Ciências Agrárias: Campo Promissor em Pesquisa 3

Jorge González Aguilera
Alan Mario Zuffo
(Organizadores)

Atena Editora
Ano 2019

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Atena Editora
2019

2019 *by Atena Editora*

Editora Executiva: Profª Drª Antonella Carvalho de Oliveira
Diagramação: Geraldo Alves
Edição de Arte: Lorena Prestes
Revisão: Os Autores

Atena Editora
Ano 2019

Dados Internacionais de Catalogação na Publicação (CIP)
(eDOC BRASIL, Belo Horizonte/MG)

C569 Ciências agrárias [recurso eletrônico] : campo promissor em pesquisa 3 / Organizadores Jorge González Aguilera, Alan Mario Zuffo. – Ponta Grossa (PR): Atena Editora, 2019. – (Ciências Agrárias. Campo Promissor em Pesquisa; v. 3)

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader.
Modo de acesso: World Wide Web.
Inclui bibliografia
ISBN 978-85-7247-417-7
DOI 10.22533/at.ed.177192006

1. Agricultura. 2. Ciências ambientais. 3. Pesquisa agrária – Brasil. I. Aguilera, Jorge González. II. Zuffo, Alan Mario. III. Série.
CDD 630

Elaborado por Maurício Amormino Júnior – CRB6/2422

Atena Editora
Ponta Grossa – Paraná - Brasil
www.atenaeditora.com.br
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Atena Editora
Ano 2019

APRESENTAÇÃO

A obra "*Ciências Agrárias Campo Promissor em Pesquisa*" aborda uma publicação da Atena Editora, apresenta seu volumem 3, em seus 23 capítulos, conhecimentos aplicados as Ciências Veterinárias.

A produção de alimentos nos dias de hoje enfrenta vários desafios e a quebra de paradigmas é uma necessidade constante. A produção sustentável de alimentos vem a ser um apelo da sociedade e do meio acadêmico, na procura de métodos, protocolos e pesquisas que contribuam no uso eficiente dos recursos naturais disponíveis e a diminuição de produtos químicos que podem gerar danos ao homem e animais. Este volume traz uma variedade de artigos alinhados com a produção de conhecimento na área de veterinária, ao tratar de temas como manejo nutricional de caprinos, peixes, cães, gatos, aves, avelhas, entre outros. São abordados temas inovadores relacionados com sistemas de produção e manejo, melhora da cadeia produtiva, qualidade e bem-estar animal. Os resultados destas pesquisas vêm a contribuir no aumento da disponibilidade de conhecimentos úteis a sociedade.

Aos autores dos diversos capítulos, pela dedicação e esforços, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos nas Ciências Veterinárias, os agradecimentos dos Organizadores e da Atena Editora.

Por fim, esperamos que este livro possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias para a área da Agronomia e, assim, contribuir na procura de novas pesquisas e tecnologias que possam solucionar os problemas que enfrentamos no dia a dia.

Jorge González Aguilera
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SUMÁRIO

CAPÍTULO 4

BONE TURNOVER MARKERS IN SHEEP AND GOAT: A REVIEW OF THE SCIENTIFIC LITERATURE

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ABSTRACT: Bone turnover markers (BTMs) are product of bone cell activity and are generally divided in bone formation and bone resorption markers. The purpose of this review was to structure the available information on the use of BTMs in studies on small ruminants, especially for monitoring their variations related to diet, exercise, gestation and metabolic lactation state, circadian and seasonal variations, and also during skeletal growth. Pre-clinical and translational studies using BTMs with sheep and goats as animal models in orthopaedic research studies to help in the evaluation of the fracture healing process and osteoporosis research are also described in this review. The available information from the reviewed studies was systematically organized in order to highlight the most promising BTMs in small ruminant research, as well as provide a wide view of the use of sheep and goat as animal models in orthopaedic research, type of markers and commercial assay kits with cross-reactivity in sheep and goat, method of sample and storage of serum and urine for bone turnover markers determination and the usefulness and limitations of bone turnover markers in the different studies, therefore an effective tool for researchers that seek answers to different questions while using BTMs in small ruminants. ["Title: **Bone turnover markers in sheep and goat: A review of a scientific literature**. Authors: José A. Camassa, Camila C. Diogo, Cristina P. Sousa, Jorge T. Azevedo, Carlos A. Viegas, Rui L. Reis, Nuno Dourado and Isabel R. Dias. An Acad Bras Cienc. March (2017) 89(1): 231-245."]
KEYWORDS: Bone formation markers; bone resorption markers; bone metabolism; small ruminants

1 I INTRODUCTION

In the last decades, small ruminants – sheep and goats – have been widely accepted as animal models in orthopaedic research (O'LOUGHLIN et al., 2008; REICHERT et al., 2009) especially due to their low cost, availability, acceptance as an experimental model, facility of handling and housing (TURNER, 2007a), compliance, and docility (NEWMAN et al., 1995).

The suitability of small ruminants as animal models for orthopaedic research results mainly from having the most similar body weight and long bones with dimensions compatible with application of implants and prostheses developed for humans (NEWMAN, et al. 1995; ANDERSON et al., 1999; VAN DER DONK et al., 2001). In this manner, compared with other species used in orthopaedic research, sheep and goats have an adequate body weight and long bones, with a macrostructure more similar to humans (NEWMAN et al., 1995), despite the bone microstructure of small ruminants being less similar to humans than other animal models such as dogs (PEARCE et al., 2007). Sheep have a predominance of plexiform bone until 3 to 4 years of age (NEWMAN et al., 1995) due to fast growth in weight and size (REINWALD and BURR, 2011) and just a predominance of secondary Haversian systems after 7 to 9 years of age with the presence of bone remodelling (NEWMAN et al., 1995). Sheep also

presents a trabecular bone density, mineralization and subsequently elevated strength relative to humans, that are variable according to skeletal location (NAFEI et al., 2000; LIEBSCHNER, 2004), nevertheless the bone mineral composition being apparently similar between small ruminants and humans (RAVAGLIOLI et al., 1996).

