BPR MCS
Bulletin of Pioneering Researches of Medical and Clinical Science

Bulletin of Pioneering Researches of Medical and Clinical Science

Available online: https://bprmcs.com
2023 | Volume 3 | Issue 2 | Page: 90-98

Embryo-Derived Hypoxanthine, Uric Acid, Nitrite, and Nitrate in Culture Media as Non-Invasive Biomarkers of Low Embryo Quality and Implantation Failure

Lucas P. Meyer[1]*, Andrea F. Conti[1], Tesfaye M. Bekele[1]

[1]Department of Clinical Sciences, University of Bern, Bern, Switzerland.

Abstract

Although ART has markedly advanced, the likelihood of achieving pregnancy from transferred human embryos remains limited. Presently, embryo evaluation relies largely on morphological characteristics, which provide weak predictive information, as only a small portion of top-graded embryos result in successful pregnancy. Research has suggested that analyzing metabolites in embryo culture media could improve embryo selection. In this investigation, 66 human embryo culture media samples were analyzed in a blinded manner, five days after in vitro fertilization, to measure compounds produced by cellular metabolism that are not normally found in culture media, including purines, pyrimidines, nitrite, and nitrate. Among purines, only hypoxanthine and uric acid were detectable in most samples, while nitrite and nitrate were consistently present. When biochemical findings were compared to morphological grades, lower-quality embryos (n = 12) showed significantly elevated levels of all measured metabolites. Further comparison based on pregnancy outcome revealed that embryos from unsuccessful pregnancies (n = 25) released higher concentrations of hypoxanthine, uric acid, nitrite, and nitrate than embryos resulting in successful pregnancies (n = 17). All embryos leading to successful pregnancies produced healthy newborns. Although performed on a limited sample set, these results suggest that metabolite analysis in embryo culture media could serve as a valuable tool for selecting embryos, potentially improving ART success rates.

Keywords: Assisted reproduction techniques, Biomarkers, Energy metabolites, Human embryo, Hypoxanthine, Infertility

Corresponding author: Lucas P. Meyer
E-mail: lucas.meyer@outlook.com

How to Cite This Article: Meyer LP, Conti AF, Bekele TM. Embryo-Derived Hypoxanthine, Uric Acid, Nitrite, and Nitrate in Culture Media as Non-Invasive Biomarkers of Low Embryo Quality and Implantation Failure. Bull Pioneer Res Med Clin Sci. 2023;3(2):90-8. https://doi.org/10.51847/ECQHuaj0ho

Introduction

Infertility, defined as the inability to achieve pregnancy after at least 12 months of regular unprotected sexual intercourse with the same partner, is becoming increasingly common, including among young couples under 30 years old [1]. For many affected couples, ART is the last available option to achieve a clinical pregnancy and give birth to a healthy child. Despite technical improvements, data from the 2018 report of the Italian Institute of Health (ISS) show that out of 97,508 ART cycles, 18,994 resulted in clinical pregnancy (19.5%), and only 14,139 produced healthy newborns, corresponding to a success rate of 14.5% [2].

One major limitation reducing ART efficiency is the lack of methods to assess human embryos based on biochemical or metabolic criteria, which would allow selection using objective, measurable parameters instead of relying solely on morphology. Standard morphological or morphokinetic assessments [3–5] do not reflect the embryo's biochemical or metabolic status. Currently,

noninvasive techniques to measure energy metabolism, mitochondrial function, oxidative stress, or nitric oxide metabolism directly in embryo cultures are unavailable, making it difficult to evaluate the metabolic quality of embryos—a key determinant of ART outcomes.

Analyzing metabolites in embryo culture media has been explored previously, initially focusing on the consumption of a few substrates [6–8], and later using more sensitive methods to detect a broader spectrum of compounds [9–11]. Recent studies mainly tracked glucose and amino acids to indirectly infer embryo metabolism and correlate it with pregnancy success.

Energy metabolism imbalance in cells is linked to increased release of ATP degradation products—oxypurines such as hypoxanthine, xanthine, and uric acid—due to mitochondrial dysfunction and altered ATP turnover, which triggers purine catabolism [12–15]. This is often accompanied by higher levels of nitrite and nitrate, indicating connections between mitochondrial impairment, energy imbalance, and nitrosative stress [16, 17]. Measuring these molecules in culture media may therefore reflect the embryo's metabolic state.

Earlier studies from our group reported elevated levels of these compounds in the follicular fluid of infertile women compared to fertile controls, suggesting that poor oocyte biochemical quality negatively affects reproductive potential [18]. To date, no studies have investigated whether these metabolites can be detected in embryo culture media or whether their concentrations relate to embryo quality or pregnancy outcome. In this study, we quantified purines, pyrimidines, nitrite, and nitrate in embryo culture media, classified embryos by morphology, and analyzed associations with successful versus unsuccessful pregnancy, highlighting the potential of metabolite profiling for optimizing embryo selection in ART.

