

AgroVet-Strickhof Conference
Vernetzung von Forschung und Praxis -
Standortangepasste Tierhaltung

Conference Proceedings
28.10.2025

ISBN Number: 978-3-907692-02-8

Editors:

M. Terranova, A. Angelozzi

Edition 1

Address: AgroVet-Strickhof
 Leitung Forschung
 Eschikon 27
 CH-8315 Lindau

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1 Single-Cell RNA-Sequencing Analysis of the Infundibulum, Ampulla, and Isthmus to Characterize Cell Populations in the Post-Ovulation Bovine Oviduct

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The oviduct is crucial for gamete transport, fertilization, and early embryonic development. It is divided into three functional parts: infundibulum, ampulla, and isthmus, with different roles in the early reproductive events. Ciliated and secretory epithelial cells are lining the lumen of each compartment to support these events. A detailed understanding of cellular heterogeneity and gene expression in these regions can shed light on the mechanisms promoting reproductive success. Therefore, this study aimed to analyze the cellular diversity and gene expression profiles within infundibulum, ampulla, and isthmus of the bovine oviduct during the post-ovulatory phase using single cell RNA sequencing (scRNA-seq).

Oviducts were collected from five cyclic Swiss Brown cows at the early post-ovulatory stage. The infundibulum, ampulla, and isthmus were separated and single cells isolated by enzymatic digestion. After cell viability assessment, single cell capture, and library preparation were performed using the Illumina Single Cell 3' RNA Prep kit. Libraries were sequenced on a 10B flow cell using the Illumina NovaSeq X Plus platform (Functional Genomics Center Zurich). Raw reads were aligned to the *Bos taurus* genome (ARS-UCD2.0) with STAR. Quality control and clustering were done using Seurat, retaining cells with 200–3,000 expressed genes and <20% mitochondrial reads. Uniform Manifold Approximation and Projection (UMAP) was applied to visualize cellular heterogeneity across all samples. The most relevant markers per cluster were identified using “*PrepSCTFindMarkers*” function, followed by annotation based on gene expression profiles and literature.

Preliminary results revealed 75,877 high-quality single cells: 13,640 from the infundibulum, 33,962 from the ampulla, and 28,275 from the isthmus. UMAP identified 32 distinct cell populations, including 10 secretory subclusters, 8 multi-ciliated subclusters, and 5 transitional epithelial subclusters. Other cell types included cells with neuronal markers, endothelial cells, fibroblasts, and immune cells. Immune cell populations consisted of 7 subclusters: 4 of T cells, 2 of macrophages, and 1 of B cells. Secretory cells were the most abundant type, with proportions of 37.2% in the infundibulum, 51.7% in the ampulla, and 47.3% in the isthmus. Multi-ciliated cells were the second most abundant cell population (25.7% in the infundibulum and 24.9% in the ampulla), except in the isthmus (9.8%), where transitioning epithelial cells (20.9%) and T cells (13.9%) were more prevalent. Cells with neuronal markers were exclusively detected in the ampulla, while fibroblasts and endothelial cells were detected in both the infundibulum and isthmus. Notably, distinctive gene expression profiles were identified in the isthmus and ampulla for secretory and multi-ciliated epithelial cells, respectively.

This study outlines a comprehensive view of the cellular diversity of the oviductal compartments for the first time and its implications in providing an optimal milieu for the success of early reproductive events. It provides the first comprehensive single-cell transcriptomic atlas of the bovine oviduct, which may be relevant to other mammals as well.

2 Evidence of extrarenal sodium storage in a large mammal: implications for comparative physiology and hypertension research

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Under conditions of dietary sodium (Na^+) excess, the kidneys may fail to adequately excrete Na^+ , potentially compromising blood pressure homeostasis. Body tissues, such as skin, can offer sites of short-term extrarenal Na^+ storage and previous research has shown that this can help guard against hypertension in small mammals (e.g., rodents). Large mammals have relatively greater Na^+ storage potential but whether an extrarenal Na^+ storage system exists for this group is unknown. Here, we provided a large pulse-dose of NaCl to four cattle (body mass: ~ 720 kg) and measured excretion of Na^+ and potassium (K^+) in urine and faeces for a period of 7 days. Following NaCl administration, Na^+ excretion spiked in both urine and faeces for ~ 48 hours before returning to and even below baseline measurements. After ~ 96 hours, however, Na^+ excretion increased again; a remarkably consistent physiological phenomenon across all individuals studied. We did not observe a pattern in urinary K^+ excretion, indicating that the mechanism of Na^+ storage does not appear to involve exchange for K^+ . However, faecal K^+ excretion was reciprocal to that of Na^+ , reflecting exchange of cations across the walls of the large intestine. We infer that during the initial period of Na^+ stress, short-term extrarenal Na^+ storage occurred and was later released when the body had returned to Na^+ homeostasis. Additional experiments are required to understand how patterns of Na^+ regulation changes across body sizes and the specific body compartments involved. Cattle may be a useful model system for examining the impact of high Na^+ intake in mammals larger than humans.

3 Environmental conditions and plant diversity show little effect on mycotoxin occurrence in European grasslands used for horse husbandry

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In light of ongoing threats to European grasslands, grazing by livestock such as horses can contribute to biodiversity conservation. An emerging field of research relevant to both grassland ecosystems and animal husbandry has focused on grass-endophyte symbioses between *Epichloe* fungi and grasses of the genera *Festuca*, *Lolium*, and *Schedonorus*. These endophytes produce toxic alkaloids and have been associated with certain fitness benefits to host grasses, possibly leading to diminished grassland diversity. However, these grass-endophyte symbioses have rarely been studied in (semi-)natural settings. In this study we explored the prevalence of mycotoxins produced by fungal endophytes in temperate European grasslands, testing for possible links between mycotoxin occurrence and ecological conditions.

We sampled 310 vegetation plots of 10 m² at seven horse farms in France, Germany, Austria, and Hungary. In a subset of 204 plots, we collected and tested 372 samples of the target grass genera for the mycotoxins ergovaline and lolitrem B using ultra-performance liquid chromatography–mass spectrometry. We calculated plant diversity measures and mean Ecological Indicator Values for Europe (EIVE). Differences in the vegetation and mycotoxin prevalence between meadows and pastures were tested using linear mixed-effects models. To explore ecological effects on mycotoxin prevalence, we calculated generalized mixed models at plot and sample level.

Plant diversity was similar to averages in the study region, with maxima and Red List species occurring in dryer and wetter conditions. Mycotoxins occurred at all seven sites under a broad range of environmental conditions. Mycotoxin occurrence was comparable to endophyte infection rates from the literature. *Festuca rubra* aggr. tested positive at rates exceeding twice that of *Lolium perenne* and *Schedonorus arundinaceus*. Mycotoxin occurrence was associated with higher soil moisture and lower nitrogen EIVEs, in contrast to the literature showing benefits to hosts primarily in dry and nutrient-rich conditions. Grass cover and host dominance showed contrasting effects on mycotoxin prevalence in host species. There was no relationship between mycotoxin prevalence and temperature, biodiversity, or land-use type.

From our results, we conclude that endophyte infection is common in semi-natural grasslands in temperate Europe but appears to currently present only a limited risk to livestock. We find no strong or consistent relationships between mycotoxin occurrence and ecological conditions or plant diversity. This is in line with evidence that the effects of infection on hosts are context-dependent and complex. Species-rich grasslands may provide a protective effect against endophyte toxicosis in livestock by diluting mycotoxin concentrations where endophyte infection occurs.

4 Antibodies from alpaca and why you need a nano-antibody for your research project

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At the Nano-antibody Service Facility we take advantage of the immune system of alpacas to produce specific and high affinity antigen-binding fragments (VHH, nano-antibodies). These small antibody fragments originate from special antibodies that occur in all camelid species and have a simpler structure than classical antibodies found in all other mammals including human, possessing only two instead of four chains.

Antibodies are proteins produced by immune cells as a reaction to foreign substances, which are known as antigens. In the body, antibodies serve to neutralize those molecules and fight off diseases, but in research they are used for a wide range of applications – for example to mark target proteins in order to identify (e.g. to visualize them), to change their function, or to deactivate them.

Conventional antibodies are complex molecules that are very difficult to genetically engineer, express recombinantly and produce in large quantities, which makes research with them challenging. The same is true for their antigen-binding domains that often fail to remain functional when produced in vitro. In contrast, antigen-binding domains originating from two chain antibodies naturally occurring in Camelidae, that we call nano-antibodies (or VHH), are considerably easier to adapt to specific requirements because of their simpler structure and much greater stability, while maintaining the specificities and affinities of the antibodies they originated from.

Another huge advantage of nano-antibodies over classical antibodies is their small size and increased solubility which enables them to easier penetrate tissues, biological barriers, and fixed specimens, but also enables them to bind recessed pockets and epitopes that are not accessible to large and complex conventional antibodies.

