BONE MINERAL DENSITY OF THE OSSEOUS PELVIC FLOOR IN COWS – A PILOT STUDY

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Abstract. Utilizing 33 cows and 13 heifers age-related changes in the bone mineral density of pelvic floor and the possible bone resorption in pregnant animals were studied. Eight cows and two heifers were in the early stage of pregnancy. The pelvic floors were scanned fresh on the day of slaughtering. For the measuring of the bone mineral density of the pelvic floor the dual absorption metre (Lunar DPX-IQ) was used. Bone density was the highest in the area of the ischial bone as ossification begins in its caudal part, while bone density was the lowest in the junction of the pubic and ischial bones. Differences between pregnant and non-pregnant animals were not statistically significant. The study revealed that the bone density was higher in cows than in heifers. Evidently the hormone-driven changes in cows during early pregnancy as well during lactation only inhibit the ossification of the bony pelvic floor and do not cause the resorption of the bone tissue.

Keywords: Bone mineral density, osseous pelvic floor, bovine pelvis

Introduction. In the bone pelvic floor, formed pairwise by pubic and ischial bones, the area of pelvic symphysis is the most variable dependent on gender and age (Schutz et al, 2009). In the pelvic symphysis the articular surfaces are covered with hyaline cartilage which in younger animals has become joined with interpubic fibrocartilage lamina. The pelvic symphysis is elastic which makes joints softer and allows limited movement. The mobility of pelvic symphyses is especially important in the females of the species who give birth to big single fetus like for example, bovines.

The changes taking place in the bone and especially the strength of bones, can be indirectly assessed by bone density. Bone density shows the content of mineral substances in the bone tissue which, however, has variations depending on the location of bones in the skeleton. Axial skeleton is more sensitive to the change of the metabolism of mineral substances than the limb skeleton (Murray et al., 1994). Shahin and co-authors have found that the bovine breed has no significant effect on the growth rate of hip bones. However, the bone density was one of the factors which determined the mass of bones in the skeleton (Shahin et al., 1992). Body weight is considered to be the most important factor influencing bone density: for example, in the same breed and gender of animals, metacarpal bones had a high correlation between bone density and body weight and age (Zotti et al., 2010). The decrease of bone density, which especially takes place in the cancellous bone, has been found most often in mammals (human, rat, monkey) in the early stage of lactation but it is passable and restores after lactating partially or fully (Vajda et al., 2001). In dairy goats and sheep the bone tissue resorbs during the late gestation and early lactation. Further the animals get adapted – the milk yield drops slowly, calcium need is covered with absorption from intestines and its reserves will be restored in bones to the prepartum level (Liesegang et al., 2006; 2007). During the second pregnancy and lactation, likely because of improved adaptivity, the bone density of goats and sheep did not decrease in comparison with the first time so much, although the animals gave more milk (Liesegang et al., 2007).

As to our knowledge the bone density of the bovine pelvic floor, the important area from the perspective of parturition, has not been studied, the aim of our work was therefore to find age-related changes in the bone density of the pelvic floor of cows and to study the resorption of the bone tissue of pregnant animals.

Material and Methods. The bone mineral densities of 33 cows in age from 4 to 11 years and 13 heifers were analysed. From all animals 31 were Estonian Holstein Friesian (EHF) cattle, five Estonian Red (ER) cattle and 10 beef breed (BC) cattle. Among cows there was eight and among heifers two pregnant animals (the length of pregnancy 2-4 months). The data were collected from the end of March until the end of May, i.e. in the spring transition period. The data about the conditions of keeping the animals, their milk yield and body weight were not available.

In the meat processing factory the pelvic floors were removed from the carcass. With this purpose an area was sawn out parallel to the pelvic symphysis with the width which cranially reaches the iliopubic eminence and caudally the ischial tuberosity. The pieces were cleaned from soft tissues because they give mistakes in measuring (Zotti et al., 2010). The pelvic floors were scanned fresh on the day of slaughtering to avoid the decrease of the bone density caused by freezing (Schalkwyk et al., 2004). For the measuring of the bone mineral density of the pelvic floor the dual absorption metre (Lunar DPX-IQ) was used. It was calibrated to be used with small animals because the bony pelvic floor was thin.

