Original Research

In Vitro Ovine Cam Impingement Model and Its Effect on Acetabular Cartilage

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Background: Femoroacetabular impingement syndrome is a condition where abnormal contact occurs between the femoral head and the acetabulum, leading to chondral damage and hip osteoarthritis. To better understand and treat femoroacetabular impingement syndrome, it is crucial to establish in vitro models that mimic the condition and assess potential interventions.

Purpose: To establish an in vitro ovine cam impingement model and assess the effectiveness of cam excision in reducing the incidence of type 3 acetabular labrum articular disruption (ALAD) (chondral flap) lesions.

Study Design: Controlled laboratory study.

Methods: Utilizing an ovine in vitro cam impingement model, 40 hips were subjected to testing across 5 groups (n = 8 per group): group 1 (control group), 750 N for 200 cycles; group 2 (cycle decrease), 750 N for 100 cycles; group 3 (load decrease), 500 N for 200 cycles; group 4 (cam excision), cam excision followed by 750 N for 200 cycles; and group 5 (halfway cam excision), 750 N for 100 cycles followed by cam excision under an additional 750 N for 100 cycles loading. Each specimen was subsequently assessed for chondral damage according to the ALAD classification, both macroscopically and microscopically.

Results: The control group (group 1) demonstrated the highest ALAD scores (2.7 ± 0.4, 2.8 ± 0.3) compared with other groups, whereas the cam excision group (group 4) exhibited lower scores (0.5 ± 0.5, 0.7 ± 0.4) than both the cycle decrease group (group 2) (1.6 ± 0.5, 1.6 ± 0.5) and the halfway cam excision group (group 5) (1.8 ± 0.6, 2 ± 0.5) ($P < .05$) in both macroscopic and microscopic gradings ($P < .05$). The load decrease group (group 3) (1 ± 0.5) also displayed lower scores compared with group 5 (2 ± 0.5) at histological grading ($P < .05$).

Conclusion: An in vitro sheep model was established that reliably induces mechanical chondrolabral damage in the hip joint. The findings show that reducing the load results in less chondrolabral damage compared with reducing the number of cycles. Furthermore, this model emphasizes the protective effect of cam excision in the management of chondral flap lesions (ALAD type 3).

Keywords: cam; FAIS; ovine model; cartilage damage

Femoroacetabular impingement syndrome (FAIS) arises from premature contact between the femur and acetabulum, leading to painful hip motions. This condition typically manifests through 2 primary morphologies: cam morphology, characterized by convexity at the femoral head-neck junction, or pincer morphology, referring to overcoverage of the acetabulum.[5,7] While conservative and surgical treatment options are available, ongoing debate persists regarding the superiority of their relative efficacy, as shown by numerous review papers. Short-term studies suggest superior outcomes with surgery.[1,2,4,6,11,22] While it is widely accepted that conservative care should be the initial approach in treating FAIS,[14,15] a lack of consensus persists.[1] Conservative treatment modalities typically involve patient education and activity modification to prevent repetitive bony impingement resulting from deep hip flexion and internal rotation.[14,15] Recent findings from a meta-analysis have highlighted supervised active core strengthening as the most effective regimen compared with non–core focused or passive modalities.[8]

The sheep hip joint, characterized by a natural cam deformity, is a valuable in vivo model for cam impingement.[18] Siebenrock et al[18] successfully induced cam-type FAIS in an ovine in vivo model through proximal varus intertrochanteric osteotomy, resulting in macroscopic

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chondrolabral lesions similar to those observed in humans. Notably, they observed a correlation between the incidence of these lesions and the duration of ambulatory periods, evident at both macroscopic and microscopic levels.[18,23] This correlation was further supported by delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC) values, a biomarker indicative of arthritic cartilage degeneration, which exhibited similar trends to those demonstrated in human studies.[3,16,17] Subsequently, an experiment was conducted to confirm the safety of performing femoral head and neck osteoplasty on ovine femurs.[10] However, they did not investigate its potential effect on the frequency of typical chondrolabral lesions.

Only 1 in vitro human cadaveric study[19] has been identified; it reports a decrease in lower intra-articular hip contact pressure after complete cam resection compared with partial resection and the intact cam model. The literature defines a dGEMRIC method,[17] which is indirectly demonstrated by magnetic resonance imaging (MRI) findings. In vivo, this model[18] involves a varus-inducing osteotomy in sheep, and it is an invasive procedure. We aim to create an in vitro model that could serve as a similar role model with its ease of access and applicability in managing cam-type FAIS.

