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Original Article

Are there differences between Sprague-Dawley and Wistar rats in long-term effects of ovariectomy as a model for postmenopausal osteoporosis?

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Abstract: Ovariectomized Sprague-Dawley (SD) and Wistar rats are widely used as animal models for postmenopausal osteoporosis. This study aimed to investigate differences in the long-term effects of ovariectomy on these two breeds of rats. 6-month-old SD and Wistar rats underwent sham operation (Ssham and Wsham) or bilateral ovariectomy (Sovx and Wovx). Body weight, absolute fat, fifth and sixth lumber vertebrae (L5-6) bone mineral density (BMD), and whole body bone mineral content (BMC) were evaluated from 14to 68 weeks after operation. Body weight significantly increased compared to shams at every point (except 68 weeks) for Sovx and at 14, 21 and 38 weeks for Wovx (all $P < 0.05$), the increases were not significantly different between breeds. Absolute fat increased significantly compared to shams at every point (except 14 and 68 weeks) for Sovx and at 30 weeks for Wovx (all $P < 0.05$), the increases were significantly different between Sovx and Wovx ($P = 0.007$), but not the shams. L5-6 BMD increased then decreased and was significantly lower than shams at 30, 38 and 50 weeks for Sovx and at 14 weeks for Wovx (all $P < 0.05$), the initial increases were significantly different between Sovx and Wovx ($P = 0.006$), but not the shams. BMC was significantly decreased compared to sham groups at every point (except 14 and 68 weeks) for Sovx and at 14, 21, 38, and 50 weeks for Wovx (all $P < 0.05$), the decreases were not significantly different between breeds. In conclusion, ovariectomy had a greater effect upon absolute fat and L5-6 BMD in SD rats.

Keywords: Bone mineral density, osteoporosis, dual-energy x-ray absorptiometry, ovariectomy, lipid metabolism, bone mineral content

Introduction

Osteoporosis results in decreased bone mineral density (BMD) and has a major impact on public health because it is the major factor in most occurrences of fracture [1]. Osteoporosis can occur in both genders but in the US women account for 71% of all reported fractures and this is related to decreased BMD resulting from postmenopausal osteoporosis (PMOP) [1]. It is estimated that in the UK 1 in 2 women over 50 years old are at risk of osteoporotic fracture [2]. In addition to the pain, expense and impact on quality of life, fractures can also be an important cause of mortality, particularly hip fractures [3].

Estrogen was introduced into skeletal research as early as the 1930s. Fuller Albright, a clinical worker with keen insight, proposed a classical concept on PMOP. He was skeptical about the "coincidence" that bone fragility in the majority of patients with PMOP either occurred naturally or through ovariectomy and was more inclined to believe that menopausal cessation of ovarian function, and the consequent sharp reduction in circulating estrogen, causes bone loss. This ultimately results in the condition he termed PMOP in women who outlive the functioning of their ovaries [4, 5]. Since breaking this new ground, Albright has inspired a multitude of researchers to challenge, confirm, or refine this idea [6]. As a popular model of osteoporosis, women's postmenopausal bone loss can be described as an accelerated, transient-phase (type I osteoporosis) and subsequent gradual, continuous bone loss (type II osteoporosis). Because estrogen withdrawal is widely accepted as the primary cause of type I osteoporosis, the quest to uncover the origin of type I osteoporosis has focused on the estrogen withdrawal-related skeletal changes at and around the time of menopause. However, consideration of the cyclical secretion of estrogen, normally beginning in adolescence and continuing over the fertile period, offers the idea that the perimenopausal period alone may be too narrow [7]. Therefore, it is important to study changes in body composition over time.

Despite this understanding, the pathological mechanisms are still not fully understood. Animal models of PMOP have helped reveal mechanisms and the development of the current methods of treatment including anti-resorptive drugs such as bisphosphonates, denosumab and calcitonin, anabolic agents such as teriparatide, strontium ranelate, and raloxifene [2]. In addition they provide important data on the maintenance of bone health by diet such as Ca and vitamin D supplementation [8]. A wide range of animal models have been developed from mice, rats, guinea pigs, rabbits, pigs, sheep and monkeys [9]. They all have different advantages such as time taken to establish the model and similarity to the human situation.

The rat model of PMOP, which was first established by Saville in 1969 and has been repeatedly utilized since that time, has become a classical animal model for the study of PMOP [10-12]. Compared with large animals, rats are able to reach a new balance in the bone formation and remodeling cycle in a shorter period of time. Among all animals, rats are most widely used to establish models of ovariectomy. Because changes in cancellous bone and the response after estrogen replacement therapy in ovariectomized female rats are similar to those in humans, rats are most often used in the research of human PMOP; in addition, rats allow for the performance of technically simple surgery with good reproducibility, and they have a short sexual cycle [11, 13]. Because of their sensitivity to estrogen and sexual cycle stability, closed colonies of Sprague-Dawley (SD) and Wistar rats have been widely used in the study of osteoporosis. However, a consensus has not been reached on the most appropriate rat model age or the most suitable breed of rat. Studies have shown that 1 month after ovariectomy, 6-month-old rats show not only a loss of bone mass and decline in bone structure, but also a decline in bone strength; these changes are very similar to those after menopause. Thus, the creation of a 6-month-old rat ovariectomy model not only provides the best animal model, but also saves time and expenditure because of the short amount of study time required [14, 15]. Therefore, our study utilized 6-month-old ovariectomized SD and Wistar rats. We observed the dynamic variation in related indicators over time to elucidate the differences between these two species of estrogen-deficient rats and provide a reference for the selection of a rat model.

Materials and methods

Animals

Six-month-old specific pathogen-free (SPF) female SD (n = 25) and Wistar (n = 24) rats with body weights of 267.7 ± 19.29 and 241.1 ± 20.91 g, respectively (license no. SCXK Guangdong 2006-0015) were supplied by the Laboratory Animal Center of South Medical University, and bred in our in-house animal facility. The rats were kept at 22°C ± 2°C and 40% humidity in the SPF laboratory animal room with a 12/12-hour light/dark cycle. All rats were provided free access to normal rat chow and tap water. All procedures and animal experiments were approved by the Animal Care and Use Committee of Jinan University.