Furthermore, not only are they more straightforward to produce, genetically modify, purify, and adapt to specific needs; because of their excellent thermal stability they are also easier to store, transport, and replicate, which makes them ideal for diagnostic applications.

Who we are/what we do: The Nano-antibody Service Facility of the University of Zurich is the core technology platform that produces individual nanobodies for research projects and helps research collaborators to make and optimize nano-antibodies for their specific needs.

Examples of research projects:

- Broad use of biomedical analyses – from immunohistochemistry to live imaging to the determination of protein structures and protein-protein interactions.
- Nanobodies are an ideal tool for functional studies as they often inhibit protein functions (enzymes, transport proteins, signaling pathways, protein knockout, etc.).
- Nanobodies are also ideal for the development of diagnostic tests – including in non-invasive cancer imaging.

5 Effects of alfalfa (*Medicago sativa*) products in organic diets on ammonia emissions and growth performance in broilers

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Livestock production faces numerous challenges, including the search for regional and sustainable protein sources for poultry feed and strategies to reduce ammonia emissions in poultry houses. An interesting plant that could provide a solution to both issues is alfalfa (*Medicago sativa*). It is a regionally adapted and widely used plant in organic farming, known for its favorable amino acid profile. It also contains antinutritive secondary plant compounds, particularly saponins, which may modulate the gut microbiota of broilers in a way that reduces ammonia emissions. Therefore, this study investigates the effects of different alfalfa products (alfalfa tops, sieved alfalfa) on broiler performance, microbiota and emissions. A total of 48 one-day-old male Hubbard JA 757 broilers were housed in four groups of 12 animals until day 14. Afterwards, they were kept in pairs (four feeding groups × six replicates). The study was carried out over three feeding phases - starter (days 1-14), grower (days 15-28), and finisher (days 28-68) - using four dietary treatments: a control diet (0/0/0% alfalfa), a low alfalfa tops diet (0/5/7.5%), a high alfalfa tops diet (0/5/10%) and a sieved alfalfa diet (0/5/10%). Weekly, individual body weights and feed intake per group were recorded. Starting from day 28, the ammonia, methane, and carbon dioxide emissions of two broilers per diet were measured simultaneously in four respiration chambers for 50 hours. These measurements were repeated twice a week for six weeks to gather an emission history of the growing broilers. Therefore, each pair of broilers was measured twice over the course of six weeks. On day 68, eight animals per feeding group were slaughtered for blood parameter analysis (albumin, total protein, uric acid, and urea) to assess also their health status. Additionally, the content of the ceca were collected for microbiota analysis via 16S rRNA sequencing. First results showed a depression in broilers performance using diets with alfalfa products.

6 The Uterine Microbiome Composition and Fertility in the Mare

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Subfertility in the mare is a major issue for horse breeding and for animal welfare due to repeated ineffective treatments. Reduced uterine receptivity is a primary cause of subfertility. Compared to humans, the effects of the uterine microbiome composition on uterine receptivity in the mare remain poorly understood. Few studies indicate changes in non-pathogenic microbiota leading to inflammation and subfertility. Understanding the microbial composition in the uterus and its impact on fertility can provide valuable insights into reproductive health in equine species. A previous study showed that fertile mares exhibited higher microbial diversity.

This study aimed to investigate the uterine microbial community of mares in relation to fertility. The study utilized 16S rRNA gene V3-V4 amplicon sequencing to compare the microbial profiles of fertile and subfertile mares, hypothesizing that differences in microbial composition may be associated with variations in fertility.

Endometrial cytobrush and uterine low volume lavage samples were collected from both fertile and subfertile mares. RNA and DNA were isolated from endometrial cytobrush samples using the AllPrep DNA/RNA/miRNA Universal kit (Qiagen). The uterine lavage samples were centrifugated at 16,000 g. The pellet was resuspended in phosphate-buffered saline and RLT Plus buffer (Qiagen) with Dithiothreitol. DNA and RNA extraction was performed with the AllPrep DNA/RNA Micro kit (Qiagen) according to the manufacturer's protocol. Universal prokaryotic Illumina Pro341F and Pro805R primers were used for 16S rRNA gene V3-V4 amplicon generation. For amplicon generation starting from RNA, first-strand cDNA was first synthesized using Pro805R primer. Amplicon generation was performed as previously described (Dyroff et al. SciRep 2025). The 16S rRNA gene V3-V4 amplicon products were confirmed by gel electrophoresis and the corresponding band isolated by gel purification. After barcoding, the 16S amplicons were sequenced on an Illumina Nextseq2000 platform (FGCZ).

Bioinformatic analysis will compare the microbial profiles between mares classified as fertile and subfertile, aiming to identify differences in microbiota composition, including alpha and beta diversity and statistical analysis of relative abundance.

We hypothesize that the analysis of the sequencing data will reveal a healthy core microbiome in the uterine samples of fertile mares compared to a disturbed microbiome in subfertile mares.

In conclusion, this study provides novel insights into the role of the 16S rRNA gene in the uterine microbiota of mares and its association with fertility. Differences in microbial composition may contribute to variations in fertility status among mares. Further research is warranted to investigate the mechanisms underlying these associations and explore potential applications of uterine microbiome analysis in reproductive management strategies for equine breeding programs.

This study was supported by the Swiss National Science Foundation (Project 200534).

7 Health assessment in pigs: is it influenced by the assessor?

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The valid, objective and repeatable assessment of animal health is essential to compare farms' health statuses over time and to evaluate the effect of management measures within a farm. For comparisons between different farms, it is also important that different assessors have high agreement, and that their assessments do not include major differences. In this study, we investigated these differences between different assessors.

With the Smart Animal Health method, a pig health assessment was established based on animal-based parameters (e.g. lameness, tail lesions, runts) and resource-based parameters (e.g. cleaning and disinfection, water supply). Overall, 57 parameters were allocated to five age categories (piglets, weaners, fattening pigs and gestating and lactating sows). This method was carried out on 94 farms by either one researcher or one out of 9 specialized pig veterinarians. The continuous and categorical results of the two assessor groups for different parameters were compared using the Mann-Whitney U test and chi-square tests, respectively.

Significant differences between assessor groups were found for the assessment of calluses (joints and shoulder) in weaners, fattening pigs, gestating and lactating sows (all $p < 0.001$). Also, the observed proportions of lame fattening pigs and lactating sows show significant differences, as the researcher assessed more lame animals in these categories ($p < 0.001$). For the categorical parameters, the cleanliness of the drinking stations in fattening pigs, gestating, and lactating sows was assessed differently in a way that the researcher counted more stations as dirty as the group of specialized veterinarians did ($p < 0.001$).

Most of the analysed parameters showed no significant difference between the two groups of assessors and the Smart Animal Health method could be carried out in practice independently of the assessor. For some parameters that either had a broader definition (callus) or were more subjective (cleanliness) a higher agreement might be reached by training.

8 Biosecurity and Animal Health Monitoring – Do we need both?

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Both animal health monitoring and the assessment of biosecurity provide important insights into potential weaknesses of pig farms. For Switzerland, comprehensive protocols and questionnaires available for both aspects are established in the field and tailored to local production conditions. In this study, a biosecurity questionnaire was compared with a health monitoring tool to determine their similarities, differences and relationships.

25 breeding and 15 fattening farms were visited and animal health was analysed using a wide range of parameters. The parameters belong to the Smart Animal Health method, which aims to assess animal health in a comprehensive, valid and practical manner. At the same time, a detailed biosecurity questionnaire of 83 questions on internal and external biosecurity was collected on these farms. The results of both methods were compared at farm level by Pearson correlation.

For the fattening farms, the animal contact rates within the farm and lack of cleaning and disinfection on the internal biosecurity side and the location, animal purchase and non-living disease transmission vectors on the external biosecurity side were rated the worst by the questionnaire. On the breeding farms, high internal and external biosecurity showed a weak positive correlation with good animal health on the farms ($r=0.12$ and $r=0.11$ respectively). On the fattening farms, both high internal and high external biosecurity showed a weak negative correlation with a good health monitoring result ($r=-0.23$ and $r=-0.02$ respectively). None of the correlation analyses were statically significant ($p>0.05$).

This study highlights two aspects of biosecurity and animal health in pigs. Firstly, both require their own appropriate recording methods and, although biosecurity can be an important risk factor, it does not fully explain animal health. Secondly, farm-specific biosecurity is the sum of various factors, some farmers can influence easily (animal contact) and others only with difficulty (location).