Results and Discussion

Metabolite profiles reflect embryo morphology

Examination of the embryo culture media, regardless of embryo grade, showed that hypoxanthine, uric acid, nitrite, and nitrate were present in most samples, while xanthine, nucleosides (inosine, guanosine, adenosine), and pyrimidines were either undetectable or below the assay's sensitivity.

Embryos were classified using the Gardner system into high-quality (A + B, n = 54) and low-quality (C + D, n = 12) groups. **Figures 1 and 2** illustrate the levels of metabolic byproducts in culture media, organized by morphological grading.

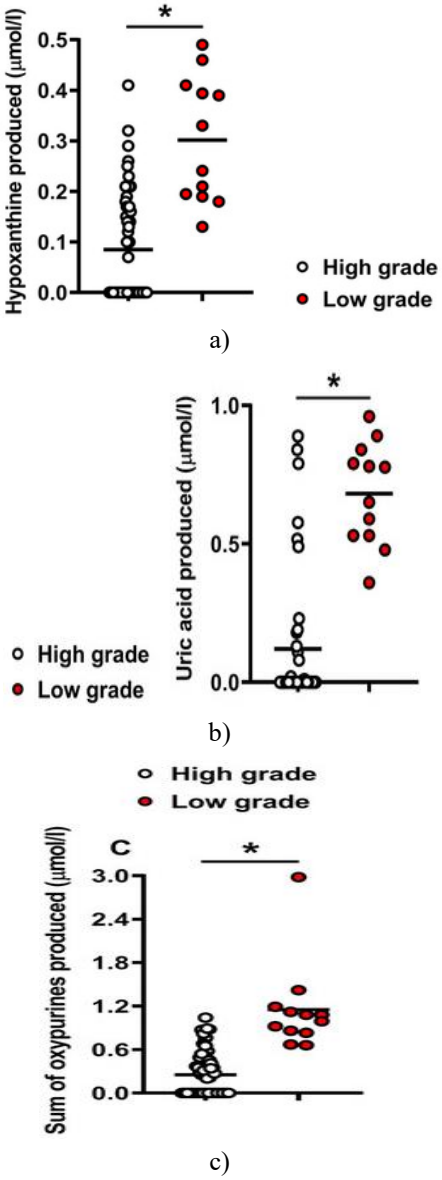
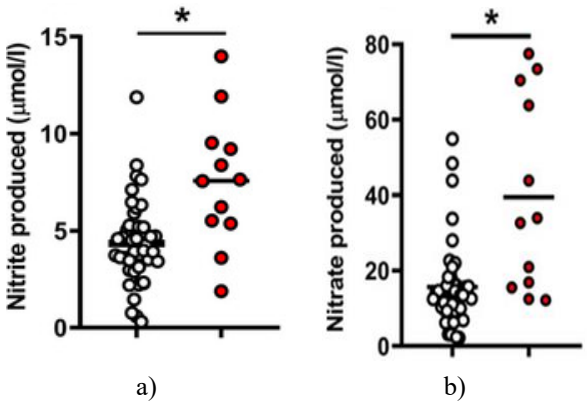


Figure 1. depicts the concentrations of hypoxanthine (a), uric acid (b), and the combined oxypurines (c) in embryos 5 days after fertilization. High-grade embryos (n = 54, empty circles) and low-grade embryos (n = 12, red circles) are distinguished according to the Gardner scale, with horizontal bars showing the mean values. *Significant differences compared to high-grade embryos, q < 0.0001 (a–c).



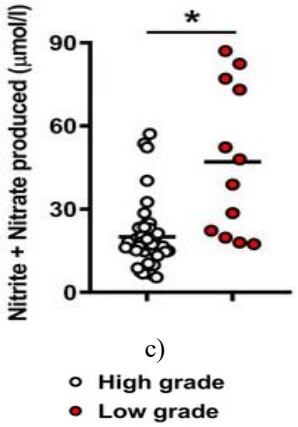


Figure 2. presents nitrite (a), nitrate (b), and their sum (c) in the same culture media. Both high- and low-quality embryos are shown, with the mean indicated by horizontal bars. *Significant differences compared to high-grade embryos, $q < 0.001$ (a–c).

As shown in **Figure 1**, embryos of lower morphological grade exhibited higher levels of hypoxanthine, uric acid, and their total (sum of oxypurines), suggesting possible disruptions in ATP balance or increased metabolic activity. Importantly, "undetectable" readings for these compounds were exclusively observed in high-quality embryos (27/54 samples), supporting a strong link between the absence of these metabolites and superior morphology.

Figure 2 demonstrates that nitrite and nitrate were measurable in nearly all embryos, but low-grade embryos consistently had higher levels of these stable nitric oxide metabolites at day 5 ($q < 0.001$).

Metabolite levels associated with pregnancy success

To assess whether metabolite concentrations could predict clinical outcomes, embryo culture media were categorized based on pregnancy occurrence, regardless of morphological grade. Among high-quality embryos, 37/54 were transferred, resulting in 15 pregnancies (40.5%). In the low-quality group, 5/12 embryos were transferred, with 3 pregnancies (60%). Overall, 42 embryos were implanted (37 A + B, 5 C + D), leading to 17 successful pregnancies (40.4%) and 25 unsuccessful pregnancies (59.5%, $p < 0.001$). All successful pregnancies produced healthy live births.

