

Report

COMBINED 2017 MEETING of the Incubation and Fertility Research Group (IFRG/WPSA Working Group 6) and the Fundamental Physiology and Perinatal Development Group (PDP/WPSA Working Group)

The 2017 meeting was organized from August 30 until September 1st in Wageningen by Marleen Boerjan (president IFRG, WPSA WG 6, Pas Reform); Glenn Baggott, (IFRG board, ed. British Poultry Science, Bristol UK) Ann Collin (IFRG board member, URA, INRA, Nouzilly France) and Barbara Tzschentke, (president of PDP/WPSA WG 12, Humboldt University Berlin).

General impression

The atmosphere of the 2017 meeting was cordial and easy-going because of the optimum mixture of PhD students, young and experienced scientists. Thanks to the active input of all participants new knowledge on basics of incubation physiology was exchanged during diner times and discussions. In addition the extra emphasis on the 7 posters presented strengthened the discussions between participants.

With the support of the WPSA Speakers Bureau we could invite two early-career scientists as keynote speaker: PhD student Bence Lázár from the Doctoral School of Animal Husbandry, Szent István University, Gödöllő, Hungary was keynote speaker in session 3: “Impact of parental effects”

Also we could invite a second keynote speaker Mylene Mariette, post-doc from the Centre of Integrative Ecology, Deakin University, Australia. Mylene Mariette was keynote speaker in session 4: “Adaptation and epigenetic alteration in birds”

Thanks to sponsors incubator manufacturers HatchTech and Pas Reform we could invite Prof Ton Groothuis from the Institute of for Evolutionary Life Sciences, dept. Behavioural Biology University Groningen. Prof Groothuis was second keynote speaker in session 3.

The organizers took several measures to increase the interaction between all participants of the meeting and students with keynote speakers Lázár and Mariette in particular.

1. In the program we organized a special Poster carousel to facilitate discussions about the posters. This was of great success we planned 30 min but discussions went on for more than 1 hour.
2. At the end of each session the speakers were invited to join a general Question&Answers. The questions not only raised a discussion between participants and speakers but also between the speakers.
3. To facilitate the discussion between keynote speakers and students a separate lunch was organized on Thursday August 31. The lunch resulted in a lively conversation between the 15 students, including the PhD students, and Keynote speakers Bence Lázár and Mylene Mariette. Barbara Tzschentke took part as representative from organizing committee.

The program

The abstracts received by the organizing committee were clustered into 6 specific themes which served to the build the program into 6 sessions. Each session addressed a specific topic related to

incubation physiology covering applied as well basic research projects. Specific attention was asked for epigenetic programming of hatchlings via the mother and incubation.

The **first session** covered the influence of breeder/parental age and management on fertility and chick health. Boerjan (Pas Reform) addressed the formation of the ‘meiotic’ oocytes during egg formation in the hen and discussed the increase of chromosomal abnormalities as the hen ages. Molenaar (HatchTEch) presented results from experiments aimed at to determine the influence of breeder age and oxygen concentration during incubation on chick performance. No interaction between flock age and incubator oxygen concentration was found, higher levels of incubation oxygen positively influenced embryonic development and increased post-hatch growth during the first week.

The next two speakers from INRA-France addressed the influence of nutrition of broiler breeders on fertility, embryonic development and chick health. Project leader Dupont summarized their biomolecular/gene expression approach to study the effects of feed-restriction and supplementation of omega-polyunsaturated fatty acids (PUFAs) breeder performance. Supplementation of PUFAs to broiler breeders feed increased progesterone production in cultured granulosa cells and *in vivo* formed egg yolks. The positive influence of PUFAs on reproductive performance (egg quality, fertility and steroidogenesis) was discussed. From the same group PhD student Mellouk summarized the effects of PUFAs in maternal feed on novel hormones (adipokines) involved in regulation of metabolism and growth of embryo and chicken. In conclusion, the research from the INRA research group shows the influence of maternal nutrition on transmission of epigenetics marks to a next generation.

The second section concentrated on storage and handling of broiler and ostrich eggs.

It is general accepted that egg storage negatively affect incubation results and chick health during the growth phase. To minimize the negative storage effects ‘old’ and ‘new’ knowledge on embryonic development is combined in new incubation practices. More and more often hatching eggs, from all species and genetic lines, are incubated for a few hours during the storage period. Benefits of these pre-storage treatments have been shown in the fifties (Coleman, 1956) and sixties (Kosin and Siegel, 1966). In this session two speakers showed very interesting results. Ferreira (Aviagen) showed many field results of repeated heat treatment during storage (SPIDES) on hatchability and chick vitality. In conclusion, SPIDES or heat treatment during storage is of benefit to commercial breeds of broilers, layers and turkey. Precautions should be taken that the cumulative heating time above 32 °C does not exceed 15 hours.

