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SERBIAN SOCIETY OF MECHANICS

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Guest Editor: Katica R. (Stevanović) Hedrih

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THEORETICAL AND APPLIED MECHANICS

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Dear Reader,

In March 2009 research team of Project ON155002 *Theoretical and Applied Mechanics of the Rigid and Solid Bodies. Mechanics of Materials (2006-2010)* and Seminar Nonlinear Dynamics - Milutin Mialnkovic, organized a one day Scientific seminar named: **Address to Mechanics: Science, Teaching and Applications**, at Mathematical Institute of SASA. Academician RAS Valery Kozlov Opening lecture. Jubilee of the 80th birthday of Professor V.A.Vujicic was included in the Seminar program and a Booklet of each two-pages abstracts was published.

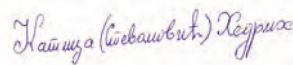
After the seminar participants and other researchers were invited to submit full papers for possible publishing in a special publication titled with the same name **Address to Mechanics: Science, Teaching and Applications**.. All submitted manuscripts for possible publishing in Special publication were evaluated by two reviewers and 19 papers were accepted for publishing.

At meeting on June 20th, 2012, Executing committee of the Serbian Society of Mechanics decided to publish a special publication titled **Address to Mechanics: Science, Teaching and Applications** in the form of **Special Issue** of the Journal **Theoretical and Applied Mechanics** with Guest editor Katica (Stevanovic) Hedrih.

This special issue contains 18 original research papers and one review paper. Research papers are grouped by area of mechanics and research results. The papers are in following areas: general mechanics and theory of stability, nonlinear dynamics, robotics, structural mechanics and damage mechanics, biomechanics, finite element method applications, control of mechanical systems, geodesic on the surface, fluid mechanics, celestial mechanics, computational mechanics, biologically inspired control in robotic systems, .fractional calculus applications in mechanics, active control in mechanical systems, estimation of life of structures, eigen sensitivity of mechanical structural systems, vector position tangent space, vector method based on the mass moment vectors applications, discrete continuum method, method of variation constants, dynamics of systems with frictions and other.

I hope, that this special issue will provoke interest and be a good addition to the quality of the Journal Theoretical and applied mechanics of our Serbian Society of Mechanics,

At Belgrade, October 2012.



Guest Editor
Katica (Stevanovic) Hedrih.

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SERBIAN SCIENTIFIC SOCIETY

THEORETICAL AND APPLIED MECHANICS TEORIJSKA I PRIMENJENA MEHANIKA

Special Issue

ADDRESS TO MECHANICS: SCIENCE, TEACHING AND APPLICATIONS

GUEST EDITOR: KATICA R. (STEVANOVIĆ) HEDRIH

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VIBRATIONAL PROPERTIES CHARACTERIZATION OF MOUSE EMBRYO DURING MICROINJECTION

UDC 519.673:531.01

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Abstract *To determine the vibration characteristics (natural frequencies and mode shapes) of a mouse embryo during microinjection the modal analysis is used. The spherical mouse embryo 60 μm in diameter is modeled as elastic finite elements biostructure consisting of 6 μm thick micromembrane and 38 μm in diameter nucleus. Embryo modeling and modal analysis were based on the use of the finite elements method in the modal analysis system of ANSYS software. The modal analysis was carried out for first six modes of embryo natural frequencies. The numerical analysis of dependence of embryo own frequencies on the boundary conditions and external loads are presented. The relevant illustrations of the typical variations of the shape, deformation and particle velocities of vibrating embryo are given.*

Key words: *modal analysis, vibration properties, mouse embryo, finite elements method.*

1. INTRODUCTION

Although papers on mechanical properties of the oocyte exist (Liu et al, 2010, [1] and on structural parts of mouse embryo (Murayama et al, 2008 [2], 2006) [3], there are very few papers that regard this structure as an oscillatory system Hedrih A. (2011) [4]). Microinjection of the mouse embryo is usually used as an experimental setup for the elastic properties of the biomembrane of the embryo (Murayama et al, 2008 [2], 2006) [3], Sun et al, 2003, [5]). Embryo is placed in a liquid medium –eg HTF (human tubal fluid), in dish. Dish is placed on a heating plate of a special microscope that maintains

the body temperature of the mouse. These are typical conditions (adequate liquid medium and temperature) to keep the embryo alive. Embryo is fixed with vacuum micropipette on one side. On the opposite side is a fine glass micro-needle. See fig.1. This experimental setup could be used for developing software for training embryologists for the special procedure that is used in artificial insemination. This procedure is called intracytoplasmic sperm injection-ICSI (one sperm cell is injected into oocyte by using a fine glass micro-needle).

