Biodynamic imaging of live porcine oocytes, zygotes and blastocysts for viability assessment in assisted reproductive technologies

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Abstract: The success of assisted reproductive technologies relies on accurate assessment of reproductive viability at successive stages of development for oocytes and embryos. The current scoring system used to select good-quality oocytes relies on morphologically observable traits and hence is indirect and subjective. Biodynamic imaging may provide an objective approach to oocyte and embryo assessment by measuring physiologically-relevant dynamics. Biodynamic imaging is a coherence-gated approach to 3D tissue imaging that uses digital holography to perform low-coherence speckle interferometry to capture dynamic light scattering from intracellular motions. The changes in intracellular activity during cumulus oocyte complex maturation, before and after in vitro fertilization, and the subsequent development of the zygote and blastocyst provide a new approach to the assessment of preimplant candidates.

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References and links

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1. Introduction

Assisted reproductive technologies (ART) are powerful tools for the treatment of infertility in human medicine or for the improvement of productivity in animal agriculture [1, 2]. Despite their potential, the efficiency of such technologies is often hindered by the difficulty of selecting the best oocytes for fertilization or identifying the embryos with the highest developmental competence for transfer [3]. Therefore, a noninvasive method that could reliably predict viability is needed. A number of methods have been developed in an effort to evaluate oocyte quality. Traditional methods are based solely on evaluation of morphological characteristics such as compactness of the cumulus investment, color and evenness of the cytoplasm, shape and integrity of the polar body, position and size of the meiotic spindle, and appearance of the zona pellucida [4–8]. These methods provide only crude information with questionable predictive value. The mitochondrial status of the oocytes is sometimes used to evaluate quality. Oocytes have a large number of mitochondria, each containing a single copy of mitochondrial DNA and the machinery to produce ATP for energy supply [9]. Data
indicate that inadequate distribution of mitochondria in the cytoplasm [10, 11], low copy number of mitochondrial DNA [12], deletions in mitochondrial DNA [13], and irregular ATP content [14] are associated with poor oocyte quality. ATP production, and hence mitochondrial function, also can be estimated by measuring oxygen consumption of the oocyte [15]. Unfortunately, these tests are either invasive or complicated and thus have had limited practical value to reliably predict oocyte viability prior to fertilization.

After fertilization and initial development of the embryo, the traditional approach to assess embryo viability is, as with oocytes, morphological evaluation. Each developmental stage has a specific set of characteristics that can be assessed using light microscopy [16]. The method is simple and practical but highly subjective, and therefore it has been unable to indicate the true developmental potential of the embryo [17]. A more complex method of visual evaluation is continuous monitoring of embryos during development using time-lapse imaging [18, 19]. This can be done while the embryo is in the incubator, but it is technically challenging, and it has not been established how the vast amount of information collected can be used to identify the best embryos.

A potential candidate for functional oocyte and embryo assessment is biodynamic imaging (BDI) that uses subcellular motion as an endogenous imaging contrast [20, 21]. It is well suited for the evaluation of oocyte and embryo quality because it captures the functional dynamics of the cells and tissues without exogenous dyes. Biodynamic imaging uses low-coherence Fourier-domain digital holography [22] to collect back-scattered light from living tissue up to 1 mm in thickness. Tissue dynamics spectroscopy [23, 24] separates the motion into frequency bands related to Doppler frequencies of the scattered light. It has been used extensively in applications related to phenotypic drug discovery [23, 25, 26].

In this paper, biodynamic imaging is used for the first time to assess the quality of cumulus-oocyte complexes (COCs) before and after maturation through hormone treatment. The dynamic activity of the matured oocyte is compared with bare oocytes prior to fertilization, and after fertilization. Changes in intracellular activity reflect changes in developmental status of the samples. Developing blastocysts exhibit especially pronounced dynamics associated with cellular proliferation and movement. The theoretical background on the role of active transport in dynamic light scattering is provided in Section 2 of the paper, in which mean transport velocities and persistence times combine to define an effective diffusion constant. Experimental procedures and handling of the delicate COC’s, oocytes and embryos are described in Section 3, as well as a description of the dynamic holography experimental system. Section 4 describes the assessment of COC quality and viability, and Section 5 explores the effects of fertilization on the intracellular dynamics of the oocyte and zygote. Multicellular blastocysts are studied using biodynamic imaging in Section 6, followed by discussion and conclusions in Section 7.

