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Literature Review

Exploring bovine three-dimensional chondrocyte culture models in osteoarthritis research: A systematic review

Explorando los modelos tridimensionales de cultivo de condrocitos bovinos en la investigación de la osteoartritis: Una revisión sistemática

Explorando modelos de cultura de condrócitos tridimensionais bovinos na pesquisa em osteoartrite: Uma revisão sistemática

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Abstract

Background: The use of different animal species for chondrocyte culture has been employed to investigate diseases affecting cartilage, including osteoarthritis. Bovine cartilage and chondrocytes can be used to establish three-dimensional cell cultures, which offer a more reliable *in vitro* model compared to conventional monolayer cultures. However, bovine chondrocytes in three-dimensional cultures have not been widely implemented, resulting in the loss of a potential source of mammal tissue that could prove valuable for preclinical studies on osteoarthritis. **Objective:** The objective of this study was to conduct a comprehensive review of the existing scientific literature employing three-dimensional cultures of bovine cartilage to investigate osteoarthritis. **Methods:** A systematic search was performed using the electronic databases PubMed and Scopus to identify studies utilizing 3D cell culture for osteoarthritis. Search terms included: ´3D culture', '3D cell culture', 'bovine cartilage' and 'chondrocyte'. A total of 59 articles were retrieved, and after screening, 12 articles were included in the final analysis. Risk-of-bias assessment was conducted by categorizing each study as having a 'low,' 'medium,' or 'high' risk of bias. **Results:** Analysis of the articles included in this review highlighted high variability in harvest sites, including carpal, metacarpal, and knee joints, as well as variability in culture methods utilizing cell passages ranging from passage zero to passage nine. Moreover, medium and high risks of bias were detected in all the articles probably due to challenges in randomization and blinding of the studies. In summary, this review critically examines three-dimensional cell culture for the investigation of cartilage disorders, with a particular emphasis on bovine cartilage. **Conclusions:** Future studies using chondrocyte culture in 3D or tissue-engineered constructs, should include consistent methods across the *in vitro* phase of the study. Factors such as chondrocyte harvest site, donor age, and passage number can significantly impact

biological characteristics and cartilage regeneration potential. Therefore, it is suggested that comparisons between relevant translational models should include age-matched conditions to avoid further confounding factors.

Keywords: *cartilage; cell culture; hydrogel; musculoskeletal tissues; osteoarthritis; risk of bias; tissue harvest; tissue procurement.*

Resumen

Antecedentes: El uso de diferentes especies animales para el cultivo de condrocitos se ha empleado para investigar las enfermedades que afectan al cartílago, incluida la osteoartritis. El cartílago bovino y los condrocitos se pueden utilizar para establecer cultivos celulares tridimensionales, que ofrecen un modelo *in vitro* más fiable en comparación con los cultivos monocapa convencionales. Sin embargo, los condrocitos bovinos en cultivos tridimensionales no se han implementado ampliamente, lo que ha llevado a la pérdida de una fuente potencial de tejido de mamíferos que podría ser útil para estudios preclínicos sobre la osteoartritis. **Objetivo:** El objetivo del presente artículo fue realizar una revisión exhaustiva de la literatura científica existente que emplea cultivos tridimensionales de cartílago bovino para investigar la osteoartritis. **Métodos:** Se realizó una búsqueda sistemática utilizando las bases de datos electrónicas PubMed y Scopus, para identificar estudios clínicos que emplearan cultivo celular 3D para la artrosis. Los términos de búsqueda incluyeron: ´3D culture', '3D cell culture', 'bovine cartilage' y 'chondrocyte'. Se recopilaron un total de 59 artículos y, tras la selección, se incluyeron 12 artículos en el análisis final. La evaluación del riesgo de sesgo se llevó a cabo categorizando cada uno de los estudios como riesgo "bajo", "medio" o "alto". **Resultados:** Se encontró que en los artículos incluidos en esta revisión existía una alta variabilidad en los sitios de aislamiento, que incluían las articulaciones del carpo, del metacarpo y de la rodilla, así como una alta variabilidad en los métodos de cultivo, empleando pasajes celulares que iban desde el pasaje cero hasta el pasaje nueve. Además, se detectó un riesgo medio y alto de sesgo en todos los artículos, probablemente debido a las dificultades en la aleatorización y el cegamiento de los estudios. En resumen, esta revisión examina críticamente el cultivo celular tridimensional para la investigación de trastornos del cartílago, con un énfasis particular en el cartílago bovino. **Conclusiones:** Los estudios futuros que utilicen el cultivo de condrocitos en 3D o construcciones de ingeniería de tejidos deben incluir métodos coherentes en toda la fase *in vitro* del estudio. Factores como el lugar de recolección de condrocitos, la edad del donante y el número de deposiciones pueden afectar significativamente las características biológicas y el potencial de regeneración del cartílago. Por lo tanto, se sugiere que la comparación de los modelos traslacionales relevantes debe incluya condiciones ajustadas a la edad para evitar factores de confusión adicionales.