Despite these macro- and micro-structural differences in bone tissue, studies with small ruminants used as animal models in orthopaedic research have increased considerably (PEARCE et al., 2007), and more recently they have also been used for studying bone turnover markers (BTMs) (SOUSA et al., 2014a). The BTMs are proteins which indicate bone metabolism (SOUSA et al., 2014b), and are generally divided into collagenous bone formation markers, bone resorption markers and osteoclast regulatory protein markers (LEEMING et al., 2006). Analysis of BTMs might supply information in a fast, effective, sensitive, specific, and low cost manner (ALLEN, 2003). Nowadays, it is used in human medicine to help evaluate fracture risk, delayed fracture healing and consolidation process, and development of metabolic bone diseases (VASIKARAN et al., 2011).

These similarities in biochemistry, biomechanics, and bone histology make BTMs a resource in sheep and goats for pre-clinical and/or translational orthopaedic research studies and veterinary and animal science studies (TURNER, 2007b). Nevertheless, the reported biological variability of BTMs among age, gender, disease, recent fractures, exercise, time (SEIBEL, 2005), diet (NICODEMO et al., 1999; LIESEGANG and RISTELI, 2005; LIESEGANG et al., 2013), seasonal changes (ARENS et al., 2007) and circadian variation (LIESEGANG et al., 2003), which can contribute substantially to the variability of these parameters (SMITH et al., 2011), are their main limitation (CREMERS et al., 2008).

Therefore, the aim of this review was to collect the studies published in scientific literature until the present date concerning the use of BTMs in small ruminant research or to investigate the clinical effectiveness of BTMs in pre-clinical or translational experimental orthopaedic research related to human medicine when sheep and goat are used as experimental animal models for this latter purpose.

2 I BONE TURNOVER MARKERS

Bone tissue undergoes turnover along the animal lifespan (SEIBEL, 2006) and that process is divided into two parts: modelling and remodelling (CLARKE, 2008).

Modelling is a longitudinal and circumferential growth process due to mechanical and/or physiological influences (CLARKE, 2008), with longitudinal growth located at the epiphyseal plates until their fusion uniting the epiphysis and metaphysis through endochondral ossification (ALTMAN et al., 2015). It also allows the adaptation of bone tissue, removing damage and maintaining its strength (SEEMAN, 2009), and requires that the process of bone formation and resorption are independent from one

another regarding time and location (RAGGATT and PARTRIDGE, 2010). Remodelling is a process of bone replacement where bone formation outpaces bone resorption (ALTMAN et al., 2015), to maintain bone strength and mineral homeostasis, regulated by osteoclasts and osteoblasts that sequentially carry out resorption of old bone and formation of new bone, keeping the new bone healthy (CLARKE, 2008). Bone remodelling predominates when bone is reaching maturity (IGLESIAS et al., 2011), but it does not influence the size and shape, although the internal architecture may have slight changes caused by external forces (HADJIDAKIS and ANDROULAKIS, 2006). Bone formation and resorption are present in same site, but not at the same time in order to maintain bone mass (RAGGATT and PARTRIDGE, 2010).

The proteins produced during bone turnover are detectable mainly in serum in bone formation markers, whereas many of the bone resorption markers are detectable in both serum and urine (ALLEN, 2003), and there are a significant number of commercial kits developed for use in humans that have cross-reactivity with other species, including sheep and goats (Tables I to III).

During the process of bone formation by osteoblasts, formation markers are represented by serum total (ALP) and the bone-specific isoform of alkaline phosphatase (BALP), serum osteocalcin (OC) and two molecules which are released during the type I collagen molecule synthesis – serum procollagen type I carboxy- and amino-terminal propeptides (PICP and PINP, respectively) (SEIBEL, 2002). In the bone resorption process there is a breakdown of type I collagen, so resorption markers are represented by serum C-terminal telopeptide of type I collagen (serum ICTP), urinary collagen type I cross-linked C- and N-telopeptide (CTx and NTx), urinary hydroxyproline (HYP), total and free urinary pyridinoline and deoxypyridinoline (PYD and DPD) and also by serum tartrate-resistant acid phosphatase (TRAP) as an enzyme produced by osteoclasts during their bone resorption activity (SEIBEL, 2002) (Figure 1).

2.1 Bone Formation Markers

2.1.1 Alkaline phosphatase

Alkaline phosphatase (ALP) is a glycoprotein that is connected to the extracellular surface of cells and is synthesized in a variety of tissues, such as intestines, placenta, and germ cells (MILLAN, 2006). Animals have four isoforms of ALP – bone-specific ALP (BALP), intestinal ALP, liver ALP, and in dogs also the corticosteroid-induced ALP. This variation would render difficult the interpretation of possible variations of the ALP isoenzymes (ALLEN, 2003). Bone ALP has been used due to its high sensitivity as bone formation marker (SEIBEL, 2006). It is produced by osteoblasts (MILLAN, 2006) and is involved in the calcification of bone matrix (MASROUR and MAHJOUB, 2012) through the hydrolysis of phosphate esters on the osteoblast cell surface, resulting in a high extracellular inorganic phosphate concentration (WHYTE, 1994).

2.1.2 Osteocalcin

Osteocalcin (OC) is synthesized by mature osteoblasts, odontoblasts, and hypertrophic chondrocytes and it is vitamin K dependent protein. It has three residues of the calcium-binding amino acid, γ - carboxyglutamic acid (Gla). Its function is poorly understood, although it is primarily deposited in the bone extracellular matrix (ECM), with a small amount present in the blood stream (CREMERS et al., 2008). Serum OC is a marker of osteoblastic activity and its serum level thus reflects the rate of bone formation (SEEBECK et al., 2005), influences bone mineralization by binding calcium and consequently hydroxyapatite (NEVE et al., 2013).