The absorption of x-rays is proportional to the density (mineral content). Bone mineral content is calculated by dividing bone mineral content by the area of the site being scanned (g/cm^2). For the measuring sites four rectangle-shape areas were chosen along pelvic symphysis including the bones on the sides – the pubic tubercles, the junction of the pubic caudal rami and rami of the ischium, the symphysial eminence and caudally the body of the interischial bone. The surface of every single rectangle was 10 cm^2. For the comparison there was an area of the same size from the ischial plate located next to the body of the interischial bone (Fig. 1). The cut pelvic floors were mounted in a holder in a way that the dorsal pubic tubercule and the caudal side of the pelvic symphysis with the symphysial eminence were on the same imaginary line. All the measurements were carried out by one person.

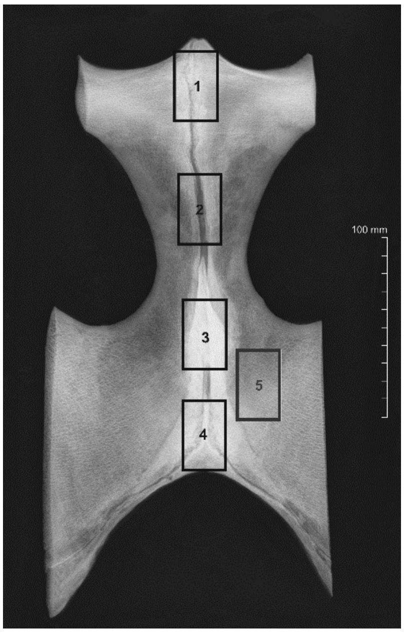

Figure 1. **X-ray image of the pelvic floor of a 6-year-old cow.** Location of the measured areas: 1 dorsal and ventral pubic tubercle; 2 ischiopubic junction; 3 symphysial eminence; 4 caudal part of pelvic symphysis; 5 tabula of ischium

The statistical analyses of the collected data were performed with SAS 9.4 software and the results were considered statistically significant at $p<0.05$. The one-way analysis of variance, followed by the Tukey *post-hoc* test, was applied to compare the breeds. The cows and heifers as well as pregnant and non-pregnant animals (separately for cows and heifers) were compared with *t*-test. The animals' age in breeds' comparison and the breed in cows and heifers comparison were not considered as the average age of animals from different breeds was not different (p=0.864, analysis of variance) and also the frequencies of heifers and cows were similar among breeds (p=0.690, Fisher exact test). The relationships between different bone mineral density measures as well the relationships between age and bone mineral densities were studied with the Pearson correlation analysis.

Results and Discussion. The bone density of the pelvic floor was higher in the caudal part of the pelvic symphysis and at the pubic tubercle (0.90-1.19 g/cm^2) and lower at the junction of pubic and ischial bone (0.36-0.54 g/cm^2), not depending on breed and physiological condition. In the latter area the cartilage between hip bones preserves longest as the ossification of pelvic symphysis starts from the ischial arc, continues in the caudal part of the ischial symphysis and after that the space between the pubic cranial rami is ossified (Nahkur et al., 2011).

There were no differences in bone mineral densities at pubic tubercles between breeds whilst at other places the bone mineral densities were the highest on BC and the lowest on EHF cattle (Table 1). As the milk yield of beef cattle is lower than in the EHF breed animals, they lose with milk less endogenous minerals which helps to preserve bone density.

There were strong or intermediate positive statistically significant correlations between bone mineral densities at the ischiopubic junction, the symphysial eminence, the caudal part of the pelvic symphysis and the ischial plate. However, the bone mineral density at the pubic tubercles was not correlated with bone mineral densities at other locations (Table 2). With the growth of age bone density increased in all the studied areas (all $p<0.05$), expect at the place of pubic tubercles where it decreased ($r= -0.11$, p=0.550; Fig. 2).