Palabras clave: *cartílago; cultivo celular; cultivo de tejido; hidrogel; obtención de tejido: osteoartritis; riesgo de sesgo; tejidos musculoesqueléticos.*

Resumo

Antecedentes: O uso de diferentes espécies animais para a cultura de condrócitos tem sido empregado para pesquisar doenças que afetam a cartilagem, incluindo osteoartrite. Cartilagem bovina e condrócitos podem ser usados para estabelecer culturas de células tridimensionais, que oferecem um modelo *in vitro* mais confiável em comparação com culturas convencionais de monocamadas. No entanto, condrócitos bovinos em culturas tridimensionais não foram amplamente implementados, resultando na ausência de uma fonte potencial de tecido de mamíferos, que poderia ser útil para estudos pré-clínicos de osteoartrite. **Objetivo:** Consequentemente, nosso objetivo foi realizar uma revisão abrangente da literatura científica existente que emprega culturas tridimensionais de cartilagem bovina para investigar osteoartrite. **Métodos:** Foi realizada uma busca sistemática, utilizando as bases de dados eletrônicas PubMed e Scopus, para identificar estudos clínicos que utilizam cultura de células 3D para osteoartrite. Os termos de pesquisa incluíram: ´3D culture', '3D cell culture', 'bovine cartilage' e 'chondrocyte'. Foram identificados 59 artigos e, após seleção, 12 artigos foram incluídos na análise final. A avaliação do risco de viés foi realizada classificando-se cada um dos estudos em "baixo", "médio" ou "alto" risco de viés. **Resultados:** Verificamos que nos artigos incluídos nesta revisão houve grande variabilidade nos sítios de isolamento, incluindo as articulações do carpo, metacarpo e joelho, bem como grande variação nos métodos de cultura, com passagens celulares que variam da passagem zero à passagem nove. Além disso, detectamos risco médio e alto de viés em todos os artigos,

provavelmente devido a dificuldades de randomização e cegamento dos estudos. Em resumo, esta revisão examina criticamente a cultura de células tridimensionais para a pesquisa de distúrbios da cartilagem, com ênfase particular na cartilagem bovina. **Conclusões:** Estudos futuros que utilizem cultura de condrócitos em construções 3D ou de engenharia de tecidos devem incluir métodos consistentes em toda a fase *in vitro* do estudo. Fatores como lugar de coleta dos condrócitos, a idade do doador e o número de passagens podem afetar significativamente as características biológicas e o potencial de regeneração da cartilagem. Portanto, sugere-se que a comparação de modelos translacionais relevantes considere condições pareadas por idade para evitar variáveis de confusão adicionais.

Palavras-chave: *cartilagem; colheita de tecidos; cultura celular; hidrogel; obtenção de tecidos; osteoartrite; risco de viés; tecidos musculoesqueléticos.*

Introduction

Musculoskeletal disorders rank highly as one of the most prevalent causes of physical disabilities worldwide (Li et al., 2021). Approximately 1.71 billion individuals suffer from conditions associated with the musculoskeletal system, encompassing ailments such as arthritis (including osteoarthritis, rheumatoid arthritis, and psoriatic arthritis), gout, and osteopenia (WHO, 2022). Osteoarthritis (OA) is characterized by cartilage degradation caused by dysregulated anabolic and catabolic responses affecting normal chondrocyte biological cues.

Traditionally, monolayer (2D) chondrocyte culture has been used *in vitro* to study cellular and pharmacological interactions with candidate molecules. However, 2D culture models exhibit a limited representation of the in vivo environment mainly due to inadequate cell-cell and cell-extracellular matrix interactions, which are crucial for maintaining the chondrocyte phenotype (Fiederlein and Evans, 2020). Three-dimensional (3D) culture provides a better model of the *in vivo* milieu compared to 2D culture, allowing for a deeper understanding of OA progression.

