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Oocyte Morphology in a Mouse Model of Collagenase-Induced Osteoarthritis

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Investigation of the effect of collagenase-induced osteoarthritis (CIOA) on mouse oocyte diameter and meiotic spindle defects showed significantly larger size and higher proportion of spindle defects in CIOA oocytes compared to the control group: detached fibers or fully disorganized spindles and microtubule asters at the spindle poles were combined with a larger diameter of CIOA oocytes.

Key words: mouse model, osteoarthritis, oocytes, meiotic spindle, oocyte diameter

Introduction

Although osteoarthritis is thought to be the "wear and tear" disease of the elderly, recent investigations reveal that certain risk factors such as genetics, obesity, sport activities and joint injuries can lead to its development in younger and actively working people. The knee joint as well as many other joints (hip, foot, ankle, toe, elbow, wrist) could be affected at a younger age by repetitive movements, overload or traumas in active and retired athletes [11]. Common sports injuries can be risk factors for post-traumatic osteoarthritis in younger patients [5]. Some of these traumas endanger predominantly young females [4]. The higher risk of cartilage damage and degeneration in these younger individuals is related to repetitive impact and loading of the joint in athletes [2, 1]. Besides sports, the group of activities associated with osteoarthritic symptoms and injuries at a younger age includes arts such as dancing (especially ballet dancers) as well as ice skating [10, 3, 7] and even musicians [13]. Because of this subset of reproductive-age patients, studying the impact of osteoarthritis on oogenesis has not only theoretical but also practical relevance.

Material and Methods

Mouse model of collagenase-induced osteoarthritis was provided by the Institute of Microbiology, Bulgarian Academy of Sciences. The oocytes (193 from CIOA and 209 from control mice) were obtained after standard hormonal ovarian stimulation of the mice by intraperitoneal injection of FSH (follicle stimulating hormone, Meriofert, IBSA Farmaceutici, Italy), 12 IU/animal at day 31 post CIOA induction and hCG (human chorionic gonadotropin, Choriomon, IBSA Farmaceutici, Italy) – 14 IU/mouse, 48 h later. The oocytes were fixed in 2% paraformaldehyde, processed for immunocytochemistry, embedded in polyvinyl alcohol, mounted onto microscopic slides and covered by coverslip. Immunofluorescence was used to visualize the microtubule cytoskeleton (monoclonal mouse anti-α-tubulin antibody and FITC-labelled anti-mouse IgG antibody, Sigma-Aldrich) and chromatin (Hoechst 33258, Sigma-Aldrich). Laser-scanning confocal microscopy of selected images was performed for a precise measurement of the oocytes mounted under coverslips. Their diameter and spindle peculiarities were analyzed for all epifluorescent images. The collected data was analyzed using IBM SPSS Statistics for Windows (Version 27.0, IBM) and p-values under 0.05 were considered statistically significant.

Results

The average diameter of metaphase I and metaphase II (M I and M II) oocytes showed larger oocytes in the CIOA group compared to the controls: 113.28±9.57μm for M I in CIOA versus 105.04±14.30μm for M I in controls ($p<0.01$**) and 111.06±11.60μm for M II in CIOA versus 107.70±15.71μm for M II in controls ($p<0.05$*), shown in **Fig. 1**.

The oocytes of CIOA group had significantly more spindle defects: detached fibers or fully disorganized spindles were more frequent in the CIOA oocytes than in controls (46.76% for CIOA and 12.79% for controls in M I; 54.90% for CIOA and 31.90% for controls in M II), see **Fig. 2**.

CIOA group had twice more oocytes with microtubule asters at their spindle poles than the control group (48.92% for CIOA and 22.09% for controls in M I; 47.06% for CIOA and 17.24% for controls in M II), as shown in **Fig. 3**.

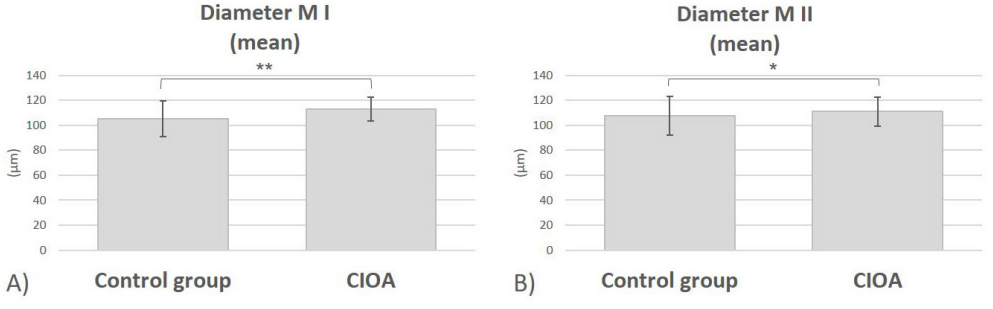

Fig. 1. Oocyte diameter in CIOA and controls: A – M I($p<0.01$**); B – M II oocytes ($p<0.05$*).