Figure 3 shows hypoxanthine (a), uric acid (b), and total oxypurines (c) in culture media from embryos with successful versus unsuccessful pregnancies. Embryos that failed to establish pregnancy had significantly higher concentrations of hypoxanthine ($q < 0.02$), uric acid ($q < 0.01$), and total oxypurines ($q < 0.001$), indicating either energy metabolism imbalance or accelerated metabolic turnover, both potentially detrimental to achieving pregnancy. Notably, "undetectable" levels of hypoxanthine and uric acid were found in 8/17 embryos that led to successful pregnancies but only 2/25 embryos from failed pregnancies, reinforcing the association between non-detectable oxypurines and positive pregnancy outcomes.

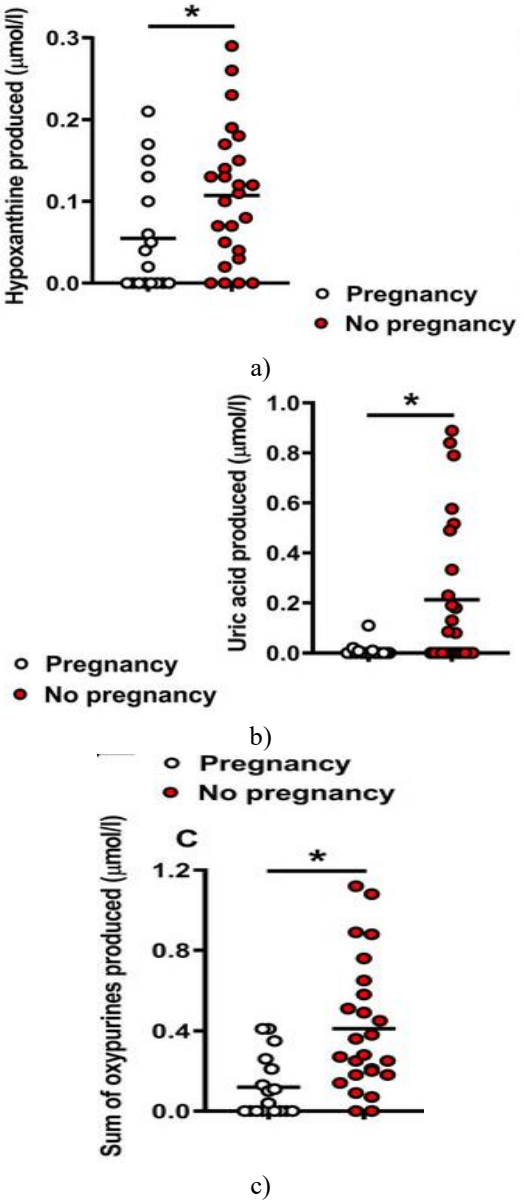


Figure 3. Levels of hypoxanthine (a), uric acid (b), and combined oxypurines (c) in the culture media of human embryos five days after fertilization. Embryos were grouped by pregnancy outcome: successful (n = 17, empty circles) and unsuccessful (n = 25, red circles), independent of morphological grade. Horizontal bars show mean values. Sum of oxypurines = hypoxanthine + uric acid. *Significant differences: $q < 0.02$ (a), $q < 0.01$ (b), $q < 0.0005$ (c).

As illustrated in **Figure 4** (panels a–c), embryos that did not lead to pregnancy exhibited higher accumulation of nitrite and nitrate in the culture medium, indicating that excessive formation of these nitric oxide metabolites—

potential markers of nitrosative stress—may compromise embryo viability and reduce the likelihood of live birth.