9 Influence of different sow and farrowing parameters on colostrum quality in a free farrowing system

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Colostrum is crucial for newborn piglets as both its quantity and quality are essential for their survival and production performance. A minimum intake of 200 grams of colostrum per piglet is recommended. Inadequate colostrum intake is considered a key factor affecting piglet health, mortality and growth rate

This study aimed to investigate the influence of various sow and farrowing parameters on immunoglobulin concentration. A total of 151 cross-bred sows (Swiss Landrace × Large White) housed in free farrowing pens were enrolled. Data collection included different sow and farrowing parameters. Before farrowing, parity, body condition score, backfat thickness, birth induction and gestation length were recorded. During farrowing, total number of piglets born (alive and stillborn), farrowing duration, obstetric intervention, placenta expulsion time, and litter and placental weights were documented. Postpartum, rectal temperature, vaginal discharge, feed intake, and number of treatments were recorded daily during the first week. Temperature and relative humidity were monitored during the entire study period.

Colostrum samples were collected immediately after the birth of the first piglet and stored frozen until analysis. IgG concentrations were estimated using a digital Brix refractometer (digital handheld pocket refractometer, MA871, Milwaukee Instruments) and quantified by ELISA (E101-104, Bethyl Laboratories, Inc.).

For the regression model, only IgG concentrations were used, as they represent the gold standard for assessing colostrum quality. A linear regression model was used to evaluate potential influencing factors, with log-transformed ELISA IgG concentrations as the outcome variable. Predictor variables for the full model were selected based on biological plausibility, descriptive statistics, and exploratory correlation analyses. The final model was defined via stepwise selection. It included total number of piglets born, parity, induction of farrowing, relative humidity before farrowing, and the interaction between parity and induction.

Brix values showed a strong positive correlation with IgG concentrations determined by ELISA (Spearman's $r = 0.66$, $p < 0.001$). The final model ($R^2 = 0.43$, $p < 0.001$) revealed significant effects of parity ($p < 0.001$), induction of parturition ($p = 0.001$), humidity ($p = 0.004$), and the interaction between parity and induction ($p = 0.032$) on IgG concentration. Higher parity was associated with increased IgG levels, suggesting a more mature immune response and stable colostrum production in older sows. Induced farrowing significantly reduced IgG concentration, particularly in gilts. This negative effect became less pronounced with increasing parity. A positive association between ambient humidity and IgG was observed, though its biological relevance is unclear.

In conclusion, this study demonstrates that parity, hormonal induction of farrowing, and environmental factors significantly influence colostrum IgG levels. The negative impact of birth induction underlines the importance of intensive birth management. On-farm colostrum assessment with the Brix refractometer is feasible and should be further integrated into sow and piglet management strategies in free farrowing systems.

10 Feeding value of high-cannabidiol (CBD) hemp flower residues in laying hens

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Circular agriculture is based on using waste from industrial processing in agriculture again. One relatively new such waste is the residue from extraction of cannabinoids, especially cannabidiol (CBD), from hemp. So far Swiss and EU feed laws prohibit feeding hemp flower to food-producing animals to prevent residues of tetrahydrocannabinol (THC), but also of CBD, in food. In addition, the feeding value of hemp residues is unclear. To investigate feeding value and potential THC and CBD residues, 45 72-weeks old Super Nick layers were kept individually in enriched cages at AgroVet-Strickhof and allocated to three groups (Control, Alfalfa, Hemp) in a 5-week experiment. Experimental feeds included a commercial compound feed (basal feed) designed for late-laying hens, included in all experimental diets, ground dried alfalfa and ground dried flower residue after CBD extraction from a variety of high-CBD-low-tetrahydrocannabinol (THC) hemp (Pure Holding AG, Zeiningen, Switzerland). In week 1 hens were familiarized to the green feed color by adding 10% alfalfa to the basal feed. The Hemp group was later familiarized with gradually increased dietary proportions of hemp residue. From days 26 to 37, the day the hens were slaughtered, Control hens received exclusively basal feed, Alfalfa and Hemp hens' basal diet were complemented with 15% alfalfa and hemp, respectively. Feed intake and eggs laid were recorded. Samples of feeds, diets, eggs, excreta, blood plasma and breast muscle were collected and analyzed. This included an LC-MS/MS analysis of CBD, THC and their inactive acid forms in hemp residue, plasma and muscle. Data were analyzed by the GLM procedure with SAS (version 9.4). Including 15% alfalfa or hemp in the diet had no effect on performance (feed intake, laying performance and feed conversion ratio) even though energy metabolisability of these two diets, compared to Control, was impaired. Egg yolks of the Hemp group were darker and redder than eggs of the Control group, and were intermediate with alfalfa. Alfalfa and hemp feeding had no effect on egg shell strength, proportions of yolk, albumen and shell as well as Haugh units, an indicator of processing quality of the albumen. The hemp residue contained substantial amounts of cannabinoids, where CBD concentration was clearly higher than that of THC (active and inactive forms). Cannabinoids were also detected in the plasma and in the muscle samples, with the concentrations being higher in plasma than muscle. The concentrations of CBD and THC in plasma and muscle were less than 1/10 respectively 1/100 of that in the diet with 15% hemp residue. Valued found in an extra group of five hens, receiving hemp only for 3 days and then alfalfa for a week, showed that most residues are excreted by a washout period. The results indicate that hemp flower residue has a feeding value similar to alfalfa, being superior to alfalfa in yolk coloration. Despite the limited transfer of cannabinoids, residues in body tissues can be detected. This may further restrict the use of hemp residues in food-producing animals.

11 **Seminal Exosomes: Potential vectors modulating sperm-to-sperm interaction for improving sperm quality and fertility**

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Cryopreservation in liquid nitrogen (-196 °C) remains the standard technique for the long-term storage of bovine semen in artificial insemination (AI) programs. Despite its widespread use, this process often causes substantial damage to sperm cells, including decreased motility, compromised membrane integrity, premature capacitation-like changes (referred to as cryo-capacitation), and disrupted molecular signaling pathways. As a result, approximately 15–20% of ejaculates are discarded by AI centers due to inadequate numbers of viable or motile sperm post-thaw (typically less than 40% in the total sperm population). While current cryopreservation protocols are generally effective, they do not sufficiently consider individual differences in sperm cryotolerance among bulls, which are likely influenced by variations in plasma membrane composition and cellular resistance to osmotic and thermal stress.

Recent studies have indicated that extracellular vesicles—particularly exosomes—within bovine seminal plasma may play a key role in protecting sperm cells during freezing and thawing. Additionally, heterospermic semen samples (those containing sperm from multiple sires) have demonstrated improved post-thaw fertility compared to homospermic samples (from a single sire); however, the biological basis of this effect remains poorly understood. Thus, our project is based on the hypothesis that seminal plasma exosomes (SE) from bulls with high cryotolerance (“good freezers”) differ in concentration, size, and microRNA (miRNA) content from those of bulls with low cryotolerance (“bad freezers”). Furthermore, we propose that we can improve sperm functional status after thawing by supplementing semen of bad freezers with SE collected from good freezers. The main objectives of this study are:

- a) To establish a model of good vs. bad freezers based on a combination of post-thaw sperm quality traits and the quantitative characteristics of exosomes identified in the seminal plasma
- b) To characterize the secretion/molecular content (miRNA and other small non-coding RNAs) of SE in sperm of good vs. bad freezers
- c) To characterize and compare the effects of whole seminal plasma, seminal plasma depleted of SE, and SE collected from good vs. bad freezers on fertility-relevant sperm quality characteristics and the developmental rates of *in vitro* produced bovine embryos
- d) To explore the role of SE in sperm-to-sperm interaction by comparing the sperm quality traits, the profile and miRNA cargo of SE in heterospermic vs. homospermic semen samples

The study will employ advanced techniques, such as computer-assisted sperm analysis and multicolor flow cytometry, for spermatological analysis. Following ultracentrifugation for SE isolation, the concentration, size and ultrastructure of SE will be assessed by means of resistive pulse sensing and transmission electron microscopy, respectively; their miRNA cargo will be explored through small RNA sequencing. Functional assessments of treated sperm and resulting embryo development will be carried out in AgroVet-Strickhof and during a research secondment at the University of Limerick, Ireland.

By identifying the role of seminal exosomes in sperm cryoprotection and intercellular communication, this research seeks to advance our understanding of male fertility and contribute to the development of more effective semen preservation strategies in cattle breeding.

12 Correctly dimensioning fermentation silos

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In addition to the silage conditions, the structural design is decisive for good quality grass and maize silage. A new information sheet summarizes the necessary information for planning tower and bunker silos. The corresponding calculation tool can be used to determine the appropriate dimensions of a silo system.

Experts from the Swiss Association for Silage Management SVS, AGRIDEA and the Swiss Working Group for Agricultural Construction ALB-CH compiled the information for the information sheet and the calculation tool. The year 2024 has shown how important the conditions during ensiling and the design of the storage facility are for quality and feed losses. In order to ensure sufficient compaction and the required feed rate, both forage cultivation and construction aspects must be considered.