Establishment of the PMOP model

The SD and Wistar rats were divided into ovariectomy (OVX) and sham-operation (Sham) groups as follows: the Sovx (n = 12), Wovx (n = 12), Ssham (n = 13), and Wsham (n = 12) groups. All rats were anesthetized by intraperitoneal injection of 2% pentobarbital sodium (0.2 ml per 100 g). The POMP model was created according to previous methods [12, 14]. In the OVX groups, an abdominal midline incision was made along the linea alba and the bilateral ovaries were ligated and ablated under aseptic conditions. To exclude the factor of surgical interference, the bilateral ovaries were identified after creation of an abdominal midline incision in the Sham groups, but they were not removed. Daily food intake was controlled in the OVX groups according to the intake in the Sham groups, and each group of rats was kept in an environment of 22°C ± 2°C and 40% humidity. All feed and bedding were provided by the Experimental Animal Center of Sun Yat-sen University.

BMC, BMD and absolute fat content

Each group of rats was tested at 14, 21, 30, 38, 50, and 68 weeks after ovariectomy for BMD (g/cm^2) and absolute fat content (g) by a dual-energy X-ray absorptiometer (Lunar Prodigy; GE Medical Systems, Madison, WI). All rats were scanned in the prone position. The indicators were analyzed by small animal analysis software. Whole body bone mineral content (BMC) were calculated according to the average of surface projection area and the body weight into the bone mineral content per gram per 53 cm^2.

Statistical analyses

Statistical analysis was performed using SPSS for Windows 19.0 (IBM SPSS, Armonk, NY, USA). The data are presented as mean ± standard deviation (SD). Time factors and interactions were analyzed by repeated measures analysis of variance (ANOVA). Comparisons of the same time point were analyzed by a *t*-test for comparison of two groups. A *P*-value of < 0.05 was considered statistically significant.

Results

Differences in long-term effects of ovariectomy on body weight between SD and Wistar rats

The body weight of the 6-month-old Sovx and Ssham groups before ovariectomy were 232.69 ± 1.97 g and 231.25 ± 10.36 g respectively and the difference between them was insignificant ($P > 0.05$). However, at 14, 21, 30, 38, 50, and 54 weeks after ovariectomy, the body weights of the Sovx group were 267.85 ± 17.44 g, 301.08 ± 27.97 g, 298.92 ± 34.24 g, 332.69 ± 34.26 g, 323.31 ± 36.74 g and 348.85 ± 35.07 g, respectively, compared with that of Ssham group at the same time point, the differences were all significant (all $P < 0.05$).

Variance analysis of repeated measures showed that the time factor was statistically significant ($F = 44.03$, $P < 0.001$), which suggested that body weight of both Sovx and Ssham groups had changed over the research period and the trend change was between the linear increase ($F = 60.43$, $P < 0.001$) and the parabolic curve ($F = 42.44$, $P < 0.001$). We used the Mauchly's test of sphericity to test the result and the hypothesis of sphericity was null ($\chi^2 = 130.77$, $P < 0.001$), the degrees of freedom adjusted by the lower-bound showed that the interaction of factors of time and treatment was also significant ($F = 5.24$, $P = 0.032$), which suggested that ovariectomy altered the trend of body weight change in the Sovx group compared to the Ssham group and that the Ssham group's body weight was significantly lower than that of Sovx group ($F = 16.05$, $P < 0.001$) (**Figure 1A**). The interaction of ovariectomized and time factors produced a parabolic curve ($F = 15.61$, $P = 0.001$) and the body weight of both Sovx and Ssham groups reached a peak of 348.85 ± 35.07 g and 290.75 ± 38.39 g at 18-months-old, respectively.

The differences in body weight of 6-month-old Wovx and Wsham groups before the ovariectomy were also insignificant ($P > 0.05$). After 14, 21, and 38 weeks the body weight of Wovx group were 275.75 ± 14.58 g, 276.25 ± 26.98 g, 290.08 ± 25.53 g and 293.67 ± 25.63 g, respectively, compared with the Wsham group at the same time points, the differences were all significant (all $P < 0.05$).

Variance analysis of repeated measures showed that the time factor was statistically significant ($F = 71.64$, $P < 0.001$), which suggested that body weight of both Wovx and Wsham groups had changed over the research period and the trend change was between the linear increase ($F = 130.34$, $P < 0.001$) and the parabolic curve ($F = 53.97$, $P < 0.001$). We used the Mauchly's test of sphericity to test the result and the hypothesis of sphericity was null ($\chi^2 = 89.58$, $P < 0.001$), the degrees of freedom adjusted by the lower-bound showed that the interaction of factors of time and treatment was also significant ($F = 8.43$, $P = 0.008$), which suggests that the ovariectomy altered the trend of body weight change in the Wovx group compared to the Wsham group and that the Wsham group's body weight was significantly lower than that of the Wovx group ($F = 9.04$, $P = 0.006$). The interaction of ovariectomized and time factors produced a parabolic curve ($F = 16.48$, $P = 0.001$) (**Figure 1A**).

