REFRACTIVE INDEX DETERMINATION OF CANINE OOCYTES USING MATRIX-OPTICS

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In this paper we report the results concerning the refractive index of canine oocytes. To determine the refractive index of canine oocytes we have used two theoretical models based on matrix optics. For this we considered the oocyte as a microlens and having a spherical shape. In our work we performed and a microscopic study using an inverted microscope Nikon Eclipse Ti-U in order to determine the oocyte dimensions and to obtain the information regarding the maturity of canine oocytes.

Keywords: canine oocyte, refractive index, matrix optics, single-mode optical fiber

1. Introduction

Knowledge of the cell characteristics is very useful in biology and medicine. Different techniques and methods can be used to characterize properties of human/animal cells. For example, Murayama Y. et al. [1] proposed micro-mechanical measurements towards identification of elastic properties of oocyte cells, but this technique can be invasive for cell. An optical non-invasive method based on the Digital Holographic Microscopy (DHM) is reported by V. Calin et al. [2] in order to evaluate the refractive index of cells.

In the literature the biology of the canine oocyte cell was usually compared with that of other mammalian females but, in contrast to oocytes of most mammals, the canine oocyte is at the germinal vesicle stage at ovulation [3,4,5]. The knowledge of the cumulus-oocyte complex characteristics plays an important role for in vitro fertilization and not only. To our knowledge, the publications have reported results regarding the dimensions, maturity and quality of the oocytes [3÷7]. Only two papers have presented results about the refractive index on human oocytes [7, 8]. Based on the same method of matrix optics, a non-invasive technique, we obtained the refractive index of the canine oocytes.

The article has the following structure: Section 2 is devoted to the study of the theoretical model, Section 3 contains the simulation results and Section 4 presents the conclusions concerning the obtained results.

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2. Theoretical considerations

To determine the refractive index of canine oocytes we used two theoretical models based on matrix optics. In the first case, we considered the oocyte (canine cumulus-oocyte complexe) being a homogeneous sphere. In fig. 1 we present the principle of this theoretical model in which the oocyte can be considered as a microlens [7]. So, the refractive index can be calculated using optical coupling analysis if the oocyte is used as a coupling element between the input and output optical fiber. This theoretical model is based on matrix optics. Thus, the refractive index may be determined using the following relation [6, 9]:

$$X_2 = M \cdot X_1, \tag{1}$$

where $X_1 = \begin{pmatrix} x_1 \\ \alpha_1 \end{pmatrix}$ is the object matrix, $X_2 = \begin{pmatrix} x_2 \\ \alpha_2 \end{pmatrix}$ represents the image matrix, x_1 is the object height, x_2 represents the image height, α_1 and α_2 are the angles given by the numerical aperture ($N.A.$) of the optical fiber (the acceptance angles) and M is a matrix which contain the transfer matrices through the culture medium and oocyte medium and the matrices which describe the phenomenon of light refraction at the separation surface between the culture medium and oocyte.

The angles α_1 and α_2 can be obtained from the numerical aperture of the optical fiber using the relation:

$$N.A. = n \cdot \sin\alpha, \tag{2}$$

where n is the refractive index of the medium outside of the optical fiber and α is the maximum acceptance angle of the fiber.

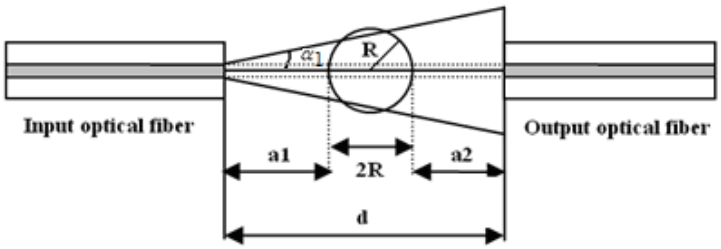

Fig. 1. Homogeneous model of the canine oocyte.