2.1.3 Pro-Collagen Type I Propeptides

Collagen type I is produced by osteoblasts in the last stage of new bone formation (ALLEN, 2003). The procollagen undergoes enzymatic cleavage producing the C- and N-terminal procollagen type I extension peptides (PICP and PINP, respectively), both extension are cleared by the liver and may be added to the bone ECM (WATTS, 1999). Nevertheless, type I collagen does not depend exclusively on the bone tissue turnover because it is also a component of other soft tissues as fibro-cartilage, tendon, skin, gum, intestine, heart valve, large vessels, and muscle. However, as the metabolism of type I collagen is faster in the bone tissue than in other tissues, changes in type I collagen are considered representative of bone collagen synthesis (CREMERS et al., 2008). It is suggested that PINP is useful in early detection of non-union processes with potential for study of the fracture healing process (COULIBALY et al., 2010), although in humans it is unknown whether there exists a correspondence between PINP and the progression of fracture healing (MOGHADDAM et al., 2011).

2.2 Bone Resorption Markers

2.2.1 Deoxypyridinoline and Pyridinoline

The collagen fibrils recently deposited in bone ECM are stabilized by intra- and intermolecular cross links helping to build the mature collagen molecule (CEPELAK and CVORIŠCEC, 2009).

The pyridinium cross links – deoxypyridinoline (DPD) and pyridinoline (PYD) are formed during extracellular maturation of fibrillar collagens (Gerrits et al. 1995). The PYD is found in bone and cartilage tissues and ligaments (Watts 1999) while DPD is found in bone and dentin (DELMAS et al., 2000), so in the bloodstream PYD is generally more abundant (CREMERS et al., 2008), although DPD is more specific as a resorption marker for bone tissue (SEIBEL et al., 1992). In a study with sheep after ovariectomy, this animal model demonstrated relevance as a model for osteoporosis due to the values of PYD and OC found (NEWTON et al., 2004).

2.2.2 Carboxy-Terminal Telopeptide of Collagen Type i and Amino-Terminal Telopeptide of Collagen Type i

The N-terminal (NTx) and C-terminal telopeptide of collagen type I (CTx) are fragments of the type I collagen molecule composed by a short peptide sequence from the non-helical domain of this molecule (CHUBB, 2012), attached by a pyridinium crosslink (ALLEN et al., 2000). Both markers are sensitive and reliable indicators of the bone resorption process (Cremers et al. 2008) and final products of the metabolism of bone ECM, amino acids, and free or peptide-bound PYD or DPD (ALLEN et al., 2000).

The CTx is not specific as a resorption marker for bone tissue since it is identified not only in bone, but also in skin, dentine, and tendon, and these peptide fragments could also be derived from other types of collagen (CHUBB, 2012). However, CTx could be used for monitoring the bone healing process because it was detected that variations in its levels corresponded to bone resorption in an experimental fracture healing study performed in dogs where two different osteosynthesis techniques were used (PASKALEV and KRASTEV, 2010).

2.2.3 Carboxy-Terminal Telopeptide of Type i Collagen – Matrix Metalloproteinase

Cleavage of the type I collagen molecule by the matrix metalloproteinases (MMP) results in the formation of cross-linked C-terminal telopeptide of type I collagen (CTX-MMP or ICTP) (CREMERS et al., 2008), suitable to represent osteoclastic activity (ALLEN et al., 2000).

The ICTP is an indicator for mobilization of bone tissue around parturition and at the beginning of lactation in sheep and goats (LIESEGANG et al., 2007). In dogs with osteosarcoma (HINTERMEISTER et al., 2008) and horses during physical training, this marker has not revealed itself suitable for determining bone resorption since it did not show correlation with other resorption markers, however it was an indicator of the rate of bone turnover (PRICE et al., 1995).

2.2.4 Tartrate-Resistant Acid Phosphatase

Tartrate-resistant acid phosphatase (TRAP) is a bone resorption marker, but not originated from the degradation of type I collagen (HANNON et al., 2004). It is a glycoprotein produced by osteoclasts, activated macrophages, and dendritic cells (LEEMING et al., 2006). There is an isoenzyme 5, from a total of 6 isoenzymes of the acid phosphate identified by electrophoresis, which through protease cleavage presents two isoforms (a, b) – the TRAP 5a is sialylated and TRAP 5b is produced by osteoclasts, and the latter proposed to reflect osteoclast activity (DELMAS et al., 2000; LEEMING et al., 2006). The TRAP could be a suitable resorption marker for detection of normal or delayed fracture healing process in sheep (SEEBECK et al., 2005) or dogs (SOUSA et al., 2011).

2.2.5 Cathepsin K

Cathepsin K is part of the cysteine protease family and has the ability to cleave both helical and telopeptide regions of collagen type I (LEEMING et al., 2006). This enzymatic cleavage is able to degrade, at low pH, several proteins of the bone ECM, namely the telopeptide and helical regions of the collagen type I molecule, the OC and osteopontin (CREMERS et al., 2008). This marker could be used as a tool to measure bone resorption, such as in canine osteosarcoma clinical cases (SCHMIT et al., 2012).

3 I VARIABILITY OF BONE TURNOVER MARKERS

The BTMs could suffer constant variation throughout the lifetime of an individual (SOUSA et al., 2014b). However, variation between individuals is also a great cause of oscillation in markers, specifically due to biological variability, together with the analytical variability introduced by the different assay techniques (VASIKARAN et al., 2011).

Biological variability can be influenced by many uncontrollable factors (CREMERS et al., 2008), such as growth (SOUSA et al., 2014a), geographical location (LIESEGANG et al., 2013), pregnancy and lactation (LIESEGANG et al., 2006, 2007), and controllable factors, such as diet (MacLeay et al. 2004a,b, Liesegang et al. 2013), and season of the year (ARENS et al., 2007), which can be mitigated in clinical studies (LIESEGANG, 2008). In short, biological variability is affected by any factor that influences the bone remodelling (WATTS, 1999).

Analytical variability has been minimized due to automated platform technology, however, there could be variations in results between different methods (CREMERS et al., 2008) and the development of new analytical techniques requires previous validation (SEIBEL et al., 2001).

The high inter-individual variability of BTMs is their main limitation for clinical use due to the difficulty to establish reference ranges for serum and urinary BTM levels (SOUBERBIELLE et al., 1999), although bone markers are an effective tool in clinical studies due to reliable, fast, non-invasive, and cost effective assays with improved sensitivity and specificity (WHEATER et al., 2013).

