Respirometric reserve capacity of cumulus cell mitochondria correlates with oocyte maturity

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Abstract

Purpose Oocyte competence is critical in success of assisted reproduction. Metabolic signaling between oocyte and cumulus cells within the cumulus-oocyte complex procure oocyte development. This study evaluated the relationship between respirometric activity of cumulus cells and maturity of corresponding oocytes.

Methods In prospective cohort study, 20 women of age 28–42 undergoing IVF procedure were involved. To evaluate oocyte maturity, the cumulus cells from individual oocytes were assessed flow cytometrically by double labeling of cells with mitochondria specific dyes. The respirometric stress analysis using ATPase inhibitor oligomycin was applied to assess mitochondria metabolic abnormalities.

Results The cumulus cells from each of 327 oocytes were analyzed. The respirometric index of cumulus cells (O'_R) strongly correlates with maternal ovarian reserve, showing to be higher in patients with higher AMH ($p < 0.0017$). Cumulus cells from immature oocytes had severe mitochondria deficiency, i.e., low O'_R , than those from mature oocytes ($p < 0.02$). No significant difference in respirometric capacity was found between cumulus cells associated with good vs poor-quality embryos.

Conclusions The oocyte maturity is potentially related to the mitochondria activity of cumulus cells.

Keywords Cumulus cells · Oocytes · Energy metabolism · Respiration · Mitochondria membrane potential

Introduction

One of the goals of assisted reproductive medicine is increasing the probability of a successful pregnancy and avoiding later complications from multiple gestations. Typically, only 15% of embryos will result in pregnancy for reasons such as

genetic abnormalities and metabolic problems [1]. Improving the embryo selection process in in vitro fertilization (IVF) procedures, particularly in a non-invasive fashion, is a key to transferring the most competent embryo to result in pregnancy. Yet, the need in biomarkers of oocyte competency is also dictated by increasing delay of women childbearing [2]. Age-related metabolic decline and adverse health conditions, including diabetes and obesity are critical for quality of eggs and reproductive ability of women [3, 4]. Thus, oocyte health is a key factor in fertility and in the development of a competent embryo. Ideally, a set of matching genomic and metabolic markers of oocyte and embryo competence is required to get a solid reference for the capacity of development to a healthy infant. Currently, the egg and embryo grading is mainly based on morphological features and cleavage rates, while quantitative parameters with better prognostic values are required. Unfortunately, most of the attempts in evaluating oocytes quality based on imaging or testing the oocytes environmental medium so far did not provide a satisfactory basis for selection [5].

Importantly, the health of oocytes is dependent on the functional activity of supportive cells, such as granulosa and

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cumulus cells [6]. Bidirectional interaction between cumulus cells and oocytes occurs via hormonal, ionic, and metabolite signaling [7–10]. Recent studies uncovered the management of multiple energetic pathways in the cumulus-oocyte complex (COC) as key for high developmental potential [6]. This reciprocal interaction is very dynamic and undergoes changes over time. At earlier stages of development, cumulus cells protect and support oocyte maturation, ovulation, and fertilization via oocyte signals directing cumulus cells homeostasis and vice versa [11–13]. Later, cumulus cells promote oocytes' aging due to declining cumulus cells processes and activation of apoptosis [14–16]. The metabolic balance in COC is greatly dependent on mitochondria. The important contribution of mitochondria to developmental processes has been demonstrated in many studies [17–21]. The mitochondria gain a central position not only in supply of cells with different energy equivalents, but also in regulation of signaling pathways and metabolite circuitries in both catabolic and anabolic processes [22, 23]. Cellular processes coordinated by mitochondria at the level of metabolic substrates and fluxes via integrated oxidative phosphorylation, glutaminolysis, and β -oxidation [24, 25] are of greater significance than the static cytosolic ATP content as commonly measured [26]. Yet, the mitochondria electron transport system is a major contributor to the complex cell redox signaling and oxidative stress, which are critical for developmental processes [27, 28]. The mitochondria instability in cumulus cells could diminish their role in COC maturation [29] and protection of oocytes against oxidative stress [30]. As a result, it could lead to development of oocyte aneuploidy as an adaptive mechanism against oxidative damage [31]. Thus, mitochondria respiratory dysfunctions both of oocytes and cumulus cells are significant risk factors for reduced fertility, pregnancy loss, and birth defects due to metabolic alterations resulting in abnormal chromosomal content.