Chondrocytes and other cell types can be cultured in 3D to mimic the *In Vivo* environment while maintaining phenotypic characteristics closely related to the native tissue. Nonetheless, the substantial array of alternatives for modeling OA involves different cell sources obtained from distinct animal species, mainly mammals. Common cell sources include those obtained from common laboratory animal species such as rodents and rabbits, which, although important for orthopedic research, may have significant biological and morphological limitations (Meng et al., 2020; Cardona-Ramirez et al., 2022). Additionally, tissues obtained from larger animals such as dogs, and sheep have also been used, revealing important morphological similarities to the human species (Mancuso et al., 2010; Oh et al., 2021; Soontararak et al., 2022). However, due to various ethical and cultural concerns, the aforementioned species may not be widely available for investigators interested in cartilage diseases (Liguori et al., 2017; Swatland, 2010).

Conversely, the bovine meat and milk industry has led to a wide availability of products for human consumption. Moreover, slaughterhouses also process a considerable quantity of tissues that may be of interest to academia and the scientific industry, emerging as a potential source of tissues and organs to study diverse musculoskeletal diseases including the potential effect of orthobiologics and cartilage preservation strategies (Camacho and Mardones, 2021; Solanki et al., 2021). Additionally, bovines represents an attractive model to study OA due to their similarity in cartilage thickness and anatomy (Bascuñán et al., 2019).

Therefore, the objective of this paper was to systematically analyze the most recent publications using bovine chondrocytes as a source of cellular material for OA studies.

Materials and Methods

Search strategy

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Page et al., 2021). The computer-assisted literature search was performed using PubMed and Scopus electronic databases to identify clinical studies using 3D cell culture for osteoarthritis. The following search terms and Boolean operators were used: ´3D culture', '3D cell culture', 'bovine cartilage', and 'chondrocyte' (Table 1). Databases were exported to bibliographic manager software files (.RIS) containing all relevant information such as author name, year of publication, title, keywords, and abstract. Bibliographic files were then imported into R Studio (R version 4.1.2), to consolidate information into a single database. Duplicated references were removed using the package ´*litsearchr*´ (Grames et al., 2019). The remaining articles were screened by two authors (M.R.J and M.P.C.) and independently reviewed, first by title for relevance and then by the Materials and methods section to include only articles that used bovine chondrocytes for 3D culture.

Table 1. Search terms and boolean operators used for the inclusion of articles.

Electronic database	Query	Results
PubMed	"3D culture" OR "3D cell culture" AND bovine cartilage OR chondrocyte	20
Scopus	"3D culture" OR "3D cell culture" AND bovine cartilage OR chondrocyte	39

Study risk of bias assessment

The risk of bias assessment was conducted using the Quality Assessment Tool For *in vitro* Studies (QUIN) (Sheth et al., 2024). Briefly, the quality assessment used a predefined set of bias domains. The final assessment involved categorizing each study feature as having a 'low,' 'medium,' or 'high' risk of bias. Two researchers (M.P.C. and M.R.J) independently conducted the assessment.

Results

Study selection and characteristics

A total of 59 studies were identified; 17 records were removed due to duplication, and 24 were removed because their title suggested that the information was not relevant for the analysis. During the screening process, three records were excluded because chondrocytes were not obtained from bovine tissues, and one record was not accessible for retrieval. Two records were excluded because the methodology did not include cell culture details. Lastly, a total of 12 articles were included in the analysis (Figure 1).

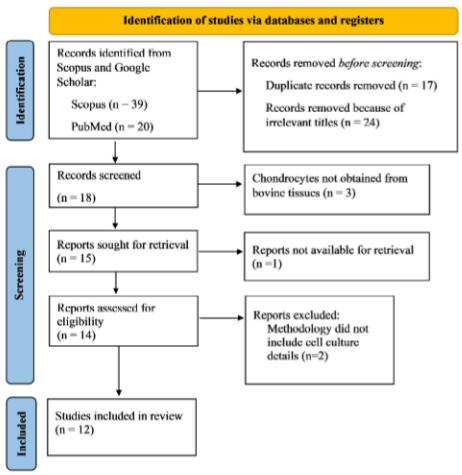

Figure 1. Identification and study selection according to the PRISMA guidelines.

