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Original Article

Impact of hip dislocation on the development of the hip joint and femoral trochlear in rats: an in vivo study

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Abstract: Background: This study was performed to investigate the effects of hip dislocation on the morphological development of the acetabulum and femoral trochlear groove utilizing an animal model. Methods: A total of 128 newborn Wistar rats were randomly assigned to a control group (CG, n=48) and an experimental group (EG, n=80). The EG underwent induced hip dislocation via one week of swaddling. At the 4 and 8-week marks, 16 rats from the CG and half from the EG were euthanized. Morphometric parameters included acetabular width (AW) and depth (AD), the diameter of the femoral head (DFH), the femoral anteversion angle (FAA), and the angle, depth, and width of the trochlear groove (ATG, DTG, and WTG, respectively). Histological analysis was performed to evaluate the maturation of the femoral head and trochlear cartilage. Results: Compared with the CG, the EG exhibited shallower trochlear grooves, smaller and more irregular femoral head morphology, and shallower acetabula. Significant differences were observed between the CG and EG in the AW, AD, DFH, and FAA (P<0.001). Additionally, the EG also showed significant differences in the WTG (P=0.045), DTG (P<0.001), and ATG (P=0.005). The FAA was positively correlated with the ATG and negatively correlated with the LCL and DTG. Histological analysis revealed notable cartilage degeneration and subchondral bone alterations in the femoral heads of the EG, whereas no significant histopathological changes were observed in the femoral trochlea. Conclusion: Hip dislocation not only leads to developmental dysplasia of the hip, but also impairs the maturation of the femoral trochlea, with the pathological changes of both DDH and trochlear dysplasia becoming more pronounced over time.

Keywords: Developmental dysplasia of the hip, femoral trochlear dysplasia, hip dislocation, traditional straight-leg swaddling, animal experiment

Introduction

Developmental dysplasia of the hip (DDH) refers to a spectrum of conditions that includes acetabular dysplasia, hip subluxation, and hip dislocation. This pathology is characterized predominantly by insufficient acetabular ossification and the presence of hip joint instability, such as subluxation or dislocation. DDH is recognized as one of the most prevalent orthopedic deformities observed in pediatric patients [1-4]. Early detection and appropriate intervention in abnormal acetabulofemoral joint relationships can facilitate normalization of joint morphology and biomechanics, preventing secondary deformities [5, 6]. Conversely, delayed diagnosis of developmental dysplasia of the hip (DDH) may lead to progressive joint deterioration and biomechanical malalignment [7, 8]. Although DDH primarily affects the hip, severe cases can result in deformities throughout the entire biomechanical alignment of the lower extremities. Studies indicates that DDH may contribute to valgus deformity of the knee joint [9]. Radiographic assessments have shown notable morphological alterations in the ipsilateral knee of individuals with dysplastic hips, including changes in the femoral condyle and patellofemoral joints [9-14]. Research demonstrates that patients with DDH often exhibit reduced femoral condyle size, increased medial-lateral condylar asymmetry, larger anterior femoral condyle angles, and shallower trochlear grooves [15]. However, the etiology of these femoral and trochlear modifications-whether congenital or acquired-remains to be conclusively determined.

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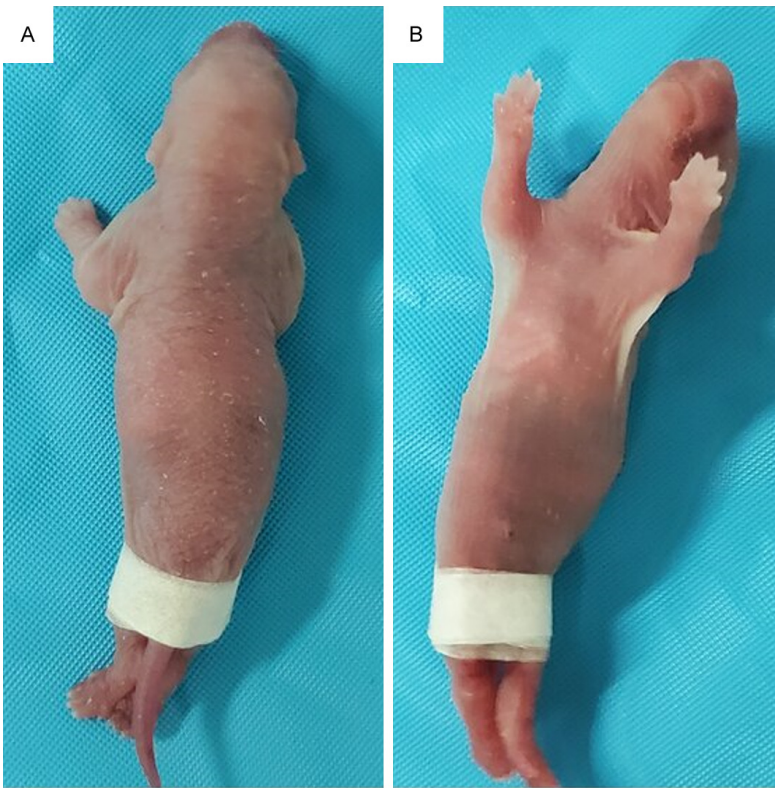


Figure 1. The straight-leg swaddling model in newborn Wistar rats. A: The dorsal view of the model; B: The abdomen view of the model.

Therefore, this research was conducted to examine the impact of hip dislocation on the development of the hip joint and femoral trochlea by establishing an animal model of hip dislocation through hip joint swaddling. We hypothesize that hip dislocation not only disrupts the development of the hip joint, leading to hip dysplasia, but can also impair the formation of the femoral trochlea, resulting in femoral trochlear dysplasia.

Methods

Study design

Following approval from the Animal Ethics Committee, a total of 128 newborn Wistar rats (64 females and 64 males) sourced from 12 litters provided by Beijing Vital River Laboratory Animal Technology were utilized in this experimental protocol. Each litter was randomly allocated into a control group (CG) (n=48, 24 females and 24 males) and an experimental group (EG) (n=80, 40 females and 40 males). The newborn rats in the EG were wrapped and secured with surgical tape (3M Durapore; 3M, Maplewood, MN, USA) to maintain hip extension [16] (**Figure 1**). This swaddling allowed for minimal hip movement (angle of motion <30°) while permitting slight flexion and extension of the knee joint. The rats were released from swaddling for approximately 30 minutes daily, after which the tape was reapplied.