The results of our study confirm the fact that by the advancing age the dorsal pubic tubercle is mostly reducing (Nahkur et al., 2011) and this is why the bone density of cows, as an exception, remains lower at the place of pubic tubercles (Fig. 1; rectangle 1). Differently from the data in literature where it is shown that calves and primipara have anabolic processes prevailing in metabolism (Starič, 2008) and the organism accumulates excessively minerals before reproduction into the skeleton (Bowman and Miller, 2001), in our study bone density was higher in cows than in heifers; statistically significantly it differed in the junction of ischial and pubic bones, at the elevation of symphysis and in the caudal part of pelvic symphysis ($p<0.001$, Table 1) and not significantly on the ischial plate (Fig. 1; rectangles 2 and 5). As cows because of repeated pregnancies and lactations were adapted to cyclic burdening of the pelvic floor, they had bigger tubercles (except the dorsal pubic tubercle) and therefore the bone density of the respective areas was higher.

Table 1. **Average (standard deviation) age and bone mineral densities by breed and age group**; p-values show the statistical significance of breed and age effect according to analysis of variance and *t*-test, respectively, different superscriptletters in breeds' columns denote statistically significantly different (p<0.05) groups according to Tukey *post-hoc* test.

	n	Age, years	Bone mineral density, g/cm^2				
			Pubic tubercles	Ischiopubic junction	Symphysial eminence	Caudal part of symphysis	Ischial plate
Breed		p = 0.864	p = 0.999	p = 0.013	p = 0.209	p = 0.261	p = 0.036
EHF	31	4.8 (2.9)	0.90 (0.15)	0.39 (0.13)^{a}	0.82 (0.34)	0.93 (0.46)	0.61 (0.19)^{a}
ER	5	4.6 (3.5)	0.90 (0.09)	0.47 (0.23)^{ab}	0.83 (0.30)	0.96 (0.35)	0.68 (0.14)^{ab}
BC	10	4.2 (2.2)	0.90 (0.18)	0.54 (0.12)^{b}	1.02 (0.28)	1.19 (0.39)	0.80 (0.20)^{b}
Age gr		p < 0.001	p = 0.499	p = 0.045	p < 0.001	p < 0.001	p = 0.109
Cow	33	5.7 (2.6)	0.89 (0.16)	0.46 (0.17)	0.98 (0.28)	1.14 (0.39)	0.69 (0.21)
Heifer	13	1.9 (0.6)	0.92 (0.13)	0.36 (0.07)	0.56 (0.23)	0.62 (0.32)	0.59 (0.16)

Table 2. **Pearson correlation coefficients between bone mineral densities**

	Pubic tubercles	Ischiopubic junction	Symphysial eminence	Caudal part of symphysis	Ischial plate
Pubic tubercles	1				
Ischiopubic junction	0.17	1			
Symphysial eminence	0.00	0.58***	1		
Caudal part of symphysis	0.08	0.51***	0.80***	1	
Ischial plate	0.24	0.61***	0.72***	0.78***	1
*** p<0.001, ** p<0.01, * p<0.05					

Although we did not have exact data about the stage of the animals' lactation, it is probable that the animals, whose production was relatively low, were brought to the slaughterhouse. This is why the bone metabolism of the studied animals was more anabolic and also the bone density was not significantly influenced by the production. According to the data of literature, in dairy cows calcium concentration in the later period of lactation (60-120 days after birth) in the rib's cortical bone is significantly lower and in total the cortical bone is thinner (Beighle, 1999). Calcium is preserved in bones from the beginning of lactation up to 30 days and later it is taken from bones particularly in the case of a bigger milk yield (more than 20 kg per day). In the pregnant dairy cows the decrease of the cortical bone in ribs takes usually place in the last trimester (Beighle, 1999).