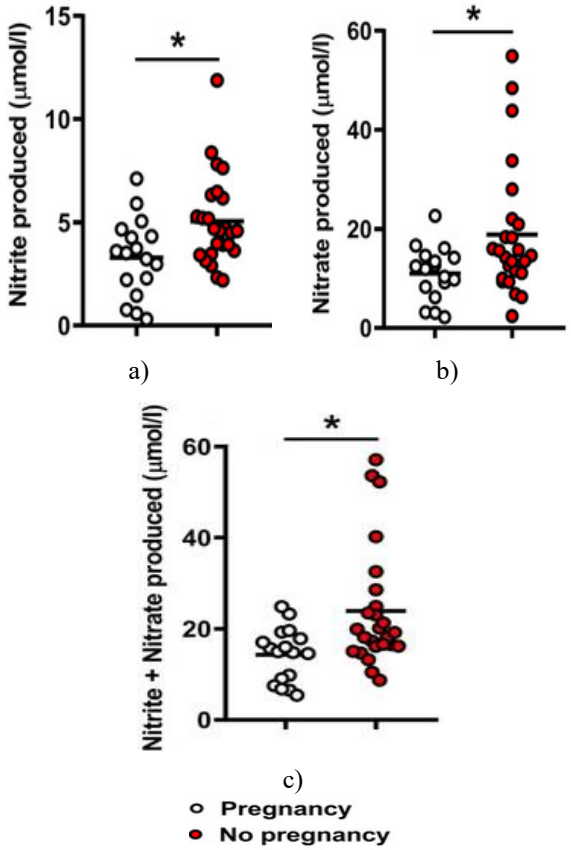


Figure 4. Nitrite (a), nitrate (b), and their sum (c) in human embryo culture media at day 5. Embryos were categorized based on pregnancy outcome: successful (n = 17, empty circles) versus unsuccessful (n = 25, red circles), regardless of morphology. Horizontal bars indicate the mean concentrations. *Significant differences: $q < 0.01$ (a), $q < 0.05$ (b), $q < 0.003$ (c).

Using metabolic profiles to guide embryo selection

To explore the potential of metabolite secretion as an objective indicator of embryo quality or implantation potential, ROC analyses were conducted for hypoxanthine, uric acid, total oxypurines, nitrite, nitrate, and nitrite + nitrate. Analyses were performed for embryos grouped by morphological quality (high vs. low according to the Gardner scale) and separately by pregnancy outcome (successful vs. unsuccessful).

Figures 5 and 6 present ROC curves for hypoxanthine (5a), uric acid (5b), total oxypurines (5c), nitrite (6a), nitrate (6b), and nitrite + nitrate (6c) in high- versus low-grade embryos. These curves demonstrate that metabolic measurements can reliably differentiate embryos with distinct morphological features. The Area Under the Curve (AUC) values indicate both high sensitivity and specificity, confirming the consistency of biochemical profiling with morphological assessment.

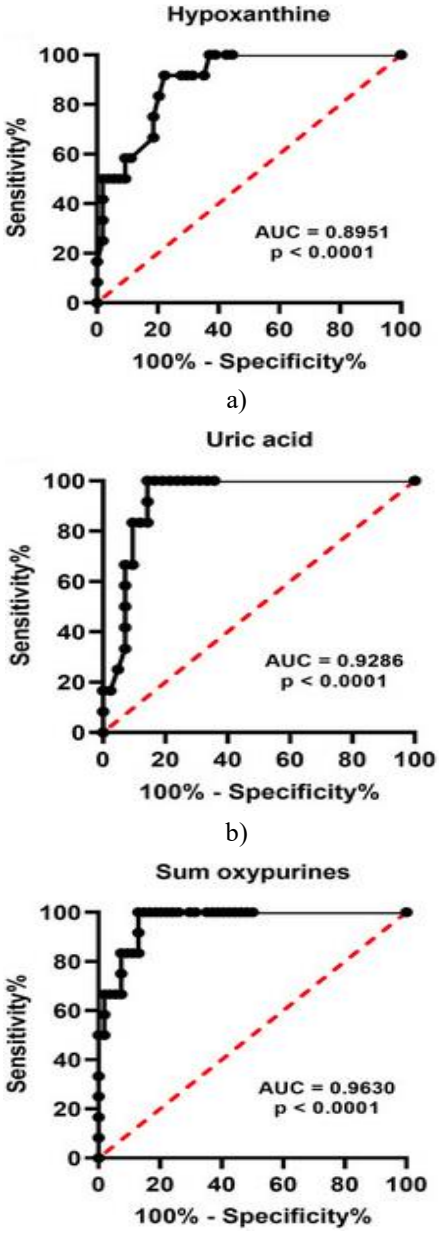
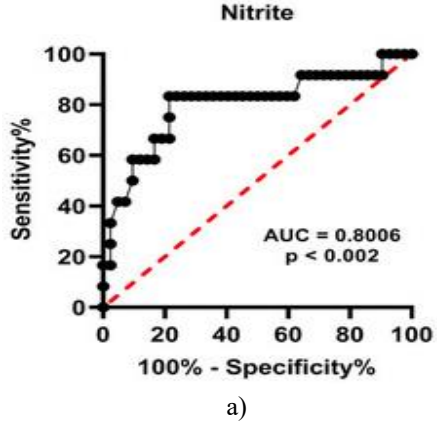


Figure 5. ROC curves for hypoxanthine (a), uric acid (b), and total oxypurines (c) in embryos classified by morphological grade. Total oxypurines = hypoxanthine + uric acid. AUC values are shown for each panel.



