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Reproduction and reproductive biotechnologies for the preservation of endangered avian species and breeds

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To address wild and domestic biodiversity loss, many programs have emerged, and attempt developing methods of ex-situ in-vitro storage of reproductive cells. These germ cells are stored in cryobanks and may be used, after thawing, to reintroduce genetic variability in breeds and species when needed. In birds, these programs must take into account the specificity of avian reproductive physiology to be efficient. Indeed, birds have developed original reproductive processes which form part of adaptive strategies of flying animals to highly variable environments. These oviparous species produce telolecitic, hard-shelled eggs, enabling the autonomous development of the embryo in a highly protected micro-environment; and the presence of sperm storage tubules (SST) in the single avian oviduct dedicated to the prolonged storage of sperm provides "adaptive freedom" to the female to sustain fertilizing potential even in the absence of males. Embryo cryopreservation is impossible up to date due to the structure of the telolecitic egg and cloning is also unsuccessful. Semen cryopreservation is an option that has been tried to be developed since many years but the success was late to obtain due to the added challenge of long semen storage in the SST before fertilization. However, since fifteen years, more and more avian species and breeds could meet this method and new methods of semen phenotyping are appearing in order to better predict its success. Another growing method is the cryopreservation after in vitro culture of primordial germ cells taken at different moment of the early embryonic life. This promising method also offers many other areas of applications. In this presentation, the different methods developed or in development for the conservation of genetic variability in rare bird resources, and their advantages and limitations will be reviewed and will be connected to the reproductive specific features of the avian species. Their use in great programs of gene conservation will also be described.

In vitro interaction of spermatozoa with hen's sperm storage tubules

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In birds, spermatozoa can be stored in specialized epithelial structures named Sperm Storage Tubules (SST) located at the utero-vaginal (UV) level. SST allow sperm cells to keep their fertilizing capacity several weeks. The aim of this work was to characterize *in vitro* the relationship between the SST cells and spermatozoa. So, we set up a primary SST cell culture method and co-incubated SST cells with spermatozoa. The utero-vaginal junction of hens (*Gallus gallus*) was dissected. UV fragments were enzymatically digested with 1µg/ml collagenase IX and 5µg/ml pronase in F-12 Ham overnight at 4ºC. SST were isolated on 2-4% Percoll gradient column. The culture was performed in 4 wells plates or in Lab-Tek Chamber Slide System with medium TCM 199 plus 10% BFS and 10 mg/ml Gentamicin. Cultures were maintained for 1, 5 and 8 days. Roosters sperm samples with more than 50% viability were pooled, seminal plasma was removed by centrifugation, then, spermatozoa were washed and resuspended with TCM 199. One million spermatozoa were added to each SST cells wells for 24h at 37ºC in 5% CO2. We observed that *in vitro* SST have the epithelial morphological characteristics expected for immunocytochemistry: cytokeratin positivity and vimentin negativity. After 5 days of culture (i.e. at 5 and 8 days), we detected a lower signal for villin protein (1:500, Polyclonal Antibody, Bioss Inc.) on SST cells, most probably indicated less microvilosities on cell surface. A higher number of sperm cells bind to SST fragments in suspension (i.e. 1 day culture) than to SST cells monolayers (i.e. 5 and 8 days cultures). We speculate that the SST cells microvilosities may have a critical role in epithelium-spermatozoa contacts and their decreasing number for prolonged culture time may compromise sperm attachment. The model of culture we set up is a very powerful research system for a better understanding of SST-spermatozoa interactions.

Exploring the role of the chicken amniotic fluid during embryonic development

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In oviparous species, all nutrients and energy are stored in the eggshell, the egg yolk and the albumen to support the development of the embryo, outside the mother's body. Additionally, extraembryonic structures, namely the amniotic, allantoic and yolk sacs, are progressively formed to ensure embryo vital functions such as breathing and digesting, since its organs are not fully functional. In many species, the amniotic sac containing the amniotic fluid (AF) contributes to protect the embryo against mechanical shocks and dehydration. In humans, proteomic studies reveal in AF effectors of innate immunity[1,2]. However, the role of AF in bird embryonic development remains unknown. Our aim was to investigate the overall protein composition and functions of chicken AF, to better appreciate its functions in the developmental biology of birds.

