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Title:

Cumulus cell acetyl-CoA metabolism from acetate is associated with maternal age but only partially with oocyte maturity

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40

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43

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Abstract

Objective: To use metabolism of cumulus cells (CCs) to predict oocyte competency.

Design: CC clumps that associate with oocytes are thought to provide the oocytes with growth and signaling factors. Thus, the metabolism of the CCs may influence oocyte function. This was a prospective and blinded cohort study that analyzed 403 individual sets of CC clumps from 36 participants. Thirty-one of the participants had paired oocyte maturity data. CCs were removed from oocytes after oocyte retrieval procedure, transported individually in vials to the research laboratory, incubated with stable isotope labeled substrates for 60 minutes, and analyzed using liquid chromatography-high resolution mass spectrometry (LC-HRMS) for isotopologue enrichment of major metabolic intermediates, including acetyl-CoA derived from the stable isotope labeled substrates.

Results: Mean enrichment of M+2 acetyl-CoA (mean, standard deviation), where M+0 is the unlabeled acetyl-CoA, M+1 contains 1 ^{13}C, M+2 contains 2 ^{13}C atoms, was for glucose (3.6, 7.7), for glutamine (9.4, 6.2), and for acetate (20.7, 13.9). Mean % enrichment of acetyl-CoA from acetate in CCs from women ≤34 (49.06, 12.73) decreased with age compared to CCs from women >34 (43.48, 16.20) ($p=0.0004$, t test). The CCs associated with the immature prophase I oocytes had significantly lower enrichment in M+2 acetyl CoA compared to the CCs associated with the metaphase I and metaphase II oocytes (difference: -6.02, CI: -1.74,-13.79, $p=0.013$). Limitations of this preliminary study include the difficulty in recovery of consistent numbers of CCs across oocytes, and the inability in this study to track oocyte function to the primary endpoint of successful birth.

Conclusion: Acetate metabolism in individual CC clumps was positively correlated with oocyte maturity and decreased with maternal age. These findings indicate that CC metabolism of short chain fatty acids like acetate should be investigated relative to oocyte function and age-related fertility.

73 **Introduction**

74

75 A major barrier in *in vitro* fertilization (IVF) remains that approximately 5% of aspirated human oocytes
76 have the competence after fertilization to implant and develop into a child (1). Oocyte developmental
77 competence decreases as a woman ages, and currently maternal age is the single best predictor of reproductive
78 outcomes in women, highlighting an acute need for non-invasive biomarkers of oocyte competency to increase
79 efficiency, lower costs, and reduce the chances of riskier multiple births (2). This has led to attempts to
80 understand the aging and maturation process of oocytes for both etiologic understanding of fertility and
81 predictive assays for oocyte function.

82 Notably, the metabolism of the oocyte *in vivo* is intimately linked to surrounding cells, such as the
83 oocyte-associated somatic cumulus cells (CCs), which are critical to oocyte function in the ovarian follicle. CCs
84 support oocyte development through the provision of essential nutrients and signaling molecules (3). CCs
85 possess specialized cytoplasmic projections that penetrate through the zona pellucida [shell] and form gap
86 junctions at their tips with the oocyte, generating an elaborate structure called the cumulus-oocyte complex
87 (COC) (4, 5). Within the COC, CCs are thought to metabolize the bulk of the glucose to supply metabolic
88 intermediates to the oocyte, where the COC glucose metabolism is pivotal in determining oocyte developmental
89 competence (6). Because of the metabolic and communication link between the cumulus and the oocyte,
90 glucose availability and metabolism within the cumulus can have a significant impact on oocyte meiotic and
91 developmental competence (7).

92 During the oocyte retrieval procedure after controlled ovarian stimulation for IVF, the oocytes within
93 specialized ovarian follicles, that support the growth and development of oocytes, are harvested. As only the
94 oocyte is used in fertilization, the remainder of the cells and fluid is remnant sample and has been used as a
95 source for non-invasive investigation of follicle function, even by destructive methods. This easy access has led

96 to insightful investigations into the metabolomics of the follicular fluid (8), but relatively few studies have been
97 conducted on the metabolism of the recovered human CCs.

98 In this study we utilized stable isotope tracing coupled with liquid chromatography-mass spectrometry
99 (LC-MS) to quantify the substrate preferences for CC metabolism. This approach for *ex vivo* metabolic studies
100 uses incubation with a stable (non-radioactive) isotope labeled metabolic substrate (e.g. $^{13}C_6$-glucose) and
101 tracking the incorporation of that isotopic label into downstream metabolites (**Figure 1**). With detection by LC-
102 MS, the isotopologues (molecules differing only in their number of isotopes), co-elute on the LC, but are resolved
103 by the MS, allowing separate quantitation of each isotopologue. After performing an adjustment for natural
104 isotope abundance, the resulting isotopologue enrichment gives the relative molar % of the product detected
105 derived from the labeled substrate in the given incubation conditions. Importantly, this isotopologue
106 enrichment is self-normalizing and is not as influenced by the number of CCs in each sample which is highly
107 variable.

