Out of sight but not out of mind

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Summary

In the last few decades, the study of maternal effects, as being strongly suggested by its name, has been substantially investigated from the perspective of mothers in terms of mechanisms and its evolutionary consequences. In addition, when it comes to the maternal effects on the offspring, the studies have been and still are more often than not focusing on the post-natal stage. However, recently, several studies have suggested that embryos are also playing an active and important role that may potentially induce a mother-offspring conflict by heavily converting maternal androgens to other hormones. Yet, this also has led to many unknowns. Therefore, in this thesis, I focused on the role of the embryo in determining its own development under the influence of the mother. In particular, in **chapter 2** to **chapter 4**, I explored 3 important unknowns about the embryonic metabolism of maternal androgens which are: 1) to what extent do embryos can affect the hormonal environment they are exposed to during early development, 2) how does such a hormonal environment affect their prenatal development, and 3) are embryos able to control their early hormonal environment depending on the context in which they develop.

In oviparous species, maternal androgens are often found being very rapidly metabolized by the embryos very early in development. This leads to a paradoxical issue of how maternal androgens affect offspring post-natal phenotypes (behaviour and physiology) while being heavily metabolized? In **chapter 2**, I proposed three hypotheses to explain this paradox: i) the back-conversion hypothesis, where the embryos metabolize the lipophilic maternal androgens into more hydrophilic metabolites to facilitate the uptake of androgens from the yolk to the embryonic circulation. Once arriving at the target tissue, the metabolites would then be converted back to its functioning lipophilic free form; ii) the diversification hypothesis, where testosterone is converted to several different hydrophilic metabolites also enabling the uptake of androgens from the yolk to the embryonic circulation yet the metabolites exert their effects directly on the target tissue; and iii) the clearance hypothesis, where the hydrophilic metabolites keep most of the active maternal androgen from reaching or affecting the embryos. To test these hypotheses, I used eggs from rock pigeons (*Columba livia*). I added stable isotope-labelled testosterone (testosterone-[D5] to the yolk of freshly laid eggs, making it possible to follow the fate of the hormone during development artificially incubated the eggs, sampled the eggs at different stages of incubation, and finally determined the hormone profiles containing the label in the different egg fractions. I found evidence for both the back-conversion hypothesis and the clearance hypothesis, whereas I cannot yet rule out the diversification hypothesis. Specifically, the presence of testosterone-[D5] in embryonic tissue and the distribution dynamics of conjugated testosterone-[D5] throughout the first 10 days of incubation provide robust evidence for the back-conversion hypothesis. The absence of etiocholanolone in embryonic tissue at day 5 and day 10 of incubation indicates an etiocholanolone-mediated clearance process. Nevertheless, the substantial amounts of etiocholanolone in the blastoderm at day 2 of incubation and the substantial amounts of unknown metabolites at day 5 and day 10 of incubation in both yolk and embryonic tissue may still mediate the observed post-natal effects from increased maternal androgens. This means I cannot rule out the diversification hypothesis. Together, the time-specific dynamics of the hormone profiles give us a better understanding of the mechanisms inducing maternal effects, it may also indicate that the embryonic metabolism of maternal androgens could be part of a mechanism that embryos use in a potential mother-offspring conflict.

In **chapter 3**, I explored the prenatal effects of maternal androgens in rock pigeons, using the