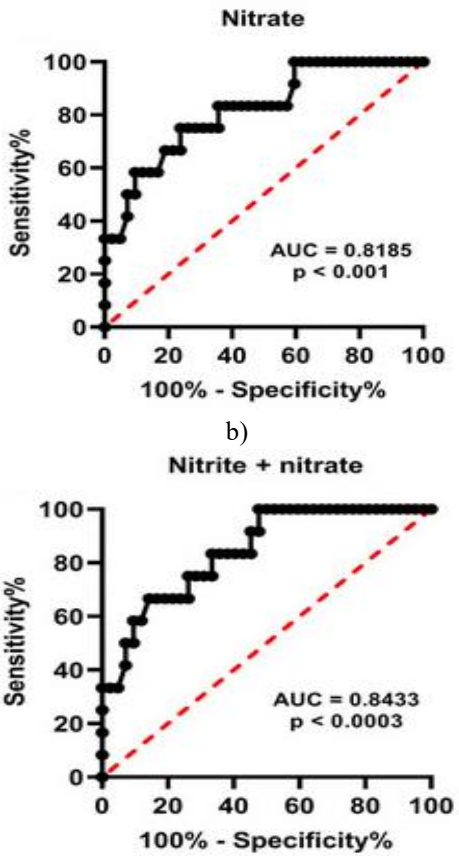
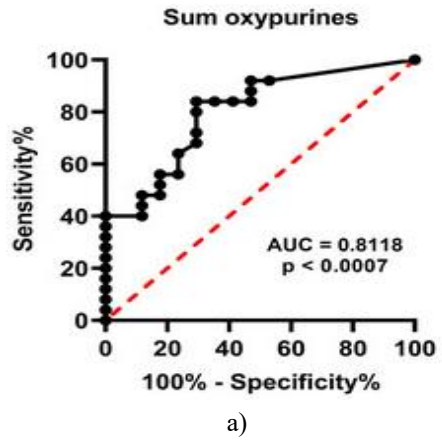


Figure 6. ROC curves for nitrite (a), nitrate (b), and nitrite + nitrate (c) in embryos classified by morphological grade. AUC significance is indicated in each panel.

To identify potential metabolic criteria for embryo selection prior to implantation, ROC analyses were also conducted for embryos grouped by pregnancy outcome. **Figure 7** shows that only total oxypurines and nitrite + nitrate reached statistically meaningful AUC values, suggesting that these metabolites may serve as useful indicators of implantation success, with adequate sensitivity and specificity for predicting pregnancy outcomes.



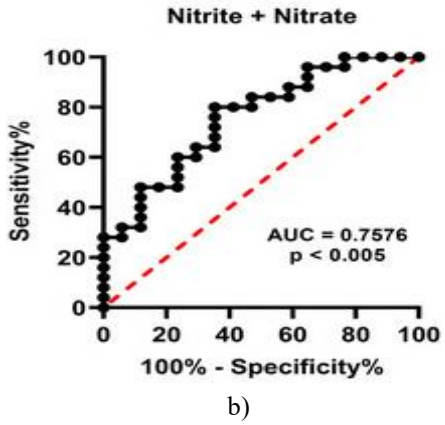


Figure 7. ROC curves for total oxypurines (a) and nitrite + nitrate (b) in embryos divided according to pregnancy success. Total oxypurines = hypoxanthine + uric acid. AUC significance is indicated for each panel.

The blinded analysis of day-5 embryo culture media after in vitro fertilization revealed that, among the targeted purines, pyrimidines, nitrite, and nitrate (the latter two representing stable nitric oxide end-products), only hypoxanthine, uric acid, nitrite, and nitrate were consistently detectable in most samples, regardless of blastocyst morphological grade according to the Gardner system. This indicates that low physiological amounts of these four metabolites are normally secreted during early human embryonic development, thereby providing a sensitive baseline from which deviations caused by metabolic disturbance can be identified.

When samples were subsequently stratified into high-grade (A+B) and low-grade (C+D) blastocysts based on standard morphological criteria, embryos with poorer morphology exhibited markedly elevated release of ATP degradation products (hypoxanthine, uric acid, and total oxypurines) as well as nitric oxide-derived metabolites (nitrite and nitrate). These findings suggest either an accelerated overall metabolic turnover in low-quality embryos—potentially driving increased purine catabolism [19, 20]—or, more likely, impaired mitochondrial performance that fails to meet cellular energy requirements, thereby triggering compensatory activation of the purine salvage/catabolic pathway and greater extracellular accumulation of oxypurines [21, 22]. The parallel increase in nitrite and nitrate further strengthens the hypothesis of mitochondrial compromise, since excessive nitric oxide generation and its oxidation products have repeatedly been linked to mitochondrial dysfunction across multiple models [23–25], despite the fact that physiological levels of nitric oxide and nitrite serve essential regulatory roles in cellular bioenergetics [26–28].

Critically, when the same metabolites were examined in the subgroup of transferred embryos (37 graded A+B and 5 graded C+D), those that failed to establish clinical

pregnancy (n=25) displayed significantly higher concentrations of all four compounds compared with those resulting in ongoing pregnancy (n=17). Notably, every embryo that achieved clinical pregnancy progressed to live birth of a healthy infant, underscoring the strong predictive value of this metabolic profile for not only implantation but also full-term healthy outcome.

Of particular interest, 37 of the 42 transferred embryos had been assigned the highest morphological scores (A+B) using routine Gardner criteria, implying equivalent developmental potential by conventional assessment. Nevertheless, within this morphologically homogeneous cohort, embryos that subsequently failed to produce pregnancy released significantly greater amounts of oxypurines and nitric oxide metabolites ($q < 0.01$ for each analyte), confirming earlier reports that morphological uniformity does not guarantee metabolic uniformity [29].