108 Based on previous literature that CCs are highly glycolytic, we expected to see utilization of glucose to
109 generate anabolic intermediates, including the central metabolic intermediate acetyl-Coenzyme A (acetyl-CoA).
110 However, initial experiments demonstrated that acetyl-CoA was poorly labeled by glucose, and that acetate,
111 not glucose or glutamine, was the preferred substrate to generate the central metabolic intermediate acetyl-
112 CoA in the recovered CCs. Little is known about the metabolism of short chain fatty acids like acetate in the CCs.
113 Thus, based on the finding of relatively high acetate usage to generate acetyl-CoA, we examined the association
114 between cumulus cell acetate metabolism to acetyl-CoA, maternal age, and oocyte maturity.

115

116

117 **Materials and Methods**

118

119 **Chemicals**

120 Water, methanol, acetonitrile, and ammonium acetate were Optima LC-MS grade solvents from Fisher
121 Scientific (Pittsburgh, PA). Trichloroacetic acid and salts used in Tyrode's buffer were from Sigma-Aldrich (St.
122 Louis, MO). Stable isotope-labeled substrates were from Cambridge Isotope Laboratories (Tewksbury, MA).
123

124 **Patient Selection**

125 Enrolled patients were scheduled for IVF at the Main Line Fertility Center (Bryn Mawr, Pennsylvania,
126 USA). Women undergoing IVF were screened for inclusion in this study and informed consent was obtained
127 from all individual participants before enrollment. Inclusion criteria included IVF patients between the ages of
128 28 and 42 years, with an anti-mullerian hormone (AMH) between 1.0 and 10 ng/ml, and follicle stimulating
129 hormone $\leq$ 10 IU/ml, luteinizing hormone (LH) < 12 IU/ml, and estradiol < 50 pg/ml on day 2-4 of the menstrual
130 cycle. A body weight $\geq$ 50 kg and a body mass index between 18 and 32 kg/m^2 were required. Patients were
131 excluded for smoking, polycystic ovarian disease, endometriosis greater than Stage I, utilizing testicular sperm
132 for IVF, and preimplantation genetic testing. This prospective study was approved by Western Institutional
133 Review Board (WIRB).
134

135 **Controlled Ovarian Stimulation Protocol**

136 Patients underwent the standardized controlled ovarian stimulation for IVF using Gonal-F RFF Redi-ject
137 pen (EMD Serono, Inc., Darmstadt, Germany). All patients received a fixed protocol of 300 IU recombinant FSH
138 (rFSH) daily for the first four days of stimulation. After, a flexible protocol was used to optimize ovarian
139 response. rFSH was adjusted by the patient's physician to 225 to 450 IU daily up to and including day of human
140 chorionic gonadotropin (hCG) trigger administration. Cycles were monitored with follicular ultrasound
141 measurements and serum estradiol concentrations throughout controlled ovarian stimulation. An antagonist
142 was used to suppress endogenous pituitary LH for the prevention of premature LH surges by administering 0.25
143 mg/day of Cetroelix Acetate (EMD Serono) when follicle size reached 12 mm and continued up to and including

144 day of hCG trigger. Subcutaneous injection of 250 µg of hCG (Ovidrel, EMD Serono) was administered when at
145 least three leading follicle sizes reached a diameter of $\geq$ 17 mm. The oocyte retrieval procedure was performed
146 36 h after hCG injection.
147
148 **Biosample Collection**
149 CCs were removed from cumulus-oocyte complexes immediately after the oocyte retrieval procedure as
150 part of routine IVF lab protocol. Instead of being discarded, CCs were placed into individually labeled micro-
151 centrifuge tubes containing one mL of transport medium, then delivered by medical courier with a 37°C heat
152 pack to a university research laboratory where metabolic tracing and analysis were conducted. A total of 403
153 individual CC clumps and their associated oocytes from 36 patients were included in this study, and 31 patients
154 and their CC clumps were paired with oocyte maturity data.
155
156 **Cumulus Cell Metabolic Tracing**
157 All tracing was conducted by laboratory analysts blinded to any sample characteristics. CC metabolism
158 was traced by centrifugation of the CC clump at 200 x g for 5 min at 25°C, removal of the supernatant, and then
159 addition of the labeling media. For labeling media we used Tyrode's buffer (139 mmol/L NaCl, 3 mmol/L KCl, 17
160 mmol/L $NaHCO_3$, 3 mmol/L $CaCl_2$, and 1 mmol/L $MgCl_2$) pH= 7.4 pre-incubated at 20% O_2 and 5% CO_2 containing
161 as a carbon source either 5 mmol/L [$^{13}C_6$]-glucose or 5 mmol/L glucose in combination with either 1 mmol/L
162 [$^{13}C_2$]-acetate or 100 µmol/L [$^{13}C_5$ $^{15}N_2$]-glutamine. Labeling was conducted by addition of labeling media, a 3
163 sec vortex, and then incubation in a 37°C water bath for 1 hour in 1.5 mL plastic Eppendorf tubes. After
164 completion of labeling incubation, cumulus cells were centrifuged at 1000 x g for 5 min at 4°C, supernatant was
165 removed, and either 1 mL of 80:20 methanol:water pre-chilled to -80°C (for lactate and pyruvate analysis) or 1
166 mL of 10% trichloroacetic acid (w/v) in water (for acyl-CoA analysis) was added. Samples were frozen at -80°C
167 until extraction and analysis. Samples were extracted by thawing at 4°C, probe tip sonication for 15 sec, and