The RNA analysis of cumulus and granulosa cells has been widely introduced as a tool to assess oocyte quality [32]. Ideally, the complex of both genomic and metabolic analyses would be required for complete information necessary to validate oocyte and embryo quality. Oocyte-associated cumulus cells are typically discarded during IVF procedures but could provide important information about oocyte developmental potential. In this work, we sought for potential markers of oocyte developmental competence through the evaluation of metabolic competence and more specifically, respirometric characteristics of cumulus cells' mitochondria. The major aim was to determine the measurable metabolic parameters that could provide criteria used to identify healthy oocytes. The ability to measure mitochondrial metabolism in individual cumulus complexes is key to understanding the relationship between metabolism of cumulus cells and oocyte competence and in the context of development of clinically relevant non-invasive assays. In this work, we examined the relationship between maternal

age, ovarian reserve indicator anti-Mullerian hormone (AMH), and corresponding cumulus cells respirometric parameters.

Materials and methods

Patients' recruitment

Twenty enrolled patients were scheduled for IVF at the Main Line Fertility Center (Bryn Mawr, Pennsylvania, USA). Women undergoing ovarian stimulation for IVF were screened for inclusion in this study, and informed consent was obtained from all individual participants included in the study before enrollment. Inclusion criteria included IVF patients between the ages of 28 and 42 years, with an AMH between 1.0 and 10 ng/ml, and a day 3 follicle stimulating hormone ≤ 10 IU/ml, luteinizing hormone < 12 IU/ml, and estradiol < 50 pg/ml on days 2–4 of the menstrual cycle. A body weight ≥ 50 kg and a body mass index between 18 and 32 kg/m² were required. Patients were excluded for smoking, polycystic ovarian disease, endometriosis greater than stage I, utilizing testicular sperm for IVF, and preimplantation genetic testing. This prospective study was approved by Western Institutional Review Board (WIRB).

Ovarian stimulation

Patients underwent the standard of care controlled ovarian stimulation for IVF using Gonal-F RFF Redi-ject pen (EMD Serono, Inc., Darmstadt, Germany). All patients received a fixed protocol of 300 IU Gonal-F daily for the first 4 days of stimulation. Thereafter, a flexible protocol was used to optimize ovarian response. rFSH was adjusted by the patient's physician to 225 to 450 IU daily up to and including day of hCG trigger administration. Cycles were monitored with follicular ultrasound measurements and serum estradiol concentrations throughout controlled ovarian stimulation. Antagonist was used to suppress endogenous pituitary LH for the prevention of premature LH surges by receiving 0.25 mg/day of Cetrorelix Acetate (EMD Serono) when follicle size reached 12 mm and continued up to and including day of hCG trigger. Subcutaneous injection of 250 μ g of human chorionic gonadotropin (hCG) (Ovidrel, EMD Serono) when at least three leading follicle sizes reached a diameter of ≥ 17 mm. The oocyte retrieval procedure was performed 36 h after hCG injection.

Clinical materials and sample preparation

(1) Collection of cumulus-oocyte complexes was performed at the time of oocyte retrieval. Total 327 individual oocytes were analyzed in the study. Cumulus cells were cut from oocytes immediately after the oocyte retrieval procedure as part of routine IVF lab procedure. Collected cumulus cell complexes

(CCCs) were individually placed into microcentrifuge tubes containing 1 ml of HTF-HEPES+5 mg/ml human serum albumin and delivered to the research laboratory. CCCs from individual oocytes were dissociated by rigorous pipetting and vortexing to form a homogeneous cell suspension examined microscopically. The medium was washed out by centrifugation at 1500 rpm for 5 min at room temperature. Then cells were rinsed with respiration buffer containing 137 mM NaCl, 2 mM KCl, 1 mM KH_2PO_4 , 2 mM CaCl_2 , 2 mM MgCl_2 , 20 mM HEPES, and pH 7.4. The final cell pellets were resuspended in respiration buffer supplemented with fluorescent probes.