Topics information sheet:

- Criteria for perfect silage
- Feed rate as a function of compaction
- How should the silage requirement and cut area be determined?
- Advantages and disadvantages of different ensiling methods
- What needs to be considered when dimensioning?
- Information on spatial planning, water protection and accident prevention

The calculation tool, associated with the information sheet, is used to determine the feed requirement; summer and winter feeding are calculated separately. Dry matter (DM) content, bulk density and feed residues can be varied. There is room in the reserve storage volume to allow for site-dependent yield fluctuations. The tool provides information on the required feedout face and storage space requirements. This results in diameter and height for tower silos and width and length for bunker silos at a certain silo height.

13 Skin bacteria as a shield from bluetongue?

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Biting midges are small blood sucking insects of major veterinary importance, as they can transmit pathogens from one infected animal to another. One such pathogen is the Bluetongue Virus, which has broken out in Europe in the last few years, affecting over 3,000 Swiss breeders in 2025. The absence control measures specific to biting midges is of great concern. It is known that insects like mosquitoes and biting midges, locate their hosts partly by detecting body odours. These body odours are largely produced by the skin microbiome, and different skin bacteria can affect the attractiveness towards a host. In our research project in collaboration with Agrovet Strickhof, presented by invited speaker Prof. Dr. Niels Verhulst, we isolate repellent bacteria from sheep, cultivate them in the lab, and produce a bacteria concentrate solution. After applying this repellent solution onto sheep, we could boost the presence of this naturally occurring bacteria and reduce by ~25% the number of bites by biting midges for multiple days. This poster introduces the spin-off "BiSafe", which aims to take this innovative and promising idea to the next step, by bringing it to the market. We highly acknowledge the Gebert RUF Stiftung and the Bundesamt für Lebensmittelsicherheit und Veterinärwesen, as sponsor of the National Center of Vector Entomology, for financing the research conducted thus far.

14 Genomic characterization of *Klebsiella* spp. from bovine mastitis: dissemination of a conserved, highly transmissible lac⁺ fec⁺ plasmid drives burden of disease

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Bovine mastitis poses a significant health concern for dairy cattle and a major economic burden on the dairy industry. *Klebsiella* spp. are important mastitis pathogens, with previous studies identifying lactose-utilization (lacacq) and iron-acquisition (fec) systems as key determinants associated with pathogenicity. To investigate how these are acquired and shared, we genomically characterized 60 mastitis-associated *Klebsiella* isolates by fully resolving their chromosomes and plasmids. The isolates were identified as *Klebsiella pneumoniae* (n = 46), *Klebsiella michiganensis* (n = 8), *Klebsiella quasipneumoniae* (n = 3), *Klebsiella grimontii* (n = 2), and *Klebsiella variicola* (n = 1). Phylogenetic analysis revealed diverse lineages, with sporadic transmission events within and across farms. Among unique isolates, 48/55 (87.3 %) and 53/55 (96.4 %) harbored lacacq and fec, respectively. In *K. pneumoniae*, the lacacq operon was consistently found on plasmids alongside fec. Both horizontal and vertical transfer of lacacq⁺ fec⁺ plasmids were observed. Many phylogenetically diverse mastitis isolates from distant farms carried an identical conjugative 132 kb plasmid, suggesting recent acquisitions of a single circulating plasmid as a major driver of mastitis. *K. pneumoniae* ST107, a globally prevalent mastitis-causing lineage, carried large non-mobilizable plasmids with fec and two lacacq operons. In contrast to *K. pneumoniae*, mastitis-associated *K. michiganensis* often carried lacacq on integrative conjugative elements and inherently harbored a chromosomal fec-like gene cluster. Few isolates possessed antimicrobial resistance genes or virulence factors linked to pathogenicity in humans. Our results provide new insights into the genomic diversity of mastitis-associated *Klebsiella* and the role of mobile genetic elements.

15 How does the supplementation of whey to a roughage-only diet affect ruminal methane formation, feed intake and blood parameters in suckler cows?

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Whey is the most important by-product of the dairy industry but disposal is problematic and expensive. Therefore, strategies on how to use the excess whey are needed.

Fresh whey consists primarily of water and lactose. As an easily fermentable feedstuff, whey might modify ruminal fermentation leading to decreases in enteric methane formation. In order to assess the potential of whey as a feedstuff, a study was performed at AgroVet-Strickhof in 2022/2023 on 27 non-lactating adult Original Brown Swiss suckler cows (mean 703±86.7 kg). The study aimed to investigate the methane mitigation potential of whey inclusion in a roughage-only diet. Additionally, dry matter intake, water intake, digestibility, rumination behaviour and blood parameters were assessed. In brief, different forms of whey (liquid, concentrate and powder) were supplemented at 2.3 kg DM/ day to a roughage-only diet (58% hay, 42% grass silage) for an adaptation period of 21 days followed by an experimental period of seven days. Data was analysed in mixed models.

Preliminary results indicate that the consumption of whey increased methane production and, to a lesser extent, methane output when corrected for dry matter intake. Dry matter intake increased with the supplementation of liquid whey and whey powder. Water intake was decreased when cows received liquid and concentrated whey but increased with supplementation of whey powder. Blood concentrations of β -hydroxybutyrate increased with supplementation of whey. Thus, our results suggest that whey did not mitigate methane. However, due to its high energy content, this finding might differ when correcting methane production for kg of energy corrected milk or body weight gain, parameters which were not assessed in our study. Furthermore, the effects on blood concentration of β -hydroxybutyrate deserve further investigation since some evidence suggests, ketone bodies might show potential health benefits in cows when increased by exogenous factors such as alimentation.

16 Sperm functional status: A multiparametric assessment of the fertilizing potential of bovine sperm

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Sperm viability is routinely assessed for the quality control of cryopreserved bovine sperm but is not always conclusive about their fertilizing potential after artificial insemination (AI). In this study, we investigated the fertility predictive value of bull sperm viability in combination with the DNA integrity as well as the functional status of viable sperm post-thaw. For this purpose, we examined two sets of 791 (set A) and 733 cryopreserved batches (set B) immediately after thawing (38 °C, 30 sec). For all batches, the non-return rate (NRR) after ≥ 100 first services was recorded as a measure of fertility after AI. Sperm viability was flow cytometrically assessed for set A and B as the percentage of plasma membrane- and acrosome-intact sperm (PMAI) after dual staining with propidium iodide and peanut agglutinin, respectively. The percentage of sperm with high DNA fragmentation index (%DFI) was determined for samples of set A with the Sperm Chromatin Structure AssayTM. The percentage of sperm with high esterase activity, intact plasma membrane and acrosome, low intracellular Ca^{2+} content and functional mitochondria ($C_{pos}PI_{neg}PNA_{neg}F_{neg}M_{pos}$) was quantified in set B with a five-color flow cytometric panel that included calcein violet, propidium iodide, the phycoerythrin-conjugated peanut agglutinin, Fluo4-AM and the cyanine stain DiIC₁(5), respectively. Using linear mixed effects models and conditional inference trees, we examined the predictive value of PMAI combined either with the %DFI or with the $C_{pos}PI_{neg}PNA_{neg}F_{neg}M_{pos}$ to predict the batch-specific NRR. Batches with %DFI $\leq 6.86\%$ were more likely to have a NRR $>60\%$, whereas %DFI values $<6.86\%$ were associated with 55%-60% or lower NRR ($P < 0.01$). In conclusion, combining PMAI with $C_{pos}PI_{neg}PNA_{neg}F_{neg}M_{pos}$ did not reliably predict the NRR of individual batches. However, the incorporation of DNA integrity assessment considerably improved sperm fertility prognostics.

This study was funded by the Dr. Dr. h.c. Karl Eibl-Foundation, Germany.

17 Viability of *Chlamydia abortus* in the environment – Lessons from an abortion storm at Frübüel

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Chlamydia abortus belongs to the most common causes of bacterial abortion in sheep and goats (ovine chlamydiosis), resulting in high rates of late-term abortion and stillbirths during the lambing season if newly introduced into a flock (abortion storms). This zoonotic pathogen may further cause miscarriage in women as well as pneumonia and sepsis in humans and has mostly been associated with direct animal contact. However, indirect infection through contaminated material such as during wool processing cannot be excluded as a possible route of infection as neither the minimal infectious dose nor the durability of *C. abortus* under different environmental conditions over time have been fully elucidated.

Here, we aimed to determine the viability of *C. abortus*-spiked placenta for up to one month if exposed to 0°C, 12°C and 20°C, using infectious doses that correlate with the expected chlamydial burden of placentas post-abortion. Viability was assessed by enhanced cell culture techniques. Samples were considered viable if *C. abortus* was successfully isolated by the end of its third passage. We found that 20°C drastically reduced chlamydial viability over time with no detectable *C. abortus* after the one-month period. In contrast, *C. abortus* remained viable for at least one month if large amounts were exposed to 0 °C or 12 °C.