Prior non-invasive metabolic approaches have predominantly relied on measuring uptake of energy substrates [30–32] or turnover of amino acids [33–35]. Such strategies are inherently challenging because these molecules are already present at millimolar concentrations in commercial culture media, forcing detection of small (micromolar or sub-micromolar) changes against a very high background and, in the case of amino acids, against a dynamic equilibrium of simultaneous consumption and production from protein turnover [35].

In contrast, the analytes selected in the current work—purine/pyrimidine catabolites—are absent (or present only as trace contaminants) in fresh culture medium, yielding an effective baseline of zero and considerably improving signal-to-noise ratio and reproducibility. Although unexpected basal contamination with nitrite (0.72–35.00 μmol/L) and nitrate (9.51–76.61 μmol/L) was observed across medium lots—likely arising from amino acid autoxidation or manufacturing impurities—all reported spent-medium values were rigorously corrected by subtracting the lot-specific day-0 concentration.

Collectively, the elevated secretion of oxypurines in lower-grade embryos and in morphologically high-grade embryos that ultimately failed suggests either unsustainably high global metabolic activity or, more plausibly, inadequate ATP generation due to dysfunctional oxidative phosphorylation, resulting in excess flux through the purine degradation pathway [36–38]. The literature remains divided on whether high metabolic rate per se is detrimental or beneficial to embryo competence [35, 39, 40], but our data align with the interpretation that disproportionate oxypurine release reflects energetic imbalance secondary to preimplantation mitochondrial insufficiency.

Thus, quantitative assessment of embryo-derived hypoxanthine, uric acid, nitrite, and nitrate in spent culture media offers a robust, objective, metabolism-based adjunctive criteria for embryo selection that appear superior to morphology alone in predicting both implantation success and the birth of healthy offspring.

Materials and Methods

Patient enrollment and ovarian stimulation

This study adhered to the Declaration of Helsinki, and all participants provided written informed consent. Twenty-eight women, aged 30–49, experiencing infertility for at least one year and with fewer than two prior ART cycles, were recruited at the Alma Res Fertility Centre (Rome, Italy) from January to November 2021. The study received approval from the Alma Res Ethical Committee (approval number AREC0319IVF). Patients underwent a controlled ovarian stimulation protocol using a GnRH antagonist. Stimulation began on cycle day 2 with recombinant FSH (Gonal-F, Merck, Australia) or urinary hMG (Meropur, Ferring, Switzerland). Follicle growth was monitored using transvaginal ultrasonography and serum estradiol, and a GnRH antagonist (Cetrotide, Merck, Australia) was administered once follicles reached ≥13 mm to prevent premature ovulation. Oocyte retrieval was performed 36 hours after r-hCG administration when at least three follicles reached ~18 mm, using ultrasound-guided aspiration and a vacuum pump.

Fertilization, embryo culture, and outcome assessment

All oocytes were fertilized using intracytoplasmic sperm injection (ICSI). Two hours after retrieval, cumulus cells were removed using hyaluronidase (Vitrolife AB, Göteborg, Sweden), and ICSI was performed. Injected oocytes were placed individually in 50 μL of Global Total Lp Medium (CooperSurgical, Inc., Trumbull, CT, USA), covered with 9 μL Ovoil (Vitrolife AB), in pre-equilibrated GPS embryo dishes. Embryos were cultured at 37 °C in a K-System incubator under 5% O_2, 6% CO_2, and 89% N_2. The time of ICSI was set as time zero for culture.

Fertilization was assessed on day 1, after which zygotes were transferred to fresh pre-equilibrated medium in GPS dishes. Embryos remained there until day 5, when blastocyst development was evaluated using the Gardner grading system [3]. Embryos scoring above BB were classified as high quality (grades A and B), and those below BB as low quality (grades C and D). Culture media were collected, stored at −20 °C, and later analyzed for metabolites. Samples contaminated with oil were excluded.

For patients undergoing transfer, clinical pregnancy and live birth outcomes were recorded. Of the 66 embryos cultured, 10 single blastocysts were transferred in fresh cycles, while the remaining embryos were vitrified in

freeze-all cycles. Embryos with superior morphology were preferentially selected for transfer. Transfers in thawed cycles were also included in outcome analyses. Biochemical pregnancies were assessed via hCG blood tests, and clinical pregnancies were confirmed at 8 weeks by ultrasound, including detection of fetal heartbeat.