168 then centrifugation at 16,000 x g for 10 min at 4°C. Supernatant was extracted with an Oasis HLB solid phase
169 extraction (SPE) cartridge washed with 1 mL methanol and then equilibrated with 1 mL water. After loading of
170 the supernatant, the SPE column was washed with 1 mL water, then eluted into a 10 mL glass tube with 1 mL
171 methanol with 25mM ammonium acetate. The eluent was evaporated to dryness under nitrogen, resuspended
172 in 50 µL 5% (w/v) 5-sulfosalicylic acid and 10 µL was injected for LC-HRMS analysis.
173

174 **Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS)**

175 Acyl-CoAs were analyzed as previously described in a quantitatively validated method (9) on an Ultimate
176 3000 Quaternary UHPLC coupled to a Q Exactive Plus mass spectrometer operating in the positive ion mode
177 with a heated electrospray ionization probe (mark II) in an IonMax Source housing. Samples were kept in a
178 temperature controlled autosampler at 6°C and LC separation was performed as previously described on a
179 Waters HSS T3 2.7 µm particle size 2.1 x 150 mm column. LC conditions were as follows; column oven
180 temperature 30°C, solvent A water with 5 mM ammonium acetate, solvent B 95:5 acetonitrile: water with 5 mM
181 ammonium acetate, solvent C (wash solvent) 80:20 acetonitrile: water with 0.1% formic acid. The gradient was
182 as follows: 0.2 mL/min flow at 98% A and 2% B for 1.5 min, 80% A 20% B at 5 min, 100% B at 12 min, 0.3 mL/min
183 100% B at 16 min, 0.2 mL/min 100% C at 17 min, held to 21 min, then re-equilibrated at 0.2 mL/min flow at 98%
184 A and 2% B from 22 to 28 min. Flow from 4-18 minutes was diverted to the instrument. Operating conditions
185 on the mass spectrometer were as follows; auxiliary gas 10 arbitrary units (arb), sheath gas 35 arb, sweep gas 2
186 arb, spray voltage 4.5 kV, capillary temperature 425°C, S-lens RF-level 50, aux gas heater temperature 400°C, in-
187 source CID 5 eV. Scan parameters were optimized during the experiments, but final conditions were alternating
188 full scan from 760-1800 *m/z* at 140,000 resolution and data independent acquisition (DIA) looped 3 times with
189 all fragment ions multiplexed at a normalized collision energy (NCE) of 20 at a resolution of 280,000. An isolation
190 width of 7 *m/z* with an offset of 3 *m/z* was used to capture all relevant isotopologues for targeted acyl-CoAs.
191 Data was processed in Xcalibur and or TraceFinder (Thermo).

192

193 **Data analysis**

194 Normalization of isotopologue distribution by natural isotopic abundance was conducted via an open
195 source resource, FluxFix (10), using experimentally derived normal isotopic distributions from cumulus cell
196 clumps labeled with non-isotopically enriched glucose. Due to this adjustment, any sample that had zero signal
197 intensity for M+0 or M+2 was excluded from analysis, since these values would not be interpretable. For
198 isotopologue analysis, summary statistics were calculated in Graph Pad Prism (v7).

199 Metabolic analysis was conducted by investigators blinded to status and identity of all samples/patients,
200 and analysis of correlation with oocyte maturity and age was conducted by an independent biostatistician, who
201 had no role in the design or collection of data, using JMP Pro 13.0 software. Unit of analysis was on each cumulus
202 cell clump sample, since we were primarily interested in the unique metabolism of the cumulus cells associated
203 with individual oocytes.

204

205 **Results**

206

207 *Cumulus cells have a high capacity to derive acetyl-CoA from acetate relative to glucose and glutamine.*

208 Cumulus cells incubated with either [$^{13}C_6$]-glucose, [$^{13}C_5$]-glutamine, [$^{13}C_2$]-acetate for one hour were
209 analyzed for isotopologue enrichment of the central carbon intermediate acetyl-CoA. Isotopologue enrichment,
210 calculated with FluxFix (10), gives the relative molar % of the product detected derived from the labeled
211 substrate in the given incubation conditions. Thus, in this context, the isotopologue notation describes how
212 many labeled carbons are in the analyte of interest corresponding to M+0 as the unlabeled acetyl-CoA, M+1
213 containing one ^{13}C, M+2 containing two ^{13}C atoms. M+2 represents the maximum labeling of the acyl-group (the
214 acetyl group) in acetyl-CoA. Mean isotopologue enrichment of M+2 acetyl-CoA (mean, standard deviation) was
215 for glucose (3.6, 7.7), for glutamine (9.4, 6.2), and for acetate (20.7, 13.9) (**Figure 2**).