Cinnamon (Hebrew university, Israel) showed his results on the development of the blastoderm in freshly laid eggs. His research is a continuation of the studies done by Eyal-Giladi and Kochav in the seventies. Cinnamon presented impressive details of the pre-incubation blastoderm using a novel 3D High Resolution Episcopic Microscopy (HREM). Using this technique he could show that the embryos from older flocks are in a more advanced stage of development compared to embryos from younger flock. Cinnamon also showed that embryos from chicken eggs are better preserved when stored at 12 °C compared to stored 18 °C.

Brand (Institute for Animal Production, South Africa; ref. Brand, 2012) presented a short overview of ostrich incubation and asked attention for high levels of late mortality (40-50%) because of low levels of weight loss. It appeared that water-loss, pipping time and day-old chick weight were independent of setting position and turning angle. A clear seasonal interaction was found with a decreasing water loss from winter to spring to summer. Pipping time became later and day-old chick weight heavier from winter to summer.

The last two presentations were related to practice of commercial incubation. Torma (Institute of Animal Sciences, Hungary) investigated whether the use of a controlled shaking of hatching eggs impact using a vibration plate, could be an alternative for turning during storage. Low levels of shaking broiler eggs (n=8100) is not an alternative for turning during storage.

Nasri (INAT University, Tunisia; WUR- Netherlands) studied the impact of hot climate on hatchability and chick performance. The main factors influencing percentage of saleable chicks were broiler breeder age and storage length. In addition, results showed decreased chick weight and percentage of heart, liver and intestine, when storage duration increased.

Session 3: impact of parental effects

Keynote speaker Lázár (Hungary) presented a new approach to increase resistance to heat stress in Transylvanian Naked Neck chicken. He studied the combined impact of heat treatment and heat shock on chicken embryos and chicken primordial germ cells (PGC). PGC cells were isolated from embryonic blood (2.5 d, HH16) and cultured *in vitro*. Three different experiment groups were analysed on sperm quality and embryo 'quality' in eggs from treated parents: (1) control (non-stressed), (2) heat stressed and (3) heat stressed +heat treatment. Definition heat treatment: of 2-day old chicks were kept at 38.5 °C for 2 hr. Definition heat stress: animals were kept for 12 weeks at 30 °C.

Reproductive performance (egg production and number of PGCS) was highest when parents received the combined heat treatment + heat stress.

This of the Hungarian research group emphasis the parental influences on offspring hatched. These observations and studies might become of great impact for breeder management practice.

The two next speakers (Institute for Evolutionary Life Sciences, dept. Behavioural Biology, University of Groningen) discussed several issues of the function of maternal steroid hormones in the yolk of hatching eggs. PhD student Kumar presented his results on detailed analysis of allocation of maternal steroid hormone in eggs from rock pigeons. Using mass spectroscopy he studied the gonadal steroids and their metabolites in yolk of pre-ovulatory follicles and yolk+albumen. Results showed a strong, independent from maternal enzymes, early embryonic steroid metabolism. This a new perception of the roles of yolk-hormones in parent-offspring communication.

Professor Groothuis, keynote speaker, presented a detailed review of steroid hormones from maternal origin in different avian species and he also made comparison with function of maternal hormones in mammals. From the enormous pile of papers published it has become clear that the discrepancy between papers can be explained by described environmental factors which influence the allocation of maternal hormones to the eggs. It was of great interest to learn, for example, that in commercial chicken the level of androgens in ejaculate of the male influence the immune response of the hatched chicken.

Session 4: Adaptation and epigenetic alteration in birds

Coustham (INRA Nouzilly, France) discussed how heat treatment of embryos inbred japaes quails. Cyclic increase of embryo temperature from E0-E13 metabolic and physiological alterations that persisted during the first weeks of age after hatch. The changes were accompanied by **transcriptome and alteration in the DNA-methylation pattern** in the hypothalami of day one TM-males quails. From this research we learned that the inbred Japanese quail might be a promising model to study the long-lasting impact of perinatal environment on bird epigenome.

Keynote speaker Mariette showed in an impressive lecture how zebra finches signal high temperatures to their embryos. The zebra finch is an example of how prenatal acoustic

communication can influence thermoregulation in offspring. The acoustic response in growth and thermoregulation of the late stage embryo was reviewed for different species including domesticated chicken. The statement: “incubation temperature programs offspring thermoregulation capacities” needs more attention from the commercial incubation industry.

