Acta fytotechn. zootechn., 17, 2014(4): 130–135

Review Paper

High heritability of egg testosterone and consequences on performance of offspring in Japanese quail. A review

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Individual phenotype is a result of an interacting genotype and environment with the most sensitive periods occurring within the late embryonic and early postnatal development. In this time, most of the signals come from the mother and such influences are termed maternal effects. In our review, we focus on hormone-mediated maternal effects in birds because the avian embryo develops without interactions with neuroendocrine milieu of the mother and the egg yolk contains biologically active substances including hormones. Thus, it is expected that the mother can adjust phenotypic development of her progeny to actual environmental conditions through differential deposition of hormones in the egg. The adaptive value of such hormone mediated maternal effects can have significant consequences for poultry performance but is still generally neglected. In the first part of our review, we summarise the data from wild living birds and urge the need to study maternal egg hormones also in poultry species. In the second part of this review, we present our experimental approach that is based on two genetic lines of Japanese quail divergently selected for yolk testosterone concentrations. We estimated relatively high heritability of yolk testosterone concentrations and proved that natural selection can shape hormone-mediated maternal effects. Our selected lines provided us with a unique model to explore mechanisms of maternal hormone transfer into the egg and epigenetic effects of maternal hormones on offspring. We expect that this approach will provide relevant data in the field of trans-generational maternal effects with potential application for poultry breeding and welfare.

Keywords: maternal effects, hormones, androgens, genetic lines

1. Introduction

Individual phenotype is a result of interactions between genotype and environment. These two factors interact with each other during the embryonic and postembryonic ontogeny. The most sensitive periods occur during the late embryonic and early postnatal development when basic physiological and neurobehavioural systems are formed and potentially exposed to epigenetic maternal influences. Therefore, maternal effects may be considered as adaptive tools by which the mother 'programs' her offspring to environmental conditions they will face after hatching (Mousseau and Fox, 1998). Although this phenomenon is generally known for a long time, only over the last two decades an active research of mechanisms, by which maternal phenotype modifies gene expression of the next generation has started. The research is motivated especially by the fact that epigenetic changes are faster than those genetically driven and enable an effective response to changing environment. In developmental biology and behavioural ecology trans-generational epigenetic changes are considered mainly from the positive (adaptive) point of view and are expected to increase probability to survive and reproductive fitness in given environment (Gil, 2008). In the medical field, they are known as maladaptive and increasing risk of affective diseases such as autism (Gardener et al., 2009). In poultry science, maternally-derived compounds in eggs are generally neglected, with an exception of vitamins A and D.

The mother has a number of ways by which she can adjust physiological and behavioural phenotype of her progeny in order to increase its reproductive success under challenging environmental conditions. Besides maternal behaviour, a growing attention is dedicated to hormone mediated maternal effects that possess a potential to permanently organize offspring's phenotype (Groothuis et al., 2005b). This mechanism is important from both, evolutionary perspective, as a rapid adaptation to unpredictable environmental conditions, as well as from the perspective of negative consequences of these adaptations for development of offspring and possible wrong programming. Maternal steroid hormones that can pass to the embryo either via the placenta in eutheria or via the chorioallantoic barrier in oviparous species

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DOI: 10.15414/afz.2014.17.04.130–135

play a dominant role. This field of research is becoming even more important with respect to problems of environmental contamination by endocrine disruptors, compounds that besides their direct negative effects can exert also indirect effects via a modification of epigenetic adaptive mechanisms (Ottinger et al., 2008).

1.1 Hormone-mediated maternal effects in birds

Recent studies show numerous advantages of the avian model, which allows to study maternal hormones in the yolk without interactions with neuroendocrine environment of the mother and possible extrapolation of the results from the evolutionary perspective (Crews and Groothuis, 2005). Moreover, the egg yolk contains substantial amounts of biologically active substances including maternal hormones, antibodies, carotenoids and other antioxidants.

Maternal effects mediated by hormone transfer into the egg yolk can influence physiology, morphology and behaviour of the next generation at each stage of development (Groothuis et al., 2005b; Navara and Mendonca, 2008). One of the most frequently used experimental approaches to the analysis of maternal androgen effects on phenotype of offspring is based on an increase of endogenous concentrations of given hormone by its injection in physiological dose into the egg yolk. The data obtained in such experiments demonstrated the effect of testosterone (T) as a dominant androgen (in some cases in combination with androstenedione) on postnatal growth (Schwabl, 1996; Eising et al., 2001), immune responses (Muller et al., 2005; Sandell et al., 2009) and behaviour (Eising and Groothuis, 2003; Strasser and Schwabl, 2004) of several wild living bird species. These published results, however, have been analysed especially by evolutionary and behavioural biologists and they revealed a large inter- and intra-individual variability in hormone mediated maternal effects (Navara and Mendonca, 2008). This variability can reflect between-species differences as well as different environmental and social factors that cannot be controlled under field conditions (Love et al., 2009).