(2) The maturity of cumulus cells associated each oocytes was assessed on the day after insemination. Oocytes were classified as being mature (metaphase II) or immature (metaphase I or prophase I) or atretic. The criteria of Society for Assisted Reproductive Technologies (SART) were used to grade embryos. Grades A and B embryos were considered good quality embryos, whereas grades C and D embryos were considered poor quality embryos. Oocytes that failed to fertilized were also classified as poor.

Evaluation of mitochondria membrane potential by flow cytometry

The mitochondria oxidative activity was assessed fluorimetrically using BD Accuri C6 flow cytometer (BD Biosciences, San Jose, CA) [33, 34] and staining of cells with two mitochondria specific fluorescent dyes, one sensitive and one insensitive to the membrane polarization. Importantly, the flow cytometer-based measurements enable reading the signal from individual cumulus cells from each corresponding oocyte, rather than from cumulus cells pooled from all oocytes together [33, 35]. This method earlier has been used to validate the linear correlation between fluorescent signal from membrane potential sensitive probe DiOC₆(3) and oxygen uptake measured using Oroboros Oxygraph-2 K [36], as oxidative phosphorylation activity is regulated by the level of polarization of mitochondria membrane.

Cells were labeled simultaneously with MitoTracker Green FM (Ex/Em 490/516 nm) (Life Technologies, Grand Island, NY) and MitoRed (Ex/Em 622/648 nm) (PromoCell GmbH, Heidelberg, Germany) by 70 nM each. The concentration was chosen from the range of 50–130 nM tested for signal saturation and responsiveness to oligomycin. In addition, cell tolerance to mechanical cell processing procedures was tested both on DU145 prostate cancer cells with known behavioral and metabolic characteristics that are routinely used in our lab [37] and cumulus cells from four patients other than those included in the study. The MitoTracker Green is accumulated in mitochondria regardless of their energization degree and enables validating the total mass of mitochondria. The MitoRed was

used to measure mitochondria inner membrane potential. The cumulus cells obtained from each egg were incubated with both probes simultaneously at room temperature for 30 min in the dark. After incubation, cells were washed with respiration buffer, and the final pellets were re-suspended in 200 μl of the same buffer. During measurements, the stained cells were protected from light. Two thousand events were set for each measurement to enable recording quick changes in membrane polarization. After reading the initial signals from both dyes, the respirometric stress analysis was applied using 2 $\mu\text{g}/\text{ml}$ oligomycin, an inhibitor of ATP synthase, typically used in conventional oxygraphic protocol of evaluation of mitochondria oxidative phosphorylation [38]. The MitoRed emission was measured to assess the changes in mitochondria membrane potential. The percent of mitochondria with high membrane potential in each sample was calculated as a ratio of non-perturbed fluorescence signals from membrane potential sensitive dye over the membrane potential insensitive dye [17].

Statistical analysis

Statistical analyses were performed using Prism program version 5.03 for Windows (GraphPad Software, San Diego, USA). Averaged data of cumulus cell mitochondria from individual oocytes are presented as mean $\pm$ S.E.M. Statistically significant differences between data were estimated by unpaired, two-tailed Student's *t* test. Differences were considered significant at $p < 0.05$. The cumulative distribution analysis was applied to evaluate correlation between respirometric and clinical parameters.

Results

Cumulus mitochondria content and membrane polarization

Due to aging dependency of metabolic and oxidative processes, the bioenergetic competence of cumulus cells was first studied with respect to patient age. We considered two groups of patients: <35 years of age ($n = 12$) and >35 years of age ($n = 8$). The patients' demographic characteristics are presented in Table 1.

Figure 1a illustrates that the >35-year-old group of patients tended to have a lower amount of mitochondria, though not statistically significant. However, the percentage of respirometrically active mitochondria, i.e., mitochondria with higher membrane potential, was significantly higher in cumulus cells in the >35-year-old group (Fig. 1b).