One week post-intervention, neonatal restraint was discontinued, and the rats were allowed unrestricted activity. Rats in the CG were not swaddled and were allowed free movement within the cage. All rats were housed in a specific pathogen-free animal room with an ambient temperature of 24°C and a humidity level of 50%. Each litter was kept in separate plastic cages with wood chip bedding. All rats received maternal nutrition, with dams managed by trained personnel to ensure proper lactation. Both experimental and control groups exhibited normal growth trajectories and comparable body weights at each developmental milestone. At 4 weeks of the experiment, X-ray examinations were conducted on the rats in the EG to determine the presence of hip dislocation. Rats without hip dislocation were excluded from further analysis (**Figure 2**).

Ethical declarations

The study was approved by the Medical Animal Ethical Committee of Harrison International Peace Hospital (ID: 2022-2-011). We confirm all methods in our study were carried out in accordance with ARRIVE guidelines.

Adverse events

In the preliminary experiment, six rats died because of too-tight swaddling. To reduce this adverse event, we permitted minor hip and knee movement (ROM of knee less than 30°), and the rats were released from swaddling for approximately 30 minutes per day.

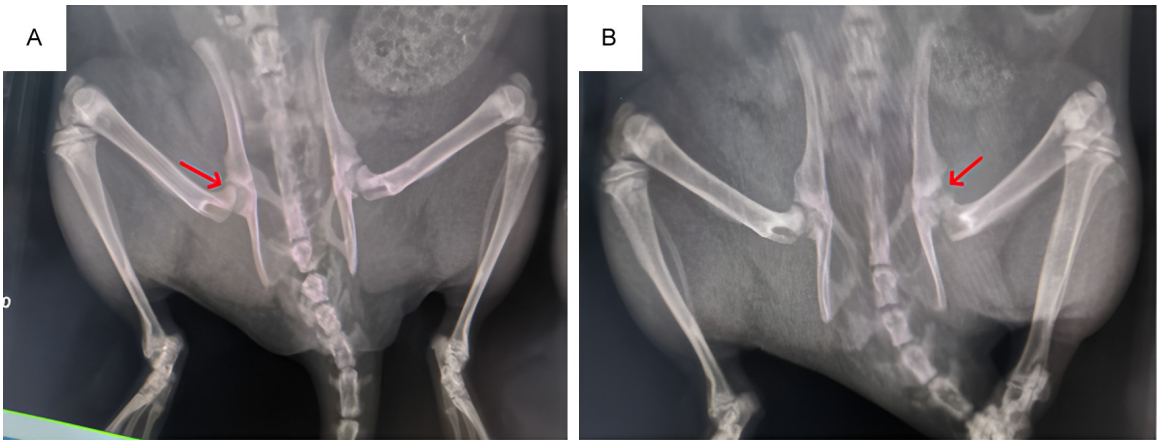


Figure 2. X-ray examination of the hip joint. A: The normal hip joint; B: The hip dislocation.

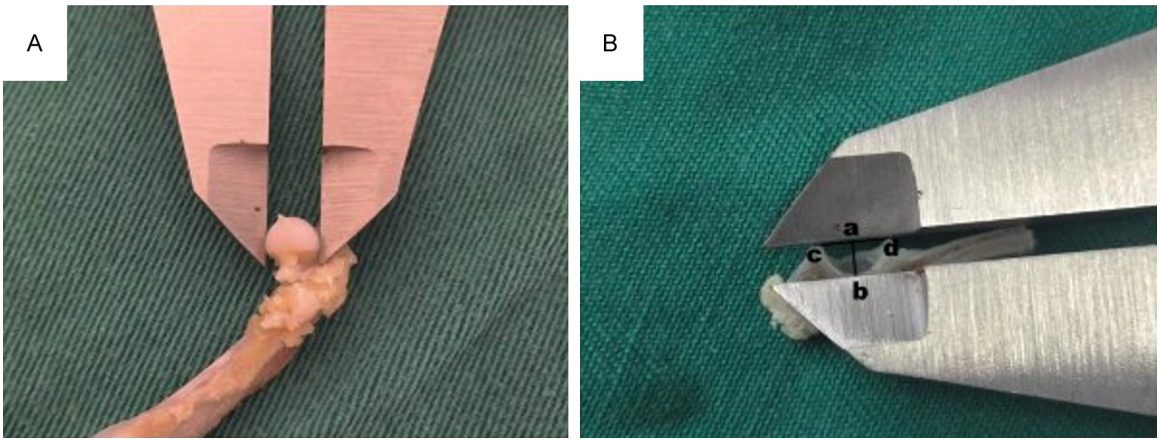


Figure 3. The assessment of the acetabulum and femoral head. A. The measurement of the femoral head's diameter. B. The measurement of the acetabular width (AW) (c, d) and the acetabular depth (AD) (a, b).

Gross observation and measurements

At 1 week, 16 rats (8 females and 8 males) from each group were euthanized using carbon dioxide inhalation in accordance with the American Veterinary Medical Association Guidelines on Euthanasia. At 4 and 8 weeks, 16 rats (8 females and 8 males) from the CG and half of the rats from the EG (with an equal distribution of males and females) were euthanized. The femoral trochlea and hip joint were isolated, and their gross appearance was visually assessed. A observer blind to grouping assessed the size and gross morphology of the hip and femoral trochlea. The development of the hip joint and femoral trochlea was observed at 1, 4, and 8 weeks. Morphometric parameters measured included the femoral head's longitudinal diameter (DFH) and acetabular width (AW) and depth (AD) (defined by the longitudinal diameter) (**Figure 3**).