Testosterone as a hormonal signal acting on various target tissues can mediate a trade-off between various functions, such as e.g. sexually selected ornaments and immune function or others (Ketterson et al., 2001). Indeed, immunosuppressive effects of yolk androgens have been demonstrated in relation to their stimulating effects on postnatal growth of offspring (Groothuis et al., 2005a; Navara et al., 2005). The trade-off, however, was not proved in many other experiments indicating that maternal androgens can influence immunity and growth independently (Muller et al., 2005). The most recent data suggest that immunomodulatory effects of yolk androgens are potentially affected context-dependently by environmental conditions or dose-dependently (Cucco et al., 2008). Dose-dependency between maternal yolk T and its effects on immunity was tested in precocial grey partridge (*Perdix perdix*). Pharmacological T dose produced inhibitory effect on both growth and immune response of offspring, while physiological dose stimulated both of these parameters (Cucco et al., 2008). Similarly in the chicken (*Gallus domesticus*), an injection of low T dose into the egg stimulated growth of the bursa Fabricii while an inhibitory effect was observed after the high dose (Norton and Wira, 1977). Moreover, long-term consequences of yolk androgens on immunity indicate their immuno-enhancing effects (Tobler et al., 2010).

1.2 Genetic differences in yolk androgen deposition

Besides evolutionary and ecological consequences, increased levels of maternal hormones can have significant consequences for poultry performance. Genetic lines of quail divergently selected for high and low sociability differed significantly in yolk T content (Gil and Faure, 2007). This can have an impact on their behavioural strategies in unpredictable environment. Several studies demonstrated that experimentally increased egg yolk T levels stimulate proactive behaviour of young quail (Daisley et al., 2005; Okuliarova et al., 2006; 2007). That implies positive effects of these manipulations on welfare. In addition, recent studies investigating yolk T concentrations in quail habituated to human support these assumptions (Bertin et al., 2008; 2009a).

1.3 Direct selection for egg testosterone content

In our experiments, we focus on Japanese quail, which has been effectively used as a model species for genetic

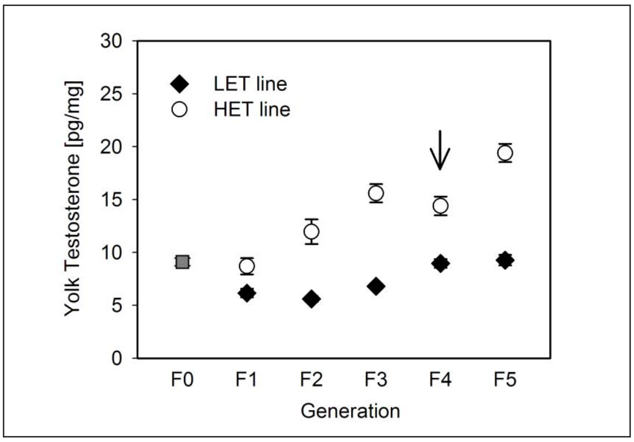

Figure 1 Generation means ±SE of yolk testosterone concentrations in the random-bred population (grey square) of Japanese quail and in the low (LET) and high (HET) egg testosterone lines in response to bidirectional selection. A black arrow indicates the response to relaxed selection
Source: Okuliarova et al., 2014

DOI: 10.15414/afz.2014.17.04.130–135

and physiological studies. On the basis of random-bred population kept at the Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences at Ivanka pri Dunaji, Slovakia, we created two genetic lines of quail, the low (LET) vs. high (HET) egg T lines that significantly differ in their yolk T levels (Okuliarova et al., 2011a; 2014; Figure 1). Our long term genetic approach was motivated by our previous results that demonstrated high repeatability of yolk T levels during the first reproductive cycle as well as among three consecutively laid eggs within a single laying sequence (Okuliarova et al., 2009). The high phenotypic repeatability of this trait corresponds with high heritability. Our results showed that realized heritability of yolk T concentrations was estimated around h^2 = 0.42 (Okuliarova et al., 2011a). The selection differential was larger in the HET than the LET line indicating a stronger selection response in an upward than downward direction (Figure 2). These data suggest that natural selection keeps egg T levels close to physiological minimum and these minimal concentrations are probably inevitable because of control of reproductive processes in maturating ovarian follicles.