216

217 *Enrichment of acetyl-CoA from acetate is decreased in cumulus cells from older women.*

218 Both measures of metabolism and fertility are known to decline with age. To examine if this was true in
219 CCs, we tested if acetyl-CoA enrichment from acetate was different from women ≤34 than >34. This cut-off is
220 based on the distribution of ages within our sample, the age categories reported by the Society for Assisted
221 Reproduction (SART), and the American College of Obstetricians and Gynecologists (ACOG) recognition of age
222 35 as a guideline for increased fertility counseling. Mean % enrichment of acetyl-CoA in CCs from women ≤34
223 (49.06, 12.73) was higher (p=0.0004, t test) than in CCs from women >34 (43.48, 16.20) (**Figure 3**).

224 Although we had pre-specified 34 as a cutoff before statistical analysis, we conducted an exploratory
225 analysis to examine the effect of treating age as a continuous variable. The equation of the regression was
226 estimated at % acetyl-CoA = 54.46 – 0.215(age), with a p-value for the slope coefficient of 0.314. The high
227 variance in acetyl-CoA labeling and the restriction of our sample population to ages 26-42 limit the utility of this
228 analysis.

229

230 *Generation of acetyl-CoA from acetate is associated with mature oocytes.*

231 Based on the discovery that cumulus cells have a high capacity to utilize acetate to generate acetyl-CoA,
232 we were interested if the enrichment in acetyl-CoA from acetate correlated with properties of the associated
233 oocyte, especially oocyte maturity. For 490 individual samples of cumulus cells from 31 patients where oocyte
234 maturity data was available, we tested if M+2 acetyl-CoA enrichment from acetate was different in cumulus
235 cells associated with oocyte maturity. Three types of oocytes are obtained at retrieval – mature metaphase II
236 oocytes, immature metaphase I oocytes (no polar body), and very immature prophase I oocytes (no polar body,
237 contain a germinal vesicle) The cumulus cells associated with the immature prophase I group had significantly
238 lower enrichment in M+2 acetyl CoA compared to the cumulus associated with the other oocytes (difference: -
239 6.02, CI: -1.74,-13.79, p=0.013) (**Figure 4**).

240 Finally, we examined if there was a difference in acetyl-CoA isotopologue enrichment between CCs that
241 were associated with embryos that were selected for transfer to the uterus versus those that were not. CCs that
242 were associated with embryos that were not selected for transfer had no significant difference in isotopologue
243 enrichment (-2.54, CI: 3.29, -8.38, p=0.38). Similarly, comparing SART scoring of embryo quality (Grade A vs
244 other grades) on day 3 of embryo culture, there was no significant difference in isotopologue enrichment (1.46,
245 CI: 4.9 to -1.97, p=0.40). Comparing differences by all embryo grades, there were no significant differences in
246 acetyl-CoA isotopologue enrichment between groups (p=0.51).
247
248 **Discussion**
249
250 Our findings indicate that cumulus cells have a high capacity to derive acetyl-CoA from acetate. This also
251 partially contrasts to previous literature, implicating cumulus cells as highly glycolytic, since labeling was
252 conducted in the presence of 5mM unlabeled glucose for glutamine and acetate tracing. Importantly, this
253 suggests that the role of glycolysis in CCs may not be to feed acetyl-CoA, via conversion to pyruvate, into the
254 Krebs cycle for oxidative phosphorylation or export as citrate to generate cytoplasmic acetyl-CoA. This
255 alternative use of glucose would be in-line with reports that CCs use glucose to generate extra-cellular matrix
256 components or increased products of the hexosamine pathway (11).
257 Strengths of this study include the novelty of applying stable isotope assisted metabolomics to individual
258 oocyte-associate cumulus cell complex metabolism. Studying the *ex vivo* metabolism of human cumulus cells is
259 difficult because of the paucity of recovered cells associated with single oocytes, variability in the numbers of
260 cells, and the effects of endogenous metabolism beyond the control of the experimentalist. With stable isotope
261 tracing, it is possible to establish a substrate/product relationship, in comparison to the metabolomics
262 experiments already conducted that only examined the contents of the follicular fluid with no regard as to their
263 origin. Isotope tracing performed *ex vivo* reduces the major confounder of diet and resulting changes in

264 circulating metabolites. Finally, isotope tracing is "self-normalizing" such that variation in cell numbers are
265 accounted for because each isotopologue is normalized to the metabolite pool within each sample (e.g. acetyl-
266 CoA M+2 is a fraction of total acetyl-CoA in that sample) (10).