Additionally, a transverse cross-section through the trochlear groove at the horizontal plane of the Roman arch (circular intercondylar notch) was observed, and the angle, width, with measurements taken for the trochlear groove's angle (ATG), width (WTG), and depth (DTG), as well as the lengths of the medial and lateral femoral condyles (MCL and LCL) (**Figure 4**). The femoral anteversion angle (FAA) was measured to evaluate femoral rotation (**Figure 5**). All measurements were independently conducted by two experienced technicians blind to group allocation, with repeat measurements after a 1-week interval. The reliability of the measurements and the agreement between the first measurements and the retests were assessed

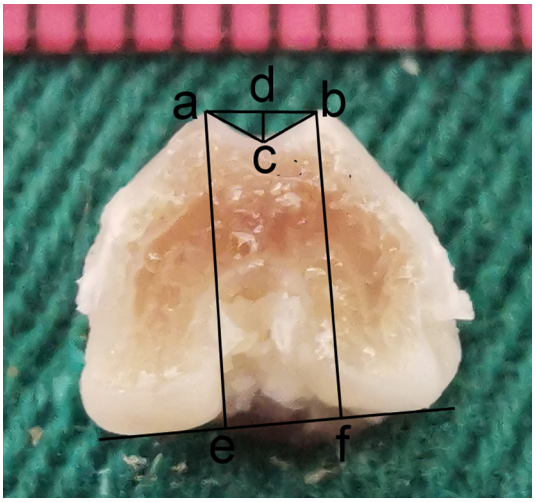


Figure 4. The measurements diagram of femoral trochlear groove. ∠(a, c, b) is the angle of the femoral trochlea, (a, b) is the width and (c, d) is the depth of femoral trochlear groove, (a, e) is the medial condyle length (MCL) and (b, f) is the lateral condyle length (LCL).

using the intraclass correlation coefficient (ICC). The measurement precision was 0.1° for angles and 0.01 mm for lengths. All descriptive data are expressed as mean ± standard deviation.

Tissue section staining and histologic analysis

After the above measurements, the tissue specimens underwent decalcification, wax embedding, sectioning, and hematoxylin and eosin (HE) staining of the femoral head and trochlea. The specimens were soaked in 4% paraformaldehyde (pH=7.40) overnight at 4°C and then immersed in 10% ethylenediaminetetraacetic acid at 4°C for decalcification over approximately 30 days. A gradient of alcohol and xylene was used for dehydration, followed by embedding in paraffin for tissue staining. A 5-mm section along the femoral axis was obtained to capture transverse images of the trochlear sulcus [15, 17, 18], while a central coronal section of the femoral head was stained with HE to assess cartilage and subchondral bone integrity. The distribution and quantity of chondrocytes in the femoral head and femoral trochlea of both groups were examined under a microscope, and the surface of the articular cartilage was evaluated.

Statistical analysis

Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). All

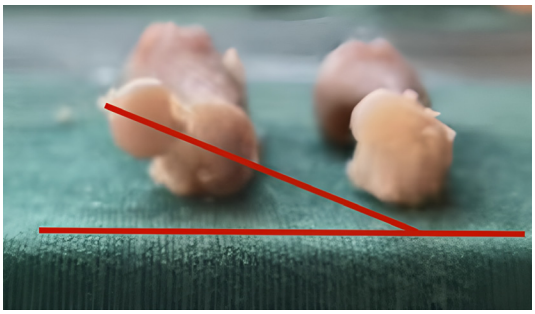


Figure 5. The measurement diagram of femoral anteversion angle (FAA).

descriptive statistical data for the hip and femoral trochlea of each rat are expressed as mean ± standard deviation. The Shapiro-Wilk test was used to assess the normality of the distribution of each variable, and the Levene test was used to evaluate the homogeneity of variance. An independent samples t-test was used to analyze parametric data. The chi-square test was applied to analyze differences between sexes and groups. Pearson's correlation coefficient (r) was used to analyze the correlation between the FAA and the femoral trochlear morphology at different developmental stages. An r value ranging from 0.75 to 1.00 indicates a strong correlation, 0.45 to 0.75 indicates a moderate correlation, 0.30 to 0.45 indicates a mild correlation, and 0.00 to 0.30 indicates no correlation. A *P*-value of <0.05 was considered statistically significant.

Results

At 4 weeks, X-ray examination revealed that 36 rats (22 females, 14 males) in the EG exhibited hip dislocation.

Gross observation

At one week, the size of the femoral distal cartilage in the EG and CG was similar, and the surface of the articular cartilage was smooth. However, some specimens in the EG had shallower trochlear grooves than those in the CG. The development of the hip joint in the CG was normal, with the femoral head and acetabulum appearing round. Conversely, the EG exhibited capsular hyperplasia of the hip, and the acetabulum had an irregular shape.

By four weeks, the height of the lateral femoral condyles had decreased in the EG, and the trochlear grooves were shallower than those in

