What age is an appropriate age to study spontaneous age-related OA in mice?

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Response/Recommendation: The age to study spontaneous OA is dependent on the strain of mice and the stage of OA being studied. For male C57BL/6 mice, 12 months of age for early OA and 18-24 months for mild to moderate OA should be considered. For female mice, minimal OA changes are expected even at advanced ages, unless menopause is induced. Given the variability of spontaneous age-related OA in mice that is sex and strain dependent, a power analysis should be performed to ensure the appropriate number of mice per experimental group are included for a given sex and strain.

Strength of evidence: Moderate

Rationale: Mice are used to study mechanisms contributing to human OA and to test potential therapeutics. For the results obtained in mice to better translate to understanding and treating human OA, it is critical to consider the age at which humans develop OA and how that age relates to the age of the mice being studied. Because knee OA is responsible for a large proportion of pain and disability in humans[1] and because the knee (stifle joint) has been the most studied joint in mice, this discussion will focus on knee OA. The prevalence of symptomatic knee OA in humans is low in adults under the age of 40 who have not had a prior history of joint injury[2], and begins to rise more rapidly around age 50-55[3]. A 50-55 year-old human is approximately equivalent to a 12 month-old mouse[4] and so 12 months of age is a reasonable age to consider studying the early stages of naturally occurring OA. However, the prevalence of spontaneous histologic OA in mice varies depending on the sex and strain and individual variability can be seen within a group of mice, even when matched for age, sex, and strain.

In most humans with knee OA, the disease develops slowly over time, and so mouse strains such as the STR/Ort mice[5] or the SAMP8 mice[6] that develop OA at a much younger age than other strains, typically by 6 months of age, may not be the best model for human OA. A systematic review was performed by Iijima et al in 2021 and identified 41 studies on mouse knee OA aging. This review was used to address the question posed in the present manuscript along with a follow-up PubMed search conducted to examine papers published between the 2021 publication and December 2025 that identified 7 additional studies (for details see response to question #14). C57BL/6 mice were found to be the most commonly studied strain[7]. Iijima et al compared the prevalence of OA in young (median age 5 months), middle-aged (median age 12 months), and aged (median age 22 months) mice and noted an age-related increase in cartilage lesions associated with changes in inflammation, autophagy, and cell senescence. A separate study examining similar ages in male and female C57BL/6 mice, found a similar age-related increase in cartilage lesions measured using the Osteoarthritis Research Society International (OARSI) score which were more severe in male than female mice[8]. Proteomic profiling further indicated that middle-aged (12-month-old) mice already exhibit shifts in protein expression, including alterations in PI3K/Akt signaling, a pathway well-characterized in human OA pathology.

An age-related increase in OARSI scores, accompanied by a reduction in walking speed, was noted in 12 month old C57BL/6JRj mice when compared to younger mice although mice older than 12 months were not included[9]. In a study on the effects of Sirt6 loss that examined naturally occurring OA at 6, 12, and 18 months of age in male mice on a C57BL/6Jx129SxFVB/NJ background, the wild type mice had an age-related increase in subchondral sclerosis measured using micro-CT{Collins, 2023 #9332}. A study examining age-related changes in synovial lymphatic function noted a decline at 19 months of age in male C57BL/6J mice compared to 3-month-old mice and this was also associated with worse OARSI scores[10]. In a study by Geraghty et al[11], male and female C57BL/6J mice at 6 months of age (approximately a 30-40 year old human) did not have histologic evidence of OA while at 20 months (60-70 year old human) cartilage lesions were seen, although they were quite variable and more severe in males, when compared to female mice. The relative protection from age-related OA in female mice has recently been shown to be related to 17β-estradiol and progesterone levels using a model of chemically induced menopause initiated at 14-16 months of age in female C57/BL6N mice[12]. Control and menopausal mice were evaluated at 5 time points starting at 25 days after menopause initiation and finishing at 195 days at which the mice were 21-22 months old providing for a longitudinal assessment of OA development. Over that time period, the OARSI scores for cartilage lesions remained relatively stable in the control mice but increased in the menopausal mice as did synovial hyperplasia.

Few studies have examined pain behavior in mice developing spontaneous age-related OA. Geraghty et al[11] noted mechanical allodynia, knee hyperalgesia, and reduced grip strength in 20-month-old male and female mice. More severe pain, relative to the histological changes of OA, was observed in the older female mice. In a study of male 21-22-month-old C57BL/6J mice, treatment with the senolytic drugs ABT263 or Dasatinib plus

Quercetin (D+Q) for 5 or 8 weeks, respectively, reduced the numbers of p16 positive senescent cells in the cartilage and synovium[13]. The reduction in senescent cells was not associated with a reduction in histologic features of OA with either senolytic. ABT263 did not change mechanical hypersensitivity measured using the Von Frey test but did decrease thermal hyperalgesia and improved grip strength as measures of pain behavior. D+Q improved mechanical hypersensitivity and grip strength but not thermal hypersensitivity. These studies in older mice illustrate the disconnect between structure and pain which has also been seen in human studies.