Biochemical assessment of embryo culture media

Embryo culture media samples, previously frozen and ranging from 25 to 45 µL, were diluted to 200 µL using HPLC-grade water and analyzed under blinded conditions. A 100 µL aliquot from each sample was processed for high-performance liquid chromatography (HPLC) to detect purines, pyrimidines, nitrite, and nitrate, employing a method validated in our laboratory and described in earlier publications [18, 41, 42]. The chromatographic separation and quantification of these compounds were performed on a Surveyor HPLC system (Thermo Fisher Scientific, Rodano, Milan, Italy) equipped with a diode array detector with a 5 cm light-path flow cell, linked to a Hypersil C-18 column (250 × 4.6 mm, 5 µm particles, 120 Å pore size) and protected by a guard column (15 × 4.6 mm). Quantification was performed using ChromQuest® software (Thermo Fisher Scientific) at 260 nm for purines and pyrimidines, and 206 nm for nitrite and nitrate, with sample dilutions accounted for in all calculations.

Statistical evaluation

All data analysis was conducted using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). The Kolmogorov–Smirnov test evaluated the distribution normality of the dataset. As the distributions of the metabolites were not normal, the Kruskal–Wallis non-parametric one-way ANOVA was used for comparisons between embryo groups (high vs. low morphological grade and successful vs. unsuccessful pregnancies). Multiple comparisons were adjusted using the two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli to control the false discovery rate. A q-value below 0.05 was considered statistically significant.

Receiver Operating Characteristic (ROC) curves were also generated to evaluate the sensitivity and specificity of each metabolite in distinguishing embryo culture media according to morphological grade or pregnancy outcome.

Conclusion

The findings from this study suggest that evaluating ATP catabolism products (oxypurines) and nitric oxide metabolites (nitrite and nitrate) in embryo culture media can provide a valuable biochemical tool for assessing embryo quality. Such assessments have the potential to improve ART outcomes by enhancing the likelihood of successful pregnancies and healthy live births. These analyses provide additional insights that are not obtainable through conventional morphological grading alone.

However, some limitations should be noted. First, the study involved a relatively small number of embryos, particularly those with lower Gardner grades, highlighting the need for confirmation in larger cohorts. Second, the biochemical analyses are time-intensive: each HPLC measurement for oxypurines, nitrite, and nitrate requires 30 minutes, followed by an additional 30 minutes before subsequent samples can be processed, allowing a maximum throughput of roughly 12 samples per instrument per working day.

In conclusion, metabolic profiling of human embryo culture media represents a promising adjunctive tool to conventional morphological evaluation, with the potential to increase successful pregnancy rates and offer significant advantages for couples undergoing ART.

Acknowledgments: None

Conflict of interest: None

Financial support: This research was funded in part by research funds from the University of Catania (PIACERI 2020 funds).

Ethics statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Alma Res Ethical Committee (approval number AREC0319IVF, date of approval 17 December 2018).

Informed consent was obtained from all subjects involved in the study.

References

1. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clin Biochem. 2018;62(1):2–10.
2. Istituto Superiore di Sanità. Report 2020: dati PMA 2018 [Internet]. Rome: ISS; 2020 [cited 2022 Sep 29]. Available from: https://www.iss.it
3. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: Towards a single blastocyst transfer. Fertil Steril. 2000;73(6):1155–8.
4. Rubio I, Galán A, Larreategui Z, Ayerdi F, Bellver J, Herrero J, et al. Clinical validation of embryo culture and selection by morphokinetic analysis: A randomized controlled trial of the EmbryoScope. Fertil Steril. 2014;102(5):1287–94.
5. Basile N, Vime P, Florensa M, Aparicio Ruiz B, Garcia-Velasco JA, Remohi J, et al. The use of morphokinetics as a predictor of implantation: A