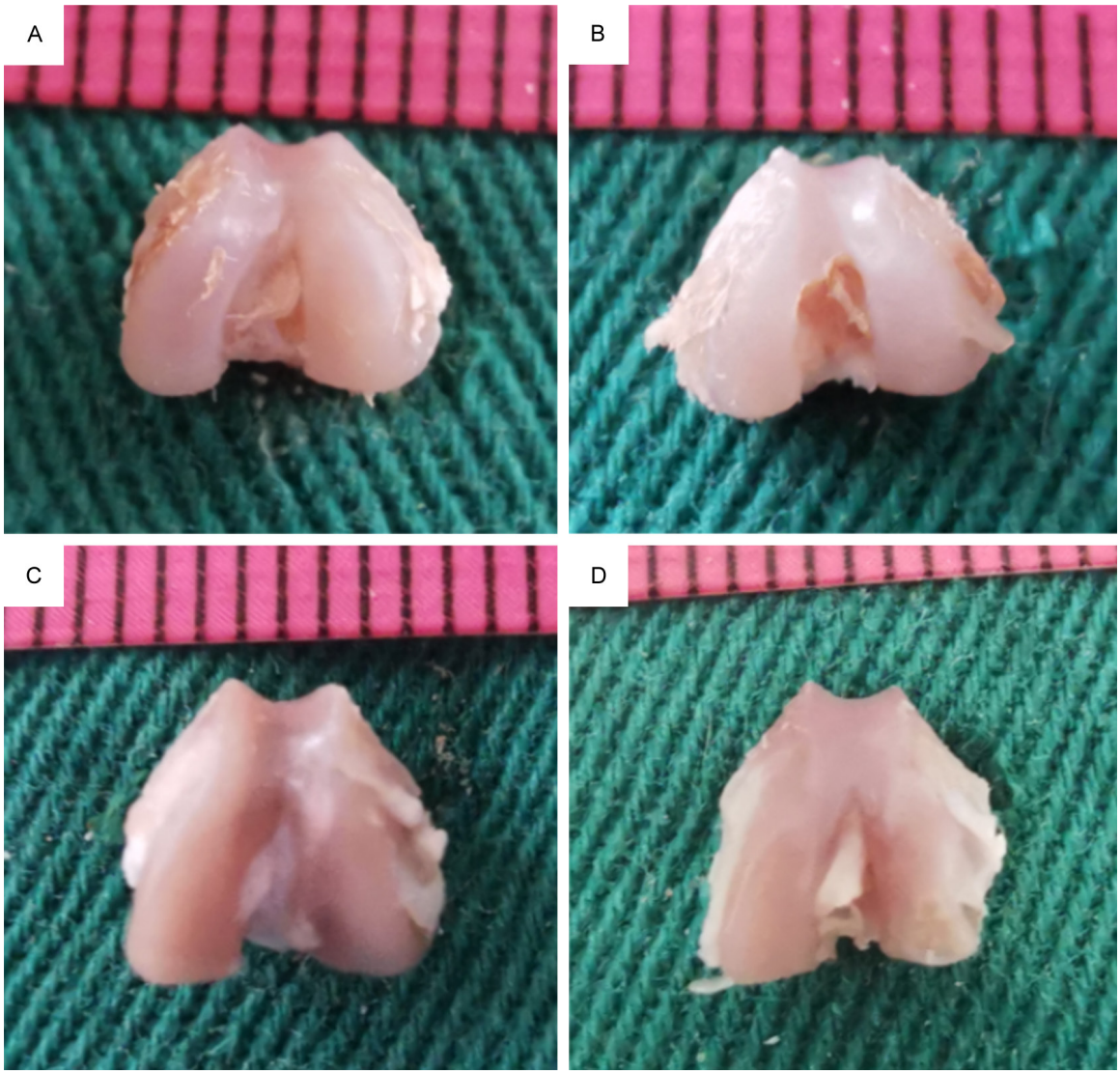


Figure 6. The gross images of the femoral trochlea. (A) is the control group at 4 weeks, while (C) depicts the control group at 8 weeks. (B) represents the experimental group at 4 weeks, and (D) shows the experimental group at 8 weeks.

the CG (**Figure 6**). The femoral head in the EG was smaller and more irregular in shape than that in the CG. The acetabulum in the EG was shallower and more irregularly shaped than that in the CG (**Figure 7**). By eight weeks, the differences in the trochlear grooves between the EG and CG were more pronounced. The femoral head and acetabulum in the EG showed further abnormalities in size and shape compared with the CG.

Measurement of hip joint and femoral trochlea

At one week, the AW in the CG and EG was 2.36±0.24 vs. 2.29±0.27 mm (P=0.277). The AD was 1.01±0.15 mm in the CG vs. 0.96±0.12 mm in the EG (P=0.146). The DFH was 1.99±0.18 mm in the CG vs. 1.95±0.19 mm in the EG (P=0.391). The FAA was 32.9°±5.4° in the CG vs. 34.3°±5.0° in the EG (P=0.286). The WTG, DTG, and ATG were 1.34±0.15 vs. 1.32±0.20 mm (P=0.652), 0.33±0.05 vs. 0.30±0.04 mm (P=0.019), and 132.6°±5.2° vs. 135.8°±5.9° (P=0.025) in the CG and EG, respectively. The MCL and LCL were 2.78±0.24 vs. 2.75±0.27 mm (P=0.640) and 2.57±0.26 vs. 2.55±0.26 mm (P=0.759) in the CG and EG, respectively (**Table 1**).

At four weeks, the AW and AD in the CG and EG were 3.65±0.25 vs. 2.52±0.31 mm (P< 0.001) and 1.56±0.22 vs. 1.06±0.26 mm

