIM

To define the population of sheep and goat industry of the Czech Republic, and to demonstrate the perspective of the cryopreservation program realized for genetic resources of sheep and goats currently running in the Czech Republic.

INTRODUCTION

Czech sheep population, with 203,612 sheep according to the latest FAO statistics, is primarily oriented to mutton production. Commonly bred intensive meat sheep used in terminal sire position are typical for our breeding conditions. These sheep represent about a third of the whole population. Approximately half of the sheep population is represented by multiple-purpose sheep breeds that are predominantly used for meat production as well. This part covers traditional sheep breeds classified among genetic resources: Wallachian and Sumava sheep. Goat management is in the majority oriented to dairy production (more than 90 % of 28,919 animals registered in the latest FAO statistics). The population of dairy goat breeds covers traditional Czech goat breeds represented by Czech white- and Czech brown-shorthaired goats. The global strategy is to protect and keep threatened animals all over the world. Aims for the preservation of original sheep and goat breeds in the Czech Republic are realized within the rescue program, which is coordinated and controlled by the National Centre for Genetic Resources of Animals (stated by the Ministry of Agriculture of the Czech Republic). This program covers *in vivo* and *in vitro* parts. *In vivo* part represents the reservoir of live animals bred under commercial flocks. Summarized statistics of live animals: 1,928 Sumava sheep kept in 28 flocks, 952 Wallachian sheep in 52 flocks, 1,895 White short-haired goats in 71 flocks, and 687 Brown short-haired goats in 60 flocks. *In vitro* part of this program determinates a minimal number of 100 insemination doses and 100 embryos for each goat and sheep breed.

REALIZATION AND PERSPECTIVE

Our current effort follows the strategy stated by *in vitro* rescue program. This covers control and assessment of the quality of insemination doses representing *in vitro* reservoirs of genetically valuable sires. The main role is to optimize and create cryopreservation schemes for these breeds. Our future effort is aimed at establishing an adequate reservoir based on suggested cryopreservation schemes and applying artificial insemination for re-introduction genetically unique sires back into the population.

Keywords: Sumava sheep, Wallachian sheep, Czech brown-shorthaired goat, Czech white-shorthaired goat, insemination dose

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Analytical approaches are an essential part of modern valuable genetic resource conservation programs: the advances of the Czech University of Life Sciences Prague

Savvulidi F.G.[1], Ptacek M.[1], Malkova A.[1], Pytlik J.[1], Petricakova K.[1], Janosikova M.[1], Zemanova K.[1], Ranna T.[1], Beranova H.[1]

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AIM

To improve the quality control of fresh and frozen-thawed sperm (obtained from rams, bucks, roosters, and honey-bee drones, and processed for the purposes of genetic resources conservation programs currently running in the Czech Republic).

MATERIAL AND METHODS

Our work uses several *in vitro* analytical approaches, such as computer-assisted sperm analysis (CASA) which is built on the iPad architecture (iSperm mCASA developed by Aidmics Biotechnology), and conventional (NovoCyte 3000, Agilent) and imaging (Amnis ImageStream, Luminex) flow cytometers. We use the artificial insemination method to verify ours *in vitro* analytical approaches.

RESULTS AND CONCLUSION

With the help of used in vitro analytical approaches, we were able to optimize the freezing and thawing rates for Wallachian ram sperm. We were able to evaluate the impact of individual motility parameters (total and progressive, VCL, VSL, VAP, STR, LIN) on the fertilizing ability of sperm from Czech short-haired bucks (confirmed by artificial transcervical insemination). The glycerol addition in the semen extender was successfully optimized for freezing of Wallachian ram sperm. The active role of pannexin channels in the process of propidium iodide and YO-PRO-1 uptake by alive Wallachian ram spermatozoa was confirmed with the use of flow cytometry. Furthermore, with the use of flow cytometry, the damaging effect of intracellular ice crystals on the viability of Wallachian ram sperm was investigated during the process of sperm freezing. Currently, we optimize the formulation of the extender for frozen storage of Wallachian ram and Czech short-haired buck's sperm. In the sperm of the Czech Golden Spotted Hen, we were able to optimize the concentration of cryoprotectants for obtaining good in vitro results of sperm freezing-thawing. For honey-bee drones' sperm, we were able to establish a new assay of HOS-test results evaluation with the use of a flow cytometer. In conclusion, the analytical approaches (at least, CASA and flow cytometry) are an essential part of any modern genetic resources conservation program. All the above-mentioned advances of the Czech University of Life Sciences Prague will help to improve the results of valuable genetic resource conservation programs currently running in the Czech Republic. We are open to international co-operation on the topic of further improvement of the results of valuable genetic resource conservation programs.

Keywords: Artificial insemination, CASA, flow cytometry, imaging cytometry, sperm freezing

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Seasonality in males and females of small ruminants and ovulation synchronization: A 20 years experience.

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AIM

In this communication, we revisited some experiments throughout last 20 years, performed at our university (41º Latitude), concerning small ruminant seasonality and ovulation synchronization mainly in local Portuguese breeds.