Embryo vibrational characterization represents very important researching subject of modern biomechanical engineering. (See ref Ladjaly et al, 2011 [6]). A modal analysis is one of possible techniques used to determine the vibration properties (natural frequencies and mode shapes) of a bio structure such as the embryo. Results of modal analysis can also serve as a starting point for another, more detailed, dynamic analysis, such as a transient dynamic analysis in different scenarios, e.g. artificial insemination of human embryo. The natural frequencies and mode shapes are important parameters in the design of a micro-robotic cell manipulation system for dynamic loading conditions [6].

Due to the nature of modal analyses any nonlinearity in material behavior are ignored. Optionally, orthotropic and temperature-dependent material properties may be used. The critical requirement is to define stiffness as well as mass in some form. Stiffness may be specified using isotropic and orthotropic elastic material models (for example, Young's modulus and Poisson's ratio), using hyper-elastic material models (they are linearized to an equivalent combination of initial bulk and shear moduli), or using spring constants, for example. Mass may derive from material density or from remote masses.

The goal activities of researching presented in this paper includes:

- Create robust finite elements model of mouse embryo and basic parts of micro-robotic cell manipulation system (holding pipette, micropipette and liquid environmental medium –human tubal fluid-HTF),
- Set the contacts and boundary conditions that affect the mouse embryo vibrations,
- Run step modal analysis to simulate vibrations of embryo alone and embryo as a part assembly with other components together.
- Determine the vibrational characteristics of mouse embryo free oscillations and embryo oscillations affected by boundary conditions.

Embryo modeling and modal analysis were based on the use of the finite elements method in the modal analysis system of ANSYS WORKBENCH® products.[7]. Parameters for modal analysis were taken from the experimental data from ref [5].

2. THEORY OF MODAL ANALYSIS APPLIED IN FEM

The equations of elastic structural systems without external excitation can be written in the following form:

$$[M]\{\ddot{u}\} + [C]\{\dot{u}\} + [K]\{u\} = \{0\} \quad (1)$$

where is: $[M]$ - structural mass matrix, $[C]$ - structural damping matrix, $[K]$ - structural

stiffness matrix, $\{\ddot{u}\}$ - nodal acceleration vector, $\{\dot{u}\}$ - nodal velocity vector, and $\{u\}$ - nodal displacement vector.

It has been recognized that performing computations in the modal subspace is more efficient than in the full eigen space. The stiffness matrix $[K]$ can be symmetrized by rearranging the asymmetric contributions; that is, the original stiffness matrix $[K]$ can be divided into symmetric and asymmetric parts. By dropping the damping matrix $[C]$ and the asymmetric contributions of $[K]$, the symmetric Block Lanczos eigen value problem is first solved to find real eigen values and the corresponding eigen vectors. In the present implementation, the asymmetric element stiffness matrix is zeroed out for Block Lanczos eigen value extraction. Following is the coordinate transformation used to transform the full eigen problem into modal subspace:

$$\{u\} = [\Phi]\{y\} \quad (2)$$

where is: $[\Phi]$ – eigen vector matrix normalized with respect to the mass matrix $[M]$ and $\{y\}$ - vector of modal coordinates

By using equation (2) in equation (1), we can write the differential equations of motion in the modal subspace as follows:

$$[I]\{\ddot{y}\} + [\Phi]^T [C][\Phi]\{\dot{y}\} + \left([\Lambda^2] + [\Phi]^T [K_{\text{asym}}][\Phi] \right)\{y\} = \{0\} \quad (3)$$

where is: $[\Lambda^2]$ - a diagonal matrix containing the first n eigen frequencies ω_i .

Classically damped systems understand the oscillatory motion of an un-forced N degree of freedom elastic structure with viscous damping and given initial conditions. The modal vectors of classically damped systems depend only on $[M]$ and $[K]$, and are independent of $[C]$, regardless of how heavily the system is damped. For classically damped systems, the modal damping matrix $[\Phi]^T [C][\Phi]$ is a diagonal matrix with the diagonal terms being $2\zeta_i \omega_i$, where ζ_i is the damping ratio of the i -th mode. In general, the damping is not classical, $[\Phi]^T [C][\Phi]$ is not a diagonal matrix, and the natural frequencies, damping ratios, and modal vectors depend on the mass, stiffness, and damping matrices of the structural system. For non-classically damped systems, the modal damping matrix is either symmetric or asymmetric. Asymmetric stiffness contributions of the original stiffness are projected onto the modal subspace to compute the reduced asymmetric modal stiffness matrix $[\Phi]^T [K_{\text{asym}}][\Phi]$.

