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Anti-müllerian hormone (AMH) as a potential predictive marker of fertility in bitches
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There are no published reports investigating AMH concentrations in bitches using a canine-specific assay. Previous reports are limited to the diagnosis of ovarian remnant syndrome (ORS) using a human AMH assay [1]. The aims of this study were: 1) to establish the normal reference interval for AMH in intact breeding bitches, and 2) to investigate whether there were any associations between AMH concentrations and reproductive outcomes in bitches, using a canine-specific AMH assay. A prospective cohort study was performed on a random sample of 155 bitches of various ages and breeds that were presented for routine breeding management over a one-year period. AMH concentrations were measured on serum harvested from blood samples that were taken for progesterone analysis used to determine insemination timing. All bitches were artificially inseminated with frozen (n=78) or fresh (n=77) semen using the transcervical insemination (TCI) technique. AMH concentrations were measured using a commercially available canine AMH ELISA (AnshLabs®, Texas, USA), which we validated prior to performing the study. The 95% reference interval (RI) for AMH was calculated using Cook’s method of outlier detection and the 90% confidence intervals of the upper and lower limits were established. The reproductive outcomes of interest were whelping rate (defined as the proportion of bitches producing at least one live pup of those inseminated), and litter size (number of pups born per litter). Multiple logistic regression was used to determine the effects of the independent variables, namely; AMH concentration, bitch age, parity, breed, size (small, medium, large, giant), semen type (fresh, frozen), sperm motility and total number of sperm inseminated on the whelping rate. The effects of the same independent variables on litter size were analyzed using multivariable linear regression. The mean age of bitches in the study was 3.7 ± 1.5 years (range 1.2 – 7.6 years). The mean whelping rate was 74.2% and the mean litter size was 5.8 pups/litter. The reference interval for AMH for all bitches in the study, regardless of body weight was 2.9 – 21.1 ng/ml. Giant breeds had significantly (P<0.01) lower AMH concentrations (1.75 - 15.6 ng/ml) than small (5.6 - 24.2 ng/ml), medium (4.3 - 23.7 ng/ml) and large (4.3 - 21.0 ng/ml) sized bitches. There was a significant effect of bitch age on AMH concentrations. The mean AMH concentration in bitches less than 4 years of age was 12.4 ng/ml, whereas the mean AMH concentration in bitches older than 4 years of age was 10.5 ng/ml (P<0.05). For each additional year of age, AMH concentrations fell by 0.5 ng/ml. The only variables remaining in the multivariable model of whelping rate were age of the bitch and size of the bitch. The whelping rate of bitches less than 4 years of age was 81.1%, compared with 64.6% in bitches older than 4 years of age (P<0.05). The whelping rates of small, medium, large and giant breeds were 80%, 78.6%, 80.3% and 57.5% respectively (P<0.01). There was no effect of AMH concentration on the whelping rate. The only variables remaining in the multivariable model for litter size were AMH concentration and bitch size; there was also a significant interaction between these two variables. Smaller breeds had smaller litters (and higher AMH levels) but within each breed size category bitches with higher AMH concentrations had significantly larger litter sizes (P<0.01). For each 1ng/ml increase in AMH, litter size increased by 0.3 pups/litter. In conclusion we are the first to report the normal reference interval for AMH in bitches using a canine-specific assay. In agreement with findings in humans and other species, we found that there is a decline in AMH concentrations with advancing age in bitches.

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