Refereed

ESTROUS CYCLE OF THE MARE EVALUATED BY FECAL STEROID METABOLITES

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SUMMARY

The estrous cycles of four domestic mares were characterized by means of urinary estrone conjugates (E_1C) and immunoreactive pregnanediol-like progesterone metabolites (iPdG). The urinary E_1C concentrations were compared to fecal E_1C concentrations, and urinary iPdG concentrations were compared to fecal iPdG and progesterone (P_4) concentrations, in order to evaluate the use of fecal steroids for assessment of ovarian function. Correlation coefficients for urinary steroid conjugates versus fecal steroid conjugates of P_4 ranged from r=0 to r=0.83, but fecal E_1C, iPdG, and P_4 all paralleled the qualitative changes in ovarian endocrine function assessed by urinary steroid conjugate analysis. Fecal P_4 provided a more accurate assessment of the luteal phase than fecal iPdG. The results indicate that ovarian endocrine activity in the mare can be evaluated through the use of fecal steroids or their metabolites, and that this approach may be useful for the study of reproduction in free-roaming feral horses.

INTRODUCTION

Characterizing endocrine events associated with reproduction in large free-roaming animals can be a difficult and dangerous task, due to the necessity of obtaining blood samples. Until recently it was necessary to capture an restrain or tranquilize wild species in order to collect blood An alternative approach to the study of reproductive endo crine events in free-roaming wildlife is based on the measure ment of urinary and fecal steroids or their metabolites. Th technology which permits remote monitoring of reproductiv endocrinology was originally developed to evaluate the re productive status of captive exotic mammals through urinar steroid metabolites.[1,2,3] The same technology was next ex tended to pregnancy detection in free-roaming feral hors es,[4,5,6] In this case, a single urine sample, collected from th ground, and the measurement of a pregnancy-specific hor mone metabolite was all that was necessary. The ovaria endocrine changes associated with the estrous cycle ar dynamic and require frequent urine sampling in order t visualize the sequential events of ovarian hormone secretion The same non-capture approaches used for pregnancy detec tion have been used for monitoring cyclic ovarian function i free-ranging wildlife.[3,7]

The collection of urine from free-roaming wildlife can b time-consuming and in some cases dangerous. The use o fecal steroid evaluation has proven successful with a numbe of domestic and captive exotic species.[8,9] Pregnancy diagno sis has been accomplished by the measurement of feca steroids or their metabolites in feral horses[5,10,11] and caribou (*Rangifer tarandus*).[12]

It is also possible to evaluate cyclic ovarian endocrin activity in wildlife by means of fecal steroids or their metab olites. Desauniers et al.[13] monitored the luteal phase of th domestic cow estrous cycle by means of fecal P_4. Our study was conducted to evaluate the estrous cycle of the domesti

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only 4.6 days in old mares. A depressing effect of progesterone on follicular growth without an alteration of FSH concentrations has been shown in cattle.[7]

In conclusion, a mean delay of 4 days in the emergence of the primary follicular wave was found in mares ≥20 years old. The delay in primary wave development accounted for the reported prolonged interovulatory interval and follicular phase in old mares. Delayed emergence was associated with a reduced number of follicles in the wave. Perhaps the follicular delay was related to a smaller pool of available follicles in the old mares or the follicles that were available may have been less sensitive to FSH stimulation. Although not critically examined, the eventual emergence of a primary wave in the oldest group seemed temporally associated with a decrease in progesterone concentrations during luteolysis.

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mare by means of fecal P_4, E_1C, and iPdG, and to compare the accuracy of fecal steroid analysis with the results of urinary steroid analysis.

MATERIAL AND METHODS

Four sexually mature, domestic, non-pregnant mares (*Equus caballus*) were used for this study. The mares were between 6 and 20 years old and were pastured on approximately 8 hectares of grass, with free-standing water available ad libitum. Matched urine and fecal samples were collected from each mare every other day from April 23 to July 1. The urine samples were removed from newly saturated soil immediately after urination as described by Kirkpatrick et al.[4] Fecal samples were collected immediately after defecation and stored in plastic bags. Both urine and fecal samples were placed on ice at the time of collection and frozen until assay.

Urine samples were diluted 1:100 in distilled water (dH_2O) for the measurement of E_1C and 1:1 for the measurement of iPdG. The E_1C enzyme immunoassay has been previously described.[14] The intra- and interassay coefficients of variation were 5.8% (n=4) and 20.7% (n=7). The iPdG enzyme immunoassay has been previously described.[15,16] The intra- and interassay coefficients of variation were 13.8% (n=4) and 14.2% (n=8). In order to account for differences in urine concentrations, the urine samples were diluted 1:100 with dH_2O and analyzed for creatinine (Cr) by the microcolorimetric method of Taussky,[17] and E_1C and iPdG were reported as ng/mg Cr. Urinary E_1C and iPdG were plotted for the duration of the study period and permitted assessment of 2 complete estrous cycles for each mare. The best defined estrous cycle for each mare was used for comparison with fecal hormone concentrations.