Cartilage harvest and chondrocyte culture

Chondrocyte sources varied significantly among studies. While some studies used adult chondrocytes, others utilized chondrocytes obtained from skeletally immature animals ranging from nine-week-old calves to twelve-old month steers (Ahmed et al., 2014; Çelik et al., 2016; Li et al., 2016; Lee et al., 2019; Antunes et al., 2020; Gawri et al., 2022).

Additionally, the site of harvest was not consistently described at the time of procurement. While the most common site of harvest was the carpal-metacarpal joint, other authors used the stifle (knee) and the fetlock joint (Lee et al., 2017; Lee et al., 2019; Antunes et al., 2020). Furthermore, there was also variation in the chondrocyte passage used for the experiments. Although most authors used a variety of passages, ranging from P0 to P4, some studies included chondrocyte passages ranging from P4 to P9 (Pizzolatti et al., 2018). Furthermore, many studies did not specify what passages were used for the experiments (Table 2).

Table 2. Chondrocyte culture characteristics.

Reference	Chondrocyte culture	Passage
Heywood *et al.* (2022)	Adult metacarpophalangeal cartilage	P0-P4
Gawri *et al.* (2022)	Metacarpal-carpal cartilage from 9 to 12-month-old steers.	N. A
Antunes *et al.* (2020)	Full-thickness fetlock joint cartilage of 4-8-month-old calves.	N. A
Müller *et al.* (2020)	Chondrocytes isolated from the metacarpal joint of 1-2-year-old cattle.	P1
Pizzolatti *et al.* (2018)	Bovine carpal joints	P4-P9
Li *et al.* (2016)	Chondrocytes were isolated from articular cartilage from the knees of a nine-week-old calf.	P2
Çelik *et al.* (2016)	Cartilage isolated from the knee joint of young calves.	N.A.
Mellor *et al.* (2014)	Hooves from 18–24-month-old steers using the metacarpophalangeal joints.	P2
Farnsworth *et al.* (2014)	Metacarpophalangeal joints of 2–3-year-old steers.	N. A
Ahmed *et al.* (2014)	Cartilage harvested from bovine metacarpo-phalangeal joints (6–9 months old).	P2
Lee *et al.* (2017)	Cartilage harvested from the patellofemoral groove of a bovine leg.	N. A

Passage number; N.A: Not available

Risk of bias assessment

The Quality Assessment Tool For *in Vitro* Studies (QUIN Tool) analyzed 12 criteria to grade the *in vitro* studies as high, medium, or low risk depending on the summed scores (Sheth et al., 2024). The first criterion, which consisted of the clarity of the objectives, was adequately specified in all the articles (Table 3). However, all the other categories showed different degrees of bias. Six studies included in the analysis exhibited a high risk of bias according to the QUIN tool assessment (Çelik et al., 2016; Li et al., 2016; Pizzolatti et al., 2018; Lee et al., 2019; Antunes et al., 2020; Heywood et al., 2022). The criteria most prone to bias were the operator details, randomization, outcome assessor details, and blinding. Moreover, sample size was not adequately described in seven articles (Farnsworth et al., 2014; Li et al., 2016; Lee et al., 2019; Antunes et al., 2020; Müller et al., 2020; Gawri et al., 2022; Heywood et al., 2022) and only one study included a detailed explanation of sample size calculation (Lee et al., 2017). Conversely, seven articles provided a detailed description of the comparison groups (Ahmed et al., 2014; Farnsworth et al., 2014; Mellor et al., 2014; Çelik et al., 2016; Lee et al., 2017; Gawri et al., 2022; Müller et al., 2020), six papers included a detailed description of the methodology (Ahmed et al., 2014; Farnsworth et al., 2014; Mellor et al., 2014; Li et al., 2016; Lee et al., 2017; Gawri et al., 2022), and only three articles provided a clear description of the sampling technique (Ahmed et al., 2014; Mellor et al., 2014; Gawri et al., 2022).

Table 3. Risk of bias assessment using QUIN tool (Sheth et al., 2024).