267 Although we found a difference in acetyl-CoA labeling by oocyte maturity, a limitation of this study is
268 that we did not follow through data collection to oocyte competence to pregnancy or to baby. Oxygen
269 consumption, a rough gauge of metabolic activity, is an existing quality marker for human oocyte competence
270 conditioned by ovarian stimulation regimens (12). Furthermore, the oxygen consumption rates of embryos have
271 been found to be associated with successful implantation and can be used to select the embryo with the best
272 developmental potential (13). However, this quantitation of metabolic function was a relatively poor predictor,
273 with an odds ratio of 1.037-1.732 (95% CI) for normal vs non-fertilized/abnormal oocytes (12). Future work may
274 be able to improve upon this prediction incorporating more specific substrates in combination with oxygen
275 consumption. The finding of high levels of acetate utilization, and significant glutamine labeling into acetyl-CoA
276 suggests that exploration of other potential substrates may have utility.

277 Our finding of decreased acetyl-CoA generation from acetate in cumulus cells from women >35 is
278 biochemically interesting. The aging process of the oocyte is complex and includes inter-related impaired
279 mitochondrial dysfunction, oxidative stress, diminished metabolic activity, potential accumulation of DNA
280 damage, and activity of several cell-signaling systems (14). Pathological perturbations of the coordinated
281 somatic cell-oocyte interactions by metabolic disease and/or maternal aging can induce molecular damage to
282 the oocyte can alter macromolecules, induce mitochondrial mutations, all of which can harm the oocyte (15). It
283 is also thought that these pathological processes harm the related CCs. Endometriosis may be associated with
284 mitochondrial dysfunction in pooled CCs (16), and subjects with endometriosis may have a defect in CC
285 mitochondrial function, which likely contribute to decreased fertilization and implantation rates (17).
286 Generation of acetyl-CoA is possible from a variety of substrates, but two major sources include glucose and
287 acetate. A switch between glucose and acetate is alternatingly dependent on the enzymes ATP citrate lyase

288 (ACLY) that generates glucose-derived acetyl-CoA from citrate exported from the mitochondria and the Acyl-
289 coenzyme A synthetase short-chain family members that form acetyl-CoA from acetate and CoASH (18). In this
290 study, our exclusion criteria limits the extrapolation of our findings to wider age ranges and pathological settings.
291 Future studies using stable isotope labeling of cumulus cells may be useful in non-invasive study of the metabolic
292 consequences of endometriosis and other diseases, especially in the context of fertility.

293

294

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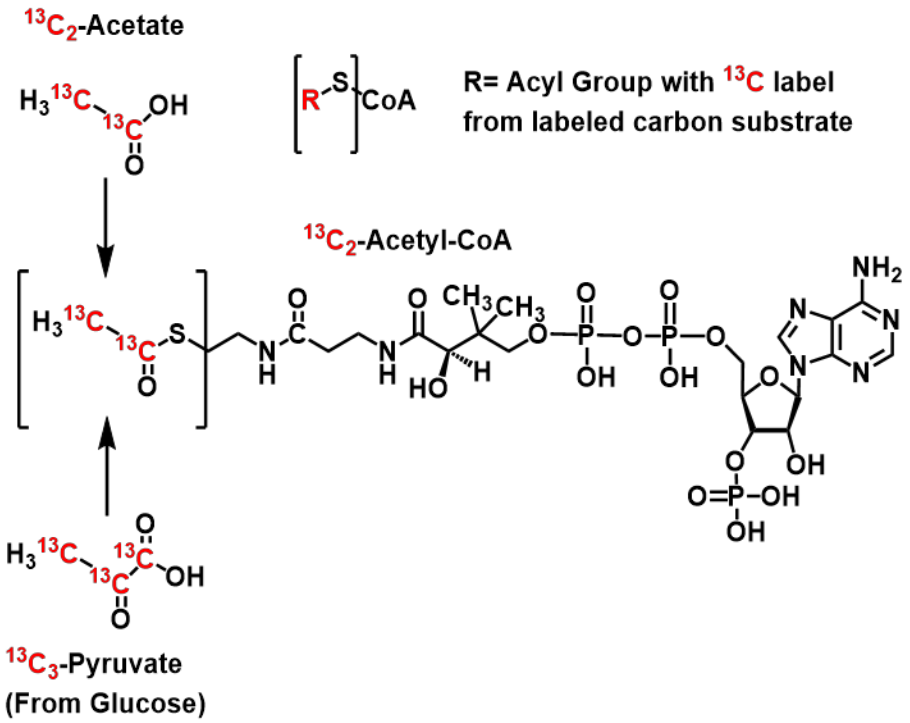
296 **References**