A first analysis of AF protein composition from day 8 to day 16 of incubation by SDS-PAGE, revealed drastic changes in protein profile when the albumen transfers into the amniotic sac at day 12. A total of 106 proteins were identified in the AF by day 11 (prior the egg white transfer), using mass spectrometry analyses. The 10 most abundant proteins represent about 80% of the total protein content. Some of them are known for being involved in innate immunity (ovotransferrin, lysozyme), in organ or tissues morphogenesis (fibronectin, gelsolin), in lipid and vitamin metabolisms (apolipoproteins A-I and B, transthyretin), or in ion and hormone transport (alpha-fetoprotein, serum albumin), suggesting a specific role of the AF in the embryo development. To further characterize the biological activities of this fluid, we explored its antimicrobial potential and its proteolytic activities. Preliminary results revealed that at day 8 and day 12 of incubation, the AF exhibits antibacterial activity against Listeria monocytogenes probably due to the presence of lysozyme. Furthermore, we showed that AF displays proteolytic activities, which reflect the capacity of AF to degrade/digest or activate proteins. Five proteases were identified in AF: transmembrane serine protease 9, suppressor of tumorigenicity 14, plasminogen, carboxypeptidase M, and astacin-like metalloendopeptidase.

Comparison with the protein content of human AF1,2 allowed us to highlight some common proteins between oviparous and viviparous species, but more interestingly some bird specificities. Altogether, these data suggest that chicken AF has more complex functions than initially reported in literature.

1 Cho et al.Molecular& cellular proteomics 6 (2007) 1406-15

2 Michaels et al. Journal of proteome research 6 (2007) 1277-85

Effect of post hatch holding and feed access time on yolk sac utilization and broiler live performance

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This study investigated the effect post-hatch holding and first feed access time on yolk sac utilization and subsequent broiler live performance.

Broiler hatching eggs were collected from commercial flocks of Ross 308 breeders at 35, and 39 wk of age in Experiment 1, and 2, respectively. Chicks that still had some wet down, indicating they had just hatched were recorded, marked, and feather sexed at 488h of incubation in both Experiments. Chicks weighed individually and received feed and water at 2, 8, 12, 16, 20, 24, 28, and 32h after hatch in Experiment 1 and at 2, 24, 26, 28, 32, 36, and 40h after hatch in Experiment 2.

Yolk sac weight was determined at 32h or 40h after hatch in all groups in Experiment 1 and 2, respectively. Feed consumption and BW was recorded at 7, 35d of age and the same age relative to placement on feed and water to evaluate the effect of post-hatch holding time on live performance. Mortality was recorded daily in both Experiments.

Feed access time did not influence yolk sac utilization in both experiments. Chicks fed within 24h after hatch exhibited greater (P<0,05) BW than those that received feed between 28 and 40h at 35d in both experiments.There were no significant differences in feed consumption at 35d of age in Experiment 1 but early feed access group (2h) consumed more feed (P<0,05) to 35d compare with groups subjected to 32 and 40h of fasting after hatch in Experiment 2. There were no significant differences in feed efficiency (FCR) and mortality at 35d of age but early feed access group (2h) tended to have higher FCR than other groups in both experiments. When birds and feed were weighed relative to actual time on feed was considered, there were no significant differences among groups in BW, feed consumption, and FCR in both Experiments.

It can be concluded that a 28-40h delay in feed access after hatch affects BW negatively compare with chicks received feed early but tend to better FCR at 35d of age.

Clearly, post-hatch holding for up to 40h after hatching had no major detrimental effects on final live performance.

Key words: feed access time, post-hatch holding time, yolk sac, BW, feed consumption, mortality

Sperm storage in hens: comprehensive proteomic analysis of uterine fluid and SST in relation to fertility length

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Domestic hens are able to store spermatozoa in their genital tract for long periods, and so to produce fertilized eggs for up to 3 weeks after one insemination. Spermatozoa are mainly stored in sperm-storage tubules (SST) located at the utero-vaginal junction of the female tract. Nevertheless, the uterine/oviductal environment influence during sperm storage remains unknown.

