CHAPTER V
DISCUSSION

Osteoarthritis is characterized by cartilage degeneration and joint effusion. It begins with injury to the articular cartilage. Because articular cartilage has no sensory nerve supply, there is no pain at this stage and the animal may continue to be active . Until the disease progresses to be advanced stage that is difficult to cure or reverse. The routine diagnostic techniques still have had some restriction in early detecting osteoarthritis or complicated to use. Immunoassay, by using monoclonal antibody against biomolecules of cartilage have been shown the pleasant ability that may be the mean of choice in early diagnosis (Alwan, *et al.*, 1990; Hazell, *et al.*, 1995; Belcher, *et al.*, 1997; Frisbrie, *et al.*, 1999) or monitoring changing of osteoarthritis. For example, keratan sulfate, chondroitin sulfate, and hyaluronan, the glycosaminoglycans found in matrix of cartilage, have been studied in order to apply them as biological markers reflecting cartilage metabolism. The study of these biomolecules in serum was known as a less invasive indirect technique than in synovial fluid or directly to the cartilage tissue. In this research, from study A, the levels of three biochemical markers (3B3 epitope, WF6 epitope, and hyaluronan) in horse serum were determined in normal horses categorized by age. From study B, they were compared between osteoarthritic and non-osteoarthritic horses. The similarity of age of these two groups of horses in study B was checked to prevent confounding from age effect. The data were checked whether they had normal distribution or not. The non-parametric and parametric statistical analyses were both used; however, they showed the same results. Thus, the parametric statistical analyses were selected to use in this thesis.

In determining these 3 biochemical markers in horse serum, competitive inhibition ELISA was used by application of 3B3 and WF6 monoclonal antibodies; the monoclonal antibodies specific for chondroitin sulfate, and hyaluronan binding protein, a protein molecule specific for hyaluronan.

Chondroitin sulfate chain mostly composes of chondroitin-6-sulfate (C-6-S), chondroitin-4-sulfate (C-4-S), and a minority of unsulfated chondroitin sulfate (C-0-S). The age-related changes in this sulfating patterns were reported in previous study (Osborne, *et al.*, 1994; Brown, *et al.*, 1998; Platt, *et al.*, 1998; Lauder, *et al.*, 2001; Uesaka, *et al.*, 2002). It was reported that C-6-S: C-4S ratio increased with age from birth until two years of horse age, then it become constant (Brown, *et al.*, 1998). However, Platt (1998) showed a slight but significant downward trend with increasing age of horse. This suggested that chondrocytes produced a lot of C-6-S at the early of life and gradually increased in synthesis of C-4-S in mature animal.

3B3 monoclonal antibody was specific to unsaturated chondroitin-6-sulfate (△di-6-S) which is the neo-epitope, the epitope on newly synthesized chondroitin sulfate chains (Caterson, *et al.*, 1995). Slater *et al.* (1995) found that 3B3 expression in normal cartilage specimen was very low or absent, occurring mainly in the young, skeletally immature subject (Slater, *et al.*, 1995). Interestingly, from this present research, the level of 3B3 epitope in serum has also shown a same trend as in cartilage specimen. There was a significant higher in foals at less than two years old, indicating a high anabolism of chondrocytes in young horses.

It was also found that C-6-S of the terminal residue of chondroitin sulfate chain was decrease in degenerative cartilage (Brown, *et al.*, 1998). Especially, in osteoarthritis that showed a strong inverse correlation between the radiographic severity and C-6-S concentration, C-6-S: C-4S and also a weak inverse correlation to C-4-S (Uesaka, *et al.*, 2002). These indicated the alteration of chondrocytes that decreased producing C-6-S or may be both of C-6-S and C-4-S in osteoarthritis. This present study has shown the result that osteoarthritic group has a significant lower △di-6-S recognized by monoclonal antibody 3B3 than in non-osteoarthritic group. These suggested that chondrocytes decreasingly produce newly synthesized chondroitin sulfate in this osteoarthritis group. Although, there were some reports found that 3B3 epitope increased in biological fluids or cartilage tissue of joint disease case than in normal (Hazell, *et al.*, 1995; Slater, *et al.*, 1995; Belcher, *et al.*, 1997; Pothacharoen, 2000), it

was suggested that the largest increased in 3B3 epitope were in sample taken early injury (<3 month) in response to compensate the degradation (Hardingham, 1995). In chronic case, the response by increase in new chondroitin sulfate chain recognized by monoclonal antibody 3B3 was low or undetectable because of the destroying of chondrocytes. In present research, however, the cases of osteoarthritic group were at late stage detected clearly by radiography. Therefore the decreasing of chondroitin sulfate may be resulted from the severe loss of chondrocytes at the advanced stage of osteoarthritis (McIlwraith and Sickle, 1981).