Ref.	Aims	Sample size	Sampling technique	Comp. group	Methods	Operator details	Rand.	Outcome measure	Outcome assessor	Blind	Statist	Result	Total score	Final score %	Risk of bias
Heywood *et al.* (2022)	2	1	1	1	1	0	0	1	0	0	2	2	11	45.8	High
Gawri *et al.* (2022)	2	1	2	2	2	0	0	1	0	0	2	2	14	58.3	Medium
Antunes *et al.* (2020)	2	1	0	1	1	0	0	2	0	0	1	1	9	37.5	High
Lee *et al.* (2019)	2	1	0	1	1	0	0	2	0	0	2	2	11	45.8	High
Müller *et al.* (2020)	2	1	1	2	1	0	1	2	0	1	1	1	13	54.2	Medium
Pizzolatti *et al.* (2018)	2	0	0	1	1	0	0	1	0	0	1	2	8	33.3	High
Li *et al.* (2016)	2	1	0	0	2	0	0	2	0	0	1	2	10	41.7	High
Çelik *et al.* (2016)	2	0	0	2	1	0	0	1	0	0	2	1	9	37.5	High
Mellor *et al.* (2014)	2	0	2	2	2	0	0	1	2	0	1	2	14	58.3	Medium
Farnsworth *et al.* (2014)	2	1	1	2	2	0	1	2	0	0	2	2	15	62.5	Medium
Ahmed *et al.* (2014)	2	0	2	2	2	0	0	2	0	0	2	2	14	58.3	Medium
Lee *et al.* (2017)	2	2	0	2	2	0	0	2	0	0	2	2	14	58.3	Medium

0 = Not Specified; **1** = Inadequately specified; **2** = Adequately specified
Aims: Clearly stated aims/objectives; **Sample size:** Detailed explanation of sample size calculation; **Sampling technique:** Detailed explanation of sampling technique; **Comp. group:** Details of comparison group; **Methods:** Detailed explanation of methodology; **Rand:** Randomization; **Outcome measure:** Method of measurement of outcome; **Outcome assessor:** Outcome assessor details; **Blind:** Blinding; **Statist:** Statistical analysis; **Result:** Presentation of results.

Discussion

Cellular sources for *in vitro* evaluation of musculoskeletal tissues vary depending on the intended application, either for basic science or translational purposes. Furthermore, animal tissues may offer advantages over human cell lines mainly by reducing costs and facilitating their availability for researchers interested in cartilage diseases.

In vitro pre-clinical research plays a crucial role in the development of new materials and techniques, providing essential information for further testing in clinical trials. The chondrocyte harvest site and the age of the donor may affect the biological characteristics of studies. In the present study, the variation in harvest site ranged from the carpus, metacarpus, and the knees of both adult and young animals. Isogai et al. (2006) compared bovine chondrocytes from different anatomical locations for tissue-engineered cartilage modeling and found that chondrocytes from different sources showed variations in cell proliferation rates, gene expression, and extracellular matrix production (Isogai et al., 2006). Interestingly, the authors found that collagen-I and aggrecan relative gene expression were highest in costal chondrocytes compared to chondrocytes isolated from articular cartilage. Similarly, Maličev et al. (2011) evaluated cell viability, proliferation, morphology, and collagen expression from chondrocytes harvested from the debrided edge of a chronic lesion of the articular surface compared to those from the edge of the lesion. The authors found differential expression and cell yield between the two harvest sites and suggested that the cultivation of chondrocytes solely from the edges of the lesion cannot be recommended for use in autologous chondrocyte implantation (Maličev et al., 2011), thus confirming the importance of considering the specific characteristics of chondrocyte types in the design of tissue-engineered cartilage models.

Different studies analyzing the effect of chondrocyte passages on cartilage formation have found that serial cell passages can cause the loss of a differentiated phenotype (Brodkin et al., 2004; Hamilton et al., 2005; Kang et al., 2007). Kang et al. (2007) found that chondrocytes cultured through various passages showed a decreased growth rate and viability, as well as increased apoptosis. Additionally, the authors showed that passage 2 chondrocytes expressed high levels of collagen type II, while passage 5 chondrocytes showed dedifferentiation with low collagen type II expression. Furthermore, when using chondrocytes for 3D culture or tissue engineered constructs, passage 1 chondrocytes exhibited mature cartilage, while tissues engineered with passage 5 chondrocytes did not have the chondrocyte morphology or cartilage-specific matrices (Kang et al., 2007). Similarly, Nam et al. (2014) compared the effects of cryopreservation and passaging on cell viability and proliferation in chondrocytes and synovium-derived mesenchymal stem cells (MSCs) used as sources for autologous chondrocyte transplantation (ACT). The authors found that passaging and cryopreservation significantly affected the ability of chondrocytes to maintain their morphology, express chondrogenic genes, and differentiate when compared to synovium-derived cells, which were not affected by passaging and cryopreservation (Nam et al., 2014).