Introducing the $2n$ -dimensional state variable vector approach, equation (3) can be written in reduced form as follows:

$$[I]\{\dot{z}\} = [D]\{z\} \quad (4)$$

where is:

$$\{z\} = \begin{Bmatrix} \{y\} \\ \{\dot{y}\} \end{Bmatrix} \quad (5)$$

and

$$[D] = \begin{bmatrix} 0 & I \\ -[\Lambda^2] - [\Phi]^T [K_{\text{asym}}] [\Phi] & -[\Phi]^T [C] [\Phi] \end{bmatrix} \quad (6)$$

The $2n$ eigen values of Equation (4) are calculated using the QR algorithm (Press et al., 1993 [8]). The inverse iteration method (Wilkinson and Reinsch, 1971 [9]) is used to calculate the complex modal subspace eigen vectors. The full complex eigen vectors, $\{\psi\}$, of original system is recovered using the following equation:

$$\{\psi\} = [\Phi]\{z\} \quad (7)$$

3. FEM MODELING

In modal analysis the embryo model was considered as three-dimensional axis-symmetric problem. The mouse embryo with basic parts of micro-robotic cell manipulation system described in [9] and shown in Fig. 1 (left) is simplified according the model setup shown in the same figure (right).

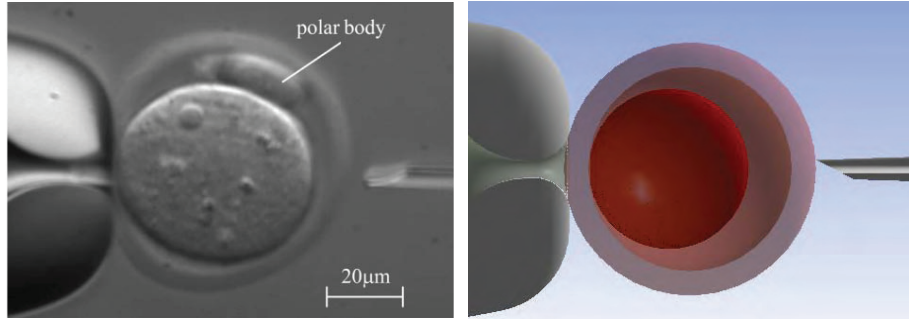


Figure 1. Photograph of cell (left) and simplified model setup of mouse embryo (right).

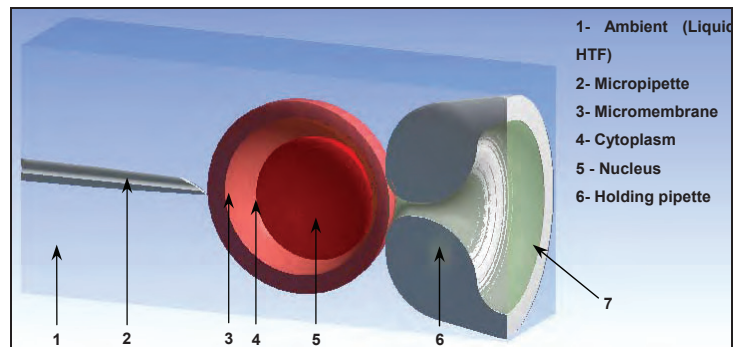


Figure 2. Axial cross-section of 3D model setup for embryo modal analysis.

3.1. Embryo model

The full model setup (Fig. 2) used in the work is consisted of embryo (micromembrane with nucleus and cytoplasm) plunged into the control volume filled with liquid medium HTF. One side of embryo is connected to the holding pipette and the second is in contact with micropipette. For all time the vacuum inside the holding pipette takes the embryo fixed independently on the way of gravity and facilitates embryo manipulation.

The review of model setup parts with used materials and basic physical characteristics is presented in Table 1. The table contains statistic data related to the number of nodes and elements for each component after medium quality meshing procedure (Fig. 3).

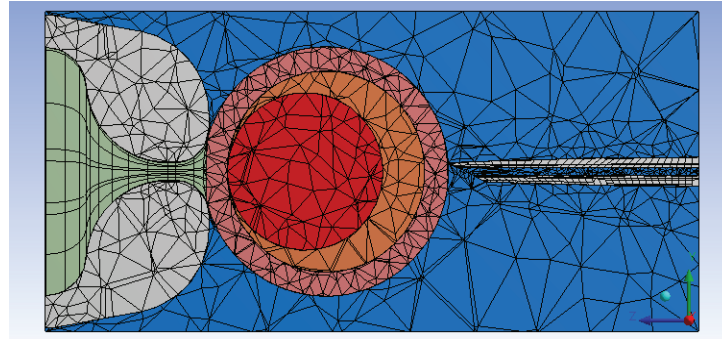


Figure 3. Details of finite elements mesh in the axial cross-section of model.

