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Serum bone biomarkers osteocalcin and pyridinoline in mares during pregnancy and lactation, and in foals during early post-natal life

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ABSTRACT

Breeding mares typically foal yearly. Little is known about the dynamics of maternal bone stores during gestation and lactation, the timing of any maternal bone mobilisation, re-accretion post-foaling, or the dynamics of bone metabolism in foals. We measured serum osteocalcin (OC) and serum pyridinoline (PYD) concentrations in 18 mares monthly from 6 months gestation to foaling, and in both mares and foals for 4 months after birth. From 6 to 11 months of gestation, there was no change in mean monthly OC. Serum PYD increased between 7 months gestation and foaling. After foaling, mean serum OC was low up to 14 days, rising to peak at 1 month. Serum PYD rose concomitantly during this period, but subsequently declined. The mare OC:PYD ratio fell to a nadir within 14 days of birth, before rising to a peak at 2 months. In foals, OC rose within the first 24 h of birth to peak at 3 months. PYD fell from birth levels by 1 month of age. Maternal bone mobilisation occurs progressively from 8 months of gestation until term, before increasing markedly in very early lactation. Net mobilisation switches to accretion by one to two months of foaling, suggesting that this is a period during which mares replenish their own bone stores. Changes in the ratio of OC to PYD indicate adaptation to the prevailing biological milieu. In foals, the increase in the OC:PYD ratio in early life reflects the dominance of bone accretion.

1. Introduction

Horses are relatively skeletally mature at birth, able to stand and gallop within a short time. In mammals, fetal skeletal mineral is derived from maternal calcium and phosphorus supplies from maternal dietary intake and bone mineral stores (Horst et al., 1997; Kovacs, 2016). In equine pregnancy, little is known about the role of maternal bone stores, nor about the timing of any maternal bone mobilisation or re-accretion during lactation, in preparation for the next pregnancy. Bone mineral in the form of hydroxyapatite is composed of calcium and phosphorus, with bone being resorbed in response to decreasing concentrations of circulating calcium, under the control of parathyroid hormone. Conversely, bone mineral is laid down in response to growth and mechanical stress. Bone turnover is a continuous, dynamic processes, where accretion and resorption are normally tightly 'coupled', associated with maintenance of calcium homeostasis, skeletal growth, weight bearing and reproduction (Rosen, 2013).

Bone turnover in animals can be assessed by measuring a number of bone biochemical markers in blood (Allen, 2003). A commonly used bone formation marker is osteocalcin (OC), a non-collagenous protein secreted by osteoblasts during bone formation, that is thought to be the site of bone mineralisation. Conversely, bone resorption markers are typically breakdown products of type I collagen in bone. Pyridinoline (PYD) is a cross-linked peptide degradation product of bone collagen that is released into circulation by osteoclast activity. Measurement of such by-products of bone metabolism in serum reflects in near real-time bone accretion and bone resorption respectively (Lepage et al., 2001).

The effects of pregnancy and lactation on markers of bone turnover have been studied in humans (Akesson et al., 2004; Ulrich et al., 2003), various production (Devkota et al., 2013; Liesegang et al., 2006) and laboratory animals (Lees et al., 1998; Sengupta et al., 2005). In many species, pregnancy and lactation are associated with depletion of maternal bone mineral stores, which are transferred to the fetus, followed by recovery post parturition during lactation. Horses are typically foal yearly with re-breeding shortly after parturition. The period during which mares replenish bone stores whilst both lactating and pregnant is unknown.

There are only a few investigations of bone metabolism during pregnancy and lactation in mares. In Croatian cold-blood breed mares, serum PYD concentrations increased from 60 days prior to foaling and

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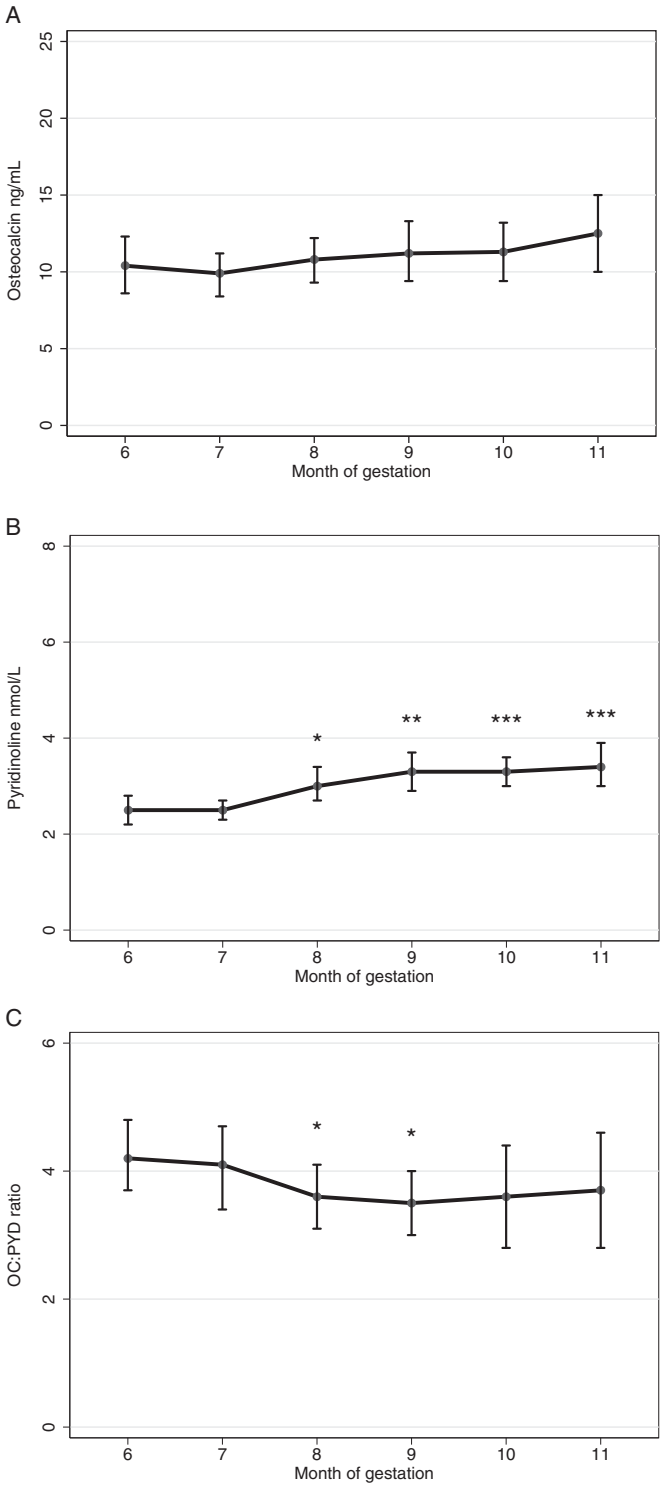