Although it was repeatedly hypothesized that natural selection for maternal androgen deposition into the eggs can negatively affect physiology and behaviour of parents (Groothuis and Schwabl, 2008) our results do not support this expectations (Okuliarova et al., 2011a; 2014). We did not find differences in plasma T levels between HET and LET females although egg T concentrations were substantially and consistently higher in HET than LET quail (Figure 1). The absence of the parallel response of egg and plasma T concentrations to selection may have several important implications. First, maternal effects mediated through egg hormones are not just an epiphenomenon of the endocrine control of the female

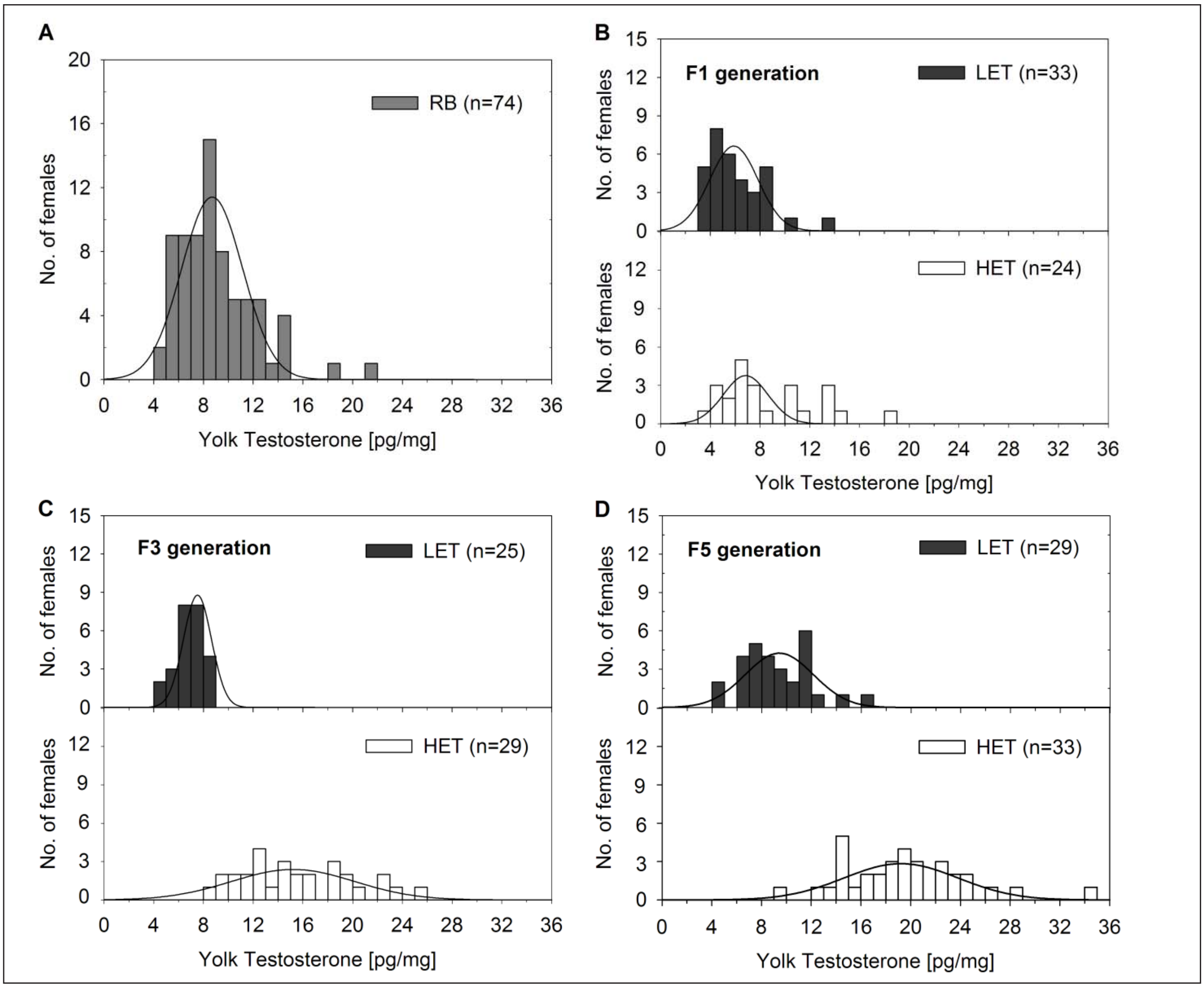

Figure 2 The frequency distribution of yolk testosterone concentrations in the initial random-bred (RB) population of Japanese quail (A) and in the low (LET) and high (HET) egg testosterone lines over the first (B), third (C) and fifth (D) generations
Source: Okuliarova et al., 2014