The present study aimed to establish an in vitro ovine cam impingement model and evaluate the effect of cam excision and other clinical scenarios on the incidence of type 3 acetabular labrum articular disruption (ALAD), characterized by chondral flap lesions.[9] The hypothesis proposed that it would be possible to induce in vitro ovine cam impingement, resulting in typical chondral lesions observed in cam impingement scenarios, achieved through consistent loading force applied over repetitive cycles during hip joint abduction. Furthermore, the study aimed to verify the effectiveness of cam excision in reducing the occurrence of these typical cartilage lesions. Last, it was hypothesized that reducing the load would be more effective than decreasing the number of cycles in preventing the occurrence of cartilage lesions.

METHODS

Specimen Description and Preparation

The study utilized 40 fresh-frozen sheep hip joints harvested from 18-month-old female sheep procured from a local abattoir within 48 hours postmortem. Since the skeletal maturity was completed, 18-month-old sheep were preferred. Female sheep were used in the study in order to provide female sheep more easily in our region and to ensure that all samples in the groups were of the same sex. After dissection of the muscular tissues around the hip, the capsule was completely excised by separating it from the femoral attachment site without damaging the labrum. The labrum, transverse acetabular ligament, and ligatmentum teres were preserved. Specimens underwent meticulous screening and were excluded if they exhibited macroscopic signs of degeneration, any evidence of trauma-related injury, or osteoarthritis. After harvesting, all specimens were promptly frozen at −20°C. Thawing of the specimens occurred at room temperature 24 hours before testing.

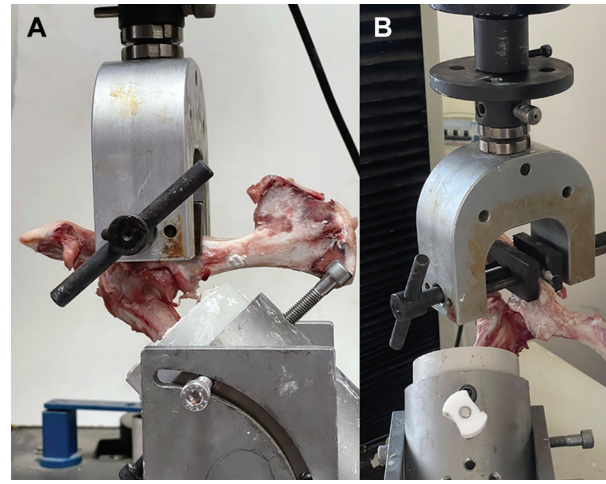

Figure 1. (A) Biomechanical setup from the sagittal view, hemipelvis parallel to the floor plane, with femur 135° hyperflexed using an adjustable actuator arm. (B) In the coronal view, the femur is 45° abducted in accordance to the hemipelvis.

Biomechanical Testing Protocol

Biomechanical testing was conducted by securing each femur at the shaft using a cemented pot and at the pelvis using Steinmann pins, with the long axis of the femur positioned at 135° to the actuator arm (AG-I 10 kN; Shimadzu) and at 45° to the pelvis (Figure 1).

It has been reported that impingement in sheep occurs in the posterior-superior acetabular region in deep

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Figure 2. Type 3 acetabular labrum articular disruption ''chondral flap'' lesion (white arrows).

flexion.[20] It has been reported that cam-type FAIS in sheep hips can be induced by hyperabduction surgically induced by medial closed-wedge varus-producing osteotomy in an in vivo study.[18] In this study, in which we aimed to create an in vitro experimental model, a pilot study was performed with a cadaveric sheep hip before determining the experimental groups to test the inducibility of cam-type FAIS and to determine the optimal abduction angle.

