

CLAIMS

1. A method for assessing the quality of an oocyte or an embryo, the method comprising
 - (a) exposing a test cell selected from the oocyte or an oocyte-associated cumulus cell or the embryo or a cell from the embryo to a fluorescence lifetime imaging microscope (FLIM) to acquire a fluorescence lifetime histogram of auto-fluorescence of endogenous NADH or FAD for the test cell;
 - (b) averaging the fluorescence lifetime histogram of NADH auto-fluorescence or FAD auto-fluorescence or both over the entire test cell, or the cytoplasm of the test cell, or mitochondria of the test cell;
 - (c) comparing the averaged fluorescence lifetime histogram from the test cell to an averaged fluorescence lifetime histogram reference value to determine if the measured averaged fluorescence lifetime histogram from the test cell differs statistically from that of the reference value; and
 - (d) if the averaged fluorescence lifetime histogram from the test cell does not differ statistically from the reference value then selecting the oocyte for *in vitro* fertilization or embryo for implantation and if the averaged fluorescence lifetime histogram from the test cell differs statistically from the reference value then excluding the oocyte from *in vitro* fertilization or embryo from implantation.
2. A method according to Claim 1 comprising the step of establishing the statistical significance by fitting lifetime histograms to a sum of two exponentials and the parameters of the measured cells are deemed to fall within or not fall within the range of parameters found in the healthy cells.
3. A method according to Claim 1 or 2, in which the parameters to be compared between the measurements and the healthy cells are alpha, defined as the ratio of the amplitude of the two exponentials, and beta, defined as the lifetime of the longer exponential.
4. A method according to Claim 1, 2 or 3, in which the maximum value of alpha from the healthy cells is a specific value within the range 1.0 - 4.0 and the corresponding maximum value of beta of healthy cells is in the range 2000 ps - 3000 ps.

5. A method according to any preceding claim, in which the FLIM is performed using a wavelength of about 740 nm in two-photon fluorescence excitation and using an emission bandpass filtered centered around about 460 nm.
6. A method according to any of Claims 1 to 4, in which the FLIM is performed using a wavelength of about 340 nm in one-photon fluorescence excitation and using an emission bandpass filtered centered around about 460 nm.
7. A method according to any preceding claim, in which the FLIM is performed in the time domain.
8. A method according to any of Claims 1 to 6, in which the FUM is performed in the frequency domain.
9. A method according to any of Claims 1 to 4, in which the FLIM is performed using a wavelength of about 900 nm in two-photon fluorescence excitation and using an emission bandpass filtered centered around about 550 nm.
10. A method according to any of Claims 1 to 4, in which the FLIM is performed using a wavelength of about 450 nm in one-photon fluorescence excitation and using an emission bandpass filtered centered around about 550 nm.

CLAIMS

1. A method for assessing the quality of an oocyte or an embryo, the method comprising
 - (a) exposing a test cell selected from the oocyte or an oocyte-associated cumulus cell or the embryo or a cell from the embryo to a fluorescence lifetime imaging microscope (FLIM) to acquire a fluorescence lifetime histogram of auto-fluorescence of endogenous NADH or FAD for the test cell;
 - (b) averaging the fluorescence lifetime histogram of NADH auto-fluorescence or FAD auto-fluorescence or both over the entire test cell, or the cytoplasm of the test cell, or mitochondria of the test cell;
 - (c) comparing the averaged fluorescence lifetime histogram from the test cell to an averaged fluorescence lifetime histogram reference value to determine if the measured averaged fluorescence lifetime histogram from the test cell differs statistically from that of the reference value; and
 - (d) if the averaged fluorescence lifetime histogram from the test cell does not differ statistically from the reference value then selecting the oocyte for *in vitro* fertilization or embryo for implantation and if the averaged fluorescence lifetime histogram from the test cell differs statistically from the reference value then excluding the oocyte from *in vitro* fertilization or embryo from implantation.
2. A method according to Claim 1 comprising the step of establishing the statistical significance by fitting lifetime histograms to a sum of two exponentials.
3. A method according to Claim 2, comprising comparing parameters between the measured cells and the healthy cells, in which the parameters comprise alpha, defined as the ratio of the amplitude of the two exponentials, and beta, defined as the lifetime of the longer exponential, and determining if the parameters of the measured cells fall within or not fall within the range of parameters found in the healthy cells.
4. A method according to Claim 3, in which the maximum value of alpha from the healthy cells is a specific value within the range 1.0 - 4.0 and the corresponding maximum value of beta of healthy cells is in the range 2000 ps - 3000 ps.

5. A method according to any preceding claim, in which the FLIM is performed using a wavelength of about 740 nm in two-photon fluorescence excitation and using an emission bandpass filtered centered around about 460 nm.
6. A method according to any of Claims 1 to 4, in which the FLIM is performed using a wavelength of about 340 nm in one-photon fluorescence excitation and using an emission bandpass filtered centered around about 460 nm.
7. A method according to any preceding claim, in which the FLIM is performed in the time domain.
8. A method according to any of Claims 1 to 6, in which the FUM is performed in the frequency domain.
9. A method according to any of Claims 1 to 4, in which the FLIM is performed using a wavelength of about 900 nm in two-photon fluorescence excitation and using an emission bandpass filtered centered around about 550 nm.
10. A method according to any of Claims 1 to 4, in which the FLIM is performed using a wavelength of about 450 nm in one-photon fluorescence excitation and using an emission bandpass filtered centered around about 550 nm.