reproductive system. Second, there is no trade-off between androgen effects on physiology of the mother and androgen deposition in the egg that can constrain evolution of hormone mediated maternal effects. Such trade-off was suggested by several authors (Groothuis and Schwabl, 2008; Moore and Johnston, 2008). However, since egg T concentrations continue to increase in the course of selection without apparent negative effects on plasma androgen levels and control of reproduction, other, still unidentified constraints, must exist. Third, it is possible that selection targets an activity of multiple genes encoding enzymes from sex steroid biosynthesis and distribution of these enzymes in the wall of ovarian follicles results in the differential transport of locally synthesized hormones predominantly either to the egg yolk or to the maternal circulation. Currently we explore all three issues, which may have a significant impact on theoretical aspects of egg hormone deposition and subsequent maternal effects.

From a practical point of view, such as aspects of poultry science and industry, it is important how hormones deposited by the mother in the egg can influence development and performance of chicks during early post-embryonic life as well as after reaching sexual maturity. In this context, our model is advantageous because we always consider endogenous maternally derived hormones that are essentially present in the egg and not exogenous hormones.

Our bidirectional selection of Japanese quail for contrasting yolk T levels affected growth and development of hatched chicks. No line differences were found in embryonic growth but HET quail exhibited higher growth rate, were heavier and displayed longer tarsi as compared with LET quail (Okuliarova et al., 2011b). These effects tended to be sex specific with distinct line differences in males from 2 weeks of age. There were no differences between lines in adult animals, although the expected higher body mass of mature females as compared to males was always recorded. Still it is not clear, which part of the body is responsible for the difference, since the slaughter yield has not been estimated yet.

Genetic differences in the yolk T content influenced deposition of maternal T in the egg during the second reproductive cycle (Zeman et al., 2013). Most HET females increased their capacity to deposit androgens in the egg while in LET quail we recorded an opposite tendency. This finding opens a possibility that HET female produce a more valuable proactive progeny in the second breeding cycle. Because of proactive behavioural changes induced by increased exogenous T in the egg (Daisley et al., 2005; Okuliarova et al., 2006) we may expect more proactive behaviour in such individuals but this assumption was still not proved and must be carefully studied. This implies that such inter-seasonal variation in the maternal hormone deposition may be important not only in free-living birds with a long lifespan but should be explored also in poultry species that are used for several breeding cycles, such as geese.

Another important aspect, which can be addressed in these two lines, is a possible impact of increased maternal hormones on behaviour of the progeny. Significant relationships between maternal egg hormones and behaviour is expected on the basis of studies performed in Japanese quail that were selected divergently for contrasting fearfulness (Bertin et al., 2009b) and social behaviour (Gil and Faure, 2007) and that substantially differ in androgen deposition into the egg. Moreover, chicks differed in their response to stressful stimuli from ambient environment (Calandreau et al., 2011). The weakened response to stressors might be important from the point of poultry welfare. If similar behavioural changes are observed between HET and LET quail, they can be attributed directly to either hormone mediated maternal effects or a closely correlated response. In any case, such results can give hints how to approach and manage welfare also from the genetic point of view.

2. Conclusions

In conclusion, our original bidirectional selection for egg T content in Japanese quail provided us with a unique model to explore mechanisms of maternal hormone transfer into the egg and epigenetic effects of maternal hormones on offspring. It is a promising tool for understanding mechanisms underlying maternal effects and their consequences on physiology and behaviour of progeny. We expect that this approach will provide relevant data to expand knowledge in the field of trans-generational maternal effects with potential application for economically important species, including egg-type and broiler chicks and in turn with consequences for poultry welfare.

3. Acknowledgement

This study was supported by the Slovak Research and Development Agency (APVV 0047-10) and the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and of the Slovak Academy of Sciences (VEGA 1/0686/12).

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