The body weight of Sovx, Ssham and Wovx groups all reached the peak at 18-months-old (54 weeks after ovariectomy). Considering the difference in body weight at 18 and 21-months-old was insignificant in Wsham group ($P > 0.05$) (**Figure 1A**), we calculated the percentage ratio of weight increase from 6 to 18-months-old for

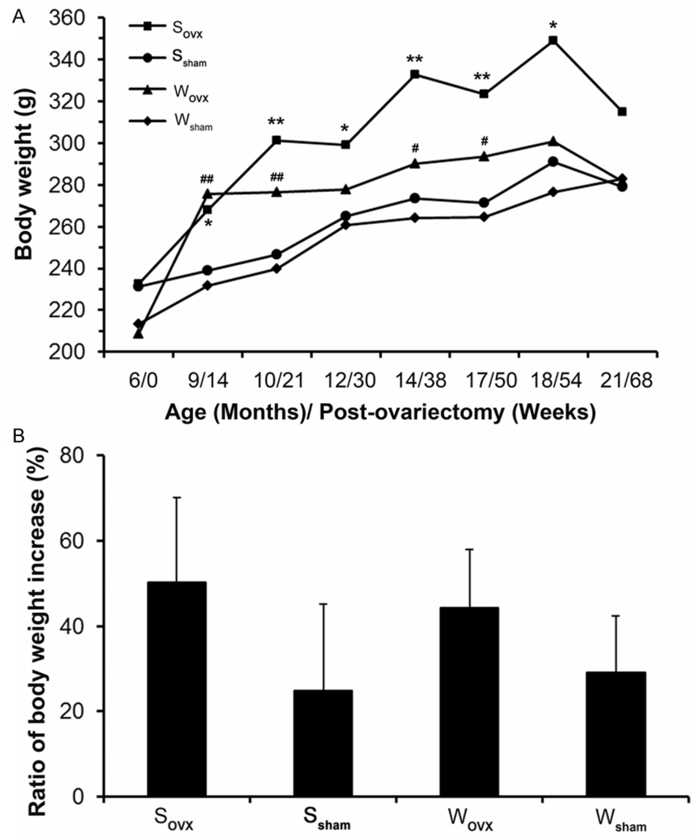


Figure 1. Differences in long-term effects of ovariectomy on body weight between Sprague-Dawley and Wistar rats. Spague-Dawley (SD) sham operation group was Ssham and bilateral ovariectomy group was Sovx, Wistar sham operaion group was Wsham and bilateral ovariectomy group was Wovx. A. Comparison of the change in body weight over the research period between SD and Wistar rats. B. Comparison of the ratios of weight increase between SD and Wistar rats from 0 to 54 weeks after ovariectomy. The data are shown as mean ± SD (SOVX: n = 13; Ssham: n = 12; WOVX: n = 12; Wsham: n = 12). $^{*}P < 0.05$, $^{**}P < 0.01$, Sovx group compared with Ssham group in the same research period; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, Wovx group compared with Wsham group in the same research period.

the Sovx, Ssham, Wovx, and Wsham groups to compare the differences and the results showed that the ratios of weight increase were 50.32 ± 19.80% and 44.3 ± 13.62% in the Sovx and Wovx groups and 24.79 ± 20.37% and 29.21 ± 13.14% in the Ssham and Wsham groups, and the differences were insignificant ($P > 0.05$) (**Figure 1B**).

Differences in long-term effects of ovariectomy on absolute fat content between SD and Wistar rats

The absolute fat content of 6-month-old rats in the Sovx and Ssham groups before ovariectomy were 30.23 ± 2.77 g and 31.92 ± 3.03 g, respectively, and the difference between them was not statistically significant ($P = 0.160$). However, at 21, 30, 38, 50, and 54 weeks after ovariectomy, the absolute fat content of rats in the Sovx group was 87.31 ± 43.62 g, 92.69 ± 49.81 g, 130.08 ± 50.16 g, 111.08 ± 54.34 g, and 153.08 ± 50.81 g, respectively; compared with the absolute fat content of rats in the Ssham group at the same time points, all differences were statistically significant ($P = 0.011$, $P = 0.024$, $P = 0.002$, $P = 0.007$, $P = 0.022$, respectively).

Analysis of variance showed that the time factor was statistically significant ($F = 25.60$, $P < 0.001$). This suggests that the absolute fat content of rats in both the Sovx and Ssham groups had changed over the research period, and the trend of the change was between a linear increase ($F = 32.65$, $P < 0.001$) and a parabolic curve ($F = 14.80$, $P = 0.001$). We used Mauchly's test of sphericity to evaluate the result, and the hypothesis of sphericity was null ($\chi^2 = 136.72$, $P < 0.001$), the degrees of freedom, adjusted by the lower-bound formula, showed the interaction of the factors of time and treatment were significant ($F = 5.94$, $P = 0.023$). These findings suggest that ovariectomy caused the absolute fat content change trend in the Sovx group to be different from the Ssham group, and the absolute fat content in the Ssham group was significantly lower than that in the Sovx group ($F = 12.81$, $P = 0.002$) (**Figure 2A**). The interaction of the factors of ovariectomy and time appeared to follow a parabolic curve ($F = 15.91$, $P = 0.001$), and the absolute fat content in the Sovx and Ssham groups reached a peak of 153.08 ± 50.81 g and 77.75 ± 39.72 g, respectively, at 18 months of age (54 weeks after ovariectomy). The difference in the peaks was statistically significant ($P = 0.022$).

The absolute fat content of 6-month-old rats in the Wovx and Wsham groups before ovariectomy was 48.83 ± 1.01 g and 48.17 ± 5.46 g, respectively, and the difference between them was not statistically significant ($P = 0.681$).

