Evaluation of salivary progesterone profiles as an indicator of reproductive status in equines
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Diagnostic assays of reproductive hormones are usually performed by veterinarians on mares to confirm the findings of trans-rectal palpation and/or reproductive ultrasound examination. Of the reproductive hormones, progesterone (P4) is one of the most commonly measured hormones in the field of equine reproduction. Serum P4 concentration in mares is usually evaluated using an extracted radioimmunoassay (RIA), which is generally considered as the gold standard in reference laboratories. However, the use of RIA for serum P4 measurement usually involves a lengthy process to extract the steroids from carrier proteins. Up to 95% of the steroid hormone binds to carrier proteins. Estimation of salivary steroid hormones presents an attractive alternative, since the salivary steroids are not protein bound and appear to be in biologically active form. The objective of this study was to compare serum and salivary progesterone values during the estrous cycle (n=13) and early pregnancy in mares and to evaluate whether saliva could serve as an alternate biological fluid to monitor the luteal phase in mares. Serum and saliva samples were collected on selected days of the estrous cycle days (1, 3, 5, 8, 14, 17 and 20) and during early pregnancy (days 1, 3, 5, 8, 14, 17, 25, 35, 45, and 65) from mares and were validated using a liquid phase RIA. Serum samples were extracted and processed using charcoal dextran adsorption before subjecting to the RIA. Salivary samples did not require solvent extraction and were subject to RIA without this step. The inter-assay coefficient of variation (CV) for low and high controls was 6.91% ± 0.81 (Mean ± S.E.) and 5.06 % ± 0.57 (Mean ± S.E.) for paired serum and saliva samples run in the same assay, while the intra-assay CV averaged 13.19% for saliva and 11.71% for serum. Mean saliva: serum ratio was elevated in cycling (35%) and pregnant (25%) mare until day 3 of the cycle and then dropped to and remained between 8 to 12% for the remaining duration in cycling (until day 17) and pregnant mares (up to day 65 ) (p > 0.05). Statistical analysis was performed using Friedman’s one way ANOVA. Serum P4 for cycling and pregnant mares differed by day of sampling (p < 0.0001). In sharp contrast there was no significant difference in salivary progesterone levels by day of sampling for cycling mares (p > 0.05). In pregnant mares it was different for only on days 1 and 3 after ovulation. A significant difference was seen with the salivary to serum ratio in cycling mares while this difference was only observed on day 1 after ovulation in the pregnant mare group. Pearson’s correlation analysis showed that the correlation between salivary and serum progesterone levels was not significant except on day 5 (after ovulation) for the cycling mare group (p >0.05), and days 5 and 8 (p < 0.05) for pregnant mares. Luteal phase saliva P4 levels were observed to be consistently above 0.5 ng/ml in both groups and could be used as a cutoff range to differentiate between the follicular and luteal phase. The study shows that salivary P4 concentration was not correlated with serum values. Nevertheless it can be utilized to monitor luteal phase of the estrous cycle and early pregnancy.

Keywords: Mare, estrous cycle, pregnancy, progesterone, saliva, serum