We elected to utilize an axial load in order to simplify the experiment and to concentrate on load and cycle. In the hip with excised muscle tissues, we gave a maximal hyperabduction of 45° in hyperflexion and found ALAD type 3 chondrolabral flaps in our control group. As a result of the pilot study, we determined that deep flexion of 135° and abduction of 45° allowed the experimental creation of an extra-articular impingement without the risk of subluxation. Mechanical impingement induced by hyperabduction achieved by varus-producing osteotomy in vivo studies was obtained by using 45° and 135° abduction under in vitro conditions in this study. After determining the experimental model, we tried to determine the load and cycle that would reliably produce chondrolabral damage. We applied 100 cycles of 100, 200, 250, 500, and 750 N load, respectively. However, we were unable to induce chondrolabral damage with this number of cycles. We then increased to 200 cycles with a same range of loads and observed ALAD type 3 (Figure 2) lesions using a 750 N load. Thus, the threshold value was determined to be 200 cycles under 750 N load. This threshold value was accepted as the control group. Five groups with 8 specimens were formed, and 40 hips were tested using the parameters determined for the specimens not used in the pilot study. Subsequently, the specimens were divided into groups:

Figure 3. Left: Cam deformity with an aspherical femoral head-neck junction. Right: Post-excision view following cam resection, demonstrating a restored femoral head-neck contour.

TABLE 1
Macroscopic and Microscopic Examination of Cadaveric Specimens for Chondral Damage With Reference to the ALAD Classification[a,9]

Grade	Description
0	Normal
1	Superficial lesions/softening
2	Early peel-back and fissures
3	Large flap or delamination
4	Complete loss of cartilage and exposed subchondral bone

[a]ALAD, acetabular labrum articular disruption.

- Group 1 (control group; n = 8): 750 N for 200 cycles
- Group 2 (cycle decrease; n = 8): 750 N for 100 cycles
- Group 3 (load decrease; n = 8): 500N for 200 cycles
- Group 4 (halfway cam excision; n = 8): cam excision (Figure 3) then 750 N for 200 cycles
- Group 5 (halfway cam excision; n = 8): 750 N for 100 cycles, then cam excision followed by an additional 750 N for 100 cycles

To ensure head and neck sphericity, the cam resections were excised by an orthopaedic surgeon (O.H.) and an assistant orthopaedic surgeon (S.A.) with experience in hip arthroscopy.

Histologic Analysis

After completing the testing, each specimen was graded according to the ALAD classification[9] (Table 1) for chondral damage, assessed both macroscopically and microscopically. Each tissue sample obtained from the specimens comprised the acetabular fossa, the articular

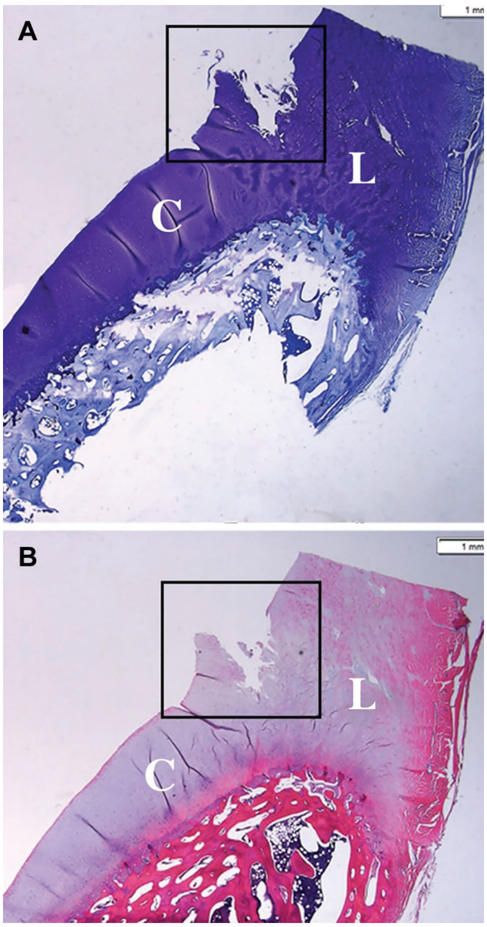

Figure 4. Histologic demonstration of ALAD type 3 lesion. (A) Constructed using toluidine blue. (B) Reproduced using hematoxylin and eosin. Cartilage (flat, left side) and labrum (joint margin, upper surface margin has been cut) are visible in both images. Boxed areas highlight the region of interest showing chondral flap formation. Scale bars represent 1 mm. *C*, cartilage; *L*, labrum.

cartilage with subchondral bone, and the acetabular rim with labrum. Fixation was achieved using 10% neutral buffered formaldehyde). After fixation, the tissues were washed in running tap water and decalcified with 10% ethylenediaminetetraacetic acid. After that, routine tissue processing was carried out, and the samples were embedded in paraffin. Transverse sections, with thicknesses of 10 μm, were obtained from the paraffin blocks. These sections were stained with hematoxylin and eosin and toluidine blue. Subsequently, they were examined under an Olympus BX53 microscope, and images were captured using an Olympus DP73 CCD color camera (Figure 4).

