LAB-ON-A-CHIP SPECTROPHOTOMETRIC “FIELD OF QUALITY” ASSESSMENT OF DOG OOCYTES

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ABSTRACT
In this paper we describe a new parametric “Field Of Quality” (FOQ) method for quality assessment of oocytes of dogs based on Lab-On-a-Chip (LOC) technique. FOQ method is based on spectrophotometric analysis of single cell and determination of two new factors AR and APP, what ensures unequivocal parametric classification of a single oocyte. In order to enable spectrophotometric analyse of the cell we developed a new lab-chip with measurement cell fitting to the oocyte diameter and with a trap for immobilization oocyte during measurement.

KEYWORDS: lab-on-a-chip, dog oocyte, spectrophotometric measurements

INTRODUCTION
Modern methods of assisted reproduction are widely used in breeding of large animals (cattle, pigs). An important aspect, proving the importance of research aimed at understanding the reproductive biology of dogs is to develop assisted reproductive technology of this species. However, applicability of these methods in case of dogs is limited. One of the reason is low ability of dog oocytes to complete the maturation process in the laboratory [1]. Current method of quality assessment is based on morphological description of the cells. The qualification is based on observation of the oocytes carried out under an optical microscope which is very subjective method. Dog oocytes compared to the other species of animals (e.g. pigs, cows) are characterized with a dark coloured cytoplasm, which makes it difficult determine the quality of cells by microscopic observation. Also, current assessment of morphological classes of the oocytes is insufficient, resulting in a small number of cells that matured and fertilization in vitro. Therefore, there is a need to develop new parametric method for classification and evaluation of the quality of female reproductive cells.

It has been proofed that optical method for assessment of reproductive material by FOQ is a non-invasive and enables definition of quality of a examined cell in a parametric way [2]. Also, the microfluidic technique allows to build a lab-on-a-chip based systems with size of microchannels similar to the size of characterized cell. The new FOQ method is combination of optical characterization and lab-on-a-chip technique.

EXPERIMENTAL
The LOC consists of silicon-glass sandwich with two glass optical fibers (Figure 1). The microfluidic channels and channels for optical fibers (120 µm – depth ) have been etched simultaneously in DRIE (Deep Reactive Ion Etching) process in the monocrystalline silicon wafer 380 µm thick. After this, a thermal silicon oxide 0,3 µm - thick is formed. ext, the wafer is anodically bonded (450 °C, 1,5 kV) to the borosilicate Borofloat (Schoot, Germany) glass with inlet and outlet via - holes. Following, optical fibers 125 µm (Ocean Optics, USA) with 100 µm core are mounted. They are perfectly aligned each to other thanking to high precision of DRIE etching. Fibers are fixed by use of UV epoxy hard glue OA 61 of THORLABS. Ends of both fibers are finished with standard SMA 905 connectors.

The measurement cell in LOC has also a trap which immobilizes the oocyte during measurement (Figure 2). The dimensions of the trap are adjusted to an average size of dog oocyte – about 120 µm (Figure 3).

The instrumentation for LOC spectrophotometric measurements consists of VIS/ IR halogen lamp (HL-2000, Ocean Optics), developed by us LOC, miniature spectrometer (USB4000Ocean Optics) and PC with original Ocean Optics soft-
ware (Figure 4). During measurement, single oocyte is introduced into the chip first by sterile pipette into glass hole and then by it is sucked capillary forces into the measurement cell. The reproductive cell is mechanically immobilized accurately between two optical fibers. Light transmitted from the source by first optical fiber passes through PBS medium and the measured oocyte, is collected by second optical fiber which is connected to the spectrometer. The spectral characteristics are recorded, normalized, and processed under Origin (USA) software. After measurement the oocyte is flushed-back to a sterile transporting container for further operations. An important issue is that dimensions of the instrumentation are small enough to have portable and “point-of-care” device which can be tested in out of laboratory places like farms.

Figure 3: View of the measurement cell

Figure 4: View of the complete instrumentation

FOQ method enables parametric evaluation of the VIS/IR absorbance spectra of a single cell. In this method, values of absorbance for $\lambda=550\text{ nm}$ ($A_{550}$) and $\lambda=850\text{ nm}$ ($A_{850}$) are determined. Then, the Absorbance Ratio (AR) - the ratio of the absorbance at a wavelength of 550 nm to the absorbance at a wavelength of 850 nm - is calculated. Ext, Absorbance Peak Position (APP) - wavelength for maximum absorbance in VIS region – is determined. Finally, these two factors forms FOQ (Figure 5). Average values of APP and AR for single measurement session divide FOQ into four quadrants.

RESULT AND DISCUSSION

In the experiments, 58 dog oocytes coming from 4 classes (according to actual veterinarian classification: class 1-very good, class 4-bad) were investigated (Figure 6). Absorbance spectra were analyzed (Figure 7). As a result of the FOQ analysis characteristic position of a single oocyte in each quadrant was obtained. In 1st quarter very good cells were located, in 3rd bad ones (Figure 8). The result of the FOQ method was correlated with the morphological assessment of oocytes (Figure 9), and allowed parametric (objective) determination of the quality of the oocyte.

Figure 6: Microscope images of examples of dog oocytes quality for 4 standard classes

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CONCLUSION

A novel parametric method FOQ for evaluation of quality of dogs oocytes has been presented here for the first time. It has been shown that FOQ method can be applied to classify dogs oocytes. Morphological differences (in relation to cytoplasmic ultrastructure) between dogs oocytes divided into 4 quality standard classes on the basis of morphological evaluation where observed as different position of the single oocytes in FOQ graph. This result may be the first step toward development of new methodology of classification of mammalian gametes. It seems that oocytes or even embryos can be characterized by this methodology. Especially, it can be useful for improvement of in vitro fertilization of farm animals and small mammals.

ACKNOWLEDGEMENTS

The work was financed by POIG 01.03.01-00-014/08 subproject 2B APOZAR, START grant of Foundation for Polish Science, 2011/03/ / Z4/00305 and 308588040.

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