fact that they lay two eggs in the same reproductive attempt, where maternal androgen levels increase steeply from the first- to the second-laid egg and embryonic hormone conversion is substantial in the first few days of embryonic development. I found that during the first half of the incubation period, embryonic heart rate was significantly higher in second-laid eggs than first-laid eggs in the control (not hormone manipulated) group, which correlates with their difference in androgen concentrations. During the same period, eggs with experimentally increased androgen levels increased embryonic heart rate in first-laid eggs to that of the control second-laid eggs, but the treatment did not affect the heart rate in second-laid eggs. Moreover, heart rate at mid-term incubation was strongly and positively correlated with the embryonic body- and heart mass near hatching. My findings indicate that maternally derived androgens stimulate embryonic metabolic rates but in a laying order dependent manner. This is possibly caused by a limitation of testosterone to increase the metabolic rate in second-laid eggs to an even higher rate. By further testing the effect of androgens on pre-hatching embryonic growth, I proposed that increasing embryonic metabolism may be a link between increased maternal androgens and its prenatal and potentially even postnatal effect on development, potentially also explaining the diverse effects of maternal androgens on offspring traits as the very early rise in metabolism can have a wide array of effects later in life.

In **chapter 4**, I investigated to what extent the embryo can metabolize maternal androgens in a context-dependent way. Mothers can differentially deposit their resources in the same reproductive attempt in relation to the offspring's position in the sibling hierarchy. However, whether embryos from different positions can be plastic in their response to the maternal signals, potentially leading to a mother-offspring conflict, is yet unclear. Here, I took the advantage of rock pigeon eggs again, who lay two eggs in one nest with the second-laid eggs having higher maternal androgen levels than the first-laid eggs. I experimentally increased the androgen levels in first-laid eggs to that present in second-laid eggs and measured the change in androgen levels and its main metabolites (etiocholanolone and conjugated testosterone) after 3.5 days of incubation. To complete the experiment, the same amount of androgens was also added to the second-laid eggs. We found that eggs with increased androgens show a different degree of androgen metabolism depending on not only the initial androgen levels but also their position in the lay sequence. In short, I found that the embryos are able to differentially convert maternal hormones in different contexts, which opens the possibility of a conflict between the mother and the offspring over the amount of androgens the latter should be exposed to under different contexts. It shows an intriguing new layer of complexity in hormone-mediated maternal effects and could explain the inconsistencies of *in ovo* androgen manipulation studies, as results may differ according to egg sequence and potentially other contextual factors influencing egg composition and or incubation patterns.

Finally, in the synthesis chapter, I summarized all my results across the chapters. To further address the biological consequences of the embryonic metabolism of maternal androgens, I also report in this chapter findings of several as yet unpublished experiments I conducted. In particular, to further disentangle the diversification hypothesis from the clearance hypothesis I proposed in **chapter 2**, I experimentally tested the effect of etiocholanolone, one of the major metabolites of maternal androgen, on embryo growth, immunity and behavior (**Box 1**). So far, increased etiocholanolone showed no effects on embryonic development which is in line with the absence of etiocholanolone in embryonic tissue at day 5 and day 10 of incubation I showed in **chapter 2**. Yet, with the substantial amount of etiocholanolone in the blastoderm

at day 2 of incubation and the substantial amount of unknown metabolites at day 5 and day 10 of incubation that may mediate the observed effects from increased maternal androgens, the diversification hypothesis remains highly possible. Moreover, to complement the study in **chapter 3**, where the embryonic heart rate data was only available after day 6 of incubation due to methodology restrictions, I applied a shell-less culture system to visually observe the effects of maternal androgens on the timing of the first heartbeat and its subsequent early beat rate (**Box 2**). I found that increased yolk androgens are already affecting embryonic heart rate at this very early stage when nucleus androgen receptors are not even likely expressed. This result further indicated the potent effect of maternal androgens while also leading to the intriguing possibility of non-genomic effect of maternal androgens.

In the end, I hope my results inspire future studies that focus on both proximate and ultimate questions addressing not only the mechanisms of embryonic metabolism of maternal hormones, but also its functions and eco-evolutionary consequences. For example, there is still a substantial amount of unknown metabolites of maternal androgens whose chemical compositions remain to be identified. As the embryos are able to differentially metabolize maternal androgens into metabolites according to the context, further testing the biological functions of these unknown metabolites under different contexts would give us a better understanding of the extent to which the embryos could regulate and optimize the hormone-mediated maternal effects.