Our objective was to perform an extensive description of the *Gallus gallus* uterine fluid (UF) and SST proteomes to provide the basis for a better understanding of long term sperm survival. UF and SST were collected from hens that displayed either a short (13d) or a long (21d) fertility length before and after artificial insemination. Bottom up proteomic approach using SDS-PAGE and nano LC-MS/MS (ultimate 3000 RSLC system coupled to LTQ Velos Orbitrap mass spectrometer) was performed with a high-low resolution MS strategy. Data were matched against NCBInr database using Mascot 2.3 and identifications were validated by the peptide and protein Prophet algorithm using Scaffold 4.0 software before bioinformatic treatment of data sets using NCBInr database, SecretomeP 2.0 and SignalP 4.1 tools, InterproScan software, and Exocarta, KEGG and UniprotKB databases. Among a total of 913 identified proteins in UF, 155 were known to be secreted and 640 were matched against exosome databases. Thus, UF exosomes were isolated and analyzed by TEM (<100 nm) and western blot. Molecular functional analysis of UF proteins revealed a majority of enzymes (34%) involved in glycolytic pathway and lipid homeostasis, whereas 55 proteins had previously been described to be implicated in reproduction. Among 1044 identified in SST, 415 are common to UF proteome. In conclusion, our proteomic analysis of UF and SST proteomes revealed a large number of novel proteins related to artificial insemination and/or fertility length that could play a key role in spermatozoa survival. Characterization of UF by functional analysis may allow a better understanding of its impact in sperm in vivo storage. Therefore, differential analysis using bottom up strategy is an efficient method to identify biomarkers of sperm survival in hen genital tract. Moreover, the presence of exosomal proteins in UF may represent a novel and exiting mechanism of epithelial cell-sperm cell interaction that may explain at least in part, the long term sperm survival. We expect and believe that the thorough catalogue of proteins presented here will serve as a valuable reference for the study of sperm interaction with the female genital tract. Moreover, it could be an interesting tool for discovering biomarkers involved in fertility.

Sperm storage in the hen oviduct: expression of progesterone receptors, structural proteins and heat shock proteins

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In avian species, sperm is stored for a quite long period in sperm storage tubules (SST) located in the utero-vaginal junction. It has been shown that progesterone (P4) regulates the release of sperm from SST for fertilization in quails (Ito *et al*., 2011). In the same context, heat shock proteins (HSP) like HSP70 family, activate sperm motility in quails (Hiyama *et al*., 2013) and may participate to the sperm release. In addition, we can hypothesize that at least some structural proteins from SST cells could play a role during sperm storage and release by interacting with sperm. Our objective was to study the potential effect of P4, HSP and structural proteins on sperm storage potential, by analyzing the expression of P4 receptors (PR and mPRa), HSPA8 and HSPB1, CNN1, TAGLN and DES genes in the avian genital tract. Uterus, vagina and utero-vaginal junctions were collected from two divergent lines of hens. One displays a long period of sperm storage (21 days, line F+, n=5), whereas the second displays a shorter period of sperm storage (10 days, line F-, n=6). Total RNA was extracted, reverse transcribed then mRNA coding for PR, mPRa, HSPA8, HSPB1, CNN1, TAGLN and DES were assessed by qPCR (CFX96 Touch Real-Time PCR Detection System, Biorad). Quantification was performed by using the relative standard curve method. Results were normalized by the geometric mean value of the two reference genes GAPDH and S17. Relative amounts of mRNA levels in each tissue were compared between lines by a Mann-Whitney test. Differences were considered significant when $p<0.05$. No significant difference was observed between lines for both P4 receptors, as well as for HSPA8, whatever the tissue. CNN1, TAGLN and DES expression was significantly higher in the utero-vaginal junction of F- than that of F+ line ($P<0.01$). HSPB1 expression was significantly higher in utero-vaginal junction of F- than that of F+ line ($P<0.05$). In conclusion, our data suggests that HSPB1 and structural proteins CNN1, TAGLN and DES are involved in the regulation of sperm storage in the utero-vaginal junction in hens.

Multicriterion analysis of embryo acclimation techniques aiming at improvingadaptive capacities in broiler chickens

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It is possible to improve poultry adaptive capacities towards changes in ambient temperature by exposing eggs to specific incubation temperature and relative humidity. Exposure of eggs for periods of 30 min. at 15°C at the end of incubation (vs. 37.8°C in standard incubation) was shown to limit the incidence of ascites in chickens subsequently exposed to cold. In the context of climate change that could increase the frequency of extreme climatic events, and of the depletion of non-renewable energy resources necessary to heat farm buildings, our goal was to improve chicken robustness and to achieve a multicriterion assessment (growth, health, production costs, welfare, meat quality ...) of the cold incubation technique depending on subsequent rearing conditions of chicks.