297 1. Ziebe S. Morphometric analysis of human embryos to predict developmental competence. Reprod
298 Fertil Dev. 2013;26(1):55-64. doi: 10.1071/RD13296. PubMed PMID: 24305177.
299 2. Keefe D, Kumar M, Kalmbach K. Oocyte competency is the key to embryo potential. Fertil Steril.
300 2015;103(2):317-22. doi: 10.1016/j.fertnstert.2014.12.115. PubMed PMID: 25639967.
301 3. Hutt KJ, Albertini DF. An oocentric view of folliculogenesis and embryogenesis. Reprod Biomed Online.
302 2007;14(6):758-64. PubMed PMID: 17579993.
303 4. Albertini DF, Combelles CM, Benecchi E, Carabatsos MJ. Cellular basis for paracrine regulation of
304 ovarian follicle development. Reproduction. 2001;121(5):647-53. PubMed PMID: 11427152.
305 5. Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and
306 oocyte quality. Hum Reprod Update. 2008;14(2):159-77. doi: 10.1093/humupd/dmm040. PubMed PMID:
307 18175787.
308 6. Sutton-McDowall ML, Gilchrist RB, Thompson JG. The pivotal role of glucose metabolism in
309 determining oocyte developmental competence. Reproduction. 2010;139(4):685-95. doi: 10.1530/REP-09-
310 0345. PubMed PMID: 20089664.
311 7. Thompson JG, Lane M, Gilchrist RB. Metabolism of the bovine cumulus-oocyte complex and influence
312 on subsequent developmental competence. Soc Reprod Fertil Suppl. 2007;64:179-90. PubMed PMID:
313 17491147.
314 8. Wallace M, Cottell E, Gibney MJ, McAuliffe FM, Wingfield M, Brennan L. An investigation into the
315 relationship between the metabolic profile of follicular fluid, oocyte developmental potential, and
316 implantation outcome. Fertil Steril. 2012;97(5):1078-84 e1-8. Epub 2012/03/01. doi:
317 10.1016/j.fertnstert.2012.01.122. PubMed PMID: 22365382.
318 9. Frey AJ, Feldman DR, Trefely S, Worth AJ, Basu SS, Snyder NW. LC-quadrupole/Orbitrap high-resolution
319 mass spectrometry enables stable isotope-resolved simultaneous quantification and (1)(3)C-isotopic labeling
320 of acyl-coenzyme A thioesters. Anal Bioanal Chem. 2016;408(13):3651-8. doi: 10.1007/s00216-016-9448-5.
321 PubMed PMID: 26968563; PMCID: PMC4838547.
322 10. Trefely S, Ashwell P, Snyder NW. FluxFix: automatic isotopologue normalization for metabolic tracer
323 analysis. BMC Bioinformatics. 2016;17(1):485. doi: 10.1186/s12859-016-1360-7. PubMed PMID: 27887574;
324 PMCID: PMC5123363.

11. Sutton-McDowall M, Thompson J. Metabolism in the pre-implantation oocyte and embryo. Animal Reproduction (AR). 2018;12(3):498-17.
12. Tejera A, Herrero J, de Los Santos MJ, Garrido N, Ramsing N, Meseguer M. Oxygen consumption is a quality marker for human oocyte competence conditioned by ovarian stimulation regimens. Fertil Steril. 2011;96(3):618-23 e2. doi: 10.1016/j.fertnstert.2011.06.059. PubMed PMID: 21782167.
13. Tejera A, Herrero J, Viloria T, Romero JL, Gamiz P, Meseguer M. Time-dependent O2 consumption patterns determined optimal time ranges for selecting viable human embryos. Fertil Steril. 2012;98(4):849-57 e1-3. doi: 10.1016/j.fertnstert.2012.06.040. PubMed PMID: 22835446.
14. Bentov Y, Yavorska T, Esfandiari N, Jurisicova A, Casper RF. The contribution of mitochondrial function to reproductive aging. J Assist Reprod Genet. 2011;28(9):773-83. doi: 10.1007/s10815-011-9588-7. PubMed PMID: 21617930; PMCID: PMC3169682.
15. Dumesic DA, Meldrum DR, Katz-Jaffe MG, Krisher RL, Schoolcraft WB. Oocyte environment: follicular fluid and cumulus cells are critical for oocyte health. Fertil Steril. 2015;103(2):303-16. doi: 10.1016/j.fertnstert.2014.11.015. PubMed PMID: 25497448.
16. Hsu AL, Townsend PM, Oehninger S, Castora FJ. Endometriosis may be associated with mitochondrial dysfunction in cumulus cells from subjects undergoing in vitro fertilization-intracytoplasmic sperm injection, as reflected by decreased adenosine triphosphate production. Fertil Steril. 2015;103(2):347-52 e1. doi: 10.1016/j.fertnstert.2014.11.002. PubMed PMID: 25516080.
17. Barnhart K, Dunsmoor-Su R, Coutifaris C. Effect of endometriosis on in vitro fertilization. Fertil Steril. 2002;77(6):1148-55. PubMed PMID: 12057720.
18. Zhao S, Torres A, Henry RA, Trefely S, Wallace M, Lee JV, Carrer A, Sengupta A, Campbell SL, Kuo YM, Frey AJ, Meurs N, Viola JM, Blair IA, Weljie AM, Metallo CM, Snyder NW, Andrews AJ, Wellen KE. ATP-Citrate Lyase Controls a Glucose-to-Acetate Metabolic Switch. Cell Rep. 2016;17(4):1037-52. Epub 2016/10/21. doi: 10.1016/j.celrep.2016.09.069. PubMed PMID: 27760311; PMCID: PMC5175409.