Table 1. Basic mechanical characteristics of model components with FE statistic data.

	Name	Assignment	Volume	Mass	Nodes	Elements
-	-	-	m ³	kg	-	-
1	Micromembrane	Biomembrane	5.3732E-14	5.4000E-11	7409	4288
2	Nucleus	Nucleus	2.8731E-14	2.9880E-11	685	350
3	Cytoplasm	Cytoplasm	3.0635E-14	3.1033E-11	1287	669
4	Holding pipette	Glass	1.0978E-13	2.7773E-10	3117	1776
5	Micropipette	Glass	1.4847E-15	3.7563E-12	3879	726
6	Vacuum	Air	2.8280E-14	3.4643E-14	1426	276
7	Liquid ambient	HTF	6.9430E-13	7.0333E-10	6953	3825

The initial contact regions and types of supports determine the boundary conditions of the model. All contacts regions of liquid medium HTF with micromembrane, vacuum pipette and micropipette are considered as frictional. For this kind of so-called wet friction the value 0.1 of frictional coefficient is accepted. The identical contact conditions are assumed on the contact surfaces of cytoplasm with nucleus and micromembrane.

From point of view of support boundary conditions, illustrated in Fig. 4, two types: fixed and frictionless supports, are used.

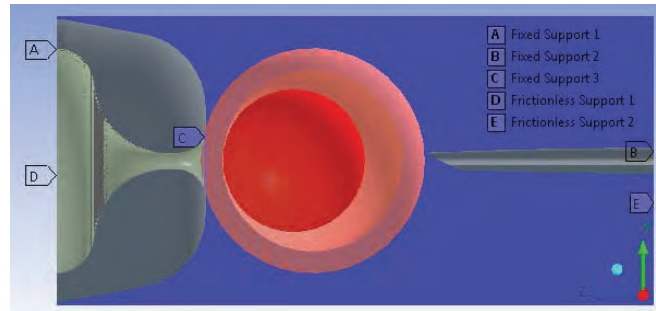


Figure 4. Details of support boundary conditions of model.

The dimensions of boundary box, represented as rectangle surface colored in dark blue in Fig. 4, filled by liquid medium HTF are $1.6\text{E-}4 \times 7.7\text{E-}5 \times 7.7\text{E-}5$ m, and affects significantly the natural frequencies of embryo. All outer free faces of box are bonded by frictionless supports (E). Free surface of vacuum inside the holding pipette is bounded by frictionless support (D). Both the holding pipette and micropipette are constrained (fixed supports A and B) from movement in axial directions (z -axis).

External loads of the embryo include conservative gravity force and surface force produced by 733.1 Pa vacuum on the air-micromembrane contact region. But in the modal analysis external loads make to be equal zero, so that the embryo is connected to holding pipette along initial contact edge (C).

3.2. Material data

According to the requirements of modal analysis, all materials, including bio materials (biomembrane, nucleus and cytoplasm), then medium materials (air and liquid medium HTF) and, finally, mechanical equipments materials (special glass for medical instruments) are considered as isotropic elasticity features materials.

Table 2. Mechanical characteristics of materials

Material	Density	Reference temperature	Young's modulus	Poisson's ratio	Bulk modulus	Shear modulus
-	kg /m ³	K	Pa	-	Pa	Pa
Biomembrane	1005	310	42400	0.499	7.067E+6	14143
Nucleus	1040	310	7200	0.250	4800	2880
Cytoplasm	1013	310	17200	0.490	2.867E+5	5771,8
Liquid HTF	1013	310	1.32E+8	0.490	2.20e+9	4.430E+7
Air (vacuum)	1.225	310	3.102E+6 ²	0.490*	5.17E+7*	1.041E+6*
Glass	2530	310	5.448E+7	0.300	4.54E+7	2.095E+7

² Given mechanical parameters of air represents the fictive values, adapted to solver requirements. It means, instead adiabatic law the linear pressure-volume dependence was assumed for small variations of air pressure up to $2\text{E}+5$ Pa.

The accepted temperature of each part of the model is same and equal to the mice body temperature of 37 °C. As was previously mention this temperature is necessary to keep the embryo alive. Although the temperature is included in the modal analysis, it doesn't take any repercussions on the final results because of the absence of thermal loads or variations of mechanical parameters that would affect the model vibrational behavior.

Mechanical characteristics of the above mentioned materials are given in Table 2.

4. RESULTS AND DISCUSSION

4.1. Natural frequencies of embryo

The numerical integration of Eq. 4 facilitates the solutions for elements of diagonal matrix $[\Lambda]$ containing the first n eigen frequencies ω_i . Computed natural (own) frequencies of embryo are given in Table 7.