In a first study, we tested incubation conditions suitable for embryonic cold acclimation of broiler chickens. The tested incubation conditions were a standard egg incubation (control IT), two acute cold exposures at 15°C at the end of incubation (IA1) or cyclic decreases in incubation temperature (36.7°C vs. 37.7°C for 6h/day for days 10-18 of embryogenesis, IA2). After hatching, chicks were reared in either control conditions (ET) or submitted to day/night variations in room temperature (EFC cyclical cold rearing) or to continuously lower ambient temperatures requiring broiler adaptation (cold rearing EF). A first experiment took place in cages to allow the individual measurement of feed consumption, followed by a second experiment in floor pens.

In our conditions, the cold incubation techniques did not affect egg hatchability. IA2 incubation (cyclic cold) degraded growth and feed consumption ratio in females reared in cages, while IA1 treatment (two rapid exposure to 15°C) was rather beneficial for growth performance. This was confirmed by the floor pen trials, where IA1 females had better starting performance and growth as compared to their controls IT, but only in standard rearing conditions ET. In males, the embryonic cold acclimation IA1 also seemed favorable, but for animals started in cold EF conditions only. Multi-criterion analysis on the floor trial data measured in floor pens is currently focused on the responses of broilers acute cold incubation IA1 or standard incubation conditions (IT) in interaction with postnatal rearing conditions. This analysis integrates economic (competitiveness, consumer expectations…), environmental (contribution to climate change, non-renewable energy consumption…) and social (welfare and animal health,

product quality…). First results point to a decrease in gas use for heating (-11%) but slightly higher litter mass when starting chicks at 28°C. Interactions were found between incubation conditions and postnatal ambient temperature on performance and meat quality at slaughter age.

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Keywords: Broilers, Cold embryo acclimation, Adaptability, Robustness, Thermotolerance, Multicriterion analysis

About the avian UF proteome: exosomes significance and biological processes in regard to eggshell mineralization, immunity and reproduction

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In avian species, spermatozoa are stored for long periods within the female reproductive tract. Oviducal environment influence during sperm storage remains unknown. An extensive description of the *Gallus gallus* uterine fluid (UF) proteome will help to provide the basis for a better understanding of long term sperm survival. UF was collected from 10 hens 10h after oviposition. Bottom up proteomic approach using SDS-PAGE and nano LC-MS/MS (ultimate 3000 RSLC system coupled to LTQ Velos Orbitrap mass spectrometer) was performed with a high-low resolution MS strategy. Data were matched against NCBInr database using Mascot 2.3 and identifications were validated by the peptide and protein Prophet algorithm using Scaffold 4.0 software. Bioinformatic treatment of data set was carried out to refine annotation of proteins using NCBInr database, and to describe UF proteins using SecretomeP 2.0 and SignalP 4.1 tools, InterproScan software, and, Exocarta, KEGG and UniprotKB databases. Among a total of 913 identified proteins, 160 were known to be secreted and 640 were matched against exosome databases. Thus we isolated UF exosomes by ultracentrifugation and analyzed by TEM (<100 nm) and western blot (exosomal markers, HSPA8, VCP). Molecular functions analysis of UF proteins revealed a majority of enzymes (34%) involved in glycolytic pathway and lipid homeostasis. Moreover, an exploration of biological processes indicated that 55 proteins had previously been described to be implicated in reproduction, 38 in mineralization and 85 in immunity. In conclusion, the UF represents a complex proteomic environment in which the presence of exosomal proteins may represent a novel and exiting mechanism of oviduct-spermatozoa interaction that may explain at least in part, the long term sperm survival. We expect and believe that the thorough catalogue of proteins presented here will be an interesting tool for biomarkers discovery involved in fertility.