361 **Figures**



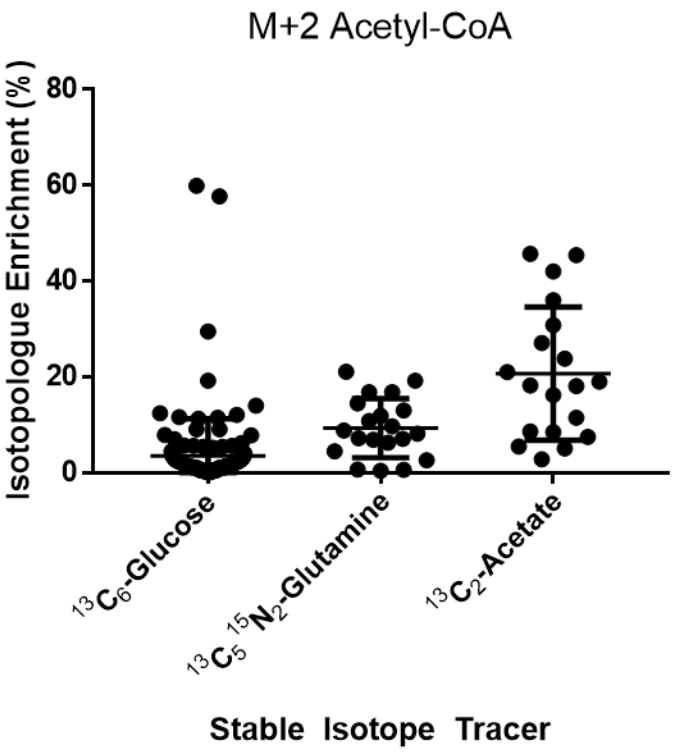
362

363 **Figure 1. Stable Isotope Metabolic Tracing.** Metabolic tracing from (**A**) Generally, acyl-CoA labeling can occur by

364 any metabolite where the R=acyl group can be variably labeled by carbon derived from substrates. Stable isotope

365 labeling into $^{13}C_2$-acetyl-CoA can be achieved through $^{13}C_2$-acetate or $^{13}C_3$-pyruvate derived from $^{13}C_6$-glucose.

