

# P1-2. ONTOGENY OF THE SYNCHRONIZATION BETWEEN ADRENAL CLOCK GENES, ADRENAL STEROIDOGENESIS-RELATED GENES AND THE CIRCADIAN RHYTHM OF THE HPA AXIS IN RATS

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## INTRODUCTION

The postnatal hypothalamic-pituitary-adrenal (HPA) axis circadian rhythm develops via increasing clock gene expression in the suprachiasmatic nucleus.

The ontogeny of adrenal clock genes and their relationship with plasma corticosterone rhythm is still unknown.

## OBJECTIVE

The aim of this study was to assess the ontogeny of daily variation of the expression of the adrenal clock genes *Clock*, *Arntl* (*Bmal1*), *Per1*, *Per2*, *Per3*, *Cry1*, *Cry2*, *Rora*, and *Nr1d1* (*Rev-Erba*), steroidogenesis-related genes (*Star* and *Mc2r*) and plasma corticosterone (B).

## MATERIALS AND METHODS

- Male *Wistar* rats were kept under a 12h light/dark cycle (lights on at 0700h, zeitgeber time-ZT0).
- Plasma and adrenal tissue samples obtained every 4h over a 24h period at ZT0, ZT4, ZT8, ZT12, ZT16 and ZT20 (n=4-14 pups/ZT) on postnatal days (P): 1, 3, 6, 12, 14, 16, 21 and 24.
- Plasma B was measured by RIA and mRNA of the adrenal clock genes and steroidogenesis-related genes were measured by qPCR.
- The results were analyzed using the Cosinor method by SAS 9.4 software. P<0.05 was adopted.

• Fig.2 shows a daily variation in the mRNA expression of *Clock*, *Bmal1*, *Per2*, *Per3*, *Cry1*, *Rev-Erba*, *Mc2r* and *Star* since P3 (P<0.05), with attenuation between nadir and acrophase at P6 and reversal of these parameters from P14, reaching adult patterns at P24.

• Synchronization between the expression of the clock genes and adrenal steroidogenesis was observed from P3. The circadian mRNA expression of *Per2*, *Per3*, *Cry1* and *Star* genes became concordant with the plasma corticosterone since neonatal period.

## RESULTS

• Plasma corticosterone circadian rhythm was identified from P1 (acrophase at ZT2). Since P14 until P24 there was a nocturnal increase of B concentrations, with acrophase at ZT16 and nadir at ZT4 (P<0.01), characterizing the well known adult rat corticosterone circadian rhythm (Fig.1).

