Introduction: The intervertebral disc is the largest avascular structure in the body. The disc consists of at least two distinct regions, the inner nucleus pulposus (NP) with a high aggregan content, surrounded by the more collagenous annulus fibrosus. At the embryonic stage, mesenchymal cells accumulate around the funicular notochord, and the notochord separates into segments during growth to form the nucleus pulposus. In humans, notochordal cells in the NP disappear during adolescence and are replaced by cartilaginous NP cells. Recently, many researchers have been attempting to achieve restoration and regeneration by biological procedures in patients with intervertebral disc degeneration. However, it is still unknown whether the process of change from notochordal to non-notochordal cells is one of early signs of intervertebral disc degeneration. Therefore, comparison of metabolic activity among these cells is considered important for determination of the type of transplanted cells to use for regeneration of intervertebral discs. In this study, we used notochordal cell of rat and rabbit, as well as non-notochordal cells of bovine. We reviewed whether proteoglycan metabolism of nucleus pulposus cells varied among these groups in vitro.

Materials and Methods: Cells were isolated from the NP of lumbar discs of 15 weeks rats, 6 month rabbits and bovine caudal discs of 18-24 month. They were cultured for 5 days in alginate beads of 4 million cells/ml in DMEM containing 6% FBS under 21% O2 with medium osmolality of 400 mOsm. Cell viability was determined by manual counting using trypan blue, lactate production was measured enzymatically [1] and glycosaminoglycan (GAG) accumulation was measured using a DMB assay [2]. And also, we examined the specimens of NP among each animals by light microscope.

Results: Notochordal cells in the NP are observed in rats (Fig.1A) and rabbits (Fig.1B) under light microscope. On the other hand, cartilaginous-like NP cells are observed in bovine discs (Fig.1C). The cell viability rate was more than 80% after culture for three days and five days in each group. The lactate production / million cells of NP shows that rat and rabbit cells produced much more lactate compared with containing non-notochordal bovine cells. The time course of GAG accumulation in cultures shows that bovine NP cells produced about 100 μg/mL of GAG after culture for 5 day (20 μg/mL/day) (Fig.2). Rat and rabbit NP cells produced about 8 and 3 times more GAG than bovine, respectively. GAG production per million cells was also higher for rat and rabbit than bovine.

Discussion: Notochordal cells are observed in rats, mice, rabbits, cats, and pigs throughout life, but in humans only up to adolescence. On the other hand, cartilaginous-like NP cells are observed in bovines, sheep, and goats, and also in humans aged 4 years or older [3]. The number of disc cells is said to decrease with aging. It is unknown, however, whether this decline is caused by differentiation of notochordal cells into non-notochordal cells, apoptosis, or lack of nutrients from the end plate. It is possible that the disappearance of notochordal cells may trigger intervertebral disc degeneration. In the future, further studies will be required to promote the development of biological therapy for intervertebral disc degeneration. It is said that a 7-10% content of GAG [4] is required to obtain regenerated intervertebral disk tissue with enough strength for clinical application. According to the results of the present study, it was calculated that it would take at least 1 year, 3 years, and 7 years for rat, rabbit, and bovine resembling human cells to produce 7% GAG, respectively (Fig.2). These results show that the rate of accumulation of GAG in three-dimensional disc cell-culture systems is also slow and is limited by the rate of GAG production per cell. In conclusion, this study demonstrated marked differences of energy metabolism and matrix production between notochordal and non-notochordal NP cells. Animals with notochordal cells in the NP, such as rats and rabbits, may not be good models for studying human disc disorders.