Sperm storage: Ultrastructural investigation of avian spermatozoa incubated in selected fractions of uterine fluid

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Hens keep spermatozoa in their genital tract for long periods, and produce fertilized eggs for up to 3 weeks after insemination. Storage mechanisms and oviducal environment impact on sperm are largely unknown. The aim of this study was to evaluate the effect of uterine fluid (UF) on semen using two different lines of hens that display a long (21 days, F+ line) or a short (10 days, F- line) period of sperm storage. UF was collected 10h after oviposition. Fresh ejaculates were used in both experiments. For the first one, spermatozoa were incubated in vitro in either PBS alone, PBS containing 25% or 50% UF, or in pure UF from both lines for 5 min, 1, 2.5, 5 and 24h at 4°C. For the second experiment, UF from both lines were depleted from proteins >3kDa (d-UF) using Vivaspin 500. Spermatozoa were incubated *in vitro* in PBS, d-UF, and pure UF for 5 min, 1 and 2.5h at 4°C. Sperm motility was assessed using computerized method at 41°C (HTM-IVOS II). Metabolites contained in d-UF from both lines were quantified by NMR and sperm morphology was analyzed using Atomic Force Microscopy (AFM). We observed that pure UF and d-UF improve sperm motility compared to PBS. Nevertheless, the effect of d-UF was lower than pure UF. In both experiments, sperm motility was higher after incubation in UF or d-UF from F-line than F+ line. NMR analysis reveals that the concentrations of 5 metabolites were higher in d-UF from F+ than F- line ($P<0.05$), including alanine, succinate, dimethylamide and N-acetyl groups. Moreover, AFM analysis clearly showed an alteration of head morphology of spermatozoa incubated with d-UF from the F- line. This study clearly demonstrates the major role of UF proteins >3kDa on the sperm motility. Nevertheless, the UF fraction (<3kDa) which mainly contains peptides and metabolites improves sperm motility and leads to ultrastructural modification of spermatozoa. Our findings demonstrate that the microenvironment dynamism and complexity is a key element during sperm storage.

Impact of embryonic heat acclimation on histone post-translational modifications in chicken

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Chickens that have been genetically selected for growth performance are challenged by temperature variations. Considering the problem of global warming, embryonic heat treatment was proposed to acclimatize chicken to heat in the long term. The embryonic thermal manipulation (TM) consists of a cyclic increase of egg incubation temperature from the embryonic day E7 up to E16 associated with an increase of the relative hygrometry (HR) (from 37,8°C and 55% HR to 39,5°C and 65% HR for 12h/d). This change in early environment has little effect on hatching and improves the survival rate of chickens when exposed to a HS at slaughter age (i.e. 5 weeks of age post-hatching). This increased thermotolerance is associated with physiological, metabolic and genes expression changes at slaughter age.

We hypothesized that the change in gene expression observed in TM chickens could be the result of epigenetic alterations established during the treatment that persist throughout development. Indeed, early environmental exposure to pollutants, drugs, diet and other factors, is able to change genes expression in the long term through epigenome alterations. For instance in Drosophila melanogaster the exposure to a heat shock (HS) during the embryogenesis leads to long-lasting epigenetic reprogramming mediated by the Polycomb Repressive Complex 2. This complex is involved in the establishment of the tri-methylation of the lysine 27 on the histone H3 (H3K27me3) which is known to play a part in the memory of a repressive state under the influence of the environment. To investigate the potential epigenetic mechanism involved in heat acclimation we performed an unbiased analysis of two histone post-translational modifications (HPTM). We analyzed H3K27me3 associated with repression of gene expression and the tri-methylation of the lysine 4 on the histone H3 (H3K4me3) associated with gene expression. The distribution of these modifications were studied by ChIP-seq on hypothalamus, the regulatory center of thermotolerance, of TM and control chicken at slaughter age. Based on the recommendations of the Encyclopedia of DNA Elements (ENCODE) we profiled both marks on four individuals per treatment (4 TM and 4 control). We performed a bio-informatic analysis to detect peaks with PePr followed by a bio-statistical analysis with DESeq2 to detect and validate differential peaks.

Effect of flock age, storage temperature and SPIDES during storage on embryonic development, and hatchability of long stored eggs

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This study investigated the effect of broiler breeder flock age, storage temperature, and SPIDES on the developmental stage of embryos and hatchability.

Hatching eggs were obtained from two Ross female line grandparent flocks at younger (28 wk) and, prime (40 wk) ages and eggs were stored for 14 days at 18°C, 15°C, or 12°C. During storage, eggs were either held continuously in the storage room (Control) or were subjected to a heat treatment regimen delivering 3.5 hours above 32°C and 2.5 hours above 35°C, in a Petersime Re-Store machine at d 5 of storage. In this experiment, 20 control eggs and 20 treated eggs were opened in each flock age at 5d of storage to examine the stage of embryonic development. All eggs were set in a single incubator and hatcher. A tray of 150 eggs constituted a replicate and 6 replicate trays (900 eggs) were set per heating treatment at each storage temperature and flock age.