A study that examined the cell senescence marker $p16^{Ink4a}$ in C57BL/6 mice noted an increase at 10-12 months of age compared to mice at 4 months of age which increased further at 18 months and was similar at 22-27 months[14]. Spontaneous OA in control C57BL/6J×129/SvJae mice at 12 and 22 months of age that were compared to MIF knockouts on the same background, revealed significant cartilage lesions and osteophytes in the controls but not the MIF knockouts at both ages but as with other studies there was significant variability within the group of control mice[15]. Even more mouse-to-mouse variability was noted in cartilage lesions, osteophytes and synovial hyperplasia, as well as cartilage and subchondral bone histomorphometric measures, with aging out to 35 months in male HET-3 mice which are a four-way cross using BALB/cByJ × C57BL/6JF1 mothers and C3H/HeJ × DBA/2JF1 fathers[16]. In that study a power calculation using a 50% effect size for an intervention on the articular cartilage structure (ACS) score and Safranin (Saf) O scores with an 80% power and an alpha of 5% revealed that 57 mice per group would be required for ACS scoring and 43 mice per group for Saf O scoring. A second study of 22–25-month-old male and female HET-3 mice also noted OA changes in cartilage, subchondral bone and the synovium in both sexes which on average was more severe in the male than female mice[17].

Conclusion: Based on the current literature, studies of spontaneous OA in C57BL/6 mice and related strains should examine at least two time points and include both male and female mice. The first time point where early disease would be expected in male mice that is relevant to human OA is 12 months of age. A second time point of 18-24 months where mild OA changes may be apparent in female mice as well as moderate OA changes in males should be included. More severe OA can be induced in female mice by inducing menopause chemically. In studies where only a single time point is feasible, then 18-24 months of age is a reasonable choice.

References:

1. DALYs GBD, Collaborators H. 2016. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388:1603-1658.
2. Whittaker JL, Toomey CM, Woodhouse LJ, et al. 2017. Association between MRI-defined osteoarthritis, pain, function and strength 3-10 years following knee joint injury in youth sport. Br J Sports Med.
3. Losina E, Weinstein AM, Reichmann WM, et al. 2013. Lifetime risk and age at diagnosis of symptomatic knee osteoarthritis in the US. Arthritis care & research 65:703-711.
4. Flurkey K CJ, Harrison DE. 2007. Mouse models in aging research. In: Fox JG BS, Davisson MT, Newcomer CE, Quimby FW, Smith AL editor. The-Mouse-in-Biomedical-Research Normative Biology, Husbandry, and Models, 2nd ed. Amsterdam, Boston: Elsevier; pp. 637-672.
5. Staines KA, Poulet B, Wentworth DN, et al. 2017. The STR/ort mouse model of spontaneous osteoarthritis - an update. Osteoarthritis Cartilage 25:802-808.
6. Sanada Y, Ikuta Y, Ding C, et al. 2022. Senescence-accelerated mice prone 8 (SAMP8) in male as a spontaneous osteoarthritis model. Arthritis Research & Therapy 24.
7. Iijima H, Gilmer G, Wang K, et al. 2022. Meta-analysis Integrated With Multi-omics Data Analysis to Elucidate Pathogenic Mechanisms of Age-Related Knee Osteoarthritis in Mice. J Gerontol A Biol Sci Med Sci 77:1321-1334.
8. Iijima H, Gilmer G, Wang K, et al. 2023. Age-related matrix stiffening epigenetically regulates α-Klotho expression and compromises chondrocyte integrity. Nature communications 14.

9. Akoum J, Tahiri K, Corvol MT, et al. 2020. Aging Cartilage in Wild-Type Mice: An Observational Study. Cartilage:1947603520926713.
10. Lin X, Bell RD, Catheline SE, et al. 2023. Targeting Synovial Lymphatic Function as a Novel Therapeutic Intervention for Age-Related Osteoarthritis in Mice. Arthritis & rheumatology 75:923-936.
11. Geraghty T, Obeidat AM, Ishihara S, et al. 2023. Age-Associated Changes in Knee Osteoarthritis, Pain-Related Behaviors, and Dorsal Root Ganglia Immunophenotyping of Male and Female Mice. Arthritis & rheumatology 75:1770-1780.
12. Gilmer G, Iijima H, Hettinger ZR, et al. 2025. Menopause-induced 17β-estradiol and progesterone loss increases senescence markers, matrix disassembly and degeneration in mouse cartilage. Nat Aging.
13. Gil TH, Zheng H, Lee HG, et al. 2022. Senolytic drugs relieve pain by reducing peripheral nociceptive signaling without modifying joint tissue damage in spontaneous osteoarthritis. Aging 14:6006-6027.
14. Diekman BO, Sessions GA, Collins JA, et al. 2018. Expression of p16(INK)(4a) is a biomarker of chondrocyte aging but does not cause osteoarthritis. Aging cell:e12771.
15. Rowe MA, Harper LR, McNulty MA, et al. 2017. Deletion of macrophage migration inhibitory factor reduces severity of osteoarthritis in aged mice. Arthritis & rheumatology 69:352-361.
16. Ewart D, Harper L, Gravely A, et al. 2020. Naturally occurring osteoarthritis in male mice with an extended lifespan. Connect Tissue Res 61:95-103.
17. Poudel SB, Ruff RR, Yildirim G, et al. 2024. Development of primary osteoarthritis during aging in genetically diverse UM-HET3 mice. Arthritis Research & Therapy 26.