Table 3. Natural frequencies of free and bonded embryo for first six modes.

Mode n	Natural frequencies of embryo ω_i , Hz				
	Free oscillations in vacuum	Free oscillations in liquid HTF	Connection with holding pipette in vacuum	Connection with holding pipette in liquid HTF	Full connection in liquid HTF
1	0	52733	2924.2	52778	52782
2	0.0282	52839	2945.7	52882	52886
3	0.0462	53321	5868.6	53486	53491
4	600.32	54242	11888	54315	54317
5	931.50	55083	19333	55113	55116
6	940.79	55112	19353	55177	55180

The modal distribution of natural frequencies of embryo vs. boundary conditions (Figs. 5 and 6) was designed based on tabular data.

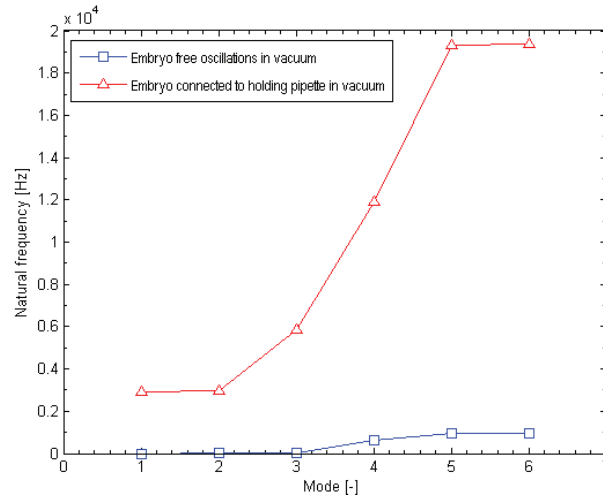


Figure 5. Modal distribution of natural frequencies of embryo vs. boundary conditions (oscillations in vacuum).

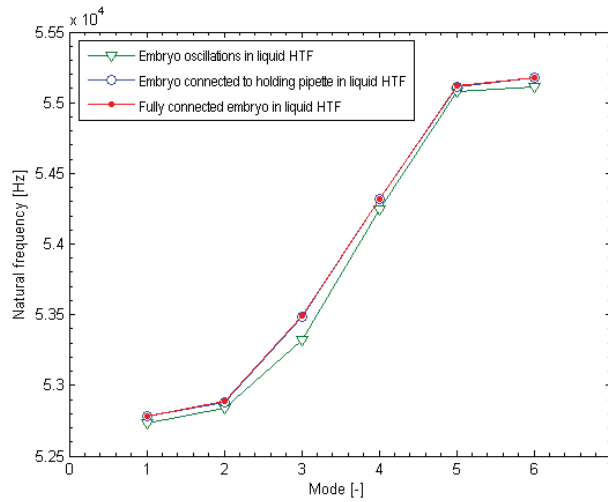


Figure 6. Modal distribution of natural frequencies of embryo vs. boundary conditions (oscillations in liquid medium HTF).

Analysis of calculated results in Table 7 and represented in Figs. 5 and 6 confirms nature of boundary conditions influence on the natural frequency of embryo. In other words, the natural frequency of embryo increases continually by involving each further boundary condition. So, in the case of contact of the embryo and liquid medium HTF the highest jump of frequency (over 52 KHz) appears and the relevant curves of frequency distribution are very close to each other (Fig. 6). Maximum frequency of 55180 Hz was reached for the embryo plunged into liquid medium and connected to micropipette and

vacuum, holding pipette. Besides the above mentioned, the computed results show that oscillations of free embryo in first mode are not practically possible ($\omega_i \approx 0$).

4.2. Typical variations of the vibrating embryo structural parameters

The appearance of scaled shape and fictive velocities distribution for first six modes of natural embryo oscillations are shown in Figs. 7-12.

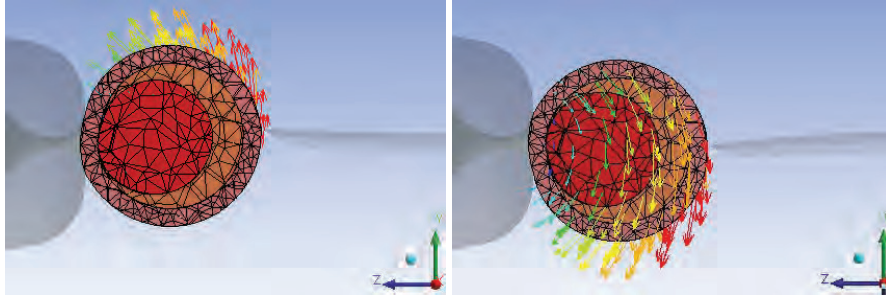


Figure 7. Shape and particle velocities distribution in extreme points of embryo vibrations in mode 1.