2. Biodynamic imaging of active transport

Random walks in intracellular environments are not limited to thermal Brownian motion, and indeed most intracellular dynamics, especially of macromolecular structures, are driven by active transport with expenditure of ATP. Approximately 10% of the energy budget of a cell goes into active transport [27], primarily driven by molecular motors (kinesin, dynein, myosin, among others), but also by polymerization forces [27]. This intracellular transport is far from thermal equilibrium, and is non-Brownian, even though the statistical properties of active transport may still be captured as a random walk.

A one-dimensional random walk can be described as the sum of uncorrelated steps \( X_i \)

\[
Z = \sum_{i=1}^{N} X_i
\]  

where \( N \) is the number of steps, \( X_i \) is a Gaussian distributed set of random steps, and \( Z \) is evaluated as statistical ensembles. Active transport is defined by velocity distributions and by
persistence times (the time of unidirectional free runs). If the velocity distribution is Gaussian, and the mean free run time (persistence time) distribution is exponential, then the rms step size is given by

\[ X_i^2 = A \int_{-\infty}^{\infty} v^2 \exp(-v^2 / 2v_0^2)dv \int_{0}^{\infty} t^2 \exp(-t / \tau)dt \]

\[ = 2v_0^2 \tau^2 \]

where it is assumed that the mean speed is zero, with a standard deviation given by the characteristic speed \( v_0 \) with a characteristic persistence time \( \tau \). The expectation of the squared displacement of the random walk is

\[ E[Z^2] = N X_i^2 = 2Nv_0^2 \tau^2 \]  

(3)

The mean number of steps taken in a measurement time \( t \) is given by \( N = t / \tau \), which yields

\[ E[Z] = 2Nv_0^2 \tau t = 2Dt \]  

(4)

where the equivalent diffusion coefficient of the actively driven random walk is defined as

\[ D = v_0^2 \tau \]  

(5)

Bare oocytes and zygotes (but not COCs that exhibit multiple light scattering) have a small optical thickness, and backscattering can be approximated through single scattering. For a given intracellular process defined by an rms speed \( v_0 \) and persistence time \( \tau \), the diffusive knee frequency in the fluctuation spectrum is

\[ f_d = \frac{q v_0^2 \lambda_b}{2\pi} \]  

(6)

where \( q \) is the momentum transfer. On the other hand, the Doppler frequency is

\[ f_D = \frac{q v_0}{2\pi} \]  

(7)

When an active transport process has a Doppler frequency smaller than the diffusive knee frequency, the spectral character is Doppler-like. Conversely, when the Doppler frequency is larger than the diffusive knee frequency, the spectral character is diffusion-like. Therefore, the criterion for the spectral behavior is

\[ \frac{q v_0 \lambda_b}{2\pi} > 1 \quad \text{Doppler-like} \]

\[ \frac{q v_0 \lambda_b}{2\pi} < 1 \quad \text{Diffusion-like} \]  

(8)

As an example, speeds of molecular motors along microtubules are on the order of 1 micron/sec and persistence times for free runs are on the order of 1 sec [28]. Active motor and organelle transport produce Doppler frequencies in the range of 3 Hz and diffusion coefficients on the order of 1 m²/sec with the diffusive knee frequency at approximately 60 Hz (in a backscattering geometry with a wavelength of 0.84 microns). Therefore, the Doppler knee frequency is typically smaller than the diffusive knee frequency, and the fluctuation spectrum caused by motor transport has Doppler character at frequencies near the Nqysist frequency. On the other hand, the nucleus has a smaller speed of about 0.1 microns per second, giving a Doppler frequency of 0.3 Hz and a diffusion frequency of 0.6 Hz which mixes the Doppler and diffusive character in the mid frequency range of the experiment. The cytoskeletal and membrane movements are even slower on the order of 10 nm/sec, which places the lowest frequencies solidly in diffusive behavior. It must be kept in mind that these are rough estimates of speeds and frequencies. Dynamic light scattering is a very broad-band form of spectroscopy that captures overall trends in behavior, but tends not to isolate specific

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mechanisms. There is an exception in the case of highly organized motion, such as chromosome separation during anaphase, or cytokinesis, or cellular motion during early development of the embryo when the persistence time can extend significantly to minutes or sometimes hours. In this situation, the light-scattering fluctuation spectrum becomes Doppler-like, and a Doppler “excess” can appear in the fluctuation spectrum.