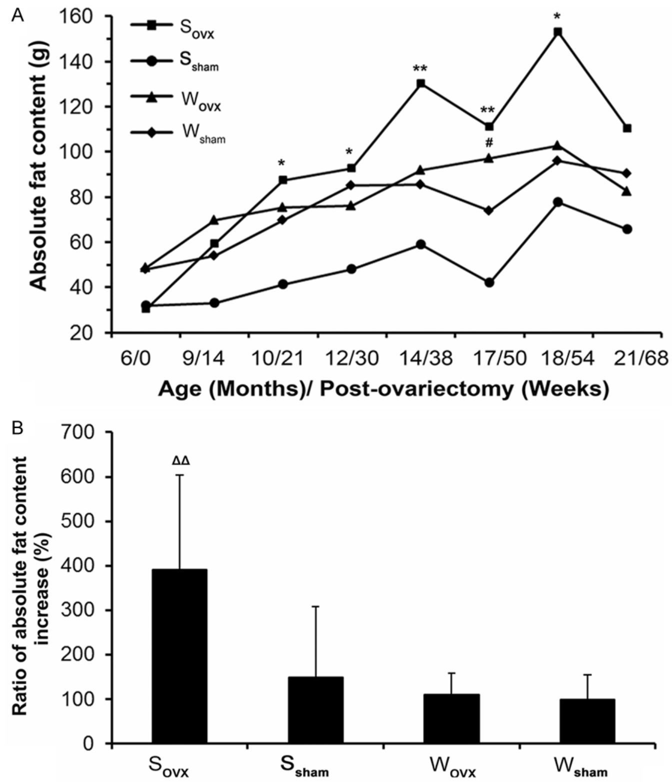


Figure 2. Differences in long-term effects of ovariectomy on absolute fat content between SD and Wistar rats. A. Comparison of the change in absolute fat content over the research period between SD and Wistar rats. B. Comparison of the ratios of the increase in the absolute fat content between SD and Wistar rats from 0 to 54 weeks after ovariectomy. The data are shown as mean ± SD (SOVX: n=13; Ssham: n = 12; WOVX: n = 12; Wsham: n = 12). $^{*}P < 0.05$, $^{**}P < 0.01$, Sovx group compared with Ssham group in the same research period; $^{\#}P < 0.05$, Wovx group compared with Wsham group in the same research period; $^{\Delta\Delta}P < 0.01$, SD rats compared with Wistar rats under the same treatment.

However, at 50 weeks after ovariectomy, the absolute fat content in the Wovx group was 97.00 ± 18.31 g; compared with the 73.73 ± 19.94 g in the Wsham group at the same time point, the difference was statistically significant (*P* = 0.016).

Analysis of variance showed that the time factor was statistically significant (F = 29.90, *P* < 0.001). This suggests that the absolute fat content of rats in both the Wovx and Wsham groups had changed over the research period, and the trend of the change was between a linear increase (F = 53.05, *P* < 0.001) and a parabolic curve (F = 26.56, *P* < 0.001). The absolute fat content of rats in the Wovx and Wsham groups reached peaks of 102.75 ± 20.62 g and 96.00 ± 28.04 g at 18 months of age (54 weeks after ovariectomy), but the difference in the peaks was not statistically significant (*P* = 0.589). We used Mauchly's test of sphericity to evaluate the result; the hypothesis of sphericity was null (χ^2 = 94.35, *P* < 0.001), the degrees of freedom, adjusted by the lower-bound formula, showed the interaction of the factors of time and treatment were not statistically significant (F = 3.38, *P* = 0.079). These findings suggest that ovariectomy did not cause the absolute fat content trend change for the Wovx group to differ from the Wsham group (**Figure 2A**).

We analyzed the changes in the absolute fat content of SD and Wistar rats over the research period by repeated measures analysis of variance. The results showed that the trend of the changes in SD and Wistar rats were between a linear increase and a parabolic curve. Ovariectomy resulted in a significantly higher absolute fat content in the Sovx group than in the Ssham group (F = 12.81, *P* = 0.002); however, ovariectomy did not appear to cause the absolute fat content changes trend for the Wovx group to differ from the Wsham group (F = 0.85, *P* = 0.367).

The absolute fat content of rats in the Sovx, Ssham, Wovx, and Wsham groups all peaked at 18 months of age (54 weeks after ovariectomy) (**Figure 2A**). We calculated the percentage ratio of the absolute fat content increase from 6 months (before ovariectomy) to 18 months of age (54 weeks after ovariectomy) in all four groups to compare the differences. The ratios of the absolute fat content increase in the Sovx and Wovx groups were 391.06 ± 213.59% and 110.49 ± 48.55%, and the difference was statistically significant (*P* = 0.007). The ratios of the absolute fat content increase in the Ssham and Wsham groups were 149.04 ± 159.72% and 99.43 ± 55.71%, but the difference was not statistically significant (*P* = 0.425) (**Figure 2B**). These findings suggest that ovariectomy resulted in a significantly higher ratio of fat content increase in SD than in Wistar rats; however, the difference in the ratio of the fat content increase in normal SD and Wistar rats was not statistically significant.

Differences in long-term effects of ovariectomy on the fifth and sixth lumber vertebrae (L5-6) bone mineral density (BMD) between SD and Wistar rats

The BMD of L5-6 in 6-month-old rats in the Sovx and Ssham groups before ovariectomy was 0.246 ± 0.006 g/cm^2 and 0.247 ± 0.017 g/cm^2, respectively. The difference between them was not statistically significant (P = 0.943). However, at 30, 38, and 50 weeks after ovariectomy, the BMD of L5-6 in the Sovx group was 0.250 ± 0.033 g/cm^2, 0.261 ± 0.030 g/cm^2, and 0.247 ± 0.032 g/cm^2, respectively; compared with the BMD in the Ssham group at the same time points, all differences were statistically significant (P = 0.015, P = 0.017, P = 0.009, respectively).