Our study further included the investigation of increased abortion rates at AgroVet Strickhof Frübüel in February and March 2025. Out of 22 pregnant ewes, four showed abortion (18.2% abortion rate) and four gave birth to smaller or weak lambs, while the rest had normal births. Where possible, placentas were collected and further processed by real-time PCR and viability assessment in cell culture. In total, fifteen placentas from aborted lambs (n=4, from three ewes), weak/smaller lambs (n=3, from two ewes) and healthy lambs (n=8, from eight ewes) were subjected to additional testing. So far, we have detected *C. abortus* in all samples, with varying loads ranging from cycle threshold (Ct)-values of 30.6-43.4 for healthy placentas, 29.8-34.5 for weak/smaller lambs and 13.6-25.1 for aborted placentas. Preliminary data from incubation at 12 °C and viability testing of *C. abortus* in placental samples indicate that the bacterium remains equally or even more viable in naturally infected placentas compared to spiked samples, for up to one month. Overall, these findings demonstrate that *C. abortus* remains infectious for extended periods under cold conditions, which highlights a sustained risk of transmission. Furthermore, the abortion storm at Frübüel further showed that the birth of healthy lambs does not exclude the possibility of viable *C. abortus* present in their placentas if ovine chlamydiosis is diagnosed in sheep of the same flock.

18 Researching PMSG: to find a standardized, animal-friendly alternative

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The hormone pregnant mare serum gonadotropin (PMSG), also called equine chorionic gonadotropin (eCG), is produced by the endometrial cups in the placentas of mares during early pregnancy. This unique gonadotropin exhibits both luteinizing hormone (LH)-like and follicle-stimulating hormone (FSH)-like effects in non-equid species, while in equines, it functions solely with LH-like activity to provide hormonal support for gestation.

PMSG is widely used in livestock farming to synchronize reproductive cycles and induce ovulation (across species like cattle, pigs, sheep.), as well as in transgenic rodent research for creating chimeras and developing new mouse models. However, the global reliance on PMSG has raised ethical and animal welfare concerns because it is exclusively sourced from the blood of pregnant mares—a practice often associated with severe health risks and high mortality rates for the animals.

The use of PMSG has sparked calls for a ban on its importation at the EU level. Although animal-free alternatives for PMSG in farming have been explored, but currently there are none available that are both effective and economically viable.

Our goal is to deepen the understanding of natural PMSG and advance the development of in-vitro produced eCG as a sustainable, animal-free substitute. This would enable the complete replacement of “horse blood PMSG” in the future, safeguarding the welfare of horses while ensuring a standardized production method for this critical hormone.

19 Validation of a standardised method to measure the ammonia emission reduction potential of stable floors

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Reducing ammonia emissions (NH₃) on farms protects the environment (less soil acidification, less eutrophication) and is beneficial to human and livestock well-being (less particulate matter pollution, less irritation to the respiratory tract; Amon et al., 2021). NH₃ is predominantly released when urine is exposed to urease producing faecal bacteria on stable floors. Different types of flooring are known to influence emissions but comparing the respective merits of various floors in on-farm experiments is difficult due to various microclimatic effects (e.g., puddling, temperature variations, wind speed) on top of biotic influences (animals, microbiota; Elzing & Monteny, 1997). Müller et al. (2025) developed a standardised procedure to measure emissions under controlled conditions, in respiration chambers for medium-sized animals at AgroVet-Strickhof (Lindau). Since only concrete floors were challenged in the initial study, the suitability of the outlined protocol to compare concrete floors with other types of floors still needed scrutiny. To this effect, 1.5 kg fresh dairy cow faeces were deposited on a rubber mat floor in a respiration chamber and left to acclimatize for 20 minutes before 1 L urine was poured over it. We measured the resulting NH₃ emissions and compared them with emissions from an identical excreta sample deposited in the same way on a concrete floor in another respiration chamber. Two different mat types (mat A and B, 3 repetitions/mat) were tested in this pilot study, at average temperatures of 24.5 ± 0.19 °C and a relative humidity of 61.2 ± 2.67%, for 24 to 144 hours.

Despite a large variability in maximal emission rates between runs (2.2 to 4.9 mL NH₃/minute), the data displayed clearly differing, consistent emission patterns between floor types. On the concrete floor and rubber mat A, for instance, emissions started with some delay and peaked 24-36h after the urine was poured onto the faeces. On rubber mat B, the emissions increased right away and peaked a lot earlier (within the first 6-18h). While critical fecal and urinary parameters should be identified and standardised to improve the long-term reproducibility of such measurements, the method appears inherently suited to compare rubber and concrete floors and explore ways to better use or clean such floors to reduce on-farm NH₃ emissions.

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20 Growth rate does not affect enamel quality in rabbit incisors

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Studies agree that the growth rate of hypselodont teeth, as those from the rabbit, adapts to wear (Müller et al., 2014). Unexplored, however, is whether a change in growth rate also alters tooth quality. This experiment therefore assessed enamel quality in rabbit incisors during long-term exposure to high wear and thus high growth rate.

This study was approved by the Cantonal Veterinary Office in Zurich, Switzerland (No. 35593; ZH041). Twelve intact female rabbits were randomly divided into two groups and managed identically. For the entirety of the study, all rabbits received the same hay for ad libitum consumption (type GR, stage 5 (Daccord et al., 2021)). The left incisor pair was cut eight times in all animals to prevent return to occlusion and maximize growth rate. Cut tooth samples were stored in tap water at room temperature prior to analysis. Enamel thickness was measured by microCT, and hardness using a 1600–6106 hardness tester. Enamel abrasion resistance was determined with a Perthometer S2 before and after automated brushing. Analysis was conducted in R v. 4.4.1 using linear mixed models.

No veterinary treatments were ever considered necessary. A pinkish discoloration, predominantly in the lower incisors was, however, noted. The analyses detected no significant change in abrasive resistance or hardness over time ($p = 0.15$ and 0.07 , respectively), between mandibular and maxillary incisors ($p = 0.86$ and $p = 0.16$) or when taking these two fixed factors together. No change in enamel thickness was noted over time ($p = 0.99$).

The results show that a high growth rate likely does not affect enamel quality of rabbit incisors. Given that growth could not compensate completely for wear induced by cutting, we suggest that maximum growth rates were reached. We hypothesize, that their limit is set by enamel quality, which is therefore not compromised. A reduced time for dentine deposition could render a larger pulpal cavity and explain the noticed pinkish discoloration. As this was not assessed, it, however, remains hypothetical.

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21 Farm structure, diet formulation and their relation to dairy herd health and productivity: a survey of the current situation in the DACH-region.

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In 2023, the average Swiss dairy cow produced 7'131 kg milk per annum for human consumption, lying in between both averages from Austria and Germany (6'775 and 9'161 kg respectively; Eurostat, 2024). This average milk yield is, however, greatly influenced by regional differences. Not including the summer pasturing farms, approximately 40% of Swiss dairy cattle are kept in mountainous areas and produce 25 % less milk per year for human consumption, compared to those kept in valleys (BLW, 2025). According to the Swiss Regulation of 7 December 1998 on the Agricultural Production Register and the Designation of Zones (Landwirtschaftliche Zonen-Verordnung, SR. 912.1, Art.2) as in force from 01.01.2025, these areas are primarily differentiated based on vegetation times. Therefore, roughage dissimilarities, assuming local vegetation is the bulk of the ration, are likely a major influence. Nevertheless, further environmental, management, dietary, and genetic factors can all play a role and might interact (Silva Neto et al., 2024). However, it remains unknown to what extent these factors differ between farms and regions.

This project therefore aims to gain more insights, using a two-section survey of 28 questions.

The first section collects data on location, farm logistics, herd health and milk performance test values, whereas the second documents diet composition and methodology of ration formulation. Data collection occurs from August to October 2025 in three languages (German, French, and Italian) and three countries (Switzerland, Germany, Austria), through national associations, various scientific institutions, magazines and social media. Results will be analyzed using generalized linear mixed models (SAS 9.4). Significance is assumed at $p < 0.05$ but might be adjusted depending on the obtained data structure and associated effect sizes.

It is hypothesized, that health and production parameters are linked to topographic, structural and dietary differences with noted interactions between the latter (i.e. structural and dietary differences would change according to region). Similar tendencies are expected in Switzerland, Austria and Germany.

The knowledge gained from this survey will provide insight into the status quo, define research priorities in dairy cow nutrition and assist consulting services in their support of farmers throughout the DACH region and beyond.

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22 Comparison of three methods for estimating dry matter intake from pasture in dairy cows at different grazing durations

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More than 90% of dairy cows in Switzerland have access to pasture during the vegetation period, while daily grazing duration varies depending on the farm, region and season (Kupper et al., 2022). However, it is very challenging to quantify the herbage dry matter intake (hDMI) from pasture.