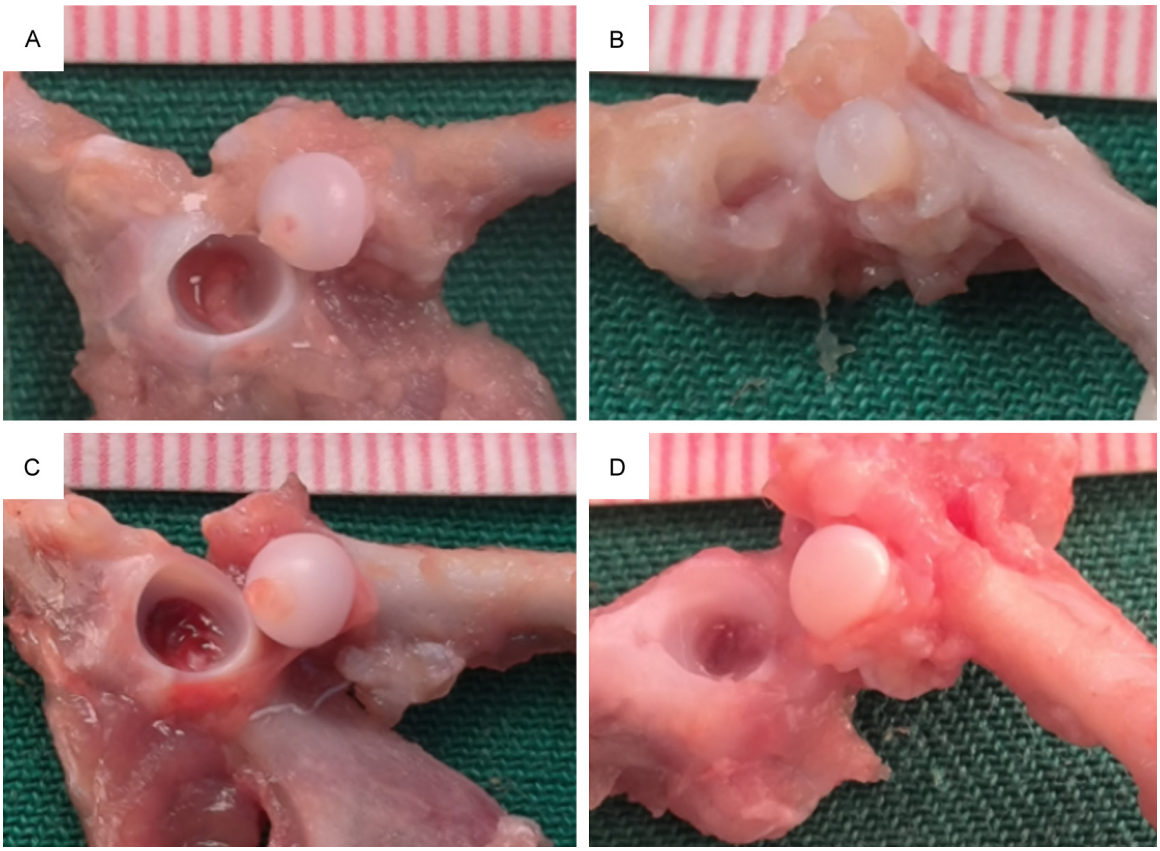


Figure 7. The macroscopic images of the hip joint. (A) is the hip joint of the control group at 4 weeks, while (C) depicts the control group at 8 weeks. (B) represents the hip joint of the experimental group at 4 weeks, and (D) shows the experimental group at 8 weeks.

Table 1. Measurements of acetabulum, femoral head and femoral trochear groove at 1 week

	Con Group (n=32)	Exp Group (n=32)	*t* Value	*P*
AW	2.36±0.24 mm	2.29±0.27 mm	1.096	0.277
AD	1.01±0.15 mm	0.96±0.12 mm	1.472	0.146
DFH	1.99±0.18 mm	1.95±0.19 mm	0.865	0.391
FAA	32.9°±5.4°	34.3°±5.0°	1.076	0.286
WTG	1.34±0.15 mm	1.32±0.20 mm	0.453	0.652
DTG	0.33±0.05 mm	0.30±0.04 mm	2.400	0.019
ATG	132.6°±5.2°	135.8°±5.9°	2.302	0.025
MCL	2.78±0.24 mm	2.75±0.27 mm	0.470	0.640
LCL	2.57±0.26 mm	2.55±0.26 mm	0.308	0.759

Data presented as mean ± SD. AW: Acetabular width; AD: Acetabular depth; DFH: Diameter of Femoral Head; FAA: Femoral anteversion Angle; WTG: Width of the Femoral Trochlea Groove; DTG: Depth of the Femoral Trochlea Groove; ATG: Angle of the Femoral Trochlea Groove; MCL: medial condyle length; LCL: lateral condyle length.

(*P*<0.001), respectively. The DFH was 3.42±0.28 mm in the CG vs. 2.32±0.24 mm in the EG (*P*<0.001). The FAA was 29.3°±5.8° in the CG vs. 39.4°±7.0° in the EG (*P*<0.001). The WTG, DTG, and ATG were 2.32±0.22 vs. 2.20±0.26 mm (*P*=0.045), 0.49±0.05 vs. 0.42±0.09 mm (*P*<0.001), and 126.2°±5.0° vs. 130.5°±6.8° (*P*=0.005) in the CG and EG, respectively. The MCL and LCL were 5.56±0.38 vs. 5.30±0.46 mm (*P*=0.014) and 5.24±0.45 vs. 4.97±0.45 mm (*P*=0.016) in the CG and EG, respectively (**Table 2**). No significant correlation was observed between the FAA and trochlear groove morphology in the CG at four weeks. In the EG, the FAA showed a moderate positive correlation with the ATG, a moderate negative correlation with the DTG, and a mild negative correlation with the LCL (**Figure 8**).

At eight weeks, the AW and AD in the CG and EG were 4.33±0.32 vs. 2.91±0.35 mm (*P*<0.001) and 2.28±0.25 vs. 0.91±0.22 mm (*P*<0.001), respectively. The DFH was 4.02±0.30 mm in

Table 2. Measurements of acetabulum, femoral head and femoral trochlear groove at 4 weeks

	Con Group (n=32)	Exp Group (n=36)	*t* Value	*P*
AW	3.65±0.25 mm	2.52±0.31 mm	16.411	<0.001
AD	1.56 mm±0.22	1.06±0.26 mm	10.03	<0.001
DFH	3.42±0.28 mm	2.32±0.24 mm	17.443	<0.001
FAA	29.3°±5.8°	39.4°±7.0°	6.367	<0.001
WTG	2.32±0.22 mm	2.20±0.26 mm	2.041	0.045
DTG	0.49±0.05 mm	0.42±0.09 mm	3.896	<0.001
ATG	126.2°±5.0°	130.5°±6.8°	2.939	0.005
MCL	5.56±0.38 mm	5.30±0.46 mm	2.522	0.014
LCL	5.24±0.45 mm	4.97±0.45 mm	2.470	0.016

Data presented as mean ± SD.

the CG vs. 2.62±0.26 mm in the EG (P< 0.001). The FAA was 24.8°±5.5° in the CG vs. 45.8°±6.5° in the EG (P<0.001). The WTG, DTG, and ATG were 2.80±0.20 vs. 2.58±0.21 mm (P<0.001), 0.76±0.11 vs. 0.59±0.14 mm (P<0.001), and 119.8°±5.6° vs. 128.4°±8.4° (P<0.001) in the CG and EG, respectively. The MCL and LCL were 7.71±0.36 vs. 7.26±0.41 mm (P<0.001) and 7.58±0.61 vs. 6.98±0.51 mm (P<0.001) in the CG and EG, respectively (**Table 3**). There was no significant correlation between the FAA and trochlear groove morphology in the CG at eight weeks. In the EG, the FAA demonstrated a moderate positive correlation with the ATG and a moderate negative correlation with both the LCL and DTG (**Figure 8**). All measurements demonstrated high reliability (ICC≥0.76), with strong agreement between initial and retest assessments (ICC≥0.79).