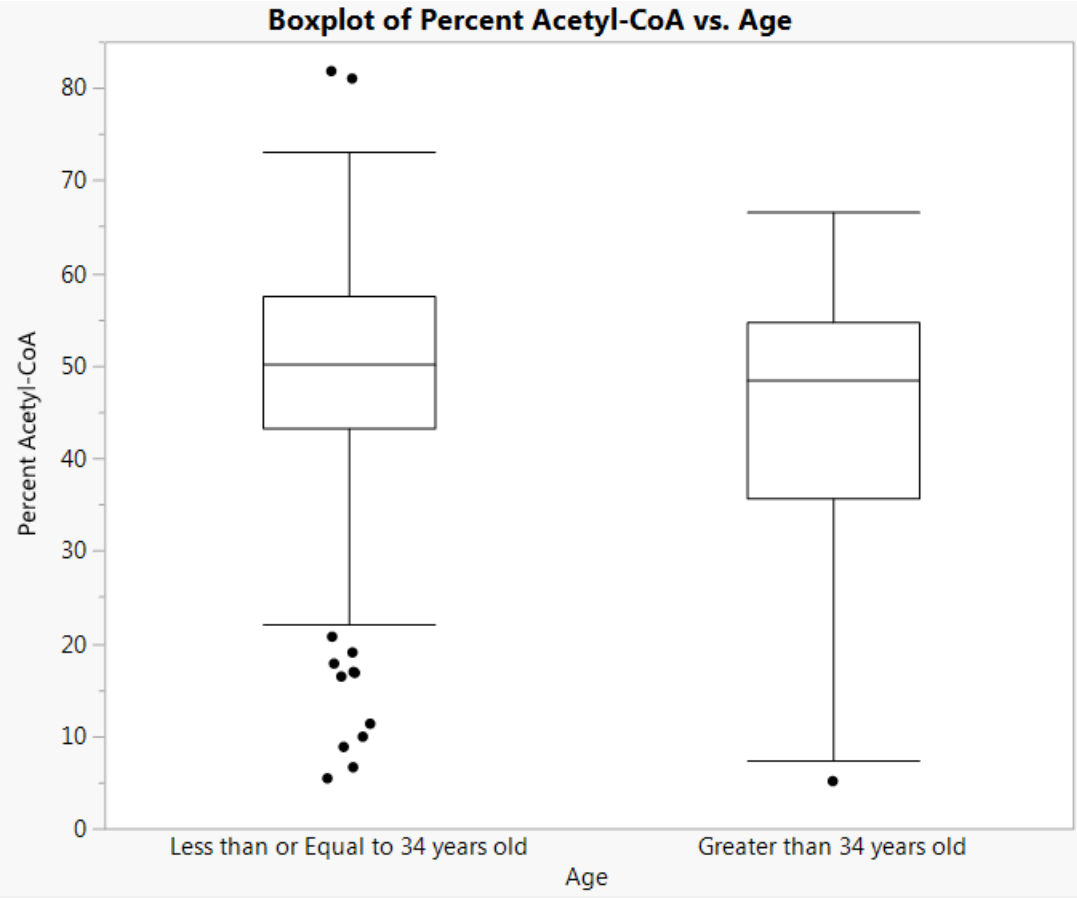
366



367

368 **Figure 2. Isotopologue enrichment in acetyl-CoA M+2 from 3 major carbon tracers in cumulus cells (CCs).** One
369 hour incubations with CC clumps from individually retrieved oocytes in stable isotope tracer media containing
370 the indicated labeled substrate. CCs have a higher capacity for generating acetyl-CoA from acetate than glucose,
371 despite the literature on the glycolytic nature of glucose.

372



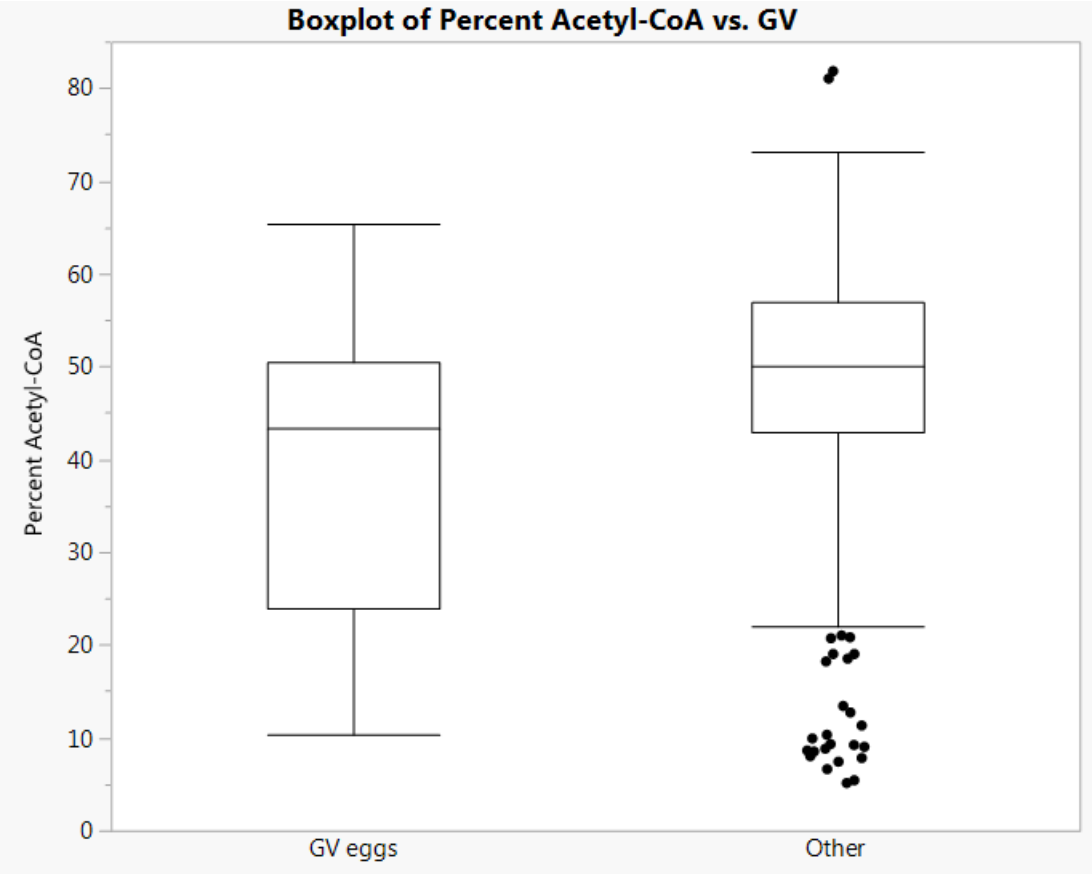
373

374 **Figure 3. Isotopologue enrichment in acetyl-CoA M+2 in cumulus cells (CCs) from participants ≤34 or >34.**

375 Isotopologue enrichment in acetyl-CoA CCs from $^{13}C_2$-acetate was 4.3% higher in women ≤34 versus >34 (p=

376 0.0004, CI: 2.3, 8.8).

377



378

379 **Figure 4. Isotopologue enrichment from CCs associated with prophase I oocytes containing germinal vesicles**

380 **(GV eggs) compared to metaphase I and II oocytes.** Isotopologue enrichment in acetyl-CoA CCs associated

381 with GV eggs was 6.02% lower than in CCs associated with metaphase I and II oocytes (p=0.013, CI: -1.74,-

382 13.79).