The aim of this study was to estimate the hDMI of lactating dairy cows from pasture at three typical grazing durations (2h, 8h and 16h per day) using different methodologies: 1) The RumiWatch System (**RWS**; Itin and Hoch GmbH, Liestal, Switzerland) to collect data on the feeding and rumination behaviour and subsequently utilizing them with the two equations described by Schori et al. (2020). 2) The net energy balance (**NEB**) to calculate the theoretical hDMI based on parameters such as body weight, milk yield and milk composition, as well as energy intake by partial mixed ration (PMR) and concentrate and energy content of the herbage, following equations described by Agroscope (2021). 3) Quantification of herbage mass by sward surface height before and after grazing using an electronic raising plate meter (Jenquip, Feilding, New Zealand) followed by a regression analysis (**Herbometer**).

40 Holstein-Friesian and Swiss Fleckvieh lactating dairy cows (on average 158 days in milk; mean milk yield: 33 kg/day) were divided into two groups of 20 cows each and housed in separate compartments. The cows were fed a PMR and were supplemented with concentrate to meet their energy and protein requirements. One group additionally had access to pasture with different grazing times: 2h, 8h or 16h per day, corresponding to the three different measurement periods. The experimental design was a crossover, meaning that after the three grazing periods, the groups switched compartments, ensuring that both groups underwent each grazing period. One measurement period lasted six consecutive days for each grazing time preceded by an adaptation phase. All cows were equipped with a RWS noseband sensor, which recorded their chewing activity continuously. The experiment was conducted in early summer and repeated in late summer.

With a 2h grazing duration, the hDMI was 1.3 and 1.6 kg per day according to the NEB and the herbometer method, respectively. Meanwhile, hDMI calculated with the two RWS equations were -10.0 and -14.0 kg per day. With 8h grazing, the estimated hDMI was 2.1 and 2.3 kg per day based on the NEL and herbometer methods, respectively, while the RWS approach yielded -7.0 and -9.1 kg per day with both equations. Finally, with 16h grazing, the three methods produced more consistent estimates: 4.6 kg per day with the NEL and herbometer methods, and 3.5 and 4.0 kg per day with the RWS equations. Our findings show that the RWS method is not applicable when cows graze for shorter times like 2h or 8h per day. This can possibly be explained by the fact that the equations are based (Rombach et al., 2019) and validated (Schori et al., 2025) on studies with cows that grazed from 16h to 19h.

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23 Food waste in the supply chain of bovine meat in Switzerland

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Food waste corresponds to material originally intended for human consumption that ultimately does not serve as food. This may occur due to overproduction, technical harvest losses, cosmetic imperfections, or too high quantities purchased and cooked, for instance. These losses accumulate along the supply chain to quantities with significant environmental impacts. First, to produce these materials, greenhouse gases are usually emitted, making food waste reduction relevant for climate change mitigation. Second, biodiversity losses are related to many types of food production, and third, water resources are consumed and sometimes polluted due to food production. In response, the Swiss Federal Office for the Environment has made an action plan to reduce food waste in Switzerland aiming to halve avoidable food losses by 2030 compared to 2017. Although meat represents a relatively small proportion of food waste by mass, it is disproportionately important in terms of greenhouse gas emissions and other negative environmental impacts of production. This study presents a Material Flow Analysis of the Swiss bovine meat sector, with a focus on food losses along the supply chain. The analysis covers the supply chain stages primary production, processing and manufacturing, retail and other distribution of food, and consumption in households and out of home. The contribution first outlines a definitional framework to transparently delineate the scope of bovine meat stages included in the analysis. Then, for one specified year, the quantities of domestically produced bovine meat, imports and exports of bovine meat, and the shares of bovine meat lost at each stage of the supply chain are calculated based on production statistics, trade statistics, and published findings on food waste rates. A visual representation of the material flows will be provided to highlight critical processes of food loss and identify leverage points to support policy and industry interventions. As the analysis is ongoing, specific figures are not yet available but will be presented at the conference.

24 AGRIDEA Climate Platform – your central hub for all key topics on agriculture and climate change

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The AGRIDEA Climate Platform on Agripedia offers clear, practical, and scientifically grounded knowledge on the complex relationship between agriculture and climate change. It provides a structured overview of key topics—from the scientific foundations of global warming and greenhouse gases to practical advice and support services. Designed to bring clarity to a multifaceted issue, the platform presents content in a user-friendly, evidence-based, and independent manner. It consolidates essential knowledge on climate change, greenhouse gas emissions, impacts, mitigation measures, and adaptation strategies, and links to key actors and initiatives from the federal government, cantons, industry, and other stakeholders. A project search tool highlights diverse cantonal efforts to foster sustainable and climate-resilient agriculture at regional and national levels.

Looking ahead, the platform will be further developed with new content and features. A dedicated exchange space for agricultural advisors is planned to facilitate knowledge sharing and networking. An interactive tool will support the selection of effective mitigation and adaptation measures, including options for site-adapted livestock farming, and will provide insights into expected impacts and co-benefits. The platform's ongoing development is guided by feedback from the Agricultural Innovation and Knowledge System (LIWIS), ensuring that it remains relevant, applicable, and grounded in practice.

25 Extracellular Vesicle Cargo Modulation after LPS Stimulation in FBS-Free Cultured Mammary Epithelial Cells

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Mastitis remains one of the costliest diseases in dairy farming worldwide. In addition to significantly reducing milk yield and quality, it imposes further costs such as an increased workload, discarded milk, veterinary costs, antibiotic treatment and premature culling. Extracellular vesicles (EVs) are lipid bilayer particles that carry functional molecular signatures from their cells of origin and are released into both the microenvironment and systemic circulation. EVs are key mediators of intercellular communication and are increasingly studied as potential disease biomarkers and therapeutic agents. Mammary gland-derived EVs are involved in both physiological and pathological processes. However, their role in bovine mammary epithelial cells (MEC) remains underexplored. The reason for this is partially due to challenges of *in vitro* systems *per se*, where foetal bovine serum (FBS)-derived EV contamination can confound results, underscoring the value of serum-free primary MEC cultures for studying MEC-derived EVs (mecEVs). In this study, we assessed the miRNA and proteomic cargo of mecEVs following stimulation with lipopolysaccharide (LPS) using an FBS-free culture system. First, we confirmed the transcriptomic response of FBS-free cultured MECs to LPS, observing differential expression of over 4,000 genes associated with inflammatory processes. We then isolated mecEVs using a combination of ultrafiltration, ultracentrifugation and size-exclusion chromatography and characterised their morphology, particle size, and concentration. No significant differences were observed between control and LPS-treated mecEVs in terms of particle concentration, mean diameter, or zeta potential, indicating that LPS treatment did not alter the physical properties of the EVs. Finally, we analysed the protein and miRNA cargo of mecEVs post-LPS stimulation. While the overall cargo was largely similar between conditions, we identified 16 differentially expressed miRNAs (FDR < 0.05) and 103 proteins with increased abundance. These proteins were primarily involved in wound healing, complement and coagulation pathways, cell adhesion, and proteasome, suggesting an active role for mecEVs during bacterial infection. Future experiments will explore the functional impact of these mecEVs on surrounding mammary epithelial cells.

26 Is a closer look always better? Evaluating scale and resolution in animal tracking studies

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Global positioning systems and remote sensing are available for some decades. Today, animal tracking and subsequent inference using spatial data sets has become standard in both grazing experiments and real-world farming. Over the years, energy efficiency and temporal frequency of tracking devices, the grain size of spatial data as well as computing techniques for their analysis have greatly improved. As technical restriction is falling as a limit of resolution, the question arises, which spatial and temporal resolution should ideally be used for animal tracking. Does a higher resolution necessarily provide better answers to the questions we are asking? What is the appropriate scale for the phenomena of interest and the uncertainty associated with them? To answer these questions, we analyzed GPS tracking data of suckler cows grazing on the heterogeneous mountain rangelands of AgroVet Strickhof mountain research station Alp Weissenstein. We applied two state-of-the-art statistical models: The first model is a log Gaussian Cox process (LGCP), which describes the observed distribution of steps in space and time. The second is a recently developed Gaussian field integrated step selection analysis (GF-iSSA), which evaluates properties of the selected location in each timestep given the area available to the animal in that timestep. To investigate effects of data resolution, we resampled the movement tracks to frequencies between 20 sec and 1 hour. Environmental covariates terrain slope and vegetation quality were resampled to grain sizes of 2, 10 and 20 meters. We found that the LGCP models were largely unaffected by temporal resolution, which was more important for the GF-iSSA models. Spatial resolution of covariance was important for both models and using too coarse grain drastically changed some of the estimated effects. We conclude that sampling effort should rather go into high-resolution co-variates than in high-resolution tracking data.

