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Short communication

Biochemical markers of bone turnover during pregnancy in horses: a longitudinal study

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Abstract

The effect of pregnancy on bone metabolism was investigated in healthy mares. Venous blood samples were collected 7 times from 19 multiparous mares starting at 20-weeks pre-parturition, continuing 6 times in 4-week intervals, including the week of parturition and one week after parturition. Serum concentrations of osteocalcin (OC) and carboxy-terminal cross-linking telopeptide of type I collagen (CTX-I) were determined. Measurement cycles and age had a significant ($p < 0.01$) influence on OC and CTX-I values. Pregnancy influenced bone metabolism with peak bone formation and resorption values around the time of parturition.

Key words: Horse, pregnancy, bone, osteocalcin, CTX-I

Introduction

The effect of pregnancy on the maternal skeleton was studied in women and in various animal species (Kritz-Silverstein et al. 1992, Liesegang et al. 2007). Despite the importance of this subject concerning sport, breeding and milk producing mares, information regarding pregnancy associated changes in bone metabolism is still missing in horses. This study evaluates the effect of pregnancy on biochemical bone markers in mares.

Materials and Methods

19 clinically sound Haflinger mares, aged 4 to 16 years (8.05 ± 4.01 years), were included in the study (Nov 2008 and April 2009). The mares had a history of one to 12 pregnancies (4.37 ± 3.49 pregnancies). They were vaccinated (tetanus, equine influenza, equine herpes virus 1 and 4) and dewormed. They were held in a herd on a pasture from April to October, thereafter in a paddock-pen. Feeding was *ad libitum* grass, respectively straw and hay, as well as

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oats and mineral supplements. Blood samples were collected by aseptic puncture of the jugular vein between 8:00am and 10:00am. Ten ml of blood was collected in serum tubes. Blood sampling was performed 7 times, starting at 20 weeks pre-parturition (T_{-20W}), continuing 6 times in four-week intervals, i.e. at T_{-16w}, T_{-12w}, T_{-8w}, T_{4w}, including the week of parturition (T_{0w}) and the week after parturition (T_{+1w}). Blood samples were held on ice and were centrifuged (3600 *x g* for 10 min) within 45 min. Serum samples were stored in polypropylen vials in aliquots of one ml at -21°C until assayed. Five ml EDTA-plasma was obtained once at T_{-20w} for hematological and biochemical analysis. Analysis of biochemical bone marker was performed in serum samples and in double. Osteocalcin (OC) concentration was analyzed utilizing an equine specific competitive radioimmunoassay (RIA) (Carstanjen et al. 2003). Carboxy-terminal cross-linking telopeptide of type I collagen (CTX-I) concentrations was analyzed by using an automated electrochemiluminescent sandwich antibody assay (ECLIA; Elecsys β-CrossLaps/serum assay, Roche Diagnostics GmbH, Penzberg, Germany) (Carstanjen et al. 2004). The EDTA-samples were analyzed to detect red and white blood cell numbers as well as plasma gamma-glutamyl-transferase (GGT) and creatinine concentrations (LT-GT 0123-Kit, LT-CR 0251-Kit, Labor und Technik, Eberhart Lehmann, Berlin, Germany). Statistical analysis utilized SAS (Version SAS 9.1.3 Service Pack 4, SAS Institute Inc., Cary, USA). A mixed model with time as fixed effect was used to test the influence of the dependent variables. Measurement cycles were regressed quadratically on the age for OC and the OC/CTX-I ratio and linearly on the age for CTX-I. Successive measurements were assumed auto-correlated and the correlation coefficient was estimated along with the other parameters of the model. The significance threshold was set to 0.05.

Results

GGT and creatinine values were in the normal range [(20.4 +/- 10.14 U/l) and (71.32 +/- 6.76 µmol/l)]. The mares' age and number of pregnancies were highly correlated (> 0.98). Age had a significant influence on CTX-I values ($p < 0.01$) and the OC/CTX-I ratio ($p < 0.05$). Measurement cycles had significant ($p < 0.0001$) influence on OC, CTX-I values and OC/CTX-I ratio. OC concentration significantly increased between T_{-4w} and T_{0w} ($p < 0.0001$). CTX-I concentration significantly increased between T-12w and T_{-8w} ($p < 0.0001$) and between T_{-4w} and T_{0w} ($p < 0.01$). The OC/CTX-I ratio did not change significantly ($p > 0.05$).

Discussion

Biochemical bone markers enable monitoring short-term changes in bone metabolism (Seibel 2005). OC was used as bone formation marker, while the maternal β-form of CTX-I was used as bone resorption marker. Creatinine and GGT levels of the study population were normal, as impaired kidney and liver function might alter bone marker concentrations (Seibel 2005). Blood samples were taken in a way to avoid circadian variations. The effect of seasonality can be limited, as mares did not foal at the same time. In order to avoid degradation processes, blood samples were held on ice until centrifuged and serum samples were frozen till analysis. Multiple pregnancies have a greater impact on bone metabolism compared to a singleton gestation (Okah et al. 1996). In this study the number of pregnancies was strongly correlated with the mares' age, excluding to test the influence of number of pregnancies on bone metabolism. Mechanisms regulating calcium metabolism are altered during pregnancy and lactation due to fetal skeleton formation and milk production. During pregnancy, absorption of calcium by the intestinal tract as well as the renal excretion rates is known to be increased. Bone marker changes at the end of pregnancy are consistent to the way maternal calcium homeostasis might adapt to liberate calcium for the fetus (Kritz-Silverstein et al. 1992). This is necessary as the maternal gastrointestinal tract might not be capable of increasing the calcium absorption sufficiently (Pitkin 1985). The observed increases in bone formation and resorption markers conform to variations described in other species (Kritz-Silverstein et al. 1992). Limitations of the study are the number of animals investigated and the number of bone markers used. Further studies with additional bone markers might give valuable information. Pregnancy however, especially towards the end does influence bone turnover in healthy adult mares.

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