Wet fecal samples were weighed and approximately 3 g were mixed with 7.0 ml of dH_2O, shaken for several hours and incubated at 10°C overnight. This slurry was centrifuged at 2,500 rpm for 15 minutes and the supernatant was recovered for measurement of E_1C and iPdG. Twenty µl of the supernatant was assayed for E_1C and iPdG. Following centrifugation, the fecal pellet was dried under a continuous stream of air at 40°C. The dried material was ground fine in a mortar and pestle and 0.25 g was rehydrated with 0.5 ml dH_2O and extracted 3 times with 3 ml of diethyl ether each time, as described by Desauinier et al.[13] The ether extracts were dried under air, the residue redissolved in 1.0 ml 95% ethyl alcohol and stored at 5°C until assay. The ethanol was diluted 1:100 and 20 µl were assayed for P_4 by enzyme immunoassay as described by Munro and Stabenfeldt.[18] Progesterone concentrations were reported as ng/g dry feces. The intra- and interassay coefficients of variation for the P_4 EIA were 9.6% (n=4) and 16.3% (n=4). Fecal P_4 and iPdG were compared to urinary iPdG concentrations, and fecal E_1C was compared to urinary E_1C concentrations for each estrous cycle.

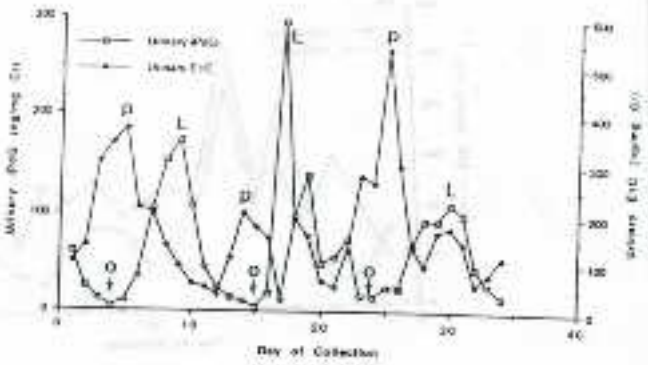

Figure 1. Three consecutive estrous cycles for the mare Granny, characterized by urinary E1C and iPdG. P=the preovulatory estrogen peaks, O=the approximate time of ovulation, based on progesterone nadirs, and L=luteal phase progesterone peaks.

RESULTS

Urinary E_1C and iPdG concentrations during the 69-day collection period revealed a pattern consistent with ovulation and a non-conceptive luteal phase P_4 elevation which is consistent with the estrous cycle of the mare.[16,19] Each estrous cycle consisted of a preovulatory E_1C peak, a concomitant iPdG nadir which is required for ovulation, and a subsequent elevation of iPdG reflecting the luteal phase of the cycle. Among the four mares, eight complete estrous cycles were identified. Three consecutive cycles, based on urinary E_1C and iPdG, for the mare Granny are shown in Figure 1. Fecal E_1C concentrations reflected the same patterns as that seen with the urinary E_1C and the correlation coefficients between urinary E_1C and fecal E_1C for different estrous cycles ranged from $r = 0.1$ to $r = 0.63$. Two estrous cycles, one with a correlation of $r = 0.1$ and one with a correlation of $r = 0.63$, characterized by urinary and fecal E1C are shown in figure 2A and B, respectively.

Fecal iPdG and Po also reflect the urinary iPdG patterns over the course of the estrous cycles. The correlation coefficients for urinary iPdG and fecal iPdG ranged from $r = 0.08$ to $r = 0.42$. an estrous cycle with a correlation of $r = 0.42$ between urinary and fecal iPdG is shown in figure 3A. The correlation coefficients for urinary iPdG and fecal P_4 ranged from $r = 0.44$ to $r = 0.83$. An estrous cycle reflecting urinary iPdG and fecal P_4 is shown in figure 3B.

DISCUSSION

The remote monitoring of ovarian function in the mare is possible by means of fecal E_1C, iPdG and P_4. There is a large quantitative difference between urinary E_1C and iPdG values and fecal E_1C and iPdG values, but the fecal values accurately reflect the qualitative changes occurring in urinary E_1C and iPdG during the estrous cycle. The quantitative differences

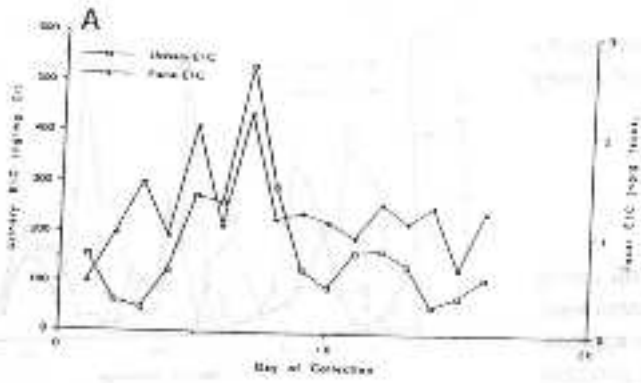

Figure 2A. A comparison of urinary E_1C with fecal E_1C.