All of these suggested that the expression of 3B3 epitope could reflect only part of the hypermetabolic response by chondrocytes, which may increase matrix synthesis and help to repair damage (Hardingham and Fosang, 1995). But at the advanced osteoarthritic stage, chondrocytes were destroyed and 3B3 epitope was decreased. So that an opportunity in appearance this epitope was reduced. Thus it is unlikely to use 3B3 epitope as a diagnostic marker of cartilage degradation disease such as osteoarthritis but may be able to use as a following cartilage healing marker.

Beside 3B3 monoclonal antibody, WF6 monoclonal antibody was also the monoclonal antibody of chondroitin sulfate but it recognizes at different part of chondroitin sulfate from 3B3. Its epitope was found to be higher in osteoarthritis group from report of Pothachareon (Pothacharoen, 2000). In present research, osteoarthritic horses also have had the higher WF6 epitope than in non-osteoarthritic horse. This indicated that WF6 can reflect the catabolism of cartilage, contrary to 3B3 epitope that reflects the anabolism. Age effect was also different between 3B3 and WF6 epitopes. While 3B3 epitope level was significantly high in young horses, there was no different of WF6 epitope level in each age group of horses. This confirms the difference specific site of both epitopes and shows the qualification of WF6 epitope that it was not reflect the anabolism as being in 3B3.

In view of hyaluronan, there are many arguments in comparison hyaluronan concentration between joint disease and normal subjects. Most of hyaluronan researches reported the decreased hyaluronan in cartilage tissue (Thonar, *et al.*, 1978),

in serum (Pothacharoen, 2000), and in synovial fluid (Tulamo, *et al.*, 1994) of joint disease subjects. However, there are a few researches that have the opposite result in serum (Sharif, *et al.*, 1995) and in synovial fluid (Dahl, *et al.*, 1985). From the present result, there was no different significance in hyaluronan concentration between osteoarthritis and non-osteoarthritis. It was suggested in previous reports that there was a high concentration hyaluronan in inflammatory joint because of a temporary disturbance of soft tissue in joint (Hilbert, *et al.*, 1984). Hascall and Laurent explained that beside the cartilage, other tissues in joint were able to produce hyaluronan. Therefore the altered hyaluronan concentration came from the inflammatory synovial tissue (Hascall and Laurent, 2002). Thus serum hyaluronan concentration provide a measure of inflammatory component in joint diseases. But the osteoarthritic horse subjects in this present study have not had joint inflammation, thus there was no alteration of serum hyaluronan concentration between non-osteoarthritic and osteoarthritic horses. However, in normal horse of this study, horses at less than five years old has a higher concentration of hyaluronan than in older age. This indicated that there was a high metabolism of joint connective tissue in young horse then it become constant when the animal become mature.

In conclusion, the osteoarthritic group has a lower concentration of 3B3 epitope and higher concentration of WF6 epitope than in non-osteoarthritic group. This indicates the different qualification of both chondroitin sulfate epitopes. 3B3 epitope is appropriate for detecting anabolism of cartilage while WF6 epitope is suitable for detecting catabolism of cartilage. According to this difference, WF6 should be applied to be a diagnostic tool of osteoarthritis while 3B3 epitope should be applied to be a monitoring tool of this disease. However, from this research, it cannot make the cut off value for making decision of osteoarthritis because the classification in stages of osteoarthritis and the history of previous treatment from the horse's owners was not detected. For further investigation, grading of osteoarthritis, history taking about previous treatment, and more clearly defined normal non-osteoarthritic horses should be done. Hyaluronan still have had many arguments because of a lot of factors effected to

its concentration in body fluids. Thus, it has not been suitable tool for detecting cartilage metabolism.