4 I SAMPLE AND STORAGE

Blood collection for measuring BTMs must be done at a specific time (morning) to avoid the influence of circadian variations (KLEIN et al., 2004; SEEBECK et al., 2005; DIAS et al., 2008; SOUSA et al., 2014a,b). Blood samples can be collected from the cephalic vein (KLEIN et al., 2004) or jugular vein (DIAS et al., 2008; SOUSA et al., 2014a,b) into serological tubes containing no anticoagulant (VERNON et al., 2010), and centrifuged (3000 rpm for 10 min) within 30 min of collection (LIESEGANG et al.,

2007). Urine can be obtained using a special external urine collector (WINDHAGEN et al., 2002) or collected by cystocentesis (ALLEN et al., 2000). Urine and serum samples should be stored at -20°C for mineral analyses (CHANETSA et al., 2000; TAYLOR et al., 2009; SOUSA et al., 2014a) and at -80°C until determination of BTMs (SEEBECK et al., 2005; TATARA, 2008; SOUSA et al., 2014b), which provides molecular stability for several months (LOMEO and BOLNER, 2000).

5 I ANIMAL AND VETERINARY SCIENCE STUDIES

Characteristics of the animal and veterinary science studies regarding population, type of studies, time, and conclusion (Table IV).

5.1 Diet

According to Liesegang and Risteli (2005), Liesegang et al. (2013), MacLeay et al. (2004a,b) and Nicodemo et al. (1999) nutritional studies using BTMs were influenced by different diets, though this influence was not statistically significant. MacLeay et al. (2004a) concluded that during the administration of a diet that induced metabolic acidosis in mature ewes, there were no significant changes in serum BALP and DPD levels. In another study by Liesegang et al. (2013) with sheep grazing at different altitudes, it was not possible to confirm the interference of diet in the serum variation of ICTP or BALP, but high bone turnover was confirmed. Also, in a study by Liesegang and Risteli (2005) where a diet with varying calcium content was used, it was not possible to demonstrate the influence of the diet on bone mineral metabolism in growing goats and sheep, possibly due to the short duration of this study, where only the sheep showed a variation in BMD due to an increase in calcium intake. However, Wilkens et al. (2010) demonstrated that sheep were a suitable model for studies with varying diets, calcium deficiency, and calcitrol.

5.2 Exercise

Liesegang and Risteli (2005) demonstrated that sheep in pasture at high altitudes had an increase in bone turnover and bone mineral content without clear cause, one possible factor being the increase in exercise. In another study in lambs, Vernon et al. (2010) concluded that the markers used were not adequate to indicate the effects of forced exercise.

5.3 Gestation And Lactation

Liesegang et al. (2006) noticed that the interval between parturition and early lactation in sheep and goats required a high nutritional value of calcium due to losses to the fetus and lactation, occurring inefficiency of calcium absorption, leading to increases in bone remodelling to help replace maternal bone loss classified as a physiological

mechanism. During a second pregnancy, bone loss was less significant compared with the first pregnancy and the lactation greater, possibly due to the adaptation of the organism (LIESEGANG et al., 2007). Finally, it was concluded that sheep were more adapted to the loss of calcium in comparison to goats, that had a lower bone mineral density and bone mineral content before parturition (LIESEGANG and RISTELI, 2005) increased bone turnover, resulting in a higher activity of bone metabolism and sensitivity to changes in calcium during pregnancy and lactation (LIESEGANG et al., 2003).

5.4 Circadian And Seasonal Variation

Chavassieux et al. (1991) reported that bone remodelling was influenced by the photoperiod, with decrease in bone remodelling occurring between spring and summer. Arens et al. (2007) confirmed that bone mass increases in summer and decreases in winter, so taking seasonal variation into account is fundamental in studies using BTMs. Liesegang et al. (2003) reported an increase in the rate of bone formation during the evening and night, indicating the influence of the circadian rhythm in bone turnover. Sousa et al. (2014b) concluded that the short-term variability should be considered during interpretation of data, such as circadian and seasonal variations, nevertheless the short-term biological variability do not represent a limitation for the use of BTMs.

5.5 Skeletal Growing

Pastoureau et al. (1991) mention that sheep are a good model to study the bone growth in growing lamb. It was reported that goats showed a more accelerated bone remodelling that sheep, which was demonstrated by ICTP, CTx (LIESEGANG et al., 2003), BALP (LIESEGANG et al., 2003; SOUSA et al., 2014a), and OC determinations in various studies (PASTOUREAU et al., 1991; LIESEGANG et al., 2003). Collignon et al. (1996) demonstrated that bone growth since the fetal stage produces alterations in serum OC and BALP, confirming the usefulness of these markers in bone formation and growth. Scott et al. (1997) reported that OC, BALP, DPD, and PYD may be useful for detection of changes in bone growth caused by deficient diets, and Wan Zahari et al. (1994) reported that high phosphorus diets resulted in increased bone resorption (increased TRAP) in lambs. However, Chanetsa et al. (2000) exposed castrated lambs to an oestrogen agonist. In this study, bone growth was observed, but no effect on markers of bone remodelling was noticed.

6 I PRE-CLINICAL AND TRANSLATIONAL ORTHOPAEDIC RESEARCH STUDIES

The characteristics of the pre-clinical and translational orthopaedic research studies, such as population, type of studies, time, and conclusion are listed in Table IV.

6.1 Fracture Healing Process

Tralman et al. (2013) and Windhagen et al. (2002) reported that the serum markers of bone formation are useful for reflecting the bone healing process, and Goebel et al. (2009) suggested that FGF23 is a good marker to indicate the healing process. Seebeck et al. (2005) stated that degradation of soft callus can be determined by serum PIIINP during the bone fracture healing process and Schmidt et al. (2008) concluded that it is possible to monitor the maturation of bone callus with the total ALP and NTx. However, without individual reference values, BTMs become a weak tool to determine the prognosis of bone consolidation (KLEIN et al., 2004).