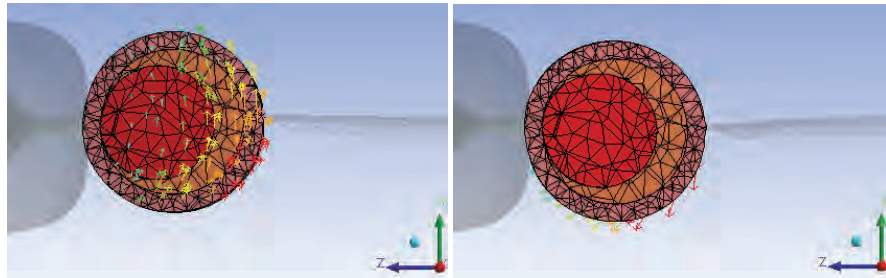


Figure 8. Shape and particle velocities distribution in extreme points of embryo vibrations in mode 2.

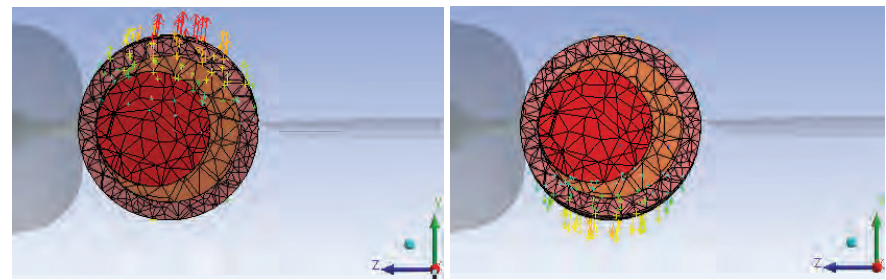


Figure 9. Shape and particle velocities distribution in extreme points of embryo vibrations in mode 3.

Based on Figs. 7 - 12 and performed 3D animations the embryo movement relative to the corresponding mode can be describes as follows:

- Mode 1: perpendicular oscillations along y-axis. Due to initial connections it looks lake rolling in yz-plane;

- Mode 2 - perpendicular oscillations along x -axis. Due to initial connections it looks like rolling in xz -plane;
- Mode 3 - rotation, i.e. torsion (due to initial connections) about z -axis;
- Mode 4 - longitudinal oscillations along z -axis;
- Mode 5 - rotation in yz -plane; and
- Mode 6 - rotation in xz -plane.

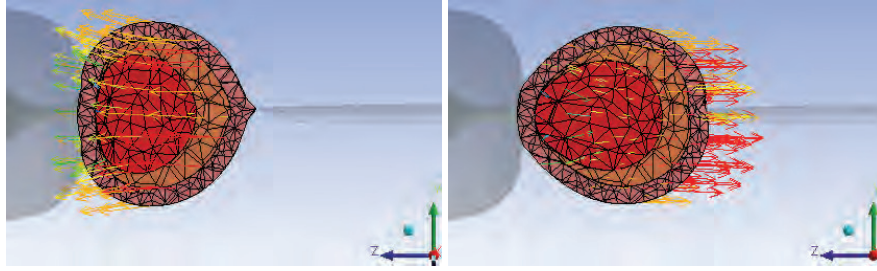


Figure 10. Shape and particle velocities distribution in extreme points of embryo vibrations in mode 4.

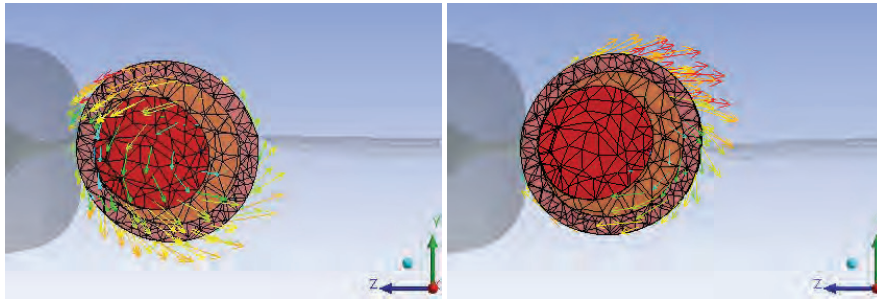


Figure 11. Shape and particle velocities distribution in extreme points of embryo vibrations in mode 5.