3. Experimental procedures

3.1 Biodynamic imaging system and tissue dynamics spectroscopy

The experimental configuration for biodynamic imaging is a Mach-Zehnder interferometer. Low coherence light is provided by a Superlum superluminescent diode with a center wavelength of 836 nm, a bandwidth of 53.7 nm and a power of 20 mW. The system setup sketch is shown in Fig. 1. The light is collimated and then divided into two paths. Waveplates and polarizing beam splitters direct most of the incoming light into the object path, and most of the backscattered signal is captured by an EMCCD camera. A lens performs an optical Fourier transform, and a 4-f system transfers the Fourier domain to a CCD screen with a 1/3x magnitude. A delay stage in the reference path is adjusted to match the optical path length of the reference relative to light scattered from specific depths inside the tissue. More details of the biodynamic imaging system have been described elsewhere [9, 21].

Fig. 1. The setup sketch of the biodynamic imaging system.

Two data collecting phases captured the fluctuation spectra of the porcine oocytes using BDI. An assessment data acquisition and analysis phase collected images from a selected oocyte at a frame rate of 40 fps in 25 seconds. The first 10 frames were the non-zero-path-matched background. The following 1000 frames contained the holographic coherence-gated data of which the first 100 frames were ignored to ensure high mechanical stability. After the first acquisition was finished, the data were analyzed, and graphs of intensity fluctuations, spatial contrast and temporal contrast were generated. The 2nd data collection phase on high-quality oocytes lasted for 13 hours and contained successive high and low frame rate collections on each oocyte.

Two experimental systems were used in this study specified as the alpha and the beta systems. For experiments performed on the alpha system 500 successive images were captured at 25 fps with an exposure time of 10 msec. For experiments performed in the beta system the capture rate was 2 fps, the exposure time was 50 msec, and the total images
collected for one oocyte was 200 frames in 100 sec. For data collection the oocytes were placed in an 8-well chamber slide coated with poly-L lysine at 39 °C filled with TCM-199 medium.

Tissue dynamics spectroscopy (TDS) captures a fluctuation spectrum as a function of time as $S(\omega, t)$. The scattering spectrum varies over three orders of magnitude in both frequency and spectral density. To capture subtle changes in the fluctuation spectrum during oocyte development a differential relative spectral density is defined as

$$D(\omega, t) = \frac{S(\omega, t) - S(\omega, t_0)}{S(\omega, t_0)}$$

which is referenced to a baseline spectrum $S(\omega, t_0)$ at the beginning of the experiment.

3.2 Porcine oocytes

The porcine oocytes were harvested from ovaries obtained at a local slaughterhouse either once or twice per week. To prepare for in vitro fertilization and early embryonic development, oocytes removed from ovarian follicles must undergo nuclear and cytoplasmic maturation. During this time they resume and complete the first meiotic division (thus creating a haploid chromosomal set) and become arrested again at the second metaphase stage of meiosis. Cytoplasmic maturation on the other hand enables the oocyte to remodel the nucleus of the fertilizing sperm and turn it into a male pronucleus after fertilization [29]. Oocytes are encased (invested) in a multilayer shell of cumulus granulosa cells. These cells produce compounds that are essential for normal oocyte development, and they transmit crucial maturation signals to the oocyte. Part of the maturation process is the expansion of the cumulus cells, meaning an increase in the thickness of the investment, in response to exposure to luteinizing hormone (LH) and follicle stimulating hormone (FSH). Cumulus cells are capable of undergoing expansion in response to these hormones in vitro in which accumulation of hyaluronan, an extracellular matrix component of cumulus cells, brings about expansion of the cumulus mass [30].

Cumulus-invested pig oocytes were harvested immature and were matured in vitro. Ovaries from slaughtered gilts were transported in a warm environment (28-33°C) to the laboratory. Ovaries were washed in 0.9% NaCl containing 0.075 g/L penicillin and 0.05 g/L streptomycin. Cumulus-oocyte complexes were aspirated from 3 to 6 mm antral follicles using a 10 ml syringe and a 20-gauge needle. For in vitro maturation, cumulus-oocyte complexes were cultured in 500 μl Tissue Culture Medium 199 (TCM-199) supplemented with 0.14% polyvinyl alcohol, 10 ng/ml epidermal growth factor, 0.57mM cysteine, 0.5 IU/ml porcine follicle stimulating hormone (FSH), and 0.5 IU/ml ovine luteinizing hormone (LH) under mineral oil at 39°C for 44 h in a 5% CO2 atmosphere [31].