6.2 Osteoporosis

Newton et al. (2004) reported that ovariectomized (OVX) ewe were a useful model due to alterations in trabecular bone architecture along with the decrease in oestrogen levels, which resemble women in early menopause and Turner (2001) suggested that old OVX ewes could be a valid model for bone loss due to oestrogen deficiency. Johnson et al. (2002) reported that 6 months after OVX in sheep there was a decrease in alveolar bone BMD which became serious during the next 6 months. However, Sigrist et al. (2007) reported that in sheep, 6 months after the OVX, the markers for formation and resorption returned to baseline, indicating that the model was not appropriate for human postmenopausal osteoporosis. Kreipke et al. (2014) reported that OVX induces the necessary changes in bone microarchitecture for studying osteoporosis, but after a year, the changes in architecture stabilize in ovine. Chavassieux et al. (2001) reported that in goats, remodelling occurred only in the cortical bone tissue regions, which was also demonstrated by increased levels of CTx one month after OVX and OC three months after OVX.

Ding et al. (2010) and Andreasen et al. (2015) stated that the induction by glucocorticoids in sheep is similar to the change in the microstructure of human bone also induced by long-term glucocorticoid treatment, therefore being a useful model. MacLeay et al. (2004b) though not knowing what the true mechanism is involved in diets that induce metabolic acidosis in bone loss, concluded that the sheep model is useful for studies of osteoporosis induced by diet.

Therefore, small ruminant models are important for the study of human osteoporosis (CHAVASSIEUX et al., 1997; LILL et al., 2002a,b; ANDREASEN et al., 2015; KIELBOWICZ et al., 2015, 2016) induced by OVX and with attention to continuous treatment with glucocorticoids to maintain the osteoporotic bone condition (DING et al., 2010).

7 I CONCLUSIONS

The suitability of the determination of BTMs in small ruminants is already confirmed in numerous animal and veterinary sciences studies and also in preclinical and/or translational studies in orthopaedic research, in addition to imaging, mechanical, histological and histomorphometric analyses. Their advantage relies on a fast and non-invasive assessment via biochemical analysis of serum or urine samples, although the referred negative aspect of using BTMs in the clinical setting is related with their high biological variability. Particularly in sheep, BTMs have been used to estimate the extent of the osteogenic response at a local level at the fracture healing site, as precocious indicators of possible bone healing disturbances. BTMs could provide important information concerning bone metabolism at a systemic level, namely about bone remodelling process during induction of osteoporosis and its treatment in experimental orthopaedic studies. Recently it was developed a study by Baharuddin et al. (2014) in sheep with osteoclast regulatory protein – receptor activator of nuclear factor NF-κB ligand (RANKL) produced by osteocytes, osteoblasts and immune system cells, its membrane-bound receptor (RANK) in the osteoclast precursor cells and osteoprotegerin (OPG) as new potential bone markers in future (SOUSA et al., 2015), nevertheless more studies would be necessary to assess the usefulness of BTMs in this scientific field.

8 I ACKNOWLEDGEMENTS

José Arthur de A. Camassa acknowledges to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil, for his PhD scholarship 202248/2015-1.

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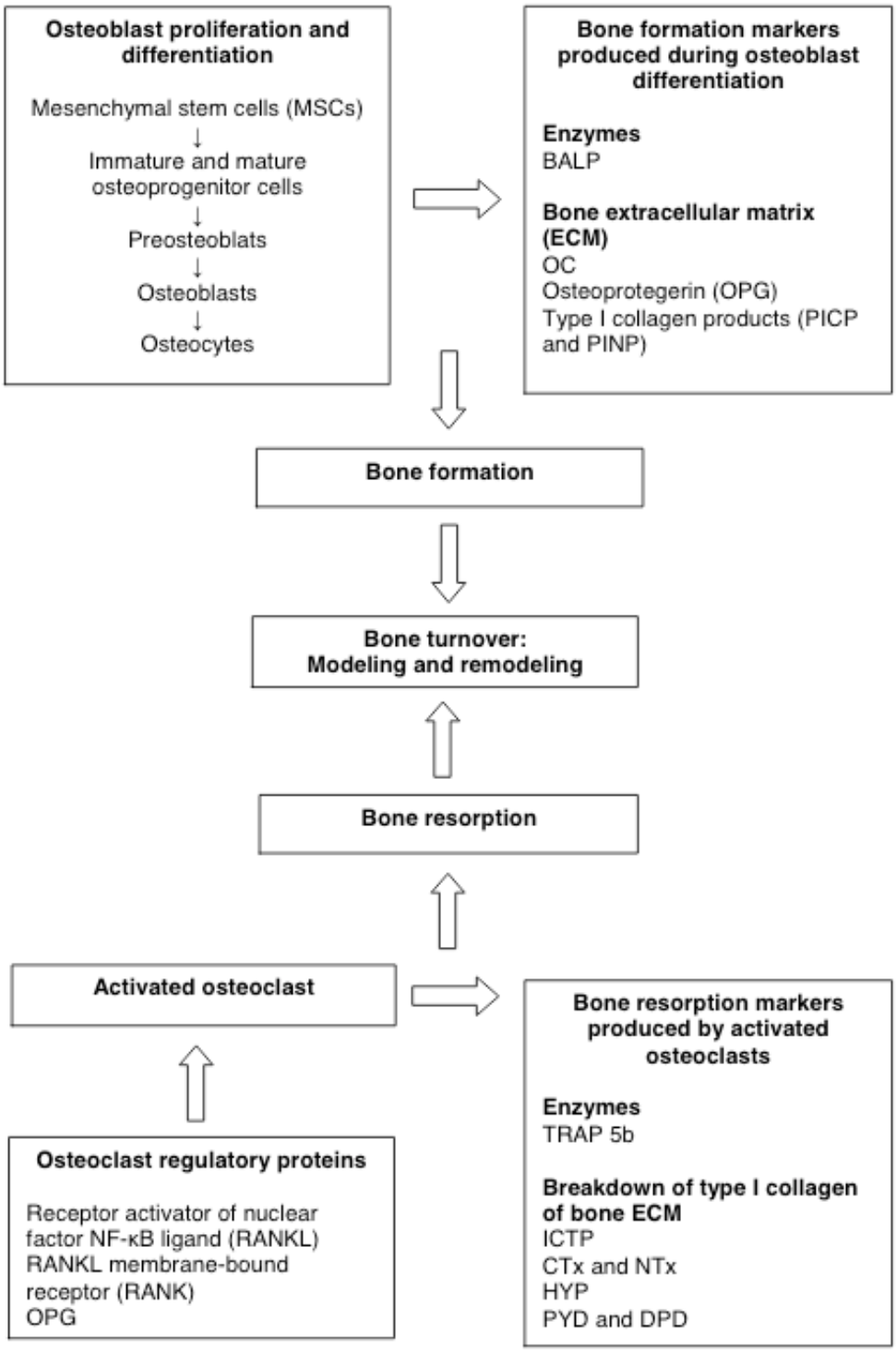