Table 1 Demographic characteristics of patients enrolled in the study

Patient	Age	AMH	BMI	No. of retrieved oocytes (mature/immature)	Cause of infertility
1	28	4.72	38.7	21 (19/1)	Tubal
2	31	2.52	25.3	4 (4/0)	Tubal
3	32	2.91	31.2	13 (13/0)	Male factor
4	32	1.96	27.4	22 (20/2)	Tubal
5	32	5.67	25.1	28 (20/7)	Unexplained
6	33	3.04	23.7	20 (20/0)	Ovulatory Dysfunction
7	33	2.31	21.9	15 (11/4)	Unexplained
8	34	5.6	29.8	27 (27/0)	Unexplained
9	34	4.19	19.7	14 (13/1)	Male factor
10	34	3.95	30.5	13 (11/2)	Unexplained
11	35	7.52	22.8	32 (31/1)	Unexplained
12	35	5.4	33.9	15 (11/4)	Tubal
13	37	1.15	31.3	10 (10/0)	Tubal
14	37	3.99	22.9	22 (18/4)	Male factor
15	37	2.63	26.6	16 (16/0)	Male factor
16	37	1.28	22.3	10 (10/0)	Donor sperm
17	38	2.24	32.9	4 (4/0)	Male factor
18	39	1.41	20	16 (16/0)	Ovulatory Dysfunction
19	39	3.17	34	11 (3/8)	Male factor
20	42	8.45	20.1	16 (15/1)	Male factor

Metabolic stress analysis of cumulus mitochondria respiration

Mitochondria generate electrical potential to adjust their respirometric and transport functions. Maintaining mitochondria energization is one of the mechanisms of metabolic control

over processes of biosynthesis, metabolic distribution, and biodegradation. Efficiency of mitochondria oxidative phosphorylation depends on coupling of electron transport with conversion of ADP to ATP. Any alterations in mitochondria membrane integrity resulting in proton leak or activation/suppression of ADP phosphorylation would cause changes in membrane potential that affect oxygen consumption rates. Therefore, perturbations in one of the parameters of mitochondria would reflect functionality of energetic system as a whole [39]. In this work, the cumulus mitochondria respiratory coupling control was assessed via evaluation of the membrane potential based on endogenous substrates under a stress test involving exposure to inhibitor of ADP phosphorylating rate of respiration, i.e., oligomycin. This antibiotic uncouples oxidative phosphorylation blocking ATPase which prevents flow of protons back to mitochondria matrix. Addition of oligomycin causes mitochondria hyperpolarization due to unidirectional electron and proton transports unless the mitochondria are already reached their respirometric capacity threshold or oppositely completely uncoupled, in both cases due to structural and catalytic abnormalities of mitochondria constituents. Therefore, the level of the membrane potential after and before oligomycin addition was used as an indicator of mitochondria respiration capacity. The mitochondrial respiration capacity index can be displayed in terms of quantity

$$O'_R = (\text{Olig}/R - 1) \times 100\%,$$

where Olig is the average membrane potential signal (MitoRed emission value) obtained after the addition of oligomycin, and R is the average membrane potential signal obtained prior to the oligomycin addition. A single oligomycin dose large enough to cause maximal inhibition of ATPase is typically used. The effect of patient age on mitochondrial respiration capacity indicator O'_R is shown in

Fig. 1 Relationship between patient age and mitochondrial mass and the % of respirometrically active cumulus mitochondria. **a** No significant difference in the cumulus cell total mitochondria mass was found between patients <35 and >35 years of age (ns, $p < 0.076$). **b** Patients

>35 years of age had a higher % of energetically active mitochondria. Data presented as mean $\pm$ S.E.M ($n=20$; 12 younger and 8 older patients); *** indicates statistically significant difference corresponding to $p < 0.0001$

Fig. 2. Even though the mean O'_R trended lower in cumulus cells from women > 35 years of age compared to that in cumulus cells from women < 35, no statistical significant difference in O'_R was found.