For fertilization, cumulus-free oocytes were placed into 100 μl of IVF medium modified Tris-buffered medium consisting of 113.1 mM NaCl, 3 mM KCl, 7.5 mM CaCl2 × 2H2O, 20 mM Tris (crystalized free base), 11 mM glucose, 5 mM sodium pyruvate, 0.1% BSA, and 1 mM caffeine [12]. Extended boar sperm was washed, resuspended with IVF medium and added to the oocytes at a concentration of 1 million sperm/ml. The gametes were co-incubated for 5 h. Heat-shocked oocytes, which were used as negative controls, were obtained by maturing the cumulus-oocyte complexes at an elevated temperature of 41 °C.

Examples of porcine cumulus-oocyte complexes (COCs) are shown in Fig. 2. Figure 2(A) is a conventional optical microscope image of an immature COC. The central oocyte is approximately 100 microns in diameter and is surrounded by the vestment of numerous cumulus cells approximately 10 microns in diameter assembled into a layer that is about 100 microns thick. The diameter of the COCs is approximately 300 microns. The immature COC is matured through the procedures described above that leads to a physical expansion of the cumulus shell, as shown in Fig. 2(B).
Fig. 2. Cumulus-oocyte complexes (COCs). A) is an optical micrograph of an immature porcine oocyte. B) is an optical micrograph of a matured porcine oocyte. C) is an optical coherence image (OCI) that is depth-gated to the center of the COC, but no oocyte is recognizable within the fully developed speckle. D) is a motility contrast image (MCI) of the COC midsection, and E) is a volumetric motility contrast image showing the high-mobility core of the complex.

4. COC viability

An optical coherence image (OCI) of a COC is shown in Fig. 2(C) at approximately the midsection of the complex. The OCI image is heavily speckled and shows no discernible oocyte at the center. The speckle character of OCI arises from the full-field coherent illumination, which generates fully-developed speckle that masks visual structure. The speckle diameter is determined by the experimental optical system and is approximately 20 microns. However, the same speckle character of OCI that prevents its use for direct imaging makes it ideally suited to dynamic imaging of dynamic speckle.

The motility contrast image (MCI) in Fig. 2(D) is a two-dimensional motility map [21] at a fixed depth in the pig oocyte. The corresponding 3D volumetric motility map is shown in Fig. 2(E). The MCI images are color coded according to the normalized standard deviation (NSD) of the fluctuating intensity. High NSD values reflect high temporal fluctuations indicated by red, and low values are indicated by blue. In Figs. 2(D) and 2(E) the COC displays a red core corresponding to the oocyte inside the cumulus vestment. Oocytes consume 3x more oxygen than cumulus tissue because of their higher metabolic activity, which is reflected in the higher motility [32,33].

We acquired motility contrast images (MCI) of two groups of COCs: 47 immature and 48 in vitro-matured COCs by incubating with follicle stimulating hormone (FSH) and luteinizing hormone (LH) defined in Section 3. To collect data, pig COCs were placed in an 8-well chamber slide coated with poly-L lysine at 39 °C filled with TCM-199 medium. For each oocyte, 500 successive images were captured at 25 fps with an exposure time of 10 msec. Figure 3 shows motility contrast images of several immature COCs and several matured COCs. During maturation, the cumulus vestment physically expands in size as the cumulus layers become less dense. This leads to irregularly shaped COCs with at least 80% larger...
area. The temporal contrast of the central oocyte is correlated with the temporal contrast of the cumulus shell in Fig. 4(A). There is a wide scatter in the data with a general trend of higher temporal contrast in the core correlating to higher contrast in the shell. Furthermore, stimulated COCs have a higher activity than unstimulated samples. Matured COCs were selected to compare the oocyte relative to the cumulus cell shell, and the data were averaged over 30 COCs. The average NSD value of the oocytes $0.93 \pm 0.002$ is higher than the averaged NSD value of the cumulus cells $0.91 \pm 0.004$ with a p value of 0.003. The change in the temporal contrast of the cumulus shell can provide a measure that distinguishes the matured from the immature samples. The receiver operator characteristic (ROC) curve shown in Fig. 4(B) is based on the temporal contrast of the cumulus shell. The area under the curve is 84% with a sensitivity of 82% and specificity of 71%. The use of a metric based on the cumulus vestment, rather than on the oocyte, as a means to assess the maturation process is because the maturation process has the most dramatic effect on the physical properties of the cumulus vestment. The cumulus cells play an important role in the metabolic nurturing of the oocyte, and hence tracking properties of the cumulus cells is an important indicator of the overall development potential of the COC.