Session 5 included several lectures on commercial incubation

Wineland started the session with asking attention for the importance of optimum incubation conditions for in-ovo vaccination. In fact the most important factor for best results is the location of vaccination. The best location is the amnion and second best are sub-cutaneous and intramuscular. In fact the location of vaccination is in fact the final step in in-ovo vaccination, the first step is to achieve a batch/trolley of eggs containing embryos in one and the same stage of development. Of course the type of incubator and whether single or multistage incubation is the routine. But, last but not least the experience of hatchery manager is of eminent importance. The experienced hatchery manager knows how to preheat, to load his/her incubators with different egg types to achieve the best uniformity for optimum in-ovo vaccination. The next speaker of the session hatchery manager Salem (Delmonte group, Amman Jordan) showed that in his hatchery thanks to good incubation practice in-ovo vaccination improved results if the highest standards of hygiene were achieved routinely.

Druyan (Volcani Center, Rehovot Israel) introduced an incubation related method to reduce the incidence of tibial dyschondroplasia (TB) in Cobb embryos. The results suggest that hypoxic exposure (13% oxygen) during the limb bud stage (E6-E9) increase angiogenesis, and improved oxygen supply to the growth plate and thereby reduced susceptibility to TB.

Van den Brand (Wageningen University Netherlands) and his group investigated the interaction between carbon dioxide concentration and eggshell temperature during the second half of incubation. From day 8 of incubation eggs were incubated in 6 different conditions: 2x3 factorial design with two egg shell temperatures (37.8 °C and 38.9 °C) and 3 concentrations of CO₂: 0.1, 0.4 or 0.8 (O₂ was kept constant). In this experiment the different CO₂ concentration had limited effects on the results, high temperatures effected hatching time and chick quality.

Maatjens presented results of incubation at different egg shell temperature from day 15, 17 or 19. Chick quality at placement was highest when the embryos were incubated at an egg shell temperature of 35.6 °C from 15 until hatch.

Van der Pol discussed the positive effects of circadian lighting (16L:8D) on embryonic bone development. A MicroCT scan was used to evaluate bone development in the different lighting experiments. Overall applying a light-dark rhythm during incubation may improve leg bone development and leg health at slaughter age compared to continuous light or continuous dark incubation.

In the last sixth session 5 lectures focussed on the “control and programming of energy balance”.

Friedman-Einat presented data from gene-expression of adipokines in visceral fat of commercial layer and broiler line. Illumina RNA-seq analysis was performed on samples from three female layers and three female broilers at the first week of lay. Results demonstrated specific adaptations of the adipose tissue gene expression achieved through selective breeding of the commercial chickens to specific traits.

Tzschentke (Humboldt University, Berlin) showed that white leghorn embryos treated in-ovo with glucose from day 14-17, developed a long-lasting, first 3 weeks after hatch, glucose resistance as observed in a decreased neuronal glucose sensitivity tested in brain slices. Also a decrease in receptor expression for insulin, leptin and a glucose-transporter. Related to this observation the risks

of in-ovo feeding were discussed.

The next speaker van der Pol (HatchTech) showed that the metabolic production increased to 521 mW/chick E21.5. In comparison the control non-fed chicks plateaued at 340 mW/chick. Based on these results the speaker discussed the need to adjusted environmental conditions during transport of early fed day old broiler chicks.

In a following lecture Tzschentke (Humboldt University, Berlin) discussed the results of a combined project with Halle (Research Institute, Celle) a peri-natal temperature training (PPT) of three different genetic lines: broiler Ross 308, Lohmann Dual and Lohmann Brown. For the PPT the incubation temperature was increased by 1 °C during in the hatching phase. It was shown that only hatchability of males from the broiler Ross 308 increased, hatchabilities of Lohmann Dual and Brown were not affected. Improved feed conversion rates and increased growing rates were observed in broiler and Dual chickens but not in Lohmann Brown.

Last speaker in the combined 2017 meeting was PhD student Tatge (Humboldt University, Berlin). Results were shown of the neuronal neuro-peptide Y (NPY) protein expression in the hypothalamus of Ross 308 broilers after perinatal temperature training at a commercial scale in a broiler hatchery. In three incubation cycles the temperature was increased by 2°F during the last days of incubation setter and one day in the hatcher. In general there were no negative effects on hatching results and performance in the farm. Results of NPY-analysis show a statistically significant lower hypothalamic PNY-expression in male chicken of PTT-group only, this indicates a lower basic metabolism for male Ross 308 chicken.