MATERIAL AND METHODS

Several studies were made in goats and sheep using ultrasonography and hormonal (P4/LH measurements), and estrous detection by active males without photoperiod or melatonin treatments. Study 1: 19 nulliparous and multiparous Serrana goats (SG) were used to determine the time of ovulation during the breeding season after induced and natural oestrus; Study 2: 42 SG were synchronized with cloprostenol (50 µg), 10 days apart (September) and oestrus detected during two sucessive oestrus cycles by bucks; Study 3: A 5-days short term progestagen treatment, with and without eCG administration, was applied to 20 Ile de France (IF) and 19 Churra da Terra Quente (CTQ) during breeding season; Study 4: 37 nuliparous or multiparous SG were synchronized with 20 or 30 mg of fluorogestone acetate during April and the oestrus behaviour, sub-oestrus and ovulation were accessed; Study 5: P4 and estrous detection was evaluated during whole the year in 10 IF and 10 CTQ.

RESULTS AND CONCLUSION

Study 1: The onset of oestrus to LH peak interval, LH peak to first ovulation interval and onset of oestrus was affected by parity and type of oestrus; Study 2: During Setember only 23.8% of SG were cyclic and 14.3% of them have short oestrous cycles. The percentage of cyclic goats increased to 100% during next month without presentig short oestrous cycles; Study 3: Ewes in oestrus, pregnancy rate and prolificacy varied between 70-90%, 35-41% and 1.2-1.6, respectively, regarding breeds and groups; Study 4: Significant differences of oestrus behaviour and sub-oestrus, but not in ovulation, were observed between groups; Study 5: Significant differences on seasonal P4 and estrous detection patterns were observed according to the genotype, where IF remain strongly seasonal. Overall, these experiments demonstrated the influence of genotypic and phenotypic factors on oestrus and ovulation variability and characterized the studied traits in Portuguese small ruminant breeds.

Keywords: breeding season, anoestrous intensity, reproduction management

Contact person: João Simões, jsimoes@utad.pt

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Use of Interspecific ICSI for the Assessment of Buck Sperm DNA Damage After Different Methods of Cryopreservation

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AIM

The interspecific ICSI is the suitable method for production of zygotes, which are formed by association of gametes of different species. This can be used to evaluate properties of genetic material. In this study, the method was used for evaluation of the quality of stored sperm originating from the buck of the white shorthair goat genetic resource. The aim of this study was to evaluate the effect of cryopreservation on the quality of goat sperm, which was frozen in three different ways. Freshly collected semen served as a control group.

MATERIAL AND METHODS

The heads of xenogeneic goat sperm were injected into mature mouse oocytes. The injected oocytes were allowed to develop to the zygote stage, in which both female (mouse) and male (buck) pronuclei were formed. In male pronuclei, DNA damage was assessed by immunofluorescence staining with anti-γH2AX antibody, a marker of DNA breaks.

RESULTS AND CONCLUSION

The results obtained by measuring the level of γH2AX fluorescence intensity in the paternal pronuclei show that the values differ between the individual groups of semen. The lowest values of fluorescence intensity, and thus the lowest DNA damage, were found in freshly collected semen (11.93 ± 8.06). Variability was also demonstrated between the experimental groups of frozen semen. The influence of different methods of semen collection and its freezing and the influence of the composition of the freezing medium were confirmed. The highest values of fluorescence intensity were found in the freezing medium supplemented with egg yolk (39.23 ± 8.06). This method of assessing the quality of cryopreserved is a relatively simple and inexpensive method that can replace previous sperm quality assessment techniques with the advantage of replacing hard-to-reach oocytes of endangered species with available mouse oocytes.

Keywords: ICSI, spermatozoa, genetic resources, DNA integrity

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CRISPR/Cas9 and its actual application in the livestock industry

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Genome editing represents a fascinating approach with enormous potential in the livestock and biomedical applications with the purpose to manipulate any gene function. It ensures the ability to improve animal quality, treat or prevent various genetic disorders through deletion/insertion, or base change at a specific location of the desired gene of interest. The ideal genome-editing approach needs to effectively alter a genomic sequence, showing high DNA sequence specificity with less or no off-target effects. This all fulfills a state-of-the-art gene editing technique called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) that has become since 2012 (2013 in livestock) used to edit any DNA of any species in practically all fields. Mankind is highly dependent on the livestock for their daily basic needs - food in the form of meat, milk, and eggs. Also no less important increased immunity and disease resistance of livestock. Therefore, gene editing by CRISPR provides a perfect opportunity for enhancing efficiency of food production, animal health and welfare. Here are some examples of how CRISPR editing is already applied in the livestock. Highly pathogenic Porcine reproductive and respiratory syndrome virus (PRRSV) causes severe significant losses to current swine production worldwide. CD163 is a cellular receptor for PRRSV that was depleted by CRISPR tool and these CD163 knockout pigs showed resistance to PRRSV. Gene NRAMP1 (natural resistance-associated macrophage protein-1) is associated with an innate resistance to pathogens like salmonella and mycobacterium (m. bovis which can lead to tuberculosis in cattle). NRAMP1 knockout cattle showed an improved immune reaction to m. bovis. To increase muscle growth and reduce fat thickness, myostatin (MSNT) knockout pigs were created. To increase the amount of cashmere producing follicles and increase the hair fibre length, FGF5 (Fibroblast growth factor 5) knockout Cashmere goats were created for this purpose. The allergenicity of milk is given by beta-lactoglobulin (BLG) therefore researchers aimed to create BLG knockout cows by CRISPR/Cas9 tool. These were just a few examples of CRISPR edited animals, their number and application will just increase with time.

Keywords: CRISPR/Cas9, gene editing, livestock

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