Fig. 1. A–C. Mean (95% confidence interval) A) serum osteocalcin (ng/mL), B) serum pyridinoline (nmol/L) and C) osteocalcin:pyridinoline ratio in mares during gestation. $*p < 0.01$, $**p < 0.001$, $***p < 0.0001$ relative to 6 months gestation.

decreased 60 days after foaling (Filipovic et al., 2010). In Haflinger mares plasma OC concentrations were found to increase during the last month of pregnancy (Greiner et al., 2012). Both these studies were confined to late pregnancy and the early post foaling period in specialised breeds.

We aimed to describe changes in bone turnover markers from mid-pregnancy through to 4 months post-foaling in mares from Thoroughbred, Standardbred or Australian Stockhorse backgrounds, and to elucidate the period post-foaling in which mares replenish their own bone stores. We hypothesised that serum OC and PYD concentrations would change during pregnancy and lactation, with 'uncoupling' of bone accretion and resorption, and that in foals in early post-natal life, bone accretion would predominate over bone resorption.

2. Materials and methods

2.1. Ethical considerations

This study was approved by University of Queensland Animal Ethics Committee 053/11 and complied with the Australian code of practice for the care and use of animals for scientific purposes (https://www.nhmrc.gov.au/guidelines-publications/ea28).

2.2. Study design

A cross-sectional panel study was designed as a pilot to investigate the levels of serum OC and PYD in mare-foal pairs through pregnancy and lactation. Study subjects were drawn as a convenience sample from mares owned by the University of Queensland, Gatton campus. Mares were eligible for inclusion based on accessibility and a positive 21 day pregnancy scan. The sampling frame included Standardbreds, Thoroughbreds and Australian Stock Horses with a mix of maiden, multiparous and dry, and multiparous and lactating mares. Horses were housed in large paddocks and fed ad-libitum grass hay.

2.3. Sample collection

Blood samples were collected from mares at monthly intervals from May 2011 to February 2012, capturing the gestation from 6 months to foaling, and the first 4 months of the post-foaling period.

Samples from foals were collected where possible in the 24 h following birth, and at monthly intervals until 4 months of age. Blood was taken via jugular venepuncture using a 20 gauge (mares) or 21 gauge (foals) Vacutainer needle (BD Vacutainer Systems, Plymouth, UK). Serum samples were collected into 10 mL Gel Separator Vacutainer tubes (BD, Franklin lakes, New Jersey, USA). Samples were collected between 9.00 am and 12:00 midday and immediately placed on ice or in a refrigerator at 4° C and allowed to sit for 30–60 min to allow for clot formation. Samples were spun in an Eppendorf centrifuge (Eppendorf South Pacific) at 4° C 3500 RPM for 10 min. Serum and plasma were pipetted off into 500 μL aliquots into Eppendorf LoBind microtubes (Eppendorf South Pacific) and stored in a −70 °C freezer until assayed.

2.4. Sample analysis – OC and PYD assays

Serum samples were assayed for OC and PYD using commercially available Quidel MicroVue Osteocalcin and Serum PYD immunoassay kits (Quidel Corporation, San Diego, California, USA www.quidel.com). The minimum detection limit of the Osteocalcin assay is 0.45 ng/mL. The coefficient of variation was < 10%.

The MicroVue Serum PYD assay provides a quantitative measure of the excretion of PYD crosslinks in serum. The minimum detection limit of the PYD assay is 0.4 nmol/L. The coefficient of variation was < 15%.

The OC:PYD ratio was calculated as a marker of relative changes in bone accretion compared to resorption.

2.5. Statistical analysis

Gestational age in days was calculated as the date of blood sampling minus the date of service. As mares' gestational age differed at each sampling date, data was corrected to the closest month of gestation relative to date of service. Month of gestation was calculated as gestation in days/30.44 (the average number of days in a month), and coded