Statistical Analysis

Kruskal-Wallis test, a nonparametric variance analysis, was used to analyze the results statistically. When an overall group difference was identified, a multiple comparisons test was implemented to pinpoint the source of the variance among the groups. The threshold for statistical significance was set at $P < .05$. All statistical analyses were performed using SPSS software for Windows, standard Version 10.0.1 (SPSS).

RESULTS

Tables 2, 3, and 4 give the macroscopic and microscopic results, classified according to the ALAD system and their comparisons.

In the study, the control group (group 1) exhibited the highest ALAD score compared with the other groups. Furthermore, the cam excision group (group 4) showed lower scores than both the cycle decrease group (group 2) and the halfway cam excision group (group 5) at both macroscopic and microscopic gradings ($P < .05$). Additionally, the load decrease group (group 3) had a lower score than group 5 at histological grading ($P < .05$). Notably, group 3 tended toward a higher macroscore compared with group 4 and a lower microscore compared with group 2, although this trend did not reach statistical significance ($P = .09$).

DISCUSSION

In this study, we successfully developed an ovine hip model to reliably induce chondrolabral damage by simulating the effects of cam-type FAIS. Our findings showed that chondrolabral damage in this model was both load and cycle dependent. Importantly, femoroplasty significantly reduced chondrolabral damage in this model, confirming the protective effect of cam excision in reducing joint stresses.

Previous studies have reported on in vivo cam impingement models.[18,23] Specifically, a proximal varus intertrochanteric osteotomy was performed to create an additional 15° of varus, leading to cam impingement due to the naturally nonspherical femoral heads in sheep.[18] In contrast to inducing varus osteotomy, we tested the compression loading at higher degrees of abduction (45°) and successfully induced typical chondrolabral separation in most control specimens, both histologically and macroscopically.[18] Subsequent findings also indicated that the degree of lesions escalated with the increased ambulatory period of the animals. Furthermore, the present study confirmed that the mean macroscopic and microscopic scores were lower in the cycle decrease group (group 2) compared with the control group (group 1). However, the direct effects of cam excision or load reduction were not explicitly tested in these models.[10,16,18,23]

Beaulé et al[3] demonstrated that cam excision improves T1ρ MRI values, indirectly indicating a positive surgical impact on joint health. Similarly, Van Houcke et al[21] observed normalized peak joint stresses in the hip joint after precise cam resection. Schmaranzer et al[17] proposed using the dGEMRIC technique as a biomarker for monitoring early cartilage degeneration and identifying patients

TABLE 2
Macroscopic Grading of Cartilage Lesions[a]

Groups (n = 8)	ALAD Type 0	ALAD Type 1	ALAD Type 2	ALAD Type 3	Mean ± SD
1			2	6	2.7 ± 0.4
2		3	5		1.6 ± 0.5
3	1	4	3		1.2 ± 0.7
4	4	4			0.5 ± 0.5
5		2	5	1	1.8 ± 0.6

[a]ALAD, acetabular labrum articular disruption.

TABLE 3
Microscopic Grading of Cartilage Lesions[a]

Groups (n = 8)	ALAD Type 0	ALAD Type 1	ALAD Type 2	ALAD Type 3	Mean ± SD
1			1	7	2.8 ± 0.3
2		3	5		1.6 ± 0.5
3	1	6	1		1 ± 0.5
4	2	6			0.7 ± 0.4
5		1	6	1	2 ± 0.5

[a]ALAD, acetabular labrum articular disruption.

TABLE 4
Statistically Significant Comparisons

Macroscopic		Microscopic	
Groups	*P*	Groups	*P*
1 vs 2	.004	1 vs 2	.000
1 vs 3	.000	1 vs 3	.000
1 vs 4	.000	1 vs 4	.000
1 vs 5	.04	1 vs 5	.008
2 vs 4	.004	2 vs 4	.008
3 vs 5	.09[a]	4 vs 5	.000
4 vs 5	.000	3 vs 5	.002
		2 vs 3	.09[a]

[a]Indicates that the value is approaching significance.

who might benefit from FAIS surgery. Interestingly, they observed a paradoxical decrease in the dGEMRIC index 1 year after joint-preserving surgery for FAIS. A similar, though less pronounced, decrease was noted in symptomatic patients who did not undergo surgery, possibly due to the simulation of the inflammatory cascade triggered by the surgery.

The study also reported decreased dGEMRIC index in the nonsurgical treatment group. However, it did not specify the conservative treatment protocols used for these patients. Further human studies are necessary to better understand the impact of load reduction on cartilage health.

The only in vitro study to demonstrate the beneficial effect of cam excision was conducted by Suppauksorn et al.[19] They reported that complete cam resection resulted in significantly lower intra-articular hip contact pressures than incomplete cam resection and native cam morphology in a cadaveric hip model. In this study, 3-dimensional formatted radial sections were evaluated before and after the excision of the cam lesion, but this method has not been fully validated.[19,12] The effect of cam excision was analyzed with intra-articular pressure maps. Total excision (superior half + inferior half) and partial excision (superior half) were performed; the lowest pressure was observed after total excision, and the highest pressure was monitored before excision. Our study investigated the formation of the ALAD type 3 chondrolabral flap, a pathognomonic and preosteoarthritic lesion associated with cam lesions. We evaluated the effects of halfway excision and other clinical scenarios on the incidence of this flap formation in sheep with natural cam lesions. The present study demonstrated the beneficial effect of cam excision through 2 comparisons. First, cam excision at the beginning (group 4) exhibited lower scores compared with the majority of the other groups (groups 1, 2, and 5). It also approached statistical significance compared with the load decrease group (group 3) (P = .09). Second, neither comparison observed any significant difference between the cycle decrease group (group 2) and the halfway excision group (group 5). These findings are consistent with the results reported by Suppauksorn et al,[19] which indicate that cam excision more effectively reduces intra-articular pressures. In our study, a similar effect was observed after halfway cam excision, with lower scores obtained at both macro and micro levels. Furthermore, considering the positive impact of load reduction on biochemical outcomes, a more detailed examination of the mechanisms of these effects and the role of cam excision and load reduction on cartilage biology will help to better explain and interpret our study's findings.

While most studies concur that conservative care should be prioritized as the initial approach in treatment,[14,15] there remains a lack of consensus regarding the rehabilitation protocol.[1] Many protocols emphasize patient education and activity modification to prevent repetitive movements.[14,15] However, a recent meta-analysis advocated supervised active core strengthening as the most effective regimen.[8] Additionally, body mass index has been shown to affect the midterm results of surgically treated patients.[13] Consistent with these findings, the present study revealed that reducing load was more beneficial than decreasing the number of cycles in reducing cartilage injury, albeit only at the microscopic level. Therefore, it can indicate that weight loss and/or muscle strengthening should form the core components of conservative treatment rather than rest or activity modification alone.

The ovine model we have developed may provide a useful platform to further investigate the biomechanical effects of both conservative and surgical treatment options. In clinical practice, different conservative treatment protocols can be investigated. The relationship of various cam resection configurations (underresection, overresection, etc) with the incidence of chondrolabral damage can be investigated. Factors affecting chondrolabral flap size can also be investigated. In addition, the healing potential of the chondrolabral flap and its relationship with the development of osteoarthritis can be evaluated with in vivo studies.

LIMITATIONS

Several limitations were present in our study. First, an ovine hip model was utilized instead of human cadaveric hip joints, potentially limiting the direct applicability of our findings to humans. Although the in vivo version of this experiment had been validated previously, utilizing fresh-frozen specimens for an in vitro study meant that our results only represent a "time-zero" snapshot and do not account for changes that may occur over time. Additionally, the sample size of 8 specimens per group, while consistent with prior biomechanical studies, may have limited the statistical power of our research. Furthermore, macroscopic examinations were performed by a single observer, albeit approved by the senior author, and microscopic examinations were performed by a separate histology consultant blinded to the groups. However, the reliability of interobserver or intraobserver assessments was not investigated. We also note that the removal of the capsule and ligaments to perform cam excision may affect joint biomechanics, which may limit the generalizability of our findings to in vivo conditions.

CONCLUSION

An in vitro sheep model was established that reliably induces mechanical chondrolabral damage in the hip joint. Our findings show that reducing the load results in less chondrolabral damage compared with reducing the number of cycles. Furthermore, this model emphasizes the protective effect of cam excision in the management of chondral flap lesions (ALAD type 3).

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