Figure 1 – Flow diagram of BTMs produced during the bone turnover process.

Marker	Tissue of origin	Sample	Method of analysis	Available commercial Assay kit	Cross-Reactivity Sheep / Goat
BALP	Bone	Serum	Colorimetric	No commercial kit available	NO / NO
			Electrophoretic	No commercial kit available	NO / NO
			Precipitation	No commercial kit available	NO / NO
			CLA	LIAISON BAP Ostase, Stillwater, MN, USA	? / ?
			ELISA	MicroVue BAP, Quidel Corporation, San Diego, CA, USA	YES / YES
			RIA	Tandem-R-Ostase, Beckman Coulter, Brea, CA, USA	YES / ?
OC	Bone	Serum	CLA	LIAISON Osteocalcin, Stillwater, MN, USA	? / ?
			RIA	BTI Human Osteocalcin RIA, Biomedical Technologies Inc, Stoughton, MA, USA	? / ?
			ELISA	MicroVue Osteocalcin, Quidel Corporation, San Diego, CA, USA	YES / YES
				BTI Intact Osteocalcin, Biomedical Technologies Inc, Stoughton, MA, USA	? / ?
				Osteocalcin, SIGMA, Saint Louis, Missouri, USA	? / ?
				Osteocalcin, GenWay Biotech, San Diego, CA, USA	NO / NO
PINP	Bone, soft tissue	Serum	CLA	PINP Roche Diagnostics, Penzberg, Germany	? / ?
			RIA	UniQ Intact PINP, Orion Corporation, Espoo, Finland	? / ?
		Serum or urine	ELISA	PINP, Neobiolab Inc, Cambridge MA, UK	YES / YES
PICP	Bone, soft tissue	Serum	RIA	PICP, Orion Corporation, Espoo, Finland	? / ?
				PICP DiaSorin, Stillwater, MN, USA	YES / ?
			ELISA	MicroVue CICP, Quidel Corporation, San Diego, CA, USA	YES / ?
		Serum or urine		PICP, Neobiolab Inc, Cambridge MA, UK	YES / YES

Table I - Bone formation markers, method of analysis and available commercial assay kits.

BALP: Bone specific alkaline phosphatase; OC: Osteocalcin; PINP: Amino-terminal procollagen propeptides of collagen type I; PICP: Carboxy-terminal procollagen propeptides of collagen type I; RIA: Radioimmunoassay; ELISA: Enzyme-linked immunosorbant assay; CLA: Chemiluminescence immunoassay; YES: presence of cross-reactivity; NO: absence of cross-reactivity; ?: no data available.

Marker	Tissue of origin	Sample	Method of analysis	Available commercial Assay kit	Cross-Reactivity Sheep / Goat
HYP	Bone, soft tissue, cartilage	Serum or urine	ELISA	HYP, Neobiolab Inc, Cambridge MA, UK	YES / YES
		Urine	Colorimetric HPLC	No commercial kit available	NO / NO
DPD	Bone, dentin	Urine	ELISA	MicroVue DPD, Quidel Corporation, San Diego, CA, USA	YES / ?
			HLPC	No commercial kit available	NO / NO
		Serum or urine	ELISA	MicroVue tDPD, Quidel Corporation, San Diego, CA, USA	YES / ?
				DPD, Neobiolab Inc, Cambridge MA, UK	YES / YES
PYD	Bone, cartilage, blood vessels	Urine	HPLC	No commercial kit available	NO / NO
			ELISA	MicroVue Serum PYD, Quidel Corporation, San Diego, CA, USA	YES / ?
			RIA	No commercial kit available	NO / NO
		Serum or urine	ELISA	PYD, Neobiolab Inc, Cambridge MA, UK	YES / YES
ICTP	Bone, skin	Serum	Colorimetric	No commercial kit available	NO / NO
			RIA	ICTP, Incstar Corporation, Stillwatter, MN, USA	? / ?
				UniQ ICTP, Orion Corporation, Espoo, Finland	? / ?
				ICTP DiaSorin, Stillwatter, MN, USA	NO / NO
			ELISA	UniQ ICTP EIA, Orion Corporation, Espoo, Finland	? / ?
		Serum or urine		ICTP, Neobiolab Inc, Cambridge MA, UK	YES / YES
CTx	Bone	Serum	CLA	β-Crosslaps Roche Diagnostics Penzberg, Germany	? / ?
			RIA	No commercial kit available	NO / NO
			ELISA	Serum CrossLaps, Biointernational, Yvette, France	YES / ?
		Urine	CLA	β-CrossLaps Roche Diagnostics Penzberg, Germany	? / ?
			RIA	CrossLaps RIA, Osteometer Biotech, Herlev, Denmark	? / ?
			ELISA	CrossLaps, Osteometer Biotech, Herlev, Denmark	YES / YES
		Serum or urine	ELISA	CTx, Neobiolab Inc, Cambridge MA, UK	YES / YES

NTx	Bone	Serum	RIA	No commercial kit available	NO / NO
			ELISA	Osteomark Ostex International Inc., Seattle, WA, USA NTx MyBioSourse, San Diego, CA, USA	YES / ?
		Urine	RIA	No commercial kit available	NO / NO
		Serum or urine	ELISA	NTx, Neobiolab Inc, Cambridge MA, UK	? / YES
Cathepsin k	Bone	Serum	ELISA	Cathepsin k ELISA Kit, Antibodies-online, Atlanta, Georgia, USA	? / ?
TRAP	Bone	Serum	RIA	No commercial kit available	NO / NO
			ELISA	MicroVue TRAP 5b, Quidel Corporation, San Diego, CA, USA	YES / YES
				Osteolink-TRAP b, Nitto Boseki Corporation, Tokio, Japan	? / ?
				Bone TRAP SBA, Science, Boldon, UK	? / ?
		Serum or urine		TRAP, Neobiolab Inc, Cambridge MA, UK	YES / YES

Table II - Bone resorption markers, method of analysis and available commercial assay kits.

