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Enzyme immunoassay of progesterone in the feces from beef cattle to monitor the ovarian cycle

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Abstract

The present study was undertaken to measure fecal progesterone concentration of beef cattle using antibody against authentic progesterone and to examine whether this method can monitor the ovarian cycle in beef cattle. Rectal fecal samples collected from 14 beef cattle were mixed with 6 ml of 100% methanol and shaken for 15 min. After centrifugation, supernatant was extracted with petroleum ether followed by an enzyme immunoassay (EIA) for progesterone. Specificity of the assay was examined by HPLC separation of fecal solution followed by the EIA in each fraction. The present assay identified only progesterone but not other metabolites in the feces sample that was extracted with petroleum ether. Sensitivity of the assay was estimated to be 0.0055 ng/ml (0.11 ng/g). Coefficient variations of intra- and inter-assay were 9.6–10.9% and 10.8–16.6%, respectively. Recovery rates ranged between 73 and 84%. Patterns in the fecal progesterone concentrations during the ovarian cycle were almost parallel to the plasma concentrations. A significant positive correlation was established between the fecal and plasma progesterone concentrations in individual animal ($r = 0.59$–0.84, $P < 0.001$, $n = 10$) as well as pooled data ($r = 0.70$, $P < 0.001$, $n = 65$). Fecal progesterone concentrations of day 0 (showing the nadir of concentration) of the ovarian cycle were less than 50 ng/g, which increased significantly toward day 9 ($P < 0.01$). From days 14 to 18, there was significant reduction of fecal progesterone concentration ($P < 0.01$). Ovarian cycles had at least 48 ng/g (mean = 74 ng/g) of difference between minimum and maximum fecal progesterone concentrations. All cattle at days 9, 11 and 14 had higher

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fecal progesterone concentrations by more than 20 ng/g compared with day 0. These results suggest that the present EIA is suitable to measure the progesterone in cattle feces and can monitor ovarian cycle.

Keywords: Beef cattle; Enzyme immunoassay; Ovarian cycle; Fecal progesterone; Corpus luteum

1. Introduction

Non-invasive methods for determining reproductive status have been developed to facilitate studies on reproductive physiology in the cattle. Milk progesterone profiles, for example, have given us useful information on the reproductive stages in lactating cows (Rajamahendran et al., 1993; Opsomer et al., 1998; Isobe et al., 2004). However, milk is not available for dairy heifers and beef cattle.

There is increasing interest in the application of fecal hormone analysis as additional or alternative approach to non-invasive endocrine assessment, primarily owing to the relative ease of sample collection from animals. The presence of measurable amounts of reproductive steroids was identified in the feces of cattle (Desaulniers et al., 1989; Larter et al., 1994; Schwarzenberger et al., 1996) and other animals (Morrow and Monfort, 1998; Morrow et al., 1999; Shaw et al., 1995; Thompson and Monfort, 1999). According to Schwarzenberger et al. (1996), since progesterone was metabolized before its fecal excretion, unmetabolized progesterone was not, or only to a limited extent, detectable in the feces. Therefore, they used specific antibody against progesterone derivative (20-oxo-pregnane) for monitoring corpus luteum function (Schwarzenberger et al., 1996; Rabiee et al., 2001). However, since progesterone assay is more universal, measurement of fecal authentic progesterone may enable the hormonal monitoring of reproductive status more practical in dairy heifers and beef cattle. However, detailed studies on fecal progesterone profiles during ovarian cycles have not been reported.

In the present study, we tried to establish an enzyme immunoassay (EIA) of progesterone in the feces from beef cattle using antibody against authentic progesterone and to examine whether this method can monitor the ovarian cycle of beef cattle.

2. Materials and methods

2.1. Animals and sample collection

Crossbred cows between Holstein–Friesian and Japanese Black ($n = 14$, age = 3–9 years) were housed at the farm of Hiroshima University. Animals were fed in accordance with the Animal Care/Ethics Committee of University. Fecal samples were collected from all animals three times a week for at least 3 weeks. Initiation of fecal sampling was based upon observation of estrus. Rectal fecal samples were taken with nylon-covered hand, put into