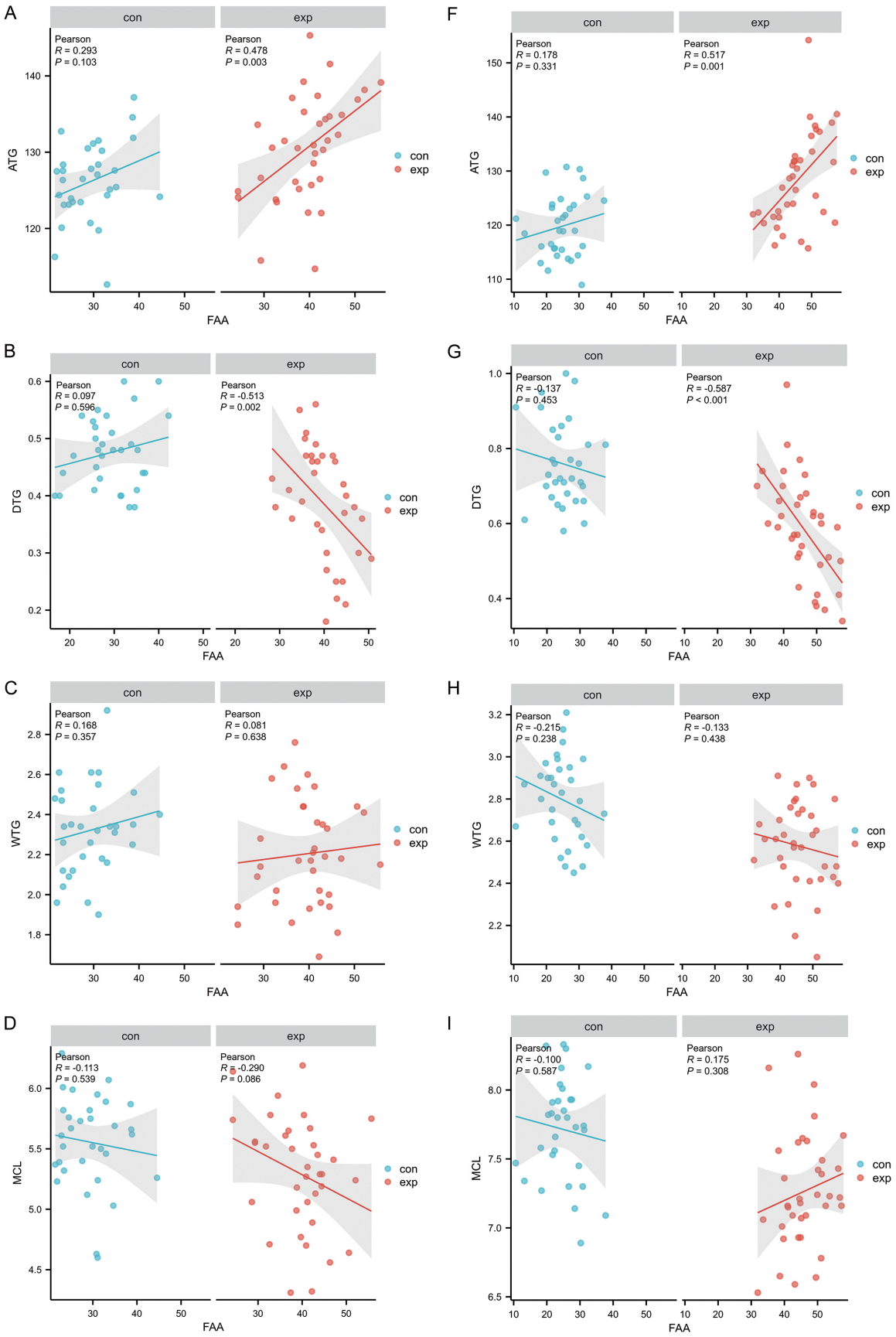
HE staining analysis

HE staining of the femoral head showed that in the CG, the femoral head exhibited a spherical and regular morphology, with a smooth articular cartilage surface and uniform chondrocyte size and distribution. The osteochondral interface cells were clear and neatly arranged as the bony structure of the femoral head gradually formed over weeks 4 and 8. By contrast, the EG displayed a flatter, irregular femoral head with reduced size compared to the CG at 4 weeks, with the malformation becoming more pronounced with further development. Although there was no obvious cartilage surface degeneration and the cartilage surface remained smooth, the chondrocyte distribution in the EG was uniform, however, the osteochondral junction cells were irregularly arranged, and the subchondral bone region was relatively osteoporotic (**Figure 9**). HE staining of the femoral trochlear groove in the EG was similar to that in the CG at weeks 4 and 8. The cartilage surface of the trochlear groove was smooth, with an orderly distribution and arrangement of chondrocytes, and the development of subchondral bone was essentially normal (**Figure 10**). Nonetheless, the trochlear grooves in the EG were shallower than those in the CG.

Discussion

This research indicates that hip dislocation may not only lead to the development of hip dysplasia but may also cause the abnormal rotation of the femur resulting in an increase in FAA. The aberrant femoral rotation further impacts the normal development of the femoral trochlea, ultimately leading to femoral trochlear dysplasia. As skeletal maturation advances, the severity of both hip dysplasia and femoral trochlear dysplasia progressively worsens.