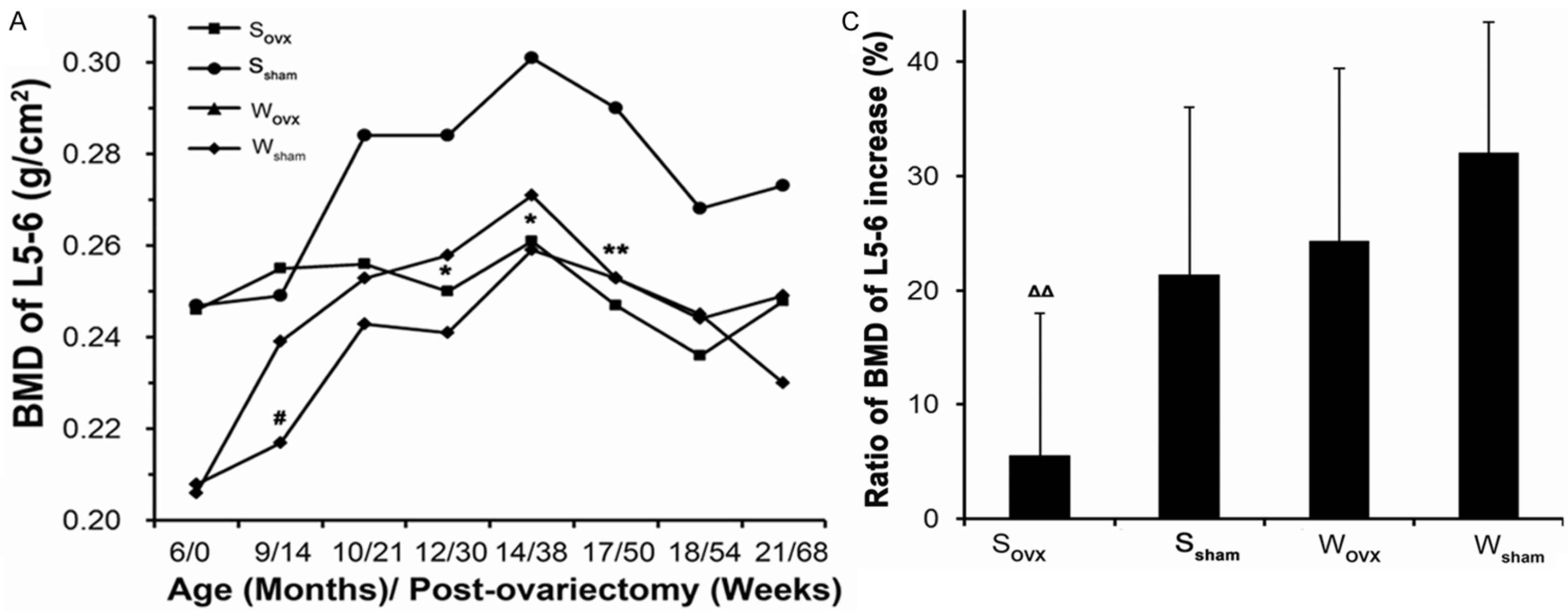
Repeated measures analysis of variance showed that the time factor was statistically significant (F = 7.19, P < 0.001). This suggests that the BMD of L5-6 in both the Sovx and Ssham groups had changed over the research period, and the trend of the change followed a parabolic curve (F = 22.27, P < 0.001). In addition, both the Sovx and Ssham groups reached a peak of 0.261 ± 0.030 g/cm^2 and 0.301 ± 0.034 g/cm^2 at 14 months of age (38 weeks after ovariectomy), and the difference in the peaks was statistically significant (P = 0.017) (**Figure 3A**). We used Mauchly's test of sphericity to evaluate the result; the hypothesis of sphericity was null (χ^2 = 43.25, P = 0.027), the degrees of freedom should be adjusted by the lower-bound formula, and the interaction of the factors of time and treatment was significant (F = 4.20, P = 0.001). These findings suggest that ovariectomy caused the change trend in the BMD of L5-6 of the Sovx group to differ from the Ssham group over the research period, and the change trend in the BMD of L5-6 was between a linear increase (F = 9.88, P = 0.005) and a parabolic curve (F = 8.40, P = 0.008). The BMD of L5-6 in the Sovx group was significantly lower than that in the Ssham group (F = 13.87, P = 0.001) (**Figure 3A**).

The BMD of L5-6 of 6-month-old rats in the Wovx and Wsham groups before ovariectomy was 0.208 ± 0.004 g/cm^2 and 0.206 ± 0.007 g/cm^2, respectively, and the difference between them was not statistically significant (P = 0.273). However, at 14 weeks after ovariectomy, the BMD of L5-6 in the Wovx group was 0.217 ± 0.008 g/cm^2; compared with that of the Wsham group at the same time point (0.239 ± 0.010 g/cm^2), the difference was significant (P = 0.019).

Repeated measures analysis of variance showed that the time factor was statistically significant (F = 28.80, P < 0.001). This suggests that the BMD of L5-6 in both the Wovx and Wsham groups had changed over the research period, and the change trend was between a linear increase (F = 93.62, P < 0.001) and a parabolic curve (F = 100.58, P < 0.001). The Wovx and Wsham groups reached a peak of 0.259 ± 0.027 g/cm^2 and 0.271 ± 0.023 g/cm^2, respectively, at 14 months of age (38 weeks after ovariectomy), but the difference was not statistically significant (P = 0.306) (**Figure 3A**). We used Mauchly's test of sphericity to evaluate the result; the hypothesis of sphericity was null (χ^2 = 44.47, P = 0.020), the degrees of freedom, adjusted by the lower-bound formula, showed the interaction of the factors of time and treatment was significant (F = 2.13, P = 0.049). These findings suggest that ovariectomy resulted in a trend toward a significantly lower BMD of L5-6 in the Wovx group than in the Wsham group over the research period, and the trend of the change in the BMD of L5-6 followed a cubic regression curve (F = 9.32, P = 0.006) (**Figure 3A**).

Repeated measures analysis of variance showed that the trends of the change in the SD and Wistar rats were between a linear increase and a parabolic curve. The interaction of the factors of ovariectomy and time in SD rats was between a linear increase and a parabolic curve, but followed a cubic regression curve in Wistar rats. The BMD of L5-6 in the Sovx, Ssham, Wovx, and Wsham groups all peaked at 14 months of age (38 weeks after ovariectomy) (**Figure 3A**); the peaks in the Sovx and Wovx groups were 0.261 ± 0.032 g/cm^2 and 0.259 ± 0.030 g/cm^2, and the difference between them was not statistically significant (P = 0.847). However, the peaks in the Ssham and Wsham groups were 0.301 ± 0.037 g/cm^2 and 0.271 ± 0.025 g/cm^2, and the difference between them was statistically significant (P = 0.039) (**Figure 3B**).