Age is certainly an important predictor of reproductive success; however, some older patients may actually perform better than some younger ones depending on reproductive health. One of the reproductive parameters that is routinely monitored in clinical IVF practices is the level of anti-Müllerian hormone (AMH) that reflects ovarian reserve and declines with aging [40]. The oocytes retrieved from the same patient with a given AMH level metabolically are never identical. The metabolic parameter (O'_R) of associated cumulus cells is also greatly scattered among oocytes within the same patient (Fig. 3).

It is well-established that the AMH level is usually lower in the older patients. We analyzed the AMH values with respect to age of patients enrolled in the study (Fig. 4a). Although in Fig. 3, the distribution of the metabolic parameter looks random, while data clustering enabled designating an arbitrary cutoff value of AMH for further correlations with cumulus respirometric parameters. The mean AMH was calculated for the < 35 and the > 35 groups. Within the range of 4.15 ± 0.49 and 2.25 ± 0.15 , the cutoff value was selected as 3.5 (Fig. 4a). While respirometric capacity values O'_R are shown to be not dependent on age (Fig. 2), but they strongly correlate with AMH cutoff value (Fig. 4b).

Cumulus cells from patients with AMH > 3.5 ng/ml had a respirometric index O'_R significantly higher than those from patients with AMH < 3.5 ng/ml indicating severe cumulus mitochondria impairment in the latter. Although the data was measured from individual cumulus cells collected from each oocyte (Fig. 3), the final data in Fig. 4 was averaged for generalization purposes.

Effect of cumulus reserve respirometric capacity on oocyte maturity and embryo quality

The respirometric parameters of cumulus cells O'_R were measured blindly and then were compared with clinically obtained grades of oocyte maturity. Among the total 327 oocytes from 20 patients enrolled in this study, 35 oocytes were classified as immature while 292 were mature. The mean respirometric capacities of cumulus cells from their corresponding mature vs immature oocytes were compared. In addition, the respirometric capacity of cumulus cells from corresponding oocytes that resulted in good quality embryos was compared to that in cumulus cells associated with oocytes that failed to fertilize or resulted in poor quality embryos. The oligomycin-based respiration index O'_R for mature oocytes was significantly higher than that of immature oocytes and negatively characterize immature oocytes as having malfunctioning mitochondria (Fig. 5a). At the same time, no difference was found in the

Fig. 2 Effect of patient age on respirometric capacity O'_R . No significant difference in respirometric capacity was found between women < 35 compared to those > 35 years of age; not significant statistical difference with $p < 0.56$

O'_R of cumulus cells from corresponding oocytes that resulted in good vs poor quality embryos and unfertilized oocytes (Fig. 5b).

Discussion

Stability of energy homeostasis is a determinant of normal physiological processes which declines by age resulting in the decrease of reproductive capacity in women. Even at younger ages, the metabolic discoordination and sustained bioenergetic deficit could result in poor quality oocytes. The network of metabolic processes is centered in mitochondria, which serve as a master controllers of different metabolic pathways [41]. Mitochondria are the terminal points of utilization of glycolytic end products, pyruvate and lactate, as well as products of β -oxidation and glutaminolysis converging at

Fig. 3 Distribution of the measured respirometric capacity values of cumulus cells obtained from same patient oocytes. Each dot is the O'_R data of an individual patient with a fixed AMH level (see Table 1).

Fig. 4 Correlation of patients' serum AMH levels with their age and effect of AMH on respirometric capacity O'_R . **a** The AMH level reflecting ovarian reserve negatively correlates with patients' ages as expected. Data are presented as mean $\pm$ S.E.M ($n = 12$ of < 35 -year old and 8 of > 35 -year-old patients), $*p < 0.0172$. **b** Respirometric capacity was higher in cumulus cells from women with AMH > 3.5 ng/ml; $*$ indicates statistically significant difference corresponding to $p < 0.0017$