![Cumulus-Oocyte Complexes](image)

**Fig. 3.** Motility contrast image collage of immature and matured COCs showing the expansion of the cumulus investment after maturation. The scale bar is 250 microns.

![Fig. 4](image)

**Fig. 4.** a) Correlation between the temporal contrast of the oocyte relative to the temporal contrast of the cumulus cells for immature and mature COCs. There is a wide scatter in the temporal contrast among the samples, but maturation increases the average temporal contrast of each subset. b) ROC based on the temporal contrast of the cumulus shell.
The fluctuation power spectra of the biological samples carries direct spectral information on the effects of COC development. Average spectra are shown in Fig. 5 that compare the oocyte spectra to the cumulus cell spectra for the immature and the matured COCs. The curve fits to the data are defined by the functional form

\[
S(\omega) = \left[ \frac{N_0}{\left(1 + (\omega/\omega_N)^s\right)^2} + (N_y)^2 \right]^{1/2}
\]

where \(N_0\) is the low-frequency amplitude, \(N_y\) is the Nyquist high-frequency floor, and \(s\) is the slope parameter. The matured oocytes show an increase by a factor of 1.9 in the knee frequency under maturation from \(f_q\) of 0.077 Hz to 0.143 Hz. The increase in the cumulus knee frequency under maturation is a similar factor of 1.8 from \(f_q\) of 0.060 Hz to 0.11 Hz. The slope parameter \(s\) is an additional metric that captures quantitative differences between fluctuation spectra. It is equal to 2 for ideal diffusive behavior, but experimentally it typically takes on values between 1.5 and 1.8. The slope is slightly larger for the cumulus shell compared with the oocyte, and slightly larger for the matured relative to the immature shell. The largest change in slope parameter upon maturation of the COC is for the matured oocyte relative to the immature oocyte. Increases in the slope parameter are caused by more directed motion rather than only random walks.

Prior to in vitro fertilization, the cumulus cells are removed from the matured COC, leaving the bare oocyte to be prepared for the fertilization process. The average power spectrum of bare unfertilized oocytes is compared in Fig. 6 to the average spectrum of cumulus-invested oocytes. The bare oocyte has a lower knee frequency by a factor of 1.4 which may reflect a lower metabolic rate of the bare oocyte. The glucose uptake of oocytes is smaller by a factor of 25 relative to cumulus cell tissue [33]. One of the roles of the cumulus vestment is to nourish the oocyte. With the cumulus vestment matured and stripped away, the oocyte metabolic activity is reduced, which may be the cause of the reduced knee frequency. On the other hand, stripping the outer shell of cells also can remove the shimmering showerglass effect in which overlying active layers contribute to the overall activity observed for deeper layers. The shimmering showerglass effect is difficult to quantify, but these data can place a limit on the effect for the first time in which the knee frequency reduces by a factor of only 1.4 upon stripping of the 100 micron cumulus layer.
5. Fertilized vs. unfertilized oocytes

Oocytes were matured after harvesting from the ovaries, and removal of the cumulus cells was performed before fertilization. The oocytes were divided into two groups. One group remained unfertilized, and the other group went through the fertilization process. However, the in vitro success rate of fertilization is about 40% - 60%, and hence some of the “fertilized” group are not actually fertilized. Both groups were measured by biodynamic imaging. A total of 51 oocytes from the fertilized group and 34 oocytes from the unfertilized group were measured. Examples of the power spectra of fertilized and unfertilized oocytes are shown in Fig. 7(A)). There is a wide variability in the power spectra among individual oocytes, but there is a systematic decrease in the knee frequency by 33% after fertilization, shown in Fig. 7(B). The averaged NSD of the fertilized oocyte group is 0.919 ± 0.001 (standard error), and the averaged NSD of the unfertilized oocyte group is 0.922 ± 0.003.