27 Establishing a multicolor flow cytometry panel for the quality control of cryo-preserved mouse sperm

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Cryopreservation of sperm provides a reliable means of preserving genetic material of valuable genetically modified mouse lines. However, prior to discontinuing the breeding, it has to be ensured that the sperm have survived the freezing-thawing process and retained the ability to produce well-developing embryos. Currently, *in vitro fertilization* (IVF) is routinely used to evaluate the fertility of cryopreserved mouse sperm, but this practice conflicts with the 3Rs principles for better animal welfare, as it requires superovulation and euthanasia of females for oocyte retrieval. As an alternative, we could use methods that assess multiple functional traits of sperm cells simultaneously. The present study aimed to establish a five-color flow cytometric assay as a quality control tool for cryopreserved mouse sperm.

Spermatozoa were collected from C57BL/6J males and cryopreserved with the Jax Sperm Cryo Kit. After thawing at 37°C for 10 minutes, sperm was diluted to a concentration of 1×10^6 cells/mL with pre-warmed (37°C) modified Krebs-Ringer's bicarbonate medium. 10 µl of diluted sperm were mixed with a staining solution to a final volume of 150 µL in a 250-µL reaction well of a 96-well plate. The fluorescent panel included Calcein Violet (1.21 µM), Fluo4-AM (2 µM), the phycoerythrin-conjugated peanut agglutinin (PE-PNA; 1 mg/mL), propidium iodide (2.99 mM) and MitoProbe™ DiIC₁(5) (0.015 µM) for the assessment of esterase activity, intracellular Ca²⁺ levels, acrosome and plasma membrane integrity, and mitochondrial membrane potential, respectively. The stained samples were incubated at 37°C for 15 minutes before analysis using a CytoFlex flow cytometer equipped with a 405 nm, a 448 nm and a 638 nm laser (Beckman Coulter Inc., Nyon, Switzerland). The bandpass filters 450/45 (violet laser), 525/40, 585/42 and 690/50 (blue laser) and the 660/20 (red laser) were used to capture the fluorescent signals of calcein violet, Fluo4-AM, PE-PNA, propidium iodide and DiIC₁(5), respectively. Targeted cells were calcein-positive, Fluo-4 AM-negative, PE-PNA-negative, propidium iodide-negative, and DiIC₁(5)-positive. Using this panel, we were able to identify the populations simultaneously exhibiting up to five functional features. The gating strategy was optimized to accurately distinguish sperm cells from debris based on their scatter properties.

Preliminary results indicated distinct cell subpopulations for each marker, demonstrating the panel's ability to detect the functional heterogeneity of mouse sperm populations. This method is a promising tool for high-throughput assessment of mouse sperm with the potential to reduce the use of live females for *in vitro* fertilization assays.

28 Comparative uterine microbiome analysis of healthy and metritis dairy cows to identify protective bacteria strains for microbiome-based metritis prevention strategies

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The uterine microbiome (UM) is one of the key drivers for uterine involution and health of postpartum dairy animals. If dysregulated, this leads to uterine disease like metritis, which is a frequent and serious postpartum disease in cows and causes substantial economic losses to the dairy industry. Microbiome-based manipulation solutions for preventing metritis can enhance dairy cow reproductive performance and also reduce antibiotic treatments, residues in meat and milk or resistance in farm animals.

This study aims to isolate commensal uterine bacterial strains from healthy dairy cows that are protective against metritis. Obtaining such isolates may be the basis for the development of probiotic-based strategies for preventing metritis through UM manipulation.

At Agrovet Strickhof Lindau 30 dairy animals after birth were selected. Clinical examination, vaginal discharge scores, uterine swab sampling, and culture were performed 3 and 10 days postpartum (DPP). At 10 DPP, the animals were classified as “healthy controls” (C, n=16) or “clinical metritis” (M, n=14) as previously reported (Sheldon et al. 2006). M animals had increased, malodorous vaginal discharge and a delayed uterine involution. Uterine swabs were used for full-length 16S rRNA sequencing to determine UM composition. UM-associated bacteria were isolated using routine bacterial culture methods and identified by MALDI TOF MS.

On clinical examination, C animals had a significantly lower rectal temperature than M animals. On the vaginal examination, the group M showed a more severe alterations in the lochia than the C cows. UM sequencing revealed that C animals harbour higher bacterial species diversity than M animals. Comparing the UM content of the high abundance bacterial taxa, showed that compared to M group, the UMs of C animals contained higher relative abundance levels of Firmicutes and Proteobacteria, and low levels of Bacteroidetes, Actinobacteria and Fusobacteria, which include major metritis pathogens *Trueperella pyogenes* and *Fusobacterium necrophorum*, respectively. The content of several bacterial genera and species significantly differed between the UMs of C and M animals. At 3 DPP, several genera (e.g. *Ruminococcus* and *Acinetobacter*) and species (e.g. *Staphylococcus chromogenes* and *Streptococcus pluranimalium*) of non-pathogenic uterine commensals were more abundant within C than M cow UMs. At 10 DPP, on the other hand, the UMs of M animals displayed increased abundance of genera (e.g. *Trueperella* and *Fusobacterium*) and species (e.g. *Trueperella pyogenes* and *Fusobacterium necrophorum*) of the common opportunistic metritis pathogens. Comparing the UMs further identified some bacteria taxa that might serve as potential biomarkers for differentiating healthy and metritis bacterial composition at 3 and 10 DPP. Finally, several species of uterine commensals species with potential metritis-protective antimicrobial activity identified by UM sequencing were recovered through culture from the uterine swabs.

UM analysis comparisons have enabled the identification and isolation of bacterial strains from healthy dairy animals postpartum that may have potential as probiotic bacterial strain candidates for preventing metritis.

29 INVESTIGATING PIG HERD HEALTH IN SWITZERLAND USING SMART ANIMAL HEALTH PARAMETERS

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Background and Objectives Monitoring and documentation of pig herd's health is an important part of veterinary service but also crucial for the pig farmers and interested parties. Monitored parameters must be both reliable and meaningful and recorded consistently. For Switzerland, such parameters and their threshold values have been specified recently (Smart Animal Health, <https://doi.org/10.3389/fvets.2023.1125806>). In this work a selection of these parameters was applied in order to describe health of pig herds in Switzerland in 2023. **Material and Methods** Data from 1456 pig herds (29% of total herds) were descriptively analysed using the thresholds (target and alarm values) of selected parameters. **Results** In 10% of the herds no alarm values were observed; 60% had 1-2 and 30% of the herds had >2 alarm values. The two most frequently achieved alarm values were management related ("AI/ AO", "Cleaning and disinfection"). In the herds, 3-28 target values/ herd were achieved. Overall, the two most frequently achieved target values were health related ("Lameness suckling piglets" and "Treatment index fatteners"). **Discussion and Conclusion** Assuming that the analysed herds and the indicators provide a proxy to pig's health, the pig herd health in Switzerland was decent. Most of the herds had <3 alarm values which was considered as biologically acceptable. Furthermore, the most frequently achieved target values were related to health and (low) antimicrobial use. However, there is room for improvement which could e.g. be achieved by veterinary support of herds with alarm values. A weakness of the analysis was the lack of data so that not all relevant parameters could be described in all herds. The analysis provides a first insight in the frequency of specific aspects of pig herd health in Switzerland. The continuous collection and analysis of the data is a valuable tool to describe herd health, observe trends and to compare health among herds.

30 Development, digestive anatomy and physiology of calves weaned on hay or concentrate diets

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Ruminants are evolutionarily adapted to utilize forages as their primary energy source. However, modern production systems often rely on high-starch diets, considered to enhance growth performance and gastrointestinal tract (GIT) development. We evaluated the effects of a high-starch concentrate versus a hay-only weaning diet on feed intake, body mass and average daily body mass gain (ADG), GIT development, digesta characteristics, and nutrient utilization in early-weaned calves. 48 newborn calves from three batches were randomly allocated to two treatments and fed milk replacer with ad libitum access to either chopped hay (HAY; crude protein 18.2% and NDF 41.9% dry matter) or a high-starch concentrate mixture with only 5% chopped hay included (STARCH; crude protein 18.0% and NDF 23.1% dry matter). Ad libitum access to the solid diet was continued for two weeks after weaning (at 9 weeks of age) during a digestibility and retention time study. Afterwards, the animals were slaughtered 2h after feeding, and various characteristics of their GIT were documented. Groups were compared using mixed models that accounted for individuals and calf batch for repeated measures, and only for batch in single measures.

Body mass and solid feed intake increased significantly over time but did not differ between groups. STARCH showed, at a similar dry matter intake, a higher dry matter digestibility and longer mean retention times for a fluid, a small particle and a large particle marker, whereas the ratio of small particle to fluid retention in the reticulorumen was higher in HAY.

At slaughter, there was no body mass difference between the groups, but STARCH had a higher empty carcass mass (68.43 ± 9.05 kg vs. 59.01 ± 5.92 kg in HAY). HAY had more digesta in the reticulorumen and the caecum, but not in any other GIT sections. GIT tissue mass was higher in STARCH for the reticulum and the caecum, and lower for the abomasum, but not different for other GIT sections – importantly, not for the rumen, the omasum nor the small intestine. The pH of rumen and caecum contents were higher in HAY (6.79 ± 0.23 and 7.26 ± 0.14 vs. 5.62 ± 0.22 and 6.43 ± 0.20 in STARCH, respectively). The spleens of STARCH were larger (379 ± 75 g vs. 276 ± 27 g in HAY).