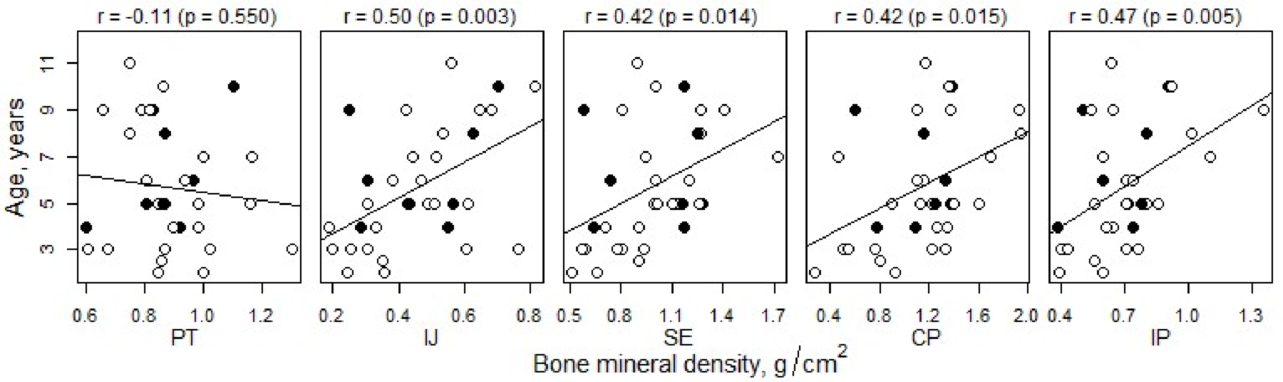

Figure 2. **Relationships between bone mineral densities (PT – Pubic tubercles, IJ – Ischiopubic junction, SE – Symphysial eminence, CP – Caudal part of pelvic symphysis, IP – Ischial plate) and the age of cows, the Pearson correlation coefficients with p-values are presented numerically, black dots in charts denote pregnant and white dots non-pregnant cows**

Comparing the pregnant and non-pregnant cows (n=8 and n=25, respectively) and pregnant and non-pregnant heifers (n=2 and n=11, respectively), it follows that the bone mineral densities at the caudal part of the pelvic symphysis and the ischial plate were higher among non-pregnant animals compared with pregnant animals for both cows and heifers. However, these differences as well as other differences between pregnant and non-pregnant animals were not statistically significant (all p-values >0.3). Such a result was evidently caused by the fact that in our study there were the animals whose pregnancy was in the initial stage and therefore in the bony pelvic floor noticeable changes had not yet taken place. According to the data in literature the growing need for calcium in pregnant animal is covered with the increased absorption from intestines (Greiner at al., 2012) and the accelerated intestinal absorption even leads to the higher bone density (Beighle, 1999).

In assessing the bone density of dairy cows with the tests of breaking strength, it has shown that the strength of metacarpal bones and caudal vertebrae did not depend on the number of parturitions. Also the breaking strength of metacarpal bones did not depend on the stage of lactation. However, the correlation between the content of mineral substances and the breaking strength was sometimes weak (Keene at al., 2005). On the contrary, the breaking strength of caudal vertebrae was significantly higher in the early lactation and until 150-250 days of milking. The authors concluded from this fact that in the weight bearing bones the fluctuation of the content of mineral substances under the influence of oestrogens was smaller; evidently it was caused by a lower metabolic activity. The caudal vertebrae, in contrary, had higher activeness. The weight bearing bones were more influenced by the environment and age than the non-bearing bones (Keene et al., 2005). As the bony pelvic floor is also a part of the skeleton, bearing the body weight, in our study no big differences were revealed between, for example, pregnant and non-pregnant animals.

Conclusion. In the present study significant correlations between the animals' age and bone density were revealed – differently from the fact in literature, bone density was higher in cows than in heifers. Bone density was the highest in the area of the ischial bone as ossification begins in its caudal part, while bone density was the lowest in the junction of the pubic and ischial bones where the cartilage tissue preserves longest. The differences between the breeds were missing at the pubic tubercles but in other measured locations the bone density of dairy cows was lower. Differences between pregnant and non-pregnant animals were not statistically significant. Evidently the hormone-driven changes in cows during early pregnancy as well during lactation only inhibit the ossification of the bony pelvic floor and do not cause the resorption of the bone tissue. Unfortunately sources about this subject are discordant in the literature as well.

To draw more substantial conclusions about the changes in the cow's bony pelvic floor during pregnancy and parturition further studies are needed with larger number of animals involved.

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