It is essential, for in vitro assisted reproductive technologies, to be able objectively to select high-quality unfertilized oocytes for fertilization and to be able to select successfully
fertilized oocytes for further culture prior to transfer into the female reproductive tract. Biodynamic imaging properties can be analyzed using principal component analysis (PCA) to distinguish between viable and unviable unfertilized oocytes, as well as between fertilized and unfertilized oocytes. To perform PCA, several quantitative metrics are considered. These are: a) backscatter brightness; 2) spatial speckle contrast; 3) temporal speckle contrast; 4) ellipticity of the denuded oocyte; 5) knee frequency of the power spectrum; and 6) slope parameter of the spectrum.

Changes in backscatter brightness relate to changing structure or densities within the sample. The spatial contrast is the relative standard deviation of the spatial intensity variability. Similar to spatial contrast, temporal contrast is the relative standard deviation of the temporal intensity. The temporal contrast parameter is the average value of the 2-D temporal contrast map of the oocyte. Ellipticity is a traditional way to measure the cellular health of an oocyte. If the oocyte has a round shape, it is more likely to be in a healthy condition, whereas irregularly shaped oocytes are usually discarded. To calculate the ellipticity parameter, the area of the oocyte is defined, and then ellipticity is obtained by calculating the ratio of the major axis and minor axis of the oocyte. The knee frequency and slope parameter are obtained by a fit of Eq. 1.10 to the spectral power density.

To create a clear distinction between viable and unviable oocytes in a PCA analysis, an experiment was performed in which half of the oocytes in a cohort were subjected to mild heat shock (see the heat-shock method in Section 3) that reduces the viability of these oocytes. A total of 37 oocytes from the normal group and 37 oocytes from the heat-shocked group were measured. The PCA results are shown in Fig. 8(A). The red data are the oocytes from the normal group, and the blue data are the oocytes from the heat-shocked group. Most of the normal and heat-shocked samples are separated into separate regions, and the healthy oocytes are more clustered than the heat-shocked ones. Although several from the normal group lie in the heat-shocked region, in any normal cohort there are non-healthy individuals due to unknown reasons. The coefficients of each original property in the first and the second principle components are given in Table 1. The first principal component takes most of its weight from backscatter brightness and temporal contrast (NSD). The second principal component takes most of its weight from the knee frequency as well as the slope parameter and the ellipticity.

Fig. 8. a) PCA on normal and heat-shocked unfertilized oocytes. b) PCA on fertilized and unfertilized oocytes.
Table 1. PCA coefficients for heat shock study

| Original property            | Coefficient on 1st PC | Coefficient on 2nd PC |
|------------------------------|-----------------------|-----------------------|
| 1 Backscatter brightness     | −0.60                 | −0.003                |
| 2 Spatial contrast           | 0.14                  | 0.15                  |
| 3 Temporal contrast          | 0.59                  | 0.28                  |
| 4 Ellipticity                | 0.21                  | 0.49                  |
| 5 Slope of spectrum          | −0.46                 | 0.48                  |
| 6 Knee frequency of spectrum | −0.10                 | 0.65                  |

Table 2. PCA coefficients for unfertilized versus fertilized oocytes

| Original property            | Coefficient on 1st PC | Coefficient on 2nd PC |
|------------------------------|-----------------------|-----------------------|
| 1 Backscatter brightness     | 0.54                  | 0.22                  |
| 2 Spatial contrast           | −0.37                 | 0.31                  |
| 3 Temporal contrast          | −0.10                 | −0.80                 |
| 4 Ellipticity                | −0.41                 | −0.19                 |
| 5 Slope of spectrum          | −0.54                 | 0.05                  |
| 6 Knee frequency of spectrum | 0.32                  | −0.41                 |

A second PCA analysis was performed to assess the fertilization state of the oocyte. The PCA results are shown in Fig. 8(B) where the red data are the 51 oocytes from the fertilized group, and the blue data are the 34 oocytes from the unfertilized group. The fertilized and unfertilized oocytes tend to separate into their own regions in which the unfertilized oocytes are more concentrated than the fertilized data. There are several purported fertilized oocytes lying in the region of the unfertilized samples because these oocytes were not successfully fertilized. To test this possibility, a group of fertilized oocytes were prepared using intracytoplasmic sperm injection for which the oocytes have a high probability of becoming fertilized. These fertilized samples are shown as red crosses that lie in the fertilized region, contrasted with unfertilized samples, denoted as the blue rectangular data that lie in the unfertilized region. There is one fertilized test oocyte that lies in the unfertilized region which may not have been fertilized successfully. The weight for each of the original properties in the first and the second principle components are given in Table 2. The principal components are different for the fertilized/unfertilized PCA analysis relative to the heat shock analysis. For fertilization, the backscatter brightness and slope parameter combine in the first principal component, while temporal contrast plays the dominant role in the second principal component.