the Q-junction. Mitochondria are the centers of production of biomass precursors like amino acids and fatty acids. Thus, impaired mitochondria play a significant role in the development of different pathologies, aging, and fertilization failure. It has been shown that the number of mtDNA copies and the amount of ATP decreases in low quality oocytes [42–44]. Importantly, the metabolic processes in oocytes are in close reciprocal coordination with those in surrounded cumulus cells. At different developmental stages, they cross-share the growth and maturation signals and later the signals of aging and degradation. Therefore, respiratory functions of cumulus cells, i.e., mitochondria membrane polarization, oxygen consumption rates, and metabolic substrates wiring, are important characteristics of oocyte microenvironment that support oocyte anabolic processes to complete meiosis, undergo fertilization, and embryogenesis. Different fertilization pathologies are proposed to be associated specifically with mitochondria dysfunctions in cumulus cells [45, 46]. Thus, the cumulus cells, which are often removed from oocyte and discarded

after the oocyte retrieval procedure, could provide a valuable information about the oocyte metabolic background and associated developmental competence.

Previous study on pooled cumulus cells applied mitochondria uncoupler as a stress input and found that cumulus cell-oocyte interactions involving metabolic exchange is associated with number of total and mature oocytes retrieved [17]. In this study, we evaluated the respirometric parameter of mitochondria, the inner membrane potential, which importance has been shown for the fertilization processes [47]. The key advantage of our approach is analysis of individual cumulus cell from each oocyte that allows accounting for the same patient oocytes heterogeneity. Secondly, preloading of cells with fluorescent probes prior to stress analysis enables reading the initial signal with accordance to mitochondria basal energization status. The most important outcome of use of such mitochondria stimuli, like uncoupler [17] and ATPase inhibitor (this work) is the demonstration of applicability of cumulus respirometric stress analysis for testing of oocytes.

Fig. 5 Oligomycin-based indicator of respirometric capacity O'_R . **a** The O'_R of cumulus cells from mature ($n = 292$) vs immature ($n = 35$) eggs. **b** The O'_R of cumulus cells from oocytes that resulted in unfertilized or poor quality embryos ($n = 261$) vs good quality embryos ($n = 66$) quality embryos. Data presented as mean $\pm$ S.E.M.; $*$ indicates statistically significant difference corresponding to $p < 0.02$; not significant statistical difference with $p < 0.69$

Our data suggested that cumulus cells from reproductively aging patients have diminished mitochondria biogenesis. The total mitochondria mass in cumulus cells from the group of patients older than 35 trended lower, though not statistically significant, than those from younger women (Fig. 1a). At the same time, the cumulus mitochondria from these older patients had higher membrane potentials compared to cumulus mitochondria from younger women (Fig. 1b). In aging mitochondria, the higher membrane potential could be an indicator of elevated but energetically inefficient oxygen consumption. For any organs and tissues too high amplitude of mitochondria polarization at cell resting, unstimulated state is a harmful situation associated with high risk of excessive ROS production [48, 49]. Thus, the higher level of basal mitochondria membrane potential of mitochondria from older women is consistent with the compromised functionality of mitochondria, whose workload could be approaching the threshold capacity. A similar situation may be relevant for cumulus cells of younger women with very low ovarian reserve. Therefore, along with age, we compared the respirometric data with AMH values.

To evaluate mitochondria membrane potential, we employed an approach that has been well-established in conventional respirometry to assess the defects of respiratory machinery by challenging cells with oligomycin, an inhibitor of ATP production [50]. The parameter O'_R reflects the changes in membrane potential upon turning off the proton transporting element of oxidative phosphorylation. The underlying causes are pathological changes of reproductive system which could significantly decrease fertility potential of even younger patients. There was no statistically significant effect of maternal age on O'_R (Fig. 2a). However, there was a significant effect of AMH on this parameter. From the grouping of patients with accordance to their AMH values, we found similar mitochondria changes within the range of 3–3.5 ng/ml AMH (Fig. 4a). In cumulus cells of patients with AMH much less than 3.5 ng/ml, addition of oligomycin caused a decrease of membrane potential instead of elevation due to a limited respiratory reserve of their mitochondria. Decline in membrane potential upon inhibition of oxidative phosphorylation occurs due to high rates of mitochondria respiration and hyperpolarization (Fig. 4b). The respirometric performance under substrate non-stimulated condition closer to the upper limit is an indication of unhealthy mitochondria. Overall, our data suggests a possible link between ovarian reserve and energy homeostasis of oocyte-associated cumulus cells.