The matrix M was numerically calculated with following relation [6]:

$$M = \begin{pmatrix} 1 & a_2 \\ 0 & 1 \end{pmatrix} \begin{pmatrix} 1 & 0 \\ \frac{n_m - n_o}{R n_m} & \frac{n_o}{n_m} \end{pmatrix} \begin{pmatrix} 1 & 2R \\ 0 & 1 \end{pmatrix} \begin{pmatrix} 1 & 0 \\ \frac{n_m - n_o}{R n_o} & \frac{n_m}{n_o} \end{pmatrix} \begin{pmatrix} 1 & a_1 \\ 0 & 1 \end{pmatrix} \tag{3}$$

where a_1 is the distance between the input optical fiber and oocyte, a_2 represents the distance between the output optical fiber and oocyte, n_m is the refractive

index of the culture medium, n_o represents the refractive index of the oocyte and R is the radius of the oocyte.

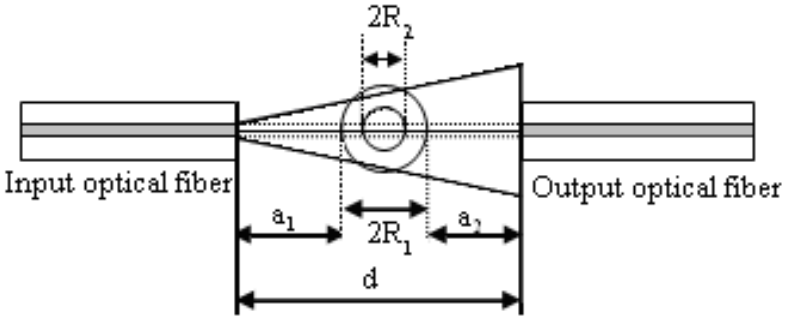

Fig.2. Inhomogeneous model of the canine oocyte.

In the second case, we consider the structured model of the canine oocyte consisting of cytoplasma and zona pellucida (Fig. 2). Thus, we considered the oocyte (canine cumulus-oocyte complexe) being an inhomogeneous sphere. The principle of this theoretical model is presented in Fig. 2. As can be seen from figure, the light passed through an optical system with spherical interfaces. For this system of microlens the optical matrix, M which describes the propagation from the input optical fiber to output optical fiber, is given by:

$$M = \begin{pmatrix} 1 & a_2 \\ 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 0 \\ \dfrac{n_m - n_{zp}}{R_1 n_m} & \dfrac{n_{zp}}{n_m} \end{pmatrix} \cdot \begin{pmatrix} 1 & R_1 - R_2 \\ 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 0 \\ \dfrac{n_{zp} - n_c}{R_2 n_{zp}} & \dfrac{n_c}{n_{zp}} \end{pmatrix} \cdot \begin{pmatrix} 1 & 2R_2 \\ 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 0 \\ \dfrac{n_{zp} - n_c}{R_2 n_c} & \dfrac{n_{zp}}{n_c} \end{pmatrix} \cdot \begin{pmatrix} 1 & R_1 - R_2 \\ 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 0 \\ \dfrac{n_m - n_{zp}}{R_1 n_{zp}} & \dfrac{n_m}{n_{zp}} \end{pmatrix} \cdot \begin{pmatrix} 1 & a_1 \\ 0 & 1 \end{pmatrix} \quad (4)$$

where a_1 is the distance between the input optical fiber and oocyte, a_2 represents the distance between the output optical fiber and oocyte, n_m is the refractive index of the culture medium, n_{zp} represents the refractive index of the zona pellucida, n_c represents the refractive index of the cytoplasma, R_1 is the radius of the oocyte and R_2 is the cytoplasma radius.

3. Simulation results

In our case, we considered a SM600 optical fiber with core diameter 4.2 μm and numerical aperture 0.12. To determine the refractive index of canine oocytes we considered the distance between input and output optical fiber being

500 μm. Also, to calculate the distance between the input/output optical fiber and oocyte we supposed that the oocyte is positioned halfway between input and output optical fibers. The culture medium was the PBS solution (phosphate buffered saline) with $n_m = 1.333$ refractive index value.