Embryonic development was advanced by SPIDES and by the warmer (18°C) storage temperature. As expected, hatchability was significantly better for the prime flock compared to the younger flock. At both flock ages, hatchability was increased by storing eggs at 15°C compared to 18°C with 12°C intermediate. Eggs stored at 18°C had the highest early embryo mortality and at 15°C the lowest, with 12°C storage intermediate.

SPIDES increased hatchability in all three storage temperature treatments. This effect was more evident for younger flock. SPIDES treatment reduced early embryonic mortality in eggs stored only at 18°C, and reduced late embryonic mortality in all 3 storage temperature treatments.

It can be concluded that the highest hatchability was observed in eggs stored at 15°C and given one SPIDES treatment at 5 days.

Key words: hatching eggs, embryonic development, SPIDES, storage temperature, flock age, hatchability

Maternal dietary protein undernutrition of breeders has a transgenerational impact on egg weight, hatch weight and hatchability

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In mammals, it's already demonstrated that maternal undernutrition can influence and program offspring performance. However, not much is known about the influence of these programming effects of breeders to their broiler progeny. Furthermore, in recent years, more and more attention is given to the large protein consumption and nitrogen excretion by broiler chickens. An interesting idea, for both environmental and economic reasons, would be to program broilers to be more nitrogen efficient by reducing the dietary protein levels in the breeder feed. Moreover, this could be beneficial for the welfare of the breeders. Indeed, as a result of the low protein diets, their feed intake can be increased to obtain the same body weight.

To gain insight in this matter, a transgenerational experiment with three generations of breeders and their offspring was performed. The F0 generation of breeder hens (pure line A) was divided in a control group (C) on a standard diet and low protein group (LP) that received a diet with 25% balanced reduction in crude protein and amino acids during their entire lifespan. The offspring of both groups of F0 was then again subdivided in a C and LP group. This resulted in four F1 breeder groups, namely C/C, C/LP, LP/C and LP/LP of which the first letter indicates the feed in the F0 generation, whereas the second one means the feed they received in the F1 generation. To obtain the same target body weight, the LP group received about 10% more feed. Progeny of these breeders was then again raised as breeders of the F2 generation, which were only fed a control diet in order to gain more insight in the true transgenerational effects instead of the direct feed influences. All breeder hens were raised according to standard management guidelines. The hens were artificially inseminated with semen of roosters on a standard diet.

Fertile eggs of the F2 breeders were incubated at 37.8°C dry bulb temperature and 29.4°C wet bulb temperature. Egg and hatch weights of the C/C/C group (the third letter indicates the control feed for the F2 generation) were significantly heavier than eggs from the other three groups ($p < 0.05$). These observations indicate that low protein feed in (grand)parent generations still reduces egg weight in the F2 generation, even though the F2 hens were all fed a standard control diet. Furthermore, hatchability of fertile eggs (HOF)

was lowest in the C/LP/C group for both the short (< 1 week) and long storage (1-2 weeks) time periods with a HOF of 74.26% and 67.19% respectively. For the short storage eggs, LP/LP/C had the highest HOF (78.81%) whereas C/C/C had the highest HOF for long storage eggs (76.39%).

To conclude, these results indicate that balanced reduced protein diets for breeders affect the egg weights and hatching performance of F2 offspring in a transgenerational way. These observations are most likely caused by epigenetic mechanisms and are being further investigated.

Microbial colonization in day old chick intestine

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This presentation will focus on the importance of the development of the gut microbiome in the early life of chicks for the development of immuno-competence and disease resistance. Events in early life, including the incubation and hatching conditions, have been shown to affect the ability of chickens to cope with infectious agents later in life. The gut is considered to be the largest immune organ of the body. At any rate, mucosal immunology is of key importance for animal health and general gut functioning. The gut microbiome shows associations with, and may be causally related to, the performance of the gut and of the whole animal. Studies have indicated that the earliest events regarding gut microbiome development or the interaction with pathogens can have longer lasting effects on gut immunological functions. The development of the gut microbiome and its effect on gut function is the result of an interplay of genetic, nutritional, and management factors. Early post-hatch access to feed and water, as well as early post-hatch colonization by germs from the environment could potentially affect early development of the gut microbiome and immune-competence.