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17- β -Estradiol and Testosterone concentrations in claws of puppies up to 60 days of age.

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The current 30% of canine perinatal mortality (Tonnessen et al., 2012) claims the need to deepen the knowledge about this phase, characterized by long-term hormonal and metabolic changes, that could benefit of long time-frame methods of investigation, reducing the quantity of samplings and thus respecting animal welfare (Veronesi et al., 2015). Indeed, apart from the well-known invasiveness of blood samplings, the necessary repeated collection of feces, urine and saliva, providing punctual information, could be stressful for the newborns and their mothers with negative effects on health status and maternal behavior.

Given that sexual steroid hormones were reported to have an influence on health outcomes and development already from the perinatal period (Frey et al., 2017), in this work 17- β -Estradiol (E2) and Testosterone (T) concentrations were assessed from the claws of dogs up to 60 days of age, to identify possible endogenous biomarkers.

Ten male and 10 female puppies, viable and healthy, born by elective cesarean section, were enrolled. Samplings were performed by trimming claws at birth, and the regrowth at 30 and 60 days of age after the breeder or the owner signed an informed consent. Then, E2 and T concentrations were analyzed by RIA (Veronesi et al., 2015) and a possible effect of gender evaluated by ANOVA.

All the hormonal claws concentrations showed a significant ($p < 0.001$) drop from birth to 30 and 60 days of age, while no significant changes were observed between 30 and at 60 days of age (Table 1). No influence of the newborns' gender was found.

Because of the higher levels of E2 and T observed at birth and at 30 days of age, it could be supposed that a source of production could be the placental and maternal compartments. However, the direct involvement of the fetus itself could not be excluded, given the reported accumulation from the nail capillary bed of those hormones (de Berker et al., 2007) and the production of sexual steroid hormones by feline fetal gonads (Braun and Jewgenow, 2017).

Table 1: Claws concentrations of 17- β -Estradiol (E2), and Testosterone (T) at birth, 30 and 60 days of age in the 20 puppies enrolled in the study.

FEMALES (n=10)	Birth	30 days	60 days
E2 (pg/mg)	9.2 \pm 3.87 ^a	3.3 \pm 1.75 ^b	1.9 \pm 0.67 ^b
T (pg/mg)	11.2 \pm 6.66 ^a	4.3 \pm 2.44 ^b	2.3 \pm 0.78 ^b
MALES (n=10)	Birth	30 days	60 days
E2 (pg/mg)	8.3 \pm 2.91 ^a	2.4 \pm 0.60 ^b	1.7 \pm 0.54 ^b
T (pg/mg)	8.7 \pm 3.12 ^a	3.7 \pm 0.98 ^b	1.9 \pm 0.75 ^b

a, b within rows denotes a significant difference with $p < 0.001$.

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