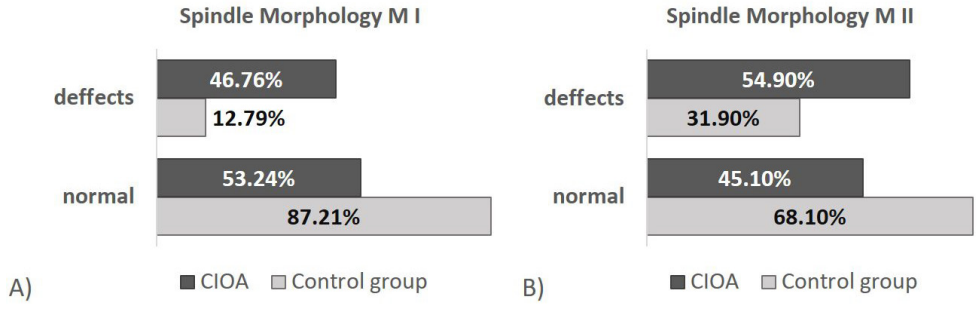

Fig. 2. Spindle normal / abnormal status of M I (A) and M II (B) – comparison of CIOA and controls.

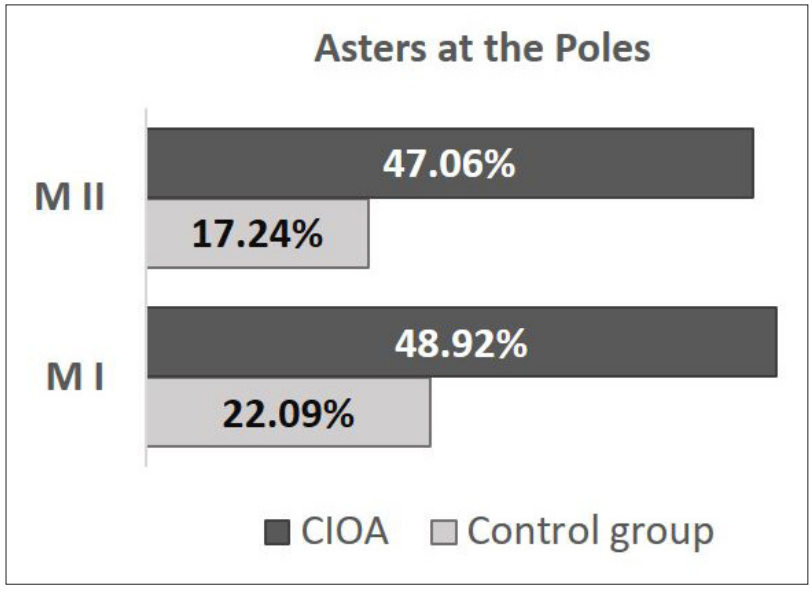

Fig. 3. Asters of microtubules at the spindle poles in M I and M II oocytes – CIOA and controls.

Discussion

Mammalian oogenesis is a complex process sensitive to its microenvironment. Osteoarthritis has recently been associated with systemic inflammation in addition to the local joint effect in both human [8] and the same mouse CIOA model [9], raising the question about its potential impact on oogenesis. In our study, the diameter of CIOA oocytes compared to controls showed an apparent enlargement, more pronounced for M I but statistically significant also for M II. It should be noted that diameters were measured when the oocytes were already under a coverslip, leading to a possibility that

any increase in observed size could be due to decreased mechanical properties of cells. The diameters of the controls were slightly larger but comparable to those reported for oocytes of outbred mice, about 100 μm [6]. Because CIOA oocytes had an increased diameter under the same coverslips, cytoplasmic factors affecting mechanical stability of the cell were supposed. We have found before cytoskeletal abnormalities in CIOA oocytes affecting the actin cap formation and spindle size [12]. These defects could provide an explanation for the supposed mechanical instability as well as the high proportion of abnormal spindles in CIOA oocytes (with detached fibers or fully disorganized, and microtubule asters at the spindle poles).

Conclusion

Osteoarthritis affects the apparent oocyte diameter and meiotic spindle formation: oocytes are significantly larger and spindle defects are significantly more frequent in CIOA mice than in controls.

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