HYP: Hydroxyproline; DPD: Deoxypyridinoline; PYD: Pyridinoline; ICTP: Carboxy-terminal telopeptide of type I collagen; CTx: cross-linked C-terminal telopeptides of type I collagen; NTx: cross- linked N-terminal telopeptides of type I collagen; TRAP 5b: Tartrate-resistant acid phosphatase isoenzyme 5b; RIA: Radioimmunoassay; ELISA: Enzyme-linked immunosorbant assay; CLA: Chemiluminescence immunoassay; HPLC: High-performance liquid chromatography.

Marker	**Tissue of origin**	**Sample**	**Method of analysis**	**Available commercial Assay kit**	**Cross-Reactivity Sheep / Goat**
RANKL	Bone, blood	Serum	ELISA	Human Serum RANKL Free ELISA Kit, Biomedica Medizinprodukte, GmbH & Co. KG, Wien, Austria	? /?
				RANKL, Immundiagnostik AG, Bensheim, Germany	? /?
		Serum or urine		RANKL, Neobiolab Inc, Cambridge MA, UK	YES / YES
RANK	Bone	Serum	ELISA	RANK R&D Systems, Minneapolis, MN, USA	? / ?
OPG	Bone	Serum	ELISA	Human Osteoprotegerin ELISA kit, BioVendo Laboratory Medicine, Inc., Labogen, Czech Republic	NO / NO
				Osteoprotegerin, Immundiagnostik AG, Bensheim, Germany	? / ?

		Osteoprotegerin R&D Systems, Minneapolis, MN, USA	? / ?
	Serum or urine	OPG, Neobiolab Inc, Cambridge MA, UK	YES / YES

Table III - Osteoclast regulatory proteins, method of analysis and available commercial assay kits.

RANKL: receptor activator of nuclear factor NF-κB ligand; RANK: receptor activator of nuclear factor NF-κB; OPG: osteoprotegerin; ELISA: Enzyme-linked immunosorbant assay.

Authors	Population	Type of Study	Markers	Time	Conclusion
Chavassieux et al. (1991)	Fourteen ewes	Determine the effects of ossein-hydroxyapatite compound on bone remodeling	Serum ALP, OC, Ca and P	90 days	Possibly the OHC is able to reduce the seasonal effect on bone turnover
Wan Zahari et al. (1994)	Ten sheep	Effects of nutrition on bone growth	Serum 1,25 Vit. D, ALP and TRAP	6 weeks	Diets rich in phosphate do not have effect on skeletal mineralization
Turner et al. (1995)	Thirty ewes	Represent changes in bone mass in ovariectomized ewes	Serum BALP	6 months	This model may be useful for estrogen deficiency studies inducing bone loss
Chavassieux et al. (1997)	Thirty-two ewes	Use of glucocorticoid for decrease of bone formation	Serum OC and BALP	7 months	Use of glucocorticoid in ewe may represent valid model for bone loss
Scott et al. (1997)	Twenty-four sheep	Effects of nutrition on bone growth	Serum TRAP, OC and BALP; Urinary PYD and DPD	90 days	Suggest that markers may be useful for diagnosis and treatment of bone disease and early detection of nutrition deficiencies
Nicodemo et al. (1999)	Twenty-four sheep	Influence of diet in bone growth	Serum BALP, OC, Ca and P; Urinary PYD and DPD	11 weeks	Markers are unsuitable for assessment of bone growth induced by different diets

Chanetsa et al. (2000)	Forty sheep	Effects of an estrogen agonist on growth and bone mineral accretion	Serum BALP, TRAP, 1,25 Vit. D, Ca, Mg and P	163 days	This study has clinical relevance for treating children with delays in growth and bone mineral accretion
Chavassieux et al. (2001)	Forty ewes	The effects of OVX in ewe associated or not with lentaron and effects of a new selective estrogen receptor modulator	Serum OC and BALP; Urinary CTx	6 months	OVX induced an increase in bone turnover and MDL may be useful for prevention of postmenopausal bone loss
Lill et al. (2002a)	Eight sheep	Which method is more effective to induce osteoporosis		6 months	The most effective method to induce osteoporosis is a combination of diet, ovariectomy, and glucocorticoid
Lill et al. (2002b)	Thirty-two sheep	Induce severe osteoporosis in an ovine model		7 months	The model may be useful for studies on osteoporosis
Windhagen et al. (2002)	Fourteen sheep	What is the response of turnover markers during distraction osteogenesis	Urinary DPD and PYD; Serum OC	74 days	Showed a pattern of osteoblast cellular activation during distraction osteogenesis
Liesegang et al. (2003)	Twelve goats and sheep	Determine the diurnal variation in bone markers	Serum BALP, ICTP, CL and OC	2 weeks	During the day there was a variation in concentration of markers, goats presented a higher bone turnover than sheep
Klein et al. (2004)	Fourteen sheep	Use of BTMs to represent callus consolidation in bone healing	Serum PICP, BALP and PIIINP	9 weeks	The markers used were not useful for represent bone healing