We calculated the percentage ratio of the increase in the BMD of L5-6 from 6 months (before ovariectomy) to 14 months of age (38



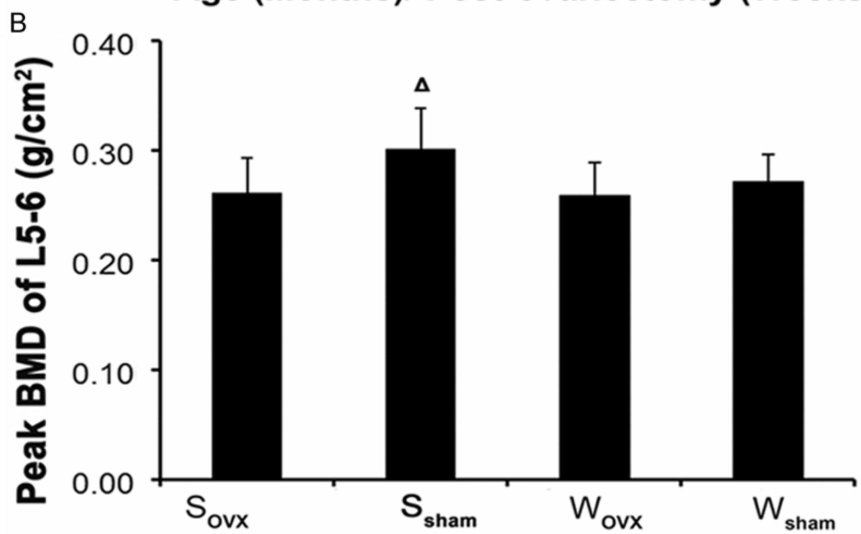


Figure 3. Differences in long-term effects of ovariectomy on the L5-6 BMD between SD and Wistar rats. A. Comparison of the change in the fifth and sixth lumber vertebrae (L5-6) bone mineral density (BMD) over the research period between SD and Wistar rats. B. Comparison of the peak of the L5-6 BMD (g/cm2) between the Sovx and Wovx groups and between the Ssham and Wsham groups at 14 months of age (38 weeks after ovariectomy). C. Comparison of the increase in the L5-6 BMD between SD and Wistar rats from 0 to 38 weeks after ovariectomy. The data are shown as mean ± SD (SOVX: n = 13; Ssham: n = 12; WOVX: n = 12; Wsham: n = 12). $^{*}P < 0.05$, $^{**}P < 0.01$, Sovx group compared with Ssham group in the same research period; $^{\#}P < 0.05$, Wovx group compared with Wsham group in the same research period; $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, SD rats compared with Wistar rats under the same treatment.

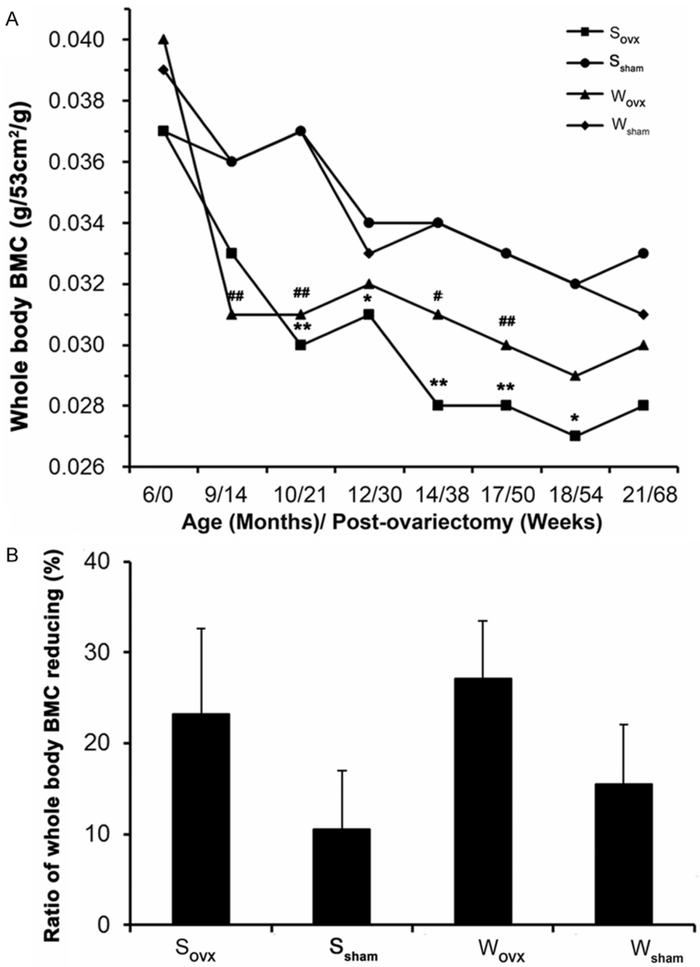


Figure 4. Differences in long-term effects of ovariectomy on BMC between SD and Wistar rats. A. Comparison of whole body bone mineral content (BMC) over the research period between SD and Wistar rats. B. Comparison of the ratio of whole body BMC reduction between SD and Wistar rats from 0 to 50 weeks after ovariectomy. The data are shown as mean ± SD (SOVX: n = 13; Ssham: n = 12; WOVX: n = 12; Wsham: n = 12). $^{*}P < 0.05$, $^{**}P < 0.01$, Sovx group compared with Ssham group in the same research period; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, Wovx group compared with Wsham group in the same research period.

weeks after ovariectomy) in the Sovx, Ssham, Wovx, and Wsham groups to compare the differences. The ratios of the increase in the BMD of L5-6 in the Sovx and Wovx groups were 5.56 ± 12.39% and 24.25 ± 15.17%, respectively, and the difference between them was statistically significant (P = 0.006). However, the ratios in the Ssham and Wsham groups were 21.37 ± 14.61% and 32.03 ± 11.39%, and the difference between them was not statistically significant (P = 0.076) (**Figure 3C**). The results suggest that ovariectomy resulted in a significantly higher ratio of increase in the BMD of L5-6 in Wistar rats than in SD rats; however, the difference in the ratio of the increase in the BMD of L5-6 in normal SD and Wistar rats was not statistically significant.