By means of an inverted microscope Nikon Eclipse Ti-U we have measured the canine oocyte diameter surrounded by compact cumulus cells. For the investigated canine oocytes we performed several measurements of the diameter. Thus, in the Table 1 and 2 we report an average value of the cytoplasma and canine cumulus-oocyte complex diameter. Also, with this microscope we performed a microscopic study. From the microscopic images we obtained the information regarding the oocyte maturity. All the oocytes investigated from the canine ovaries were at the germinal vesicle stage, so immature oocytes. According to the literature [3,4,5], in contrast to oocytes of most mammals, the canine oocyte is at the germinal vesicle stage at ovulation. In Fig. 3 we report an image with an immature canine oocytes surrounded by compact cumulus cells.

Thus, considering the relationship (1), we have determined the refractive index of canine oocytes. In Table 1 we present the values of refractive index obtained and the average diameter of the canine oocyte for a number of ten canine oocytes.

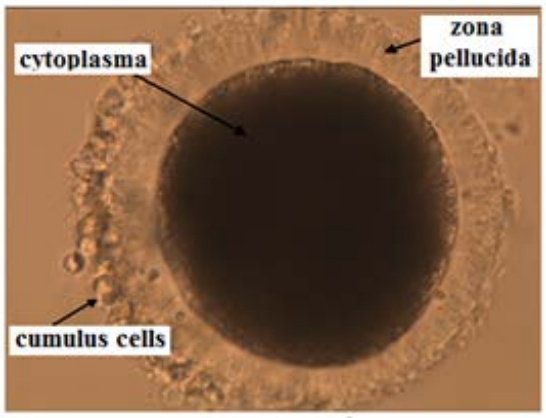

Fig. 3. Microscopic image of a canine oocyte. the structure of an oocyte.

Taking into account of the theoretical considerations presented, the numerical values of the refractive index of studied canine oocytes were determined from Eq. (1). Therefore, we obtained the 1.3462 ± 0.0005 refractive index of canine oocytes in germinal vesicle stage (mean value).

Also, using the relation (4), one can determine, from Eq. (1), the refractive index values for zona pellucida and cytoplasma. The results of simulations are presented in Table 2. For the investigated canine oocytes the mean refractive

index was 1.3168 ± 0.0005 for zona pellucida and 1.3655 ± 0.0008 for cytoplasma.

Table 1

Results of refractive index of canine oocytes obtained using first theoretical model

Canine oocytes	Average diameter of the canine cumulus-oocyte complexes [μm]	Refractive index
1	155.146	1.3447
2	150.733	1.3444
3	171.880	1.3460
4	186.760	1.3471
5	196.612	1.3479
6	184.165	1.3469
7	168.055	1.3457
8	138.573	1.3435
9	172.576	1.3461
10	213.515	1.3492

Table 2

Results of refractive index of canine oocytes obtained using the second theoretical model

Canine oocytes	Average diameter of the canine cumulus-oocyte complexes [μm]	Average diameter of the cytoplasma [μm]	Refractive index of zona pellucida	Refractive index of cytoplasma
1	155.146	114.173	1.3178	1.3674
2	150.733	115.035	1.3181	1.3679
3	171.880	129.560	1.3189	1.3679
4	186.760	115.910	1.3168	1.3652
5	196.612	115.355	1.3165	1.3645
6	184.165	95.510	1.3143	1.3622
7	168.055	108.513	1.3166	1.3655
8	138.573	108.498	1.3179	1.3681
9	172.576	111.796	1.3168	1.3656
10	213.515	99.580	1.3139	1.3610

4. Conclusions

Based on two theoretical models we determined the refractive index of canine immature oocytes. In the first case, the oocyte (canine cumulus-oocyte complexe) was considered being a homogeneous sphere. By means of this model we obtained the 1.3462 ± 0.0005 refractive index of canine oocytes in germinal

vesicle stage (mean value). In the second case, we consider the structured model of the canine oocyte consisting of cytoplasma and zona pellucida. In this case the oocyte was considered an inhomogeneous sphere. Taking into account by the second model we obtained the 1.3168 ± 0.0005 refractive index for zona pellucida and the 1.3655 ± 0.0008 refractive index for cytoplasma. The cytoplasma had a higher refractive index than the zona pellucida.

In conclusion, the characteristics obtained through these theoretical models may be used for in vitro fertilization.

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