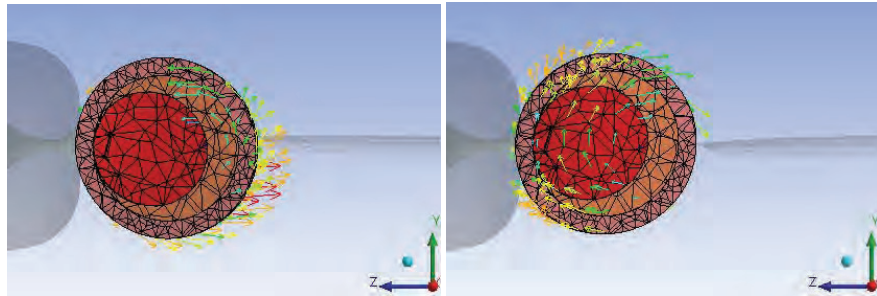


Figure 12. Shape and particle velocities distribution in extreme points of embryo vibrations in mode 6.

Finally, the appearance of scaled shape and fictive deformations for first six modes of natural embryo oscillations are illustrated in Figs. 13-18.

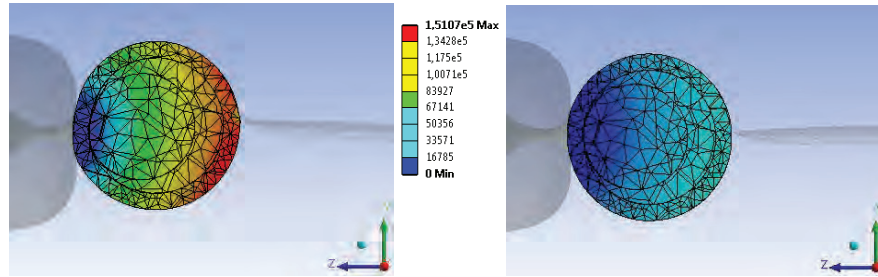


Figure 13. Total deformations in extreme points of embryo oscillations for mode 1 ($\times 1.65\text{E-}11$ m).

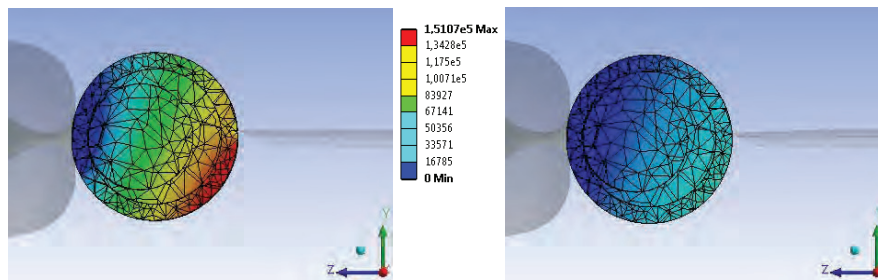


Figure 14. Total deformations in extreme points of embryo oscillations for mode 2 ($\times 1.60\text{E-}11$ m).

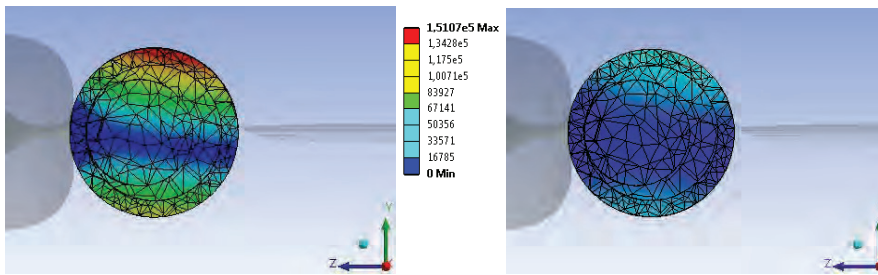


Figure 15. Total deformations in extreme points of embryo oscillations for mode 3 ($\times 1.40\text{E-}11$ m).

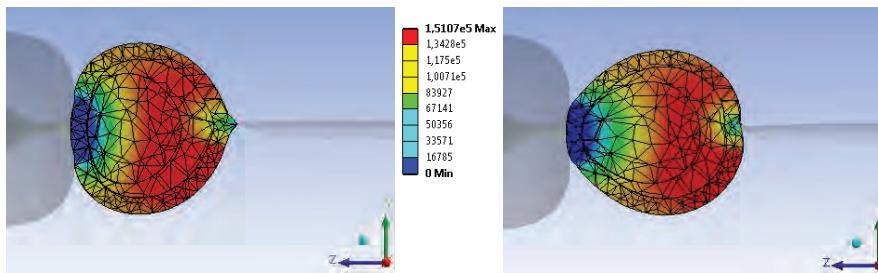


Figure 16. Total deformations in extreme points of embryo oscillations for mode 4 ($\times 2.15\text{E-}11$ m).