6. Multi-cell zygotes and blastocysts

After fertilization, a cascade of cell division begins within 24 hours through first-cleavage into 2-cell zygotes, and then 4-cell zygotes and beyond. Traditionally, in human assisted reproduction, 2 to 3 embryos at the 8-cell stage are transplanted into the uterus three days after fertilization. Motility contrast images of healthy 2-cell and 4-cell zygotes are shown in Fig. 9 compared to healthy single-cell zygotes. The multi-cell zygotes have extremely high temporal contrasts (normalized standard deviations) that exceed unity. These are the highest temporal contrast values that have been observed using motility contrast imaging to date, which may reflect the large energies expended in the early developing embryo.
The blastocyst is an embryo with a blastocoelic cavity and two discernible cell types forming the trophoblast and the inner cell mass. In mammals it appears about five to six days after fertilization depending on the species. Recently a method has been developed which involves the transfer of blastocysts into surrogates, rather than 8-cell zygotes, in an effort to improve the selection of viable embryos and to minimize the complications related to multiple pregnancies. Therefore, the ability to measure blastocysts using biodynamic imaging could be important. Figure 10 shows a preliminary data set of BDI performed on two healthy and one unhealthy blastocyst. The frames at the top of Fig. 10 are successive OCI frames from a movie that shows rapid migration of speckle in the developing blastocysts, but static speckle in the unhealthy sample. In Fig. 10(B) the movie data set is cut along one time axis and one space axis showing the holographic intensity fluctuation at a fixed depth of the blastocyst as a function of position (horizontal axis) and time (vertical axis) over a 100 second scan. The color is on a log scale showing the intensity value. The speckle intensity shows a very interesting pattern which has never before been seen using biodynamic imaging in which bands of speckle intensity “swirl” across the field of view. This pronounced motion may be capturing the organized movement of groups of cells in the developing embryo.
Fig. 10. a) successive OCI images of two healthy blastocysts and one unhealthy blastocyst; b) The intensity change fluctuation over time of the same healthy blastocysts and unhealthy blastocyst; c) the max intensity map of the same healthy blastocysts and unhealthy blastocyst.

9. Discussion and conclusions

The intracellular dynamics of cells and tissues are a central property of all living systems. The wide array of motions that take place during the natural operation of cellular machinery is directly connected to the functioning of that cellular machinery. Therefore, an experimental technique that accesses those motions can probe the functional operation of the living system and can track changes in motions as the cells and tissues develop naturally, or are perturbed by external influences. Developmental biology is an especially dynamic stage of living systems, as rapid changes occur over short times and large distances, and is particularly well suited to study with a dynamics-based technique such as biodynamic imaging. In this paper, biodynamic imaging was applied to successive stages in porcine embryology, beginning with cumulus-oocyte complexes with eggs surrounded and nurtured by the shell of cumulus cells. Maturation of the complexes was followed by removal of the cumulus vestment and subsequent fertilization and division into multicell zygotes and blastocysts.

At each step in the embryogenesis, specific changes in intracellular dynamics takes place as captured by biodynamic imaging. The immature COCs tended to have a lower state of energy and activity compared to matured COCs whose internal molecular machinery becomes primed to accept fertilization by a sperm. An interesting finding of the biodynamic imaging study was the decrease in intracellular activity immediately after fertilization. The early zygote displayed lower knee frequencies and lower slope parameters in the fluctuation power spectra than the prefertilized oocyte. However, leading up to first cleavage and subsequent multicell development, the zygote becomes more active than any other tissue type previously studied using biodynamic imaging. In the blastocyst stage, large-scale organized motion of speckle was observed for the first time. These results of biodynamic imaging, across the range of embryo development from immature to mature COCs, from invested to bare oocytes, from unfertilized oocytes to fertilized zygotes and subsequent cleavage, point to the potential utility of biodynamic imaging for applications in assisted reproductive technologies.

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