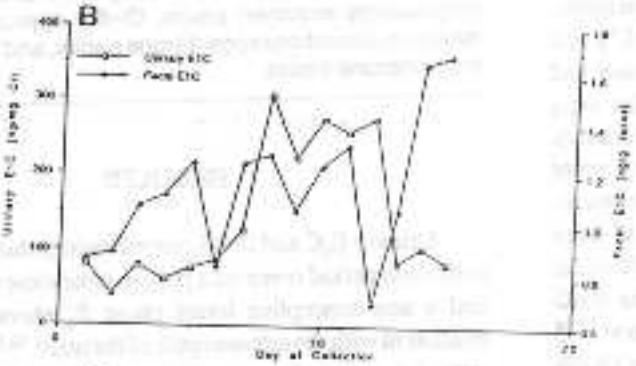

Figure 2B has a correlation of r = 0.1 and 2B has a correlation of r = 0.63.

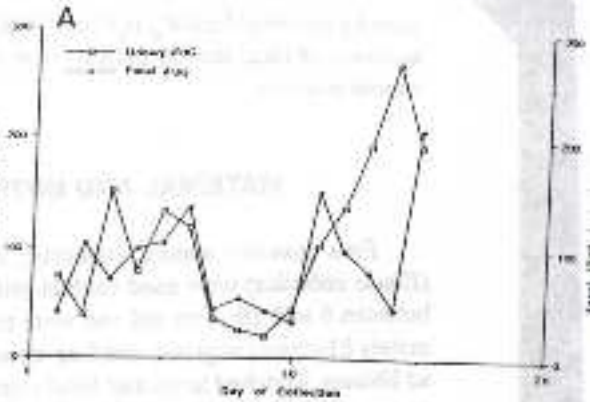

Figure 3A. A comparison of urinary and fecal iPdG with a correlation of r = 0.42.

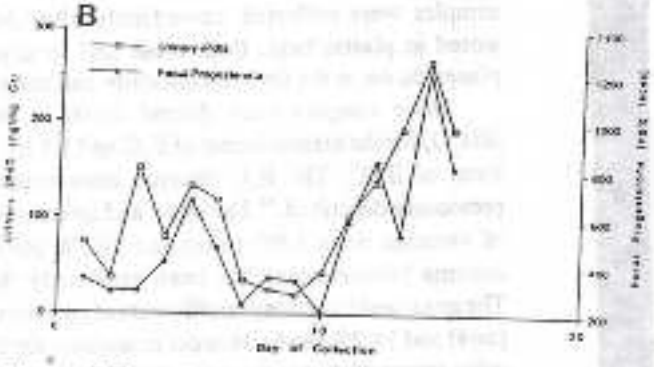

Figure 3B. A comparison of urinary iPdG and fecal progesterone (r = 0.83).

are explained by the fact that the majority of estrogen and P_4 metabolites in the mare are excreted in the urine rather than the feces. Estrogens in the vascular space are metabolized in the liver and conjugated to either sulfate or glucuronic acid.[19] From the liver, these conjugates pass into the gastrointestinal tract where the majority are resorbed back into the blood, transported to the kidney and excreted in the urine. However, a smaller portion of the E_1C is trapped in the gastrointestinal tract and excreted in the feces.[10] Also, the kidney is a concentrating organ and therefore urine concentrations are significantly higher than fecal concentrations.

The precise nature of the iPdG in the mare is not known, but consists of three progesterone metabolites, all of which are more polar than pregnanediol-3-glucuronide (PdG) and which are highly correlated with plasma P_4.[18] It is probable that the appearance of iPdG in the feces occurs in the same manner as fecal E_1C. Fecal P_4 concentrations in the mare are considerably lower than those found in the cow,[13] and may reflect differences in P_4 metabolism between the two species. The primary P_4 metabolite in the domestic cow (*Bos taurus*)[20] and the North American Bison (*Bison bison*)[21] is PdG, however, PdG is absent in the mare.[16] Conversely, iPdG, the primary P_4 metabolite in the mare, is absent in the cow and bison.

The correlation coefficients between urinary steroid metabolites and fecal metabolites or progesterone were the lowest for iPdG, and the strongest for P_4. This suggests that fecal progesterone is a more accurate measure of luteal function in mares than iPdG. Despite some very low correlation coefficients, particularly for urinary vs fecal E_1C and iPdG, all 3 fecal hormones provided a qualitative view of ovarian endocrine activity that was useful for identifying the time of estrus, the occurrence of ovulation, and the presence of a luteal phase in the mare. This provides the field biologist with a relatively simple approach to monitoring ovarian function among feral mares, without the physiological stresses of capture and the costs of immobilization. The same basic techniques should be applicable to a variety of free-roaming exotic ungulates, although care must be taken to identify the specific fecal steroids or their metabolites and to validate their accuracy in identifying reproductive events in each species.

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