Differences in long-term effects of ovariectomy on whole body bone mineral content (BMC) between SD and Wistar rats

The whole body BMC of 6-month-old Sovx and Ssham groups before ovariectomy were 0.037 ± 0.001 g/53 cm^2/g and 0.037 ± 0.001 g/53 cm^2/g, respectively. The difference between them was insignificant ($P > 0.05$). However, at 21, 30, 38, 50, and 54 weeks after ovariectomy, whole body BMCs of the Sovx group were 0.030 ± 0.003 g/53 cm^2/g, 0.031 ± 0.003 g/53 cm^2/g, 0.028 ± 0.003 g/53 cm^2/g, 0.028 ± 0.003 g/53 cm^2/g, and 0.027 ± 0.003 g/53 cm^2/g, respectively, which when compared with the Ssham group at the same time points, the differences were all significant ($P < 0.05$).

Variance analysis of repeated measures showed that the time factor was statistically significant (F = 32.93, $P < 0.001$), which suggested that whole body BMC of both the Sovx and Ssham groups had changed over the research period and the change trend was reducing linearity (F = 71.54, $P < 0.001$). We used the Mauchly's test of sphericity to test the result and the hypothesis of sphericity was null ($\chi^2 = 87.27$, $P < 0.001$), the degrees of freedom, adjusted by the lower-bound, showed that the interaction of factors of time and treatment was also significant (F = 5.89, P = 0.023), which suggested that the ovariectomy made the change trend of whole body BMC in the Sovx group significantly lower than that in the Ssham group over the research period (F = 24.77, $P < 0.001$) (**Figure 4A**).

The whole body BMC of the 6-month-old Wovx and Wsham groups before ovariectomy were 0.040 ± 0.001 g/53 cm^2/g and 0.039 ± 0.001 g/53 cm^2/g, respectively, and the difference between them was insignificant ($P > 0.05$). However, at the 14, 21, 38, and 50 weeks after ovariectomy, the whole body BMC of the Wovx group were 0.031 ± 0.001 g/53 cm^2/g, 0.031 ± 0.003 g/53 cm^2/g, 0.031 ± 0.003 g/53 cm^2/g and 0.030 ± 0.002 g/53 cm^2/g, which when compared with that of the Wsham group at the same time points, the differences were significant ($P < 0.05$).

Variance analysis of repeated measures showed that the time factor was statistically significant ($F = 87.91$, $P < 0.001$), which suggested that whole body BMC of both Wovx and Wsham groups had changed over the research period and the change trend was reducing linearity ($F = 224.60$, $P < 0.001$). We used the Mauchly's test of sphericity to test the result and the hypothesis of sphericity was null ($\chi^2 = 53.53$, $P = 0.002$), the degrees of freedom, adjusted by the lower-bound, showed that the interaction of factors of time and treatment was also significant ($F = 10.59$, $P = 0.004$), which suggested that the ovariectomy made the change trend of whole body BMC in the Wovx group significantly lower than that in the Wsham group over the research period ($F = 13.69$, $P = 0.001$) (**Figure 4A**).

Variance analysis of repeated measures showed that the change trends of either SD or Wistar rats were reducing linearity. The difference of whole body BMC were significant at 21, 30, 38, 50, and 54 weeks after ovarietomy on SD rats and at 14, 21, 38, and 50 weeks after ovarietomy on Wistar rats. At 50 week after ovariectomy, differences of whole body BMC between either Sovx and Ssham groups or Wovx and Wsham groups were significant ($P < 0.01$), so we calculated the percentage ratio of whole body BMC reduction from 6 months (before the ovariectomy) to 17-months-old (50 weeks after ovariectomy) of Sovx, Ssham, Wovx, and Wsham groups to compare the differences and the results showed that the ratio of whole body BMC reduction in the Sovx and Wovx groups were 23.24 ± 9.38% and 27.18 ± 6.27%, and the Ssham and Wsham groups were 10.50 ± 6.53% and 15.49 ± 6.57%, respectively. The differences between them were all insignificant (**Figure 4B**).

Discussion

Both SD and Wistar rats are used as models for PMOP. The aim of this investigation was to evaluate whether there were any differences between the two breeds in terms of typical measures of PMOP. The results show that there are differences between the breeds in all the evaluations of L5-6 BMD, body weight, absolute fat, and whole body BMC. In general there were more significant differences with SD rats than with Wistar rats. However, there were only significant differences between the breeds in terms of the change in ratios of absolute fat and L5-6 BMD. This provides important information on the use of rats as models for PMOP, as the effects of ovariectomy may be more pronounced in SD rats.

In this study, the absolute fat content of ovariectomized SD and Wistar rats were all significantly increased, in line with previous reports [16-20]. However, fat accumulation was more significant in ovariectomized SD than in Wistar rats: compared with the Ssham group, SD rats from 21 weeks (10 months old) to 54 weeks (18 months old) after ovariectomy, the difference was significant, and over the research period, the trend between the Sovx and Ssham groups differed. Regardless of the fact that the absolute fat content in the Sovx group was significantly higher than that in the Ssham group, both of these groups reached a peak at 54 weeks after ovariectomy (18 months of age), and the peak in the Sovx was higher than that in the Ssham group. However, in Wistar rats, fat accumulation was not obvious. A significant difference between the Wsham and Wovx groups was only exhibited at 50 weeks after ovariectomy (17 months of age), and the difference subsequently disappeared; the difference between these two groups had no statistical significance throughout the research period. In addition, a comparison of SD and Wistar rats showed that the rate of increase in the absolute fat contents in the Sovx group was significantly higher than that in the Wovx group. However, in the sham groups, the difference between SD and Wistar rats had no statistical significance, which suggests that fat accumulation induced by estrogen deficiency in SD rats is more obvious than that in Wistar rats and this may suggest that the fat metabolism disorder induced by estrogen deficiency in SD rats is more serious than in Wistar rats, which is similar to the findings by Gao et al [21]. However, in both SD and Wistar rats, fat accumulation showed a significant difference within a certain period of time between the OVX and Sham groups, suggesting that fat metabolism induced by estrogen deficiency might be only temporary and unstable [12].