In our research, early swaddling fixation resulted in hip dislocation in the EG, with developmental dysplasia of the hip worsening over time. The acetabulum in the EG was significantly smaller and shallower than that in the CG, and the femoral head was also significantly reduced in size. Studies have shown that swaddling is associated with an increased risk of hip dysplasia during critical periods of musculoskeletal development [19-21]. Histological analysis revealed disorganized chondrocyte arrangement within the osteochondral junction, with the subchondral bone region displaying osteoporotic changes. We believe that the dysplasia observed in the EG was primarily due to the weakened interaction between the femoral head and acetabulum caused by hip dislocation. Previous studies have indicated that biomechanical stimulation promotes bone and cartilage formation [10, 22]. In addition to stimulating osteogenesis, biomechanical pressure likely induces localized bone and cartilage deposition of joints, contributing significantly to the morphogenesis of articulations, including both the hip joint and femoral trochlea.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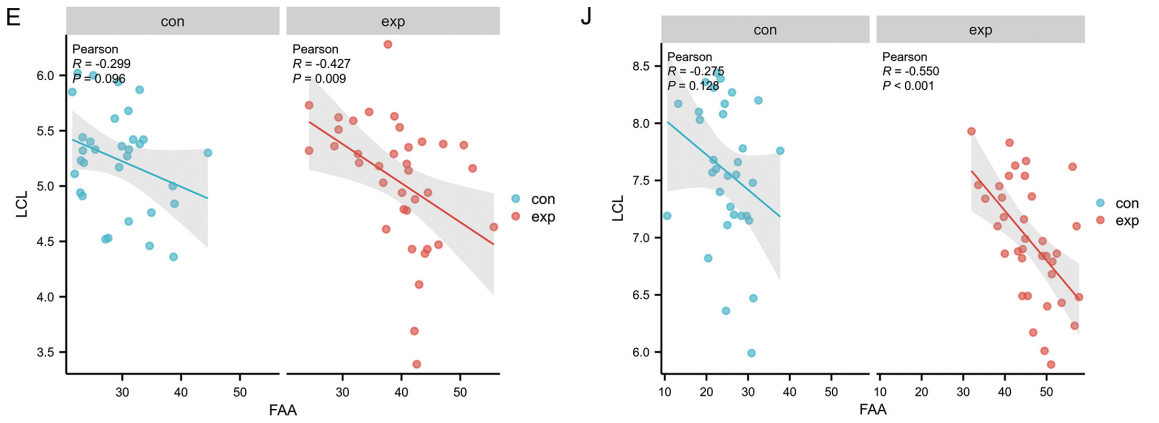

Figure 8. The diagram of Pearson correlation coefficient (r) analysis between FAA and femoral trochlea morphology at different developmental stages. (A-E) were the correlation between FAA and ATG, DTG, WTG, MCL and LCL at 4 weeks, and (F-J) were the correlation between FAA and ATG, DTG, WTG, MCL and LCL at 8 weeks.

Table 3. Measurements of acetabulum, femoral head and femoral trochlear groove at 8 weeks

	Con Group (n=32)	Exp Group (n=36)	*t* Value	*P*
AW	4.33±0.32 mm	2.91±0.35 mm	17.382	<0.001
AD	2.28±0.25 mm	0.91±0.22 mm	24.039	<0.001
DFH	4.02±0.30 mm	2.62±0.26 mm	20.617	<0.001
FAA	24.8°±5.5°	45.8°±6.5°	14.285	<0.001
WTG	2.80±0.20 mm	2.58±0.21 mm	4.093	<0.001
DTG	0.76±0.11 mm	0.59±0.14 mm	5.518	<0.001
ATG	119.8°±5.6°	128.4°±8.4°	4.902	<0.001
MCL	7.71±0.36 mm	7.26±0.41 mm	4.782	<0.001
LCL	7.58±0.61 mm	6.98±0.51 mm	4.416	<0.001

Data presented as mean ± SD.

Although DDH primarily affects the hip, severe cases may result in malformation of the entire biomechanical alignment of the lower extremities. Studies have shown that patients with DDH often exhibit abnormal lower limb rotation, which may affect the development of the patellofemoral joint, leading to abnormal movement of this joint [12, 15, 23, 24]. Li et al. [15] reported that DDH was associated with morphological changes in the knee joint. Fithian et al. [25] observed decreased trochlear depth in DDH patients, indicating a higher prevalence of trochlear dysplasia in the patients with DDH. In our study, although knee joint activity in EG rats was normal, the biomechanical alterations caused by hip dislocation likely impacted the normal development of the femoral trochlea. Abnormal torsional alignment of the lower limb could weaken the interaction between the patella and femoral trochlea and affect bone metabolism in the femoral trochlear groove.

Several studies have shown that the FAA is larger in patients with acetabular dysplasia [26-28]. Increased FAA and excessive lower limb pronation are also recognized as significant biomechanical risk factors contributing to patellar instability and dislocation [29-32]. Our findings confirm that hip dislocation significantly increases the FAA and femoral internal rotation, with these changes becoming more pronounced over time. Pearson correlation analysis shows a moderate positive correlation between the FAA and ATG and a moderate negative correlation between the FAA and the DTG, and a mild negative correlation between the FAA and LCL at four weeks. By eight weeks, there is a moderate positive correlation between the FAA and ATG and a moderate negative correlation between the FAA and both the LCL and DTG. Chen et al. [33] and Hao et al. [34] reported that there was correlation between the FAA and the distal femoral morphology in patients with patellar dislocation and trochlear dysplasia. Fan et al. [35] found that adult patients with femoral trochlear dysplasia and patellar dislocation also exhibited acetabular dysplasia, suggesting a correlation between femoral trochlear dysplasia and hip dysplasia during growth and development, consistent with our findings.