Earlier studies have shown that in older patients, the incidence of cumulus cells apoptosis is higher, and this factor can be used as a predictive factor of age-related decline in oocytes quality [15, 16, 51]. In our experiments, the elevated membrane potential of cumulus mitochondria in older patients was not statistically significant. However, the higher membrane potential of cumulus mitochondria in patients with AMH less

than 3.5 ng/ml may be associated with accelerated apoptosis associated with lower ovarian reserve because programmed cell death is an energy consuming process.

The O'_R of cumulus cells from mature oocytes was significantly higher than that from immature oocytes. Therefore, a relationship was found between the mitochondrial energetics of cumulus cells and oocyte maturity. At the same time, we did not find that the O'_R of cumulus complexes that resulted in good quality embryos was significantly higher than that in cumulus complexes that were associated with oocytes that did not fertilize or resulted in poor quality embryos. Perhaps this is because the sperm also affects fertilization and embryo quality, and more data needs to be collected to discover any relationship between cumulus mitochondrial respiration and embryo quality.

The statistical analysis of data using cumulative distribution function (CDF) enabled to further explore the predictive power of the respirometric capacity of cumulus cells for evaluating the maturity of their associated oocytes (see Fig. 4). In Fig. 6a, the distribution of AMH values does not resemble theoretical behavior of a typical CDF, but the distribution of O'_R values does. For the small values of respirometric capacity, the CDF exhibits a lot of variation due to a low number of data available (Fig. 6b). However, starting from zero, the behavior of this parameter approaches a theoretical trend of CDF (dashed circle). A considerable amount of data in a bigger scope of study is still required for complete smooth approximation of cumulative distribution. However, the 20 patient's cohort with cumulus cells from total 327 oocytes studied in this pilot work provided a proof of concept that the respirometric capacity of cumulus cells may have a power of predicting the probability of oocyte metabolic maturity.

These data provide a basis for further larger studies utilizing the translational method described herein to further elucidate the relationship between mitochondrial metabolism of individual cumulus complexes and corresponding oocyte competence and embryo quality.

Study limitations

Due to a small number of patients involved in the study, this data needs to be taken with some caution, even though a sufficient number of oocytes and associated cumulus cells were analyzed. This study does not link the data from individual cumulus cells/oocytes with pregnancy or live birth outcome.

The work aimed to analyze individual oocyte-specific parameters. For that the respirometric capacity of cumulus cells from each oocyte was measured rather than pooled cells. The yield of cumulus cells from individual COC is very small varying from 9000 to 68,000 cells. Technically, this limited number of cells hardly can be processed by several methods concurrently to obtain multiple characteristics. Therefore,

Fig. 6 Cumulative distribution plots comparing distribution of AMH (a) and respirometric capacity O'_R (b) as a function of oocytes maturity. The data on plot b demonstrate the probability of prediction of oocyte maturation based on associated cumulus cells respirometric capacity.

only one metabolic parameter was measured by combining the flow cytometry and respirometric stress protocol. The data of this study provided a proof-of-concept and encourage the larger-scope investigation to be done to account for respirometric and possibly other measures and multiple patient-specific variables.

Conclusions

Our data has shown that the respirometric activity of cumulus cells is potentially related to oocyte maturity. We found that the reserve respirometric capacity of cumulus cells is higher in mature oocytes compared to immature oocytes, but no difference was found with respect to embryo quality. The translational method used in this pilot study to evaluate the respirometric capacity of individual cumulus complexes could be highly valuable to further study the relationship between individual cumulus complexes and their corresponding oocyte competence and resulting embryo quality.

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Compliance with ethical standards

Conflict of interest Michael Glassner is a founding partner and the medical director at the Main Line Fertility Center. Other authors have no conflicts of interest to declare.

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