Moreover, the age of the donor is also an important factor in ECM production capability. Son and Levenston (2017) evaluated phenotypic changes in juvenile and adult articular chondrocytes as well as fibrochondrocytes across multiple passages and subsequent 3D culture and found that Col-1 expression increased with passage in adult cells, but decreased in juvenile cells, whereas 3D gel culture reversed this increase in adult cells (Son and Levenston, 2017). Therefore, besides considering factors such as the place of harvest, chondrocyte passage, and donor age, using cell therapy for surgical treatments may require additional experimental conditions to direct the cell phenotype, such as the use of growth factors in the culture medium and various cell sources for tissue-engineering strategies.

When analyzing the summed scores for the risk of bias, we found that most papers were classified as having a medium or high risk of bias, probably due to the lack of a complete description of procedures, specifically regarding the randomization and blinding criteria, leading to a poor grade in the risk of bias assessment tool. Whether the grading instructions for each criterion in the assessment tool were clear remains unknown. It would be valuable to compare different grading strategies to see which achieve better consensus. Consequently, various authors have developed different guidelines for reporting *in vitro* studies based on the CONSORT checklist for reporting randomized clinical trials (Faggion, 2012; Krithikadatta et al., 2014). Additionally, implementing good standards for reporting preclinical research is necessary for improving efficiency and ensuring the reliability of study findings. However, further refinements of the current risk of bias reporting tools are still needed. The QUIN tool utilized in this paper provided adequate information on the descriptions of relevant characteristics of *in vitro* studies, including randomization and blinding processes. It is important to note that the twelve criteria evaluated by the QUIN tool only indicate whether the authors of the articles included such descriptions.

Importantly, the use of animal tissues for *in vitro* studies can enhance the understanding of cellular behavior in cartilage integration. Research has shown that bioengineered cartilage derived from bovine chondrocytes can effectively migrate and integrate with native cartilage when treated with platelet-rich plasma (PRP) (Wu et al., 2022). Furthermore, the mechanical properties of engineered cartilage constructs may be modulated by modifying the osmolarity of the culture medium (Oswald et al., 2011). Additionally, the expression and secretion of appropriate extracellular matrix (ECM) components may also be affected by the viscosity of the cell culture medium, with higher levels of cartilaginous gene expression observed in low-viscosity medium (Zheng et al., 2023), providing further evidence of the importance of culture conditions for the mechanical properties of cartilage constructs.

In conclusion, having examined the most relevant evidence for the use of bovine chondrocytes in 3D culture, we suggest that future studies adopt consistent methods across the *in vitro* phase of the study, such as uniform harvest sites (based on previous molecular analysis of ECM yield), and maintaining chondrocyte passages between passage zero (P0) and passage four (P4) to preserve the cellular phenotype, especially for cartilage transplantation purposes. Furthermore, comparisons of relevant translational models should include age-matched conditions (either for pediatric or adult cartilage diseases) to improve model accuracy and avoid additional confounding factors.

Declarations

Conflicts of interest

The authors declare they have no conflicts of interest regarding the work presented in this report.

Author contributions

Cardona-Ramírez S, Conceived and designed the manuscript, contributed with data analysis, wrote, and edited the manuscript. Ramírez-Jaramillo M, and Currea-Gómez MP wrote and prepared the manuscript, collected the data, and contributed to data analysis. All authors provided critical feedback during writing and editing.

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Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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Variables	Categories	N	%	Mean
Age	–	177	100	41.3
Gender	Female	126	71.2	41.2
	Male	51	28.8	41.5
Race	African - American	47	26.65	–
	Mixed - race	130	73.5	–
Indoor rodents	Yes	100	56.5	–
	No	77	43.5	–
Peridomiciliary rodents	Yes	99	55.9	–
	No	78	44.1	–

NA: Not applicable