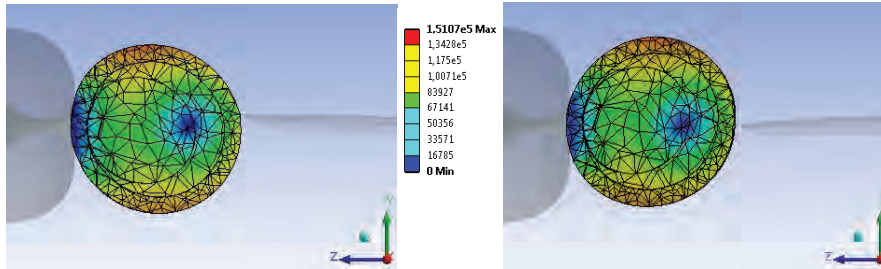


Figure 17. Total deformations in extreme points of embryo oscillations for mode 5 ($\times 1.55\text{E-}11$ m).

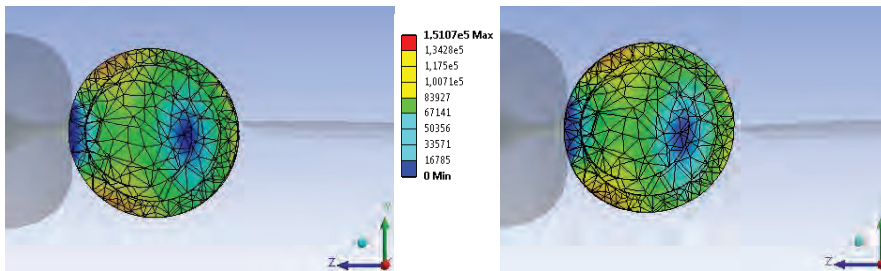


Figure 18. Total deformations in extreme points of embryo oscillations for mode 6 ($\times 1.60\text{E-}11$ m).

Real rate of total deformations are varying from zero up to maximum $3.348 \mu\text{m}$ (Max $1.5107\text{E}+5 \times 2.15\text{E-}11 \text{ m} = 3.348\text{E-}6 \text{ m}$) in mode 4.

Ladjaly *et al*, 2011[6], used the method of finite elements in modelling the Microrobotic Simulator for Assisted Biological Cell Injection, but they regarded the cell as a unified structure. Our model approximates the real phenomenon better as the cell is modelled as a three layer structure (biomembrane, cytoplasm, nucleus). Parameters that were used for the mouse embryo nucleus are approximative since a search of the literature yielded no adequate data on the subject. Data that were available to us refer to nuclei of mouse embryo fibroblasts (Rowat *et al*, 2008 [10]) or to the nucleus of amphibian egg cell (Schaöpe *et al*, 2009 [11]).

5. CONCLUSION

Based on the results of numerical analysis given in the paper it is shown that the robust finite elements model of mouse embryo with basic parts of ICSI system (holding pipette and micropipette) were correctly created. All necessary contacts and boundary conditions were regularly involved facilitating the modal analysis and numerical simulation of all situations of the embryo vibrations. The determinations of the vibrational characteristics of mouse embryo free oscillations and embryo oscillations affected by boundary conditions for first six modes were successfully carried out.

To summarize, the work presented in the paper confirms possibility to use the finite elements method coupled with numerical modal analysis as powerful tool in the

vibrational characterization of bio structures such as the mouse embryo. This method can be used to analyze vibrational properties of embryos of both mice and humans, and not only in physiological conditions, but also under pathological conditions, for example when artificial insemination is unsuccessful, or when the implantation of the embryo does not occur. This opens new possibilities for developing an oscillation theory of reproduction.

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VIBRACIONE KARAKTERISTIKE EMBRIONA MIŠA TOKOM UBODA MIKROIGLOM

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Sažetak. Vibracione karakteristike embriona mogu biti od značaja za procenu njegove vitalnosti. Da bi odredili sopstvene učestalosti i oblike modova oscilovanja embriona miša tokom mikroinjekcije staklenom iglom koristili smo metod konačnih elemenata u okviru modalne analize pomoću ANSYS softvera. Embrion miša dijametra 60 μm modelovan je kao elastična biostruktura sa konačnim brojem elemenata koja se sastoji od biomembrane debljine 6 μm i jedra dijametra 38 μm. U radu je prikazano prvih šest modova sopstvenih učestalosti embriona miša kao i zavisnost sopstveih učestaosti embriona od konturnih uslova i spoljašnjeg opterećenja. Tipične varijacije oblika, deformacije i raspodele brzine oscilovanja embriona date su u vidu ilustracija

Ključne reči: modalna analiza, vibracione karakteristike, embrion miša, metod konačnih elemenata.

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