A similar pattern was seen in terms of body weight in the two breeds of rat investigated in this study. This is probably not surprising as body weight and fat accumulation are obviously related. However, the number of significantly different time points occurred more often in the Wistar rats than in the fat accumulation results and the differences between the Ssham and Sovx groups were not as large as in the fat accumulation. This resulted in no significant difference in weight gain between the SD and Wistar rats.

A definite impact on BMD of L5-6 was induced by ovariectomy in SD and Wistar rats over the research period. The BMD of L5-6 showed a trend characterized by an increase followed by a decrease from preovariectomy (6 months of age) to 68 weeks postovariectomy (21 months of age) in both the OVX and Sham groups, and all reached a peak at 38 weeks postovariectomy (14 months of age). The peak in the OVX groups was not as high as the peak in the Sham groups. In addition, the peak of L5-6 in the Ssham group (normal group of SD rats) was significantly higher than that in the Wsham group; however, in the OVX groups, the difference in the peaks between the SD and Wistar rats showed no statistical significance. Six-month-old SD rats were ovariectomized, and a significant difference in the BMD of L5-6 between the Sovx and Ssham groups was shown 30 weeks later. This result differs from that reported at only 4 weeks, which may have occurred because of the different tested body part. At 54 weeks after ovariectomy, the significant difference between the Sovx and Ssham groups disappeared. However, in Wistar rats, the BMD of L5-6 showed a significant difference between the Wovx and Wsham groups at 14 weeks after ovariectomy and disappeared at 21 weeks. This finding suggests that the effect of estrogen withdrawal on the BMD of L5-6 in SD rats appeared later and lasted longer than in Wistar rats. In comparison, this effect appeared earlier but was maintained for a shorter period of time in Wistar rats, possibly because of the sensitivity to estrogen and other stronger compensatory mechanisms excluding increased weight. The significant difference in the BMD of L5-6 in the SD and Wistar rats of the OVX and Sham groups subsequently disappeared, suggesting that the effect of estrogen deficiency was temporary and unstable [12]. The change in the BMD of L5-6 in SD rats in both the OVX and Sham groups was either linear or parabolic as time went on, but in Wistar rats, the change in the BMD of L5-6 between the OVX and Sham groups showed cubic regression. These findings are similar to those of Wu et al [22]. In terms of the increase in the ratio of the BMD of L5-6 between SD and Wistar rats, we found that the increase in the ratio of the BMD of L5-6 in the Sovx group was significantly lower than that in the Wovx group, but the difference in the ratio of the increase in the BMD of L5-6 between the Ssham and Wsham groups had no statistical significance. This suggests that the Wistar rats have more sensitivity to and stronger compensatory mechanisms for estrogen withdrawal. Combined with the results of the absolute fat content changes, the bone loss in L5-6 of SD rats was more obvious and lasted longer than that in Wistar rats after estrogen withdrawal, which suggests that SD rats are more susceptible to osteoporosis than are Wistar rats at L5-6. In addition, we found that the peak of the BMD at L5-6 in the Ssham group (14 months of age) was significantly higher than that in the Wsham group; however, from 6 to 14 months of age, the ratio of the increase in the BMD of L5-6 in the Wovx group was significantly higher than that in the Sovx group. Overall, in the end, the Wistar rats did not readily develop osteoporosis. It is uncertain whether the above results suggest that in the process of reaching the peak bone mass, a lower risk of PMOP is associated with either a higher ratio of bone mass increase or with the peak bone mass. In other words, before reaching the peak bone mass, as the bone mass fills more quickly, there may be a lower probability of the occurrence of PMOP.

There was a general downward trend for whole body BMC over the research period that became slower as time progressed. Once again the SD rats demonstrated significant differences at more time points than the Wistar rats after ovariectomy, but this was less striking than in the other evaluations, because both rat breeds demonstrated significant differences over many time periods from 14 to 54 in the SD rats and 14 to 50 in the Wistar rats. There were no significant differences between the breeds in terms of the decreases in BMC. Thus, of all the evaluations undertaken in this study BMC appears to be the most consistent across rat breeds.

It seems that estrogen deficiency can more readily cause the L5-6's bone loss in SD rats, in combination with the increased fat mass induced by estrogen deficiency in the SD rats was more obvious than in the Wistar rats. Thus, it is uncertain whether the increase in body weight or, to be accurate, the increase in body weight caused by the increase in fat mass is the compensatory mechanism for bone loss in the body. Activity is reduced secondary to estrogen deficiency, resulting in a decreased energy consumption and accumulation of excess subcutaneous fat. An increase in body weight can result in an increase in body mechanical loading, which helps to inhibit bone resorption and increase bone mass and density [23]. In addition, an increased fat mass may be a compensatory mechanism for bone loss, possibly protecting the bone from fracture caused by an increase in bone fragility. The surrounding excess fat mass will help to protect bones from fractures caused by external shocks. Thus, we can postulate that estrogen deficiency causes osteoporosis to more readily occur in SD rats than in Wistar rats; correspondingly, the body has excess fat mass to protect fragile bones from fracture, which demonstrates a more serious fat metabolism disorder and is a specific compensatory mechanism and concrete manifestation of bone protection. Could the severity of fat metabolism disorders be associated with the occurrence of PMOP?

This study has some limitations; we undertook a preliminary study to look at the potential differences in SD and Wistar rats, but we did not measure the levels of hormones to compare levels between the rats and relate that to the findings. We also did not attempt to investigate other background factors that might have caused the differences between the rats, such as exercise and genetic factors. These and many other questions will have to be investigated in further studies.

In summary, we found that the increase in the fat mass of SD rats was more obvious than that of Wistar rats. Also the occurrence of osteoporosis as measured by BMD of L5-6 occurred more readily in SD rats than in Wistar rats. These results show that the two rat breeds are not equivalent as models for PMOP, and that these differences should be considered when selecting the most suitable rat model. Further research will reveal the reasons for these differences between rat breeds.

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Disclosure of conflict of interest

None.

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