PS19 - Assessing the age-related relationship between plasma anti-Müllerian Hormone (AMH) and ovarian follicle population in prepubertal ewes

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Anti-Müllerian Hormone (AMH) is mainly produced by the granulosa cells of small growing antral follicles and has been proposed in many species as a reliable marker of the ovarian follicle population. Despite the advanced knowledge of this hormone in adults, information during the prepubertal period is scarce. The aim of this study was to characterize plasma AMH profile in ewe lambs from birth to 5 months of age and to establish its relationship with ovarian follicular population. With this aim, blood samples from 13 Rasa Aragonesa ewe lambs were taken weekly from birth until slaughtered, either at 1 (M1; n=6) or 5 (M5; n=7) months. Ovaries were dissected to isolate all the antral follicles larger than 1 mm in diameter. For each animal, follicles were classified according to their size (small: 1-<3 mm; medium: 3-5 mm; large: >5 mm) and follicular fluid and granulosa cells (GCs) were recovered. Plasma and intrafollicular AMH levels were determined with an ELISA kit (Beckman Coulter, Roissy CDG, France). For evaluation of in vivo AMH expression, RNA was extracted from GCs and reverse transcription was carried out to finally perform quantitative RT-PCR. Plasma AMH concentrations peaked at 1 month and then declined gradually to reach stable values along time, so that levels at 1 month (2059 ± 440 pg/ml) were significantly higher than those at 5 months (107 ± 41 pg/ml; $P < 0.001$). Total follicle number per animal was also higher in M1 (205 ± 47) than in M5 (34 ± 5) groups ($P < 0.005$), mainly due to increased number of small follicles in M1 when compared with the older counterparts (+170 follicles; $P < 0.005$). In both M1 and M5 ewes, plasma AMH was highly correlated with total follicle number ($r = 0.96$ and 0.89, respectively; both $P < 0.01$). Regarding intrafollicular AMH concentration, it was significantly higher in small (917 ± 64 ng/ml) than in medium (250 ± 70 ng/ml) or large follicles (30 ± 11 ng/ml; $P < 0.0001$ for both), independently of age group. Concerning small follicles, while no significant differences were found in AMH concentrations between M1 and M5, differences among age-groups where highly significant when the total AMH produced per this class was estimated taking into account the number and volume of these follicles (839 ± 201 ng vs. 126 ± 25 ng, $P < 0.005$). No differences were found in AMH gene expression between M1 and M5 small follicles. Finally, total AMH produced per each pair of ovaries was also estimated and related with total AMH measured in plasma per ewe, taking into account body weight and blood volume. The correlation coefficient found ($r = 0.94$; $P < 0.001$) demonstrated a great relationship between AMH determination in the ovaries and blood levels. In conclusion, altogether, our results indicate that great variations in follicle number and size are responsible for the major AMH peak found very soon after birth. Independently of age, plasma AMH concentrations in ewe lambs reliably reflect the ovarian follicle population.

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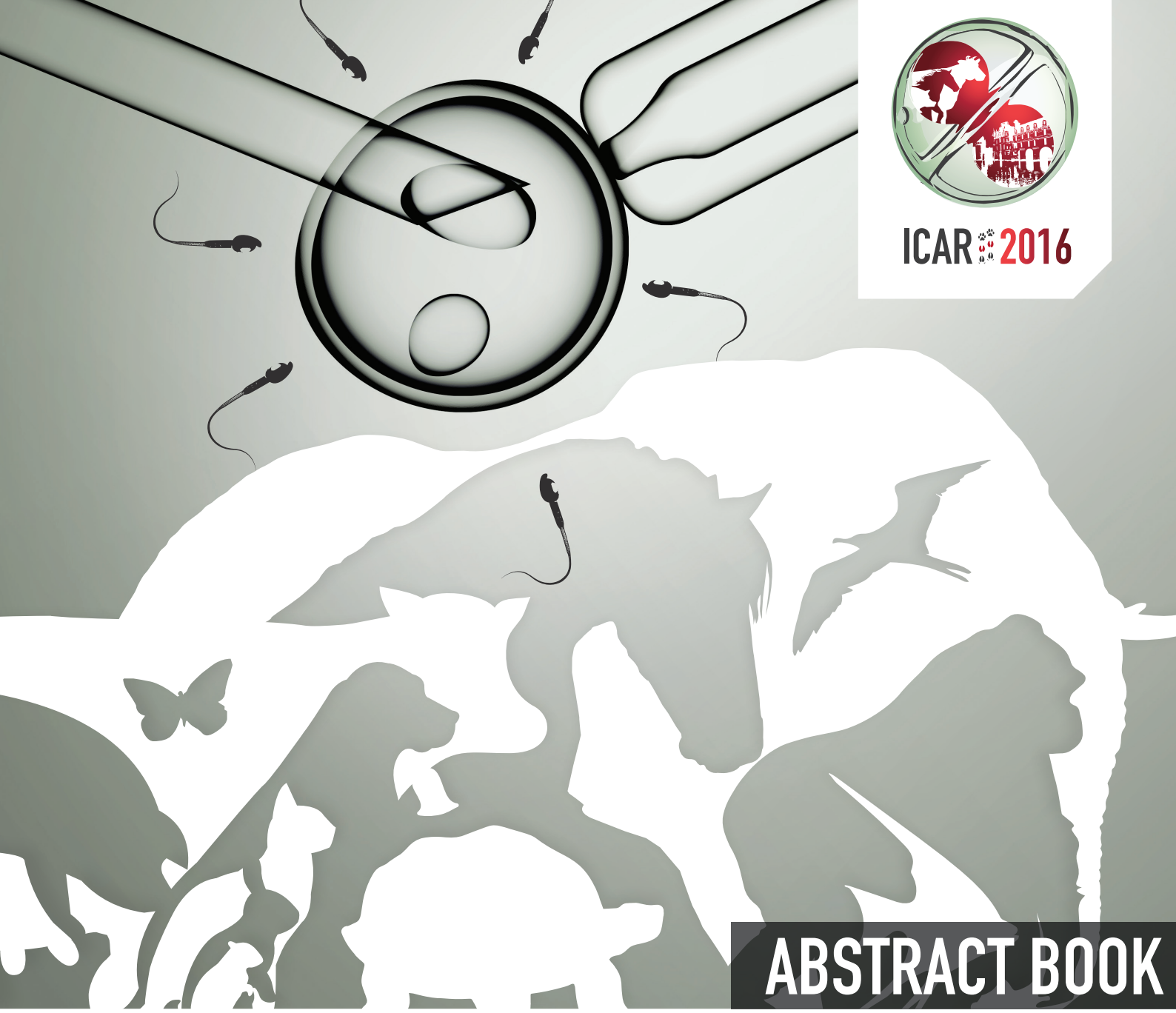

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