MacLeay et al. (2004a)	Fifty-two ewes	Develop an animal model for human postmenopausal osteoporosis		90 days	Model is sensitive for bone loss due to a dietary metabolic acidosis
MacLeay et al. (2004b)	Twenty-four sheep	Influence of diet and OVX on bone turnover	Urinary DPD; Serum BALP, Ca and P	180 days	Model is sensitive for bone loss due to a dietary metabolic acidosis
Newton et al. (2004)	Twelve ewes	Effects of ovariectomy on the trabeculae of ovine iliac bone	Serum OC; Urinary PYD	12 months	The ovine model is adequate for changes in trabecular bone architecture studies
Liesegang and Risteli (2005)	Six sheep and six goat	Influence of diet	Serum ICTP, BALP, OC, Ca, CL and 1,25 Vit. D	8 weeks	Due to short duration, it is difficult to associate the diet with bone turnover
Seebeck et al. (2005)	Sixteen sheep	Use bone markers to represent callus formation during fracture healing	Serum PICP, ALP, BALP, PIIINP, Ca and P	9 weeks	The markers used were not useful in representing callus formation
Liesegang et al. (2006)	Twelve goat and sheep	Determine the effects of pregnancy and lactation on markers	Milk and serum Ca; Serum OC, BALP, CTx, ICTP, and 1,25 Vit. D	11 months	Markers showed that bone turnover occurred during gestation and lactation
Arens et al. (2007)	Eight sheep	Measure the seasonal variations in quantity and quality of bone turnover	Serum BALP, PYD; Urinary DPD	18 months	Seasonal variation must be considered when using an ovine model in osteoporosis studies

Liesegang et al. (2007)	Twelve goats and sheep	Determine the effects of a second pregnancy and lactation on markers in comparison with a first	Milk and serum Ca; Serum OC, BALP, CTx, ICTP, and 1,25 Vit. D	11 months	The bone loss in the second pregnancy and lactation is lower than in the first, possibly due to an adaptation of the organism
Sigrist et al. (2007)	Fourteen sheep	The effect of ovariectomy on bone metabolism in sheep	Serum BALP, PYD; Urinary DPD	18 months	The ovine model is not an appropriate model for human postmenopausal osteoporosis
Dias et al. (2008)	Eighteen ewes	Measurement of bone markers in ewes under controlled environmental factors, and the study of their correlation with serum minerals	Serum ALP, BALP, OC, Ca, P, Mg and Ca^{2+}	6 weeks	References for the serum values of bone turnover parameters in sheep could be of great value, possibly for obtaining an early prognosis of fracture healing
Goebel et al. (2009)	Eighty-five sheep	Verification of FGF23 as a possible marker of bone healing and regeneration	Serum ALP, Ca and P; Urinary P	42 days	FGF23 is a promising marker for indicating bone healing
Ding et al. (2010)	Eighteen sheep	Use of glucocorticoid for inducing osteopenia in cancellous bone		10 months	This method is useful for induced osteoporosis in sheep
Vernon et al. (2010)	Twenty sheep	Influence of exercise on the degradation of the articular cartilage	Serum LOX and C2C	5 months	Markers were unable to demonstrate the effects of forced exercise
Liesegang et al. (2013)	Twenty-four sheep	Influence of diet	Serum ICTP, BALP, Ca, P and 1,25 Vit. D	4 months	Changes in bone turnover associated with diet

Tralman et al. (2013)	Seven sheep	Compare two methods of osteosynthesis in sheep	Serum ALP and OC	10 weeks	Use of RTP fixator is more effective than plate fixation in osteotomies of long bones in sheep
Kreipke et al. (2014)	Thirteen sheep	The effect of ovariectomy on vertebral bodies and femoral condyles in sheep after 1 and 2 years		24 months	The vertebral bodies are preferable for trabecular microarchitecture studies
Sousa et al. (2014a)	Ninety sheep	Measure the values of bone markers and evaluate the correlation between those and serum minerals in sheep of various ages and different physiologic stages	Serum ALP, BALP, Ca, Mg and P	1 day	The measure of lifespan in sheep is useful in preclinical orthopedic research and provide information complementary for other analyses with imaging
Sousa et al. (2014b)	Eighteen sheep	Assessment of the short-term variation in the BTMs serum levels	Serum ALP, BALP, OC, PIIINP, DPD, TRAP, Ca and P	12 weeks	The variability in short-term does not seem to be a limitation for studies with bone markers
Andreasen et al. (2015)	Twenty ewes	Assessment of cellular events during the remodeling process induced by glucocorticoid in ovariectomised sheep	Serum CTx and OC	7 months	It is a useful animal model due to significant bone loss compared to osteoporosis in postmenopausal women
Kielbowicz et al. (2015, 2016)	Forty-nine ewes	Assessment of different factors after osteoporosis induction with glucocorticoid	Serum BALP, CTx, estradiol, cortisol, progesterone and parathormone	3.7 months	Glucocorticoid treatment in ovariectomised sheep was considered by the authors as a very appropriate method for osteoporosis induction

Table IV - Characteristics of the animal and veterinary science studies that reported the use of BTMs in different types of research.

SOBRE OS ORGANIZADORES

Jorge González Aguilera - Engenheiro Agrônomo (Instituto Superior de Ciências Agrícolas de Bayamo (ISCA-B) hoje Universidad de Granma (UG)), Especialista em Biotecnologia pela Universidad de Oriente (UO), CUBA (2002), Mestre em Fitotecnia (UFV/2007) e Doutorado em Genética e Melhoramento (UFV/2011). Atualmente, é professor visitante na Universidade Federal de Mato Grosso do Sul (UFMS) no Campus Chapadão do Sul. Têm experiência na área de melhoramento de plantas e aplicação de campos magnéticos na agricultura, com especialização em Biotecnologia Vegetal, atuando principalmente nos seguintes temas: pre-melhoramento, fitotecnia e cultivo de hortaliças, estudo de fontes de resistência para estres abiótico e biótico, marcadoresmoleculares, associação de características e adaptação e obtenção de vitroplantas. Tem experiência na multiplicação “on farm” de insumos biológicos (fungos em suporte sólido; Trichoderma, Beauveria e Metharrizum, assim como bactérias em suporte líquido) para o controle de doenças e insetos nas lavouras, principalmente de soja, milho e feijão. E-mail para contato: jorge.aguilera@ufms.br

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Agência Brasileira do ISBN
ISBN 978-85-7247-417-7