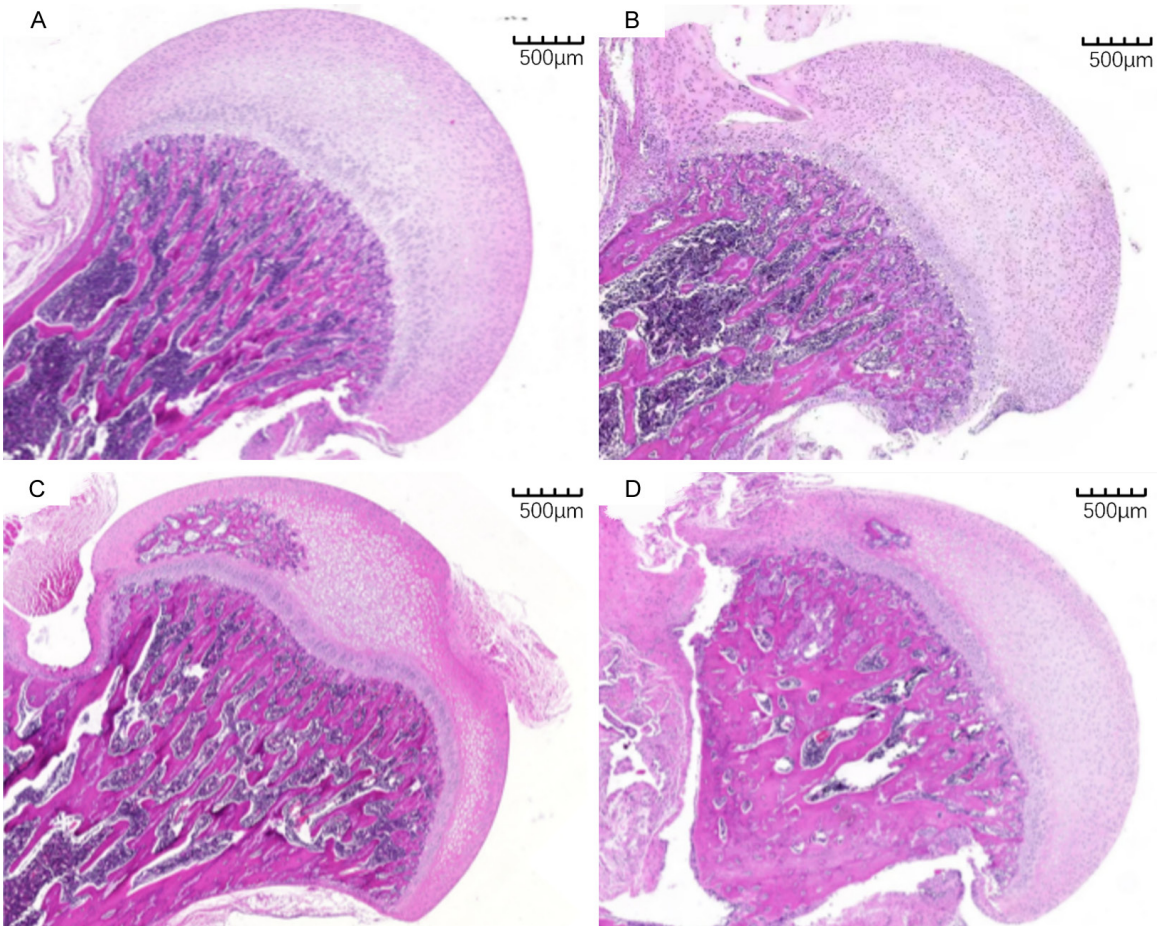

Figure 9. The HE staining of the femoral head. (A) illustrates the femoral head of the control group at 4 weeks, while (C) depicts the control group at 8 weeks. (B) represents the femoral head of the experimental group at 4 weeks, and (D) shows the experimental group at 8 weeks. The magnification of the staining figures was 2×.

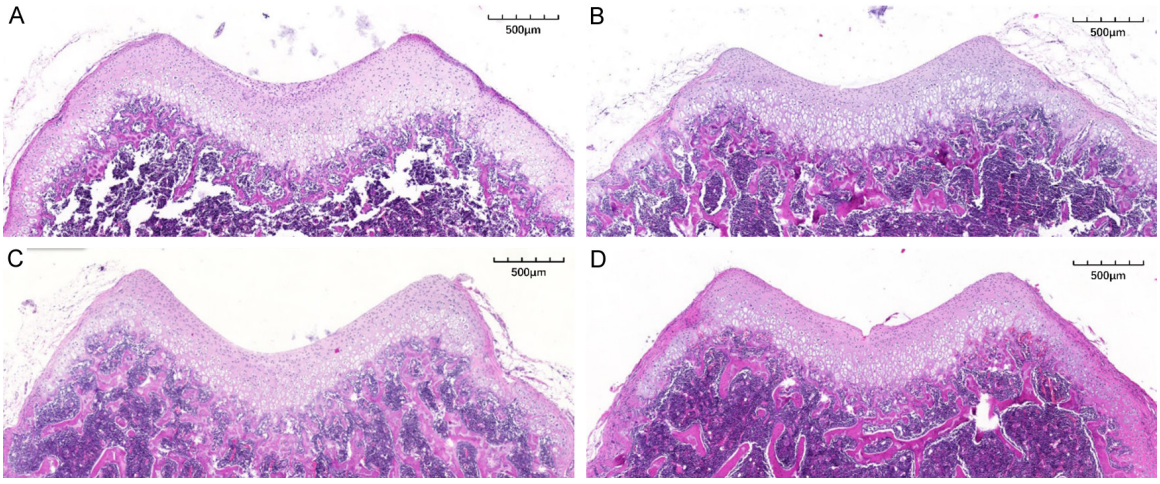

Figure 10. The HE staining of the femoral trochlear groove. (A) illustrates the femoral trochlea of the control group at 4 weeks, while (C) depicts the control group at 8 weeks. (B) represents the femoral trochlea of the experimental group at 4 weeks, and (D) shows the experimental group at 8 weeks. The magnification of the staining figures was 2×.

This study has some limitations. First, it was conducted using an animal model, which exhibits notable anatomical and physiological disparities from humans. Therefore, the results may not entirely reflect the clinical situation in humans. Second, the study utilized rats rather than larger mammalian models such as pigs or sheep, which are more similar to humans anatomically. Nonetheless, rats were chosen because of their strong fertility, lower cost, and shorter growth cycle, making it easier to observe hip and femoral trochlear development at different stages. Third, we could not completely eliminate other risk factors for hip and femoral trochlear dysplasia, such as individual differences among rats and variations in the tightness of fixation.

Conclusions

Our findings indicate that hip dislocation may not only lead to developmental dysplasia of the hip, but also impair the maturation of the femoral trochlea, with the pathological changes of both DDH and trochlear dysplasia becoming more pronounced over time.

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Disclosure of conflict of interest

None.

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