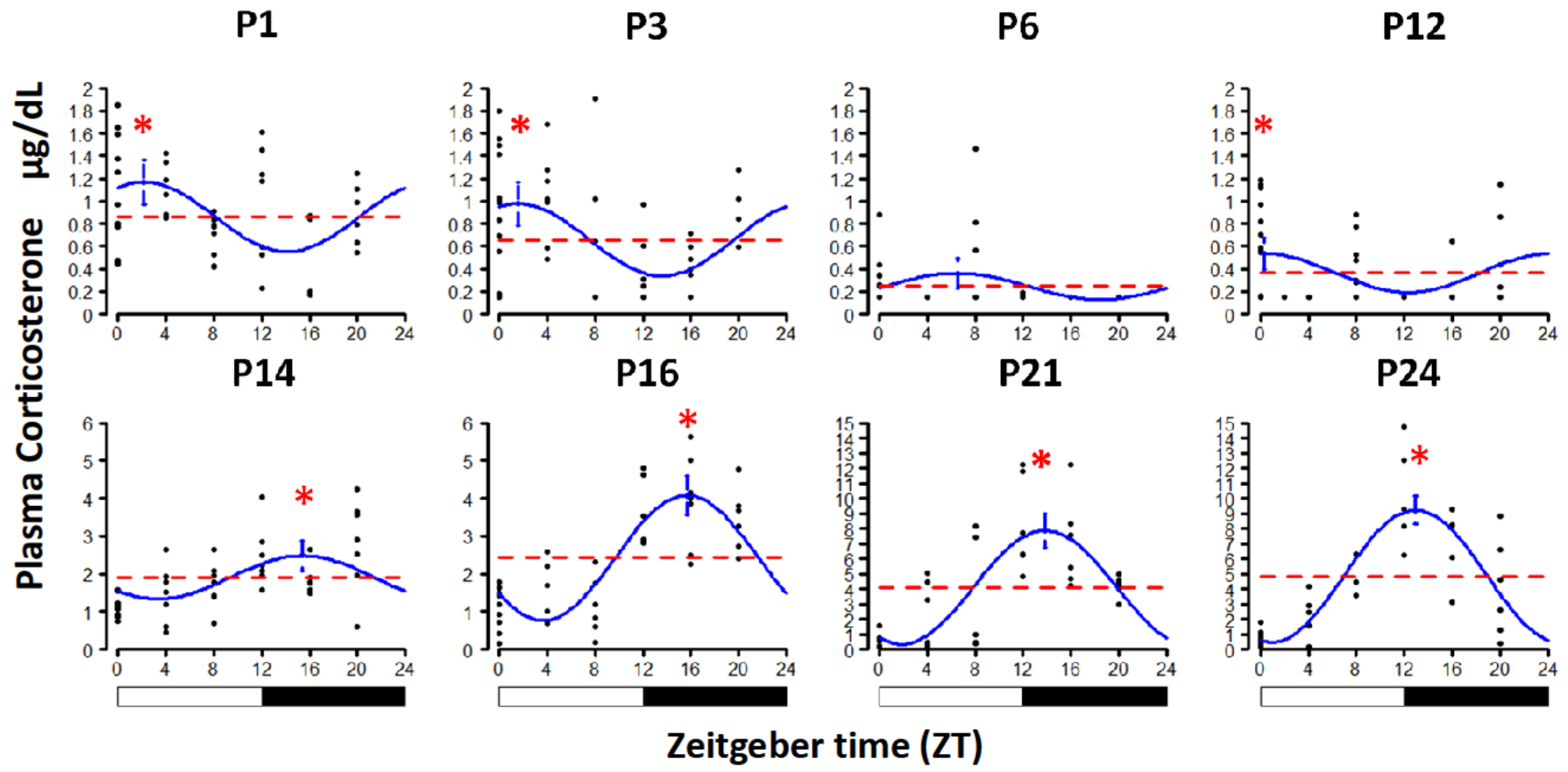


Fig.1. Plasma corticosterone values at ZT 0, 4, 8, 12, 16 and 20 at postnatal days P1, P3, P6, P12, P14, P16, P21 and P24. — Cosinor curve; - - - Mesor. (\*), differences between plasma B nadir and acrophase concentrations.

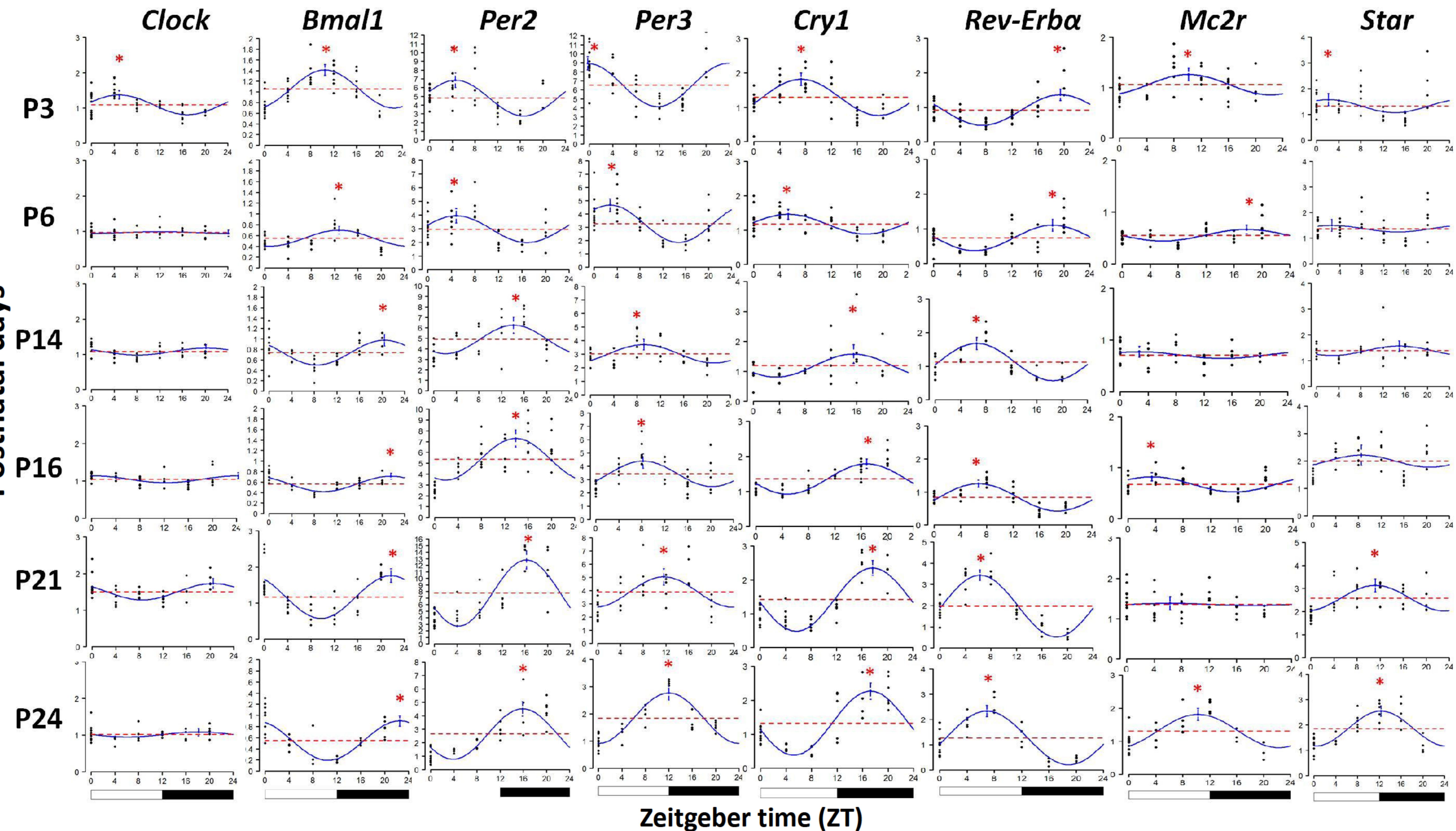


Fig. 2. Circadian profiles of gene expression ( $2^{\Delta\Delta Ct}$ ) in the adrenal at postnatal days P3, P6, P14, P16, P21 and P24. (\*) differences between nadir and acrophase.

## CONCLUSIONS

- The ontogenetic daily variations of adrenal gene expression were synchronous, concordant (*Per2*, *Per3*, *Cry1* and *Star*) or antagonistic (*Bmal1* and *Rev-Erba*) with circadian plasma corticosterone concentrations.
- The adult circadian profiles of the adrenal clock gene expression are observed from P14, and probably contribute to the appearance of the adult circadian corticosterone rhythm.

Support: