INTRODUCTION: The long-term efficacy of osteochondral allografts (OCA) is thought to be due to the presence of viable chondrocytes within graft cartilage. OCA are routinely used after storage at 4°C for prolonged durations (10-43d) to accommodate regulatory screening for infectious diseases. Chondrocytes, particularly in the superficial zone of cartilage, succumb with increasing storage duration, and decreased cellularity within graft cartilage. However, the long-term in vivo performance of such stored allografts, in association with variable cellularity, is unclear. The objectives of the study were to determine the effects of allograft storage (FROZEN, FRESH, 4°C/14d, 4°C/28d) on the (i) macroscopic structure, (ii) cellular and matrix composition, and (iii) biomechanical function of cartilage within OCA retrieved at 12 months in a goat model, and (iv) to assess the associations between cartilage function and cell and matrix components.

METHODS: Studies were IACUC approved. Donor Storage. Donor OCA were prepared from both knees of adult Boer goats (n=8, 2-4yo). Each knee was divided into medial femoral condyle (MFC) and trochlea fragments, and each fragment was stored either FROZEN (~70°C), or at 4°C in tissue culture medium (Dulbecco’s modified Eagle medium with 10% fetal bovine serum) for 3d (FRESH), 14d (4°C/14d), or 28d (4°C/28d). Allograft Surgery. Adult Boer goats (n=15, 3yo) were OPerated in one knee, with two site-matched OCA (d=8mm, h=5mm) of different storage conditions, implanted into alternating MFC and lateral trochlea (LT) sites. Contralateral knees were Non-OPerated controls. Each knee received (Exp. 1, n=7) FROZEN and FRESH or (Exp. 2, n=8) 4°C/14d and 4°C/28d stored OCA. At 12 months, animals were euthanized and both knees analyzed. Repair Site Analysis. Intact knee joints were received on wet ice within 24h of sacrifice. Distal femurs were harvested, photographed, and examined grossly for surface structure (smooth, slightly uneven, irregular in the central and edge regions). Cartilage load-bearing function was assessed by indentation testing at the center of each repair site; material stiffness was determined as the peak load divided by indentation depth, normalized to cartilage testing at the center of each repair site; material stiffness was determined as the peak load divided by indentation depth, normalized to cartilage thickness and indenter tip area. Next, OC slabs (d=15mm, h=8-10mm, w=2mm), cut centrally from proximal to distal, were isolated and analyzed. Matrix content in cartilage was assessed by HEXabrix-Enhanced micro-computed tomography at (18mM), and Safranin-O histology; HEX [%] was calculated within a 2mm region of segmented cartilage surrounding the site of indentation. HEX, an ionic contrast agent, distributes inversely to the fixed charge density in soft tissue, and is therefore a sensitive (inverse) indicator of proteoglycan content. Cellularity was assessed with depth from the articular surface (Superficial=top 10%, Middle=next 35%, Deep=remaining 50%) from images of fluorescently labeled cell nuclei, normalized to cartilage area. Statistics. Data are expressed as mean ± SEM. Effect of allograft storage and site were assessed by 2-way ANOVA & Tukey post-hoc tests. Linear regression was used to analyze the relationships between cellularity and stiffness or HEX. ~p<0.05, **p<0.01, ***p<0.001.

RESULTS: Exp. 1 Frozen vs. Fresh. FRESH OCA maintained cartilage load-bearing function, while also maintaining cartilage cellularity, compared to FROZEN OCA. The cartilage surface (Figure 1A,B) of FRESH OCA was smooth (5/7 allografts) or slightly uneven (2/7), whereas that of FROZEN OCA was visually deteriorated (7/7). Cellularity was ~95-99% lower in FROZEN than Non-OP and FRESH OCA (each, p<0.01, Figure 2A,B). Stiffness in Non-OP was similar to FRESH (p<0.4). Stiffness was lower in FROZEN by ~70-80% than Non-OP and FRESH at MFC (each, p<0.01, Figure 2C), and by ~93% than FRESH (p<0.05) at LT (Figure 2D). Matrix content at the MFC was similar between Non-OP and FRESH (p>0.9), and tended to be lower for FROZEN vs. Non-OP (~30% increase in HEX, p=0.1, Figure 1E,F). Matrix content at the LT was lower for FROZEN vs. Non-OP and FRESH (~80% increase in HEX, p<0.001, Figure 1E,F).

Exp. 2 4°C/14d vs. 4°C/28d. Both 4°C/14d and 4°C/28d stored OCA displayed cartilage surfaces (Figure 1CDGH) that were relatively smooth at the graft center (13/16) and irregular at the edges (15/16). Cellularity varied with storage and site (each, p<0.05), and tended to be reduced in 4°C stored allografts vs. Non-OP by ~20-35%, p<0.05-0.2, Figure 3A,B). Stiffness varied with storage (p=0.01) and site (p<0.001), with decreased stiffness (~25-45%, p<0.05 and 0.18-0.30, Figure 3C,D). Matrix content was not detectably altered (Figure 3E,F).

Correlations. Cartilage stiffness correlated with cartilage cellularity (p<0.001, Figure 4A), as did matrix content, taken as the inverse of %HEX (p<0.01, Figure 4B).

DISCUSSION: The reduced cartilage load-bearing function in FROZEN and 4°C/28d OCA, in association with cellularity within the OCA cartilage suggests that reduced chondrocyte cellularity as a result of allograft storage is related to in vivo performance.

SIGNIFICANCE: High chondrocyte density in OCA in vivo is important for maintaining long-term cartilage function and matrix content. Chondrocyte viability may be a useful predictor or marker of biological performance.