These results indicate that early-life concentrate feeding need not be advantageous in terms of growth and GIT development if high-quality forage is available. Consequences of low digesta pH, especially in the rumen, for health later in life remain to be investigated.

31 Detection and isolation of *Chlamydia suis* in pig feces and manure

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Chlamydia suis is the only known chlamydial species to have acquired a tetracycline resistance gene, specifically the *tetA(C)* gene. Found abundantly in pigs, *C. suis* typically causes inapparent intestinal infections, though there is association with clinical signs such as conjunctivitis and diarrhea. While the abundance of *C. suis* in conjunctival and rectal swab samples of pigs is already known, this study aims to assess its occurrence and viability after shedding, as well as to look for the presence of *tetA(C)* in these *C. suis* strains. For this purpose, three types of samples representing different stages of manure processing were collected: fresh fecal samples from old weaners or pigs at the beginning of the fattening period, slurry from channels where manure is stored for up to two weeks and manure from silos where it is stored for several months before field application. Across Switzerland, samples were collected from 20 conventional and 10 organic farms. Using real-time PCR (qPCR), the presence of *C. suis* was confirmed in all farms ($n = 30$) with 93% ($n = 84$) of all samples testing positive. To assess pathogen viability, all qPCR-positive samples are isolated in cell culture and investigated by viability PCR (v-PCR). While cell culture remains the only established method to confirm viability, v-PCR has been considered as a potential alter-native or supplementary method to determine bacterial viability. For v-PCR, samples are pre-treated or not with propidium monoazide (PMA) and exposed to a light source, which results in covalent binding to free DNA. Consequently, bacterial cells that lost membrane integrity ("dead") are bound by PMA prior to DNA extraction, and only the DNA of bacteria with intact membranes is obtained for subsequent qPCR analysis. Here, subsamples for v-PCR and isolation are obtained and processed in parallel. If v-PCR yields reliable results, this method could replace the labor-intensive and time-consuming process of isolation by cell culture. Preliminary data demonstrate the viability of *C. suis* at all three stages of manure processing (feces, slurry channel and manure silo, respectively). For the detection of *tetA(C)* gene, a specific, conventional PCR will be performed on both native samples and isolates. Investigation of individual isolates is necessary considering that *tetA(C)* gene, albeit comparatively less frequent than other *tet* genes, is not exclusive for *C. suis*. Moreover, the study will compare whether the type of husbandry (conventional and organic, respectively) influences the occurrence of antibiotic resistance as well as the viability of this opportunistic pathogen. Overall, this study demonstrates the high abundance of *Chlamydia suis* in Swiss pig farms. It has so far confirmed the survival of *C. suis* throughout the manure processing chain, including in long-term storage in manure silos. The risk of spreading viable, tetracycline-resistant *C. suis* on the field is therefore not eliminated by current manure management practices.

32 Suckling piglet monitoring – a retrospective study on mortality in the farrowing unit

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Since 2007, only free farrowing has been used in Switzerland. According to Suisag, characteristics such as piglet rearing rate and breeding on 8/8 teats are crucial. Consequently, pre-weaning mortality (PWM) is below 10% in piglet production (the best quarter even at 9.3%; Suisag 2023). Efforts are continuously being made to reduce PWM through research and the formulation of new industry guidelines. For economic reasons, it is crucial for pig farmers that piglet losses are reduced. For that reason, the piglet welfare and the sow performance must be taken into account (Koketsu et al. 2021). Influencing factors are litter size, the vitality of the piglets at birth, the behavior of sows and the quality of colostrum, as well as the design of the farrowing pen and husbandry during the first days of the piglets lives. The first few days are particularly crucial for the survival rate (Tuchscherer et al. 2000; Tucker et al. 2021) and monitoring is essential in order to be able to make optimizations immediately. For example specific supplementary feeding or split nursing could be used for early fostering.

In the pig barn at AgroVet-Strickhof (Lindau, Switzerland), the pen systems used are stocked alternately in a three-week cycle. One is a single-area pen and the other is a two-area pen with separate lying and dunging areas and piglet nests arranged to the side or front respectively.

The litter data from 2020 to 2024 will be analyzed retrospectively. A total of 874 litters are included, with an average of 14.1 (± 3.4 SD) live births per litter and 11.2 (± 2.3 SD) weaned piglets per litter with 8.4 kg body weight (± 1.6) at weaning. The two pen types are compared, seasonal fluctuations, breed effects, and structural adjustments are also taken into account in order to examine PWM. Subsequently, specific recommendations can be made to optimize piglet management.

Contrary to expectations, initial evaluations indicate that pen types have no influence on PWM over the years. The seasonal effect, correlation analyses of mortality causes and number of litters per sow will be examined in the next step as well as a comparison to Swiss and global production, including the benchmark.

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33 Impact of 3-nitrooxypropanol and whey on technological properties of milk and cheese in Holstein cows

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Feeding 3-nitrooxypropanol (3-NOP) is known to reduce methane emissions in dairy cows; however, its impact on milk's technological properties remains largely unexplored. Previously, the inclusion of whey in dairy cow diets supplemented with 3-NOP has been shown to enhance methane mitigation effects in vitro. This study aimed to investigate the effects of 3-NOP, with or without dietary whey powder, on milk composition and cheese-making quality in Holstein cows.

A 2×2 factorial design was used in a 15-week trial involving 24 cows. Animals were assigned to one of four dietary treatments: a basal total mixed ration (BAS) or a whey-supplemented ration (WHM; 15% DM), combined with either 3-NOP (80 mg/kg DM) or a placebo (PCB). Milk evaluations and cheese-making trials were conducted in weeks 9 and 13. Morning and evening milk samples were collected over two consecutive days, pooled daily by treatment, thermized, and processed into Appenzeller-type cheeses, resulting in 16 cheeses.

Milk pH was recorded before rennet addition, and cheese pH was measured at 2, 4, and 24 hours post-coagulation. Cheeses at 24 hours were analyzed for yield, galactose, and lactic acid content. After 120 days of ripening, a trained sensory panel conducted a blind descriptive sensory analysis, complemented by fatty acids profile analysis. Data were analyzed using mixed models in R, including 3-NOP, whey, their interaction, period, κ-casein variant (AA, AB, AE, BE), and cow as a random effect.

Results indicated that 3-NOP had no effect on milk clotting parameters (r, k20, A30) or on fat, protein, and urea content, but it significantly reduced lactose concentration (−3.3%, $P=0.04$). In contrast, whey supplementation increased milk lactose content (+3.8%, $P=0.01$) and tended to improve cheese-making potential by reducing k20 ($P=0.06$) and increasing A30 ($P=0.09$). Notably, cheeses from cows fed the BAS + 3-NOP diet were perceived as more bitter ($P=0.02$) and showed the highest levels of aroma defects ($P=0.10$), compared to the BAS PCB group. However, these sensory defects potentially determined by 3-NOP appeared to be masked when whey was included in the diet (WHM). This study offers new insights into how 3-NOP and dietary whey inclusion influence milk composition and cheese-making quality, with potential implications for methane mitigation strategies in dairy production.

34 AgroVet-Strickhof as a Compartmentalized One Health Ecosystem for AMR Research

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One Health villages are increasingly used to study antimicrobial resistance (AMR) at the interface of humans, animals, and the environment. However, real-world communities are difficult to control, often heterogeneous, and subject to confounding social, ecological, and economic factors. The AgroVet-Strickhof campus provides a unique alternative: a compartmentalized One Health ecosystem that mirrors the complexity of a rural community but in a controlled, research-ready setting. Livestock (dairy cattle, pigs, poultry), crop production, manure management, water run-off, and regular human occupational exposure all co-exist on site. Yet unlike natural villages, AgroVet-Strickhof offers structured compartments where targeted sampling, interventions, and longitudinal follow-up can be carried out with reduced noise and clear traceability. Our project leverages this “village in miniature” to monitor the spatiotemporal dynamics of resistant bacteria and resistance genes across animal, environmental, and human niches. We conducted active air sampling by liquid impingement in a cow barn and a pig facility and, in parallel, deployed torpedo-shaped passive water samplers with electronegative membranes along a campus stream transect for 72 h. DNA extracts from air concentrates and recovered membranes were subjected to nanopore shotgun sequencing, enabling rapid resistome and pathogen profiling. This layout supports targeted sampling, controlled comparisons, and repeat measurements with clear traceability. As a village in miniature, the campus provides a practical model for tracking AMR across animal, environmental, and human settings and for scaling One Health surveillance from a single site to regional and national networks.