multicentric study. Hum Reprod. 2015;30(2):276–83.
6. Gott A, Hardy K, Winston R, Leese H. Non-invasive measurement of pyruvate and glucose uptake and lactate production by single human preimplantation embryos. Hum Reprod. 1990;5(1):104–8.
7. Guerif F, McKeegan P, Leese HJ, Sturmey RG. A simple approach for consumption and release analysis of metabolic activity in single mammalian embryos. PLoS One. 2013;8(8):e67834.
8. Gardner DK, Wale PL, Collins R, Lane M. Glucose consumption of single post-compaction human embryos is predictive of embryo sex and live birth outcome. Hum Reprod. 2011;26(8):1981–6.
9. Brison DR, Houghton FD, Falconer D, Roberts SA, Hawkhead J, Humpherson PG, et al. Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover. Hum Reprod. 2004;19(10):2319–24.
10. Picton HM, Elder K, Houghton FD, Hawkhead JA, Rutherford AJ, Hogg JE, et al. Association between amino acid turnover and chromosome aneuploidy during human preimplantation embryo development. Mol Hum Reprod. 2010;16(8):557–69.
11. Donohoe DR, Bultman SJ. Metaboloepigenetics: Interrelationships between energy metabolism and epigenetic control of gene expression. J Cell Physiol. 2012;227(9):3169–77.
12. Rambo LM, Ribeiro LR, Della-Pace ID, Stamm DN, da Rosa Gerbatin R, Prigol M, et al. Acute creatine administration improves mitochondrial membrane potential. Amino Acids. 2013;44(3):857–68.
13. Bracko O, Di Pietro V, Lazzarino G, Amorini AM, Tavazzi B, Artmann J, et al. 3-Nitropropionic acid-induced ischemia tolerance in rat brain. J Cereb Blood Flow Metab. 2014;34(9):1522–30.
14. Yakoub KM, Lazzarino G, Amorini AM, Caruso G, Scazzone C, Ciaccio M, et al. Fructose-1,6-bisphosphate protects hippocampal rat slices from NMDA excitotoxicity. Int J Mol Sci. 2019;20(9):2239.
15. Lazzarino G, Amorini AM, Petzold A, Gasperini C, Ruggieri S, Quartuccio ME, et al. Serum compounds of energy metabolism impairment in multiple sclerosis. Mol Neurobiol. 2017;54(9):7520–33.
16. Tavazzi B, Vagnozzi R, Signoretti S, Amorini AM, Belli A, Cimatti M, et al. Temporal window of metabolic brain vulnerability to concussions. Neurosurgery. 2007;61(2):390–5.
17. Caruso G, Fresta CG, Fidilio A, O'Donnell F, Musso N, Lazzarino G, et al. Carnosine decreases PMA-induced oxidative stress in murine macrophages. Antioxidants. 2019;8(8):281.
18. Lazzarino G, Pallisco R, Bilotta G, Listorti I, Mangione R, Saab MW, et al. Altered follicular fluid metabolic pattern correlates with female infertility. Int J Mol Sci. 2021;22(16):8735.
19. Abd-Elfattah AS, Jessen ME, Lekven J, Wechsler AS. Differential cardioprotection with selective inhibitors of adenosine metabolism. Mol Cell Biochem. 1998;180(1-2):179–91.
20. Lee WH, Gounarides JS, Roos ES, Wolin MS. Influence of peroxynitrite on cardiac energy metabolism. Am J Physiol Heart Circ Physiol. 2003;285(4):H1385–95.
21. Lazzarino G, Raatikainen P, Nuutinen M, Nissinen J, Tavazzi B, Di Pierro D, et al. Myocardial release of malondialdehyde and purine compounds during coronary bypass surgery. Circulation. 1994;90(1):291–7.
22. Igaki Y, Tanno M, Sato T, Kouzu H, Ogawa T, Osanami A, et al. Xanthine oxidoreductase-mediated injury is amplified by upregulated AMP deaminase in type 2 diabetic rat hearts. J Mol Cell Cardiol. 2021;154(1):21–31.
23. Bagnoli E, Diviney T, FitzGerald U. Dysregulation of astrocytic mitochondrial function following exposure to a dopamine metabolite. Eur J Neurosci. 2021;53(9):2960–72.
24. Strifler G, Tuboly E, Görbe A, Boros M, Pécz D, Hartmann P. Targeting mitochondrial dysfunction with L-alpha glycerylphosphorylcholine. PLoS One. 2016;11(11):e0166682.
25. Fresta CG, Chakraborty A, Wijesinghe MB, Amorini AM, Lazzarino G, Tavazzi B, et al. Non-toxic engineered carbon nanodiamond concentrations induce oxidative/nitrosative stress. Cell Death Dis. 2018;9(3):245.
26. Zhang G, Han H, Zhuge Z, Dong F, Jiang S, Wang W, et al. Renovascular effects of inorganic nitrate following ischemia-reperfusion of the kidney. Redox Biol. 2021;39(1):101836.
27. Fiorello ML, Treweeke AT, Macfarlane DP, Megson IL. Impact of glucose exposure on bioenergetics and endothelial cell function. Sci Rep. 2020;10(1):19547.
28. Laranjinha J, Nunes C, Ledo A, Lourenço C, Rocha B, Barbosa RM. Nitric oxide as a cellular messenger in brain bioenergetics. Neurochem Res. 2021;46(1):64–76.
29. Gardner DK, Lane M, Stevens J, Schoolcraft WB. Noninvasive assessment of human embryo nutrient consumption. Fertil Steril. 2001;76(6):1175–80.
30. Leese HJ, Barton AM. Pyruvate and glucose uptake by mouse ova and preimplantation embryos. J Reprod Fertil. 1984;72(1):9–13.
31. Lee YSL, Thouas GA, Gardner DK. Developmental kinetics of cleavage stage mouse embryos and

nutrient utilization. Hum Reprod. 2015;30(3):543–52.
32. Wale PL, Gardner DK. Oxygen regulates amino acid turnover and carbohydrate uptake in mouse embryos. Biol Reprod. 2012;87(1):21–8.
33. Gardner DK. Changes in nutrient requirements during mammalian preimplantation embryo development. Theriogenology. 1998;49(1):83–102.
34. Houghton FD, Hawkhead JA, Humpherson PG, Hogg JE, Balen AH, Rutherford AJ, et al. Non-invasive amino acid turnover predicts human embryo developmental capacity. Hum Reprod. 2002;17(4):999–1005.
35. Ferrick L, Lee YSL, Gardner DK. Metabolic activity of human blastocysts correlates with morphokinetics and KIDScore. Hum Reprod. 2020;35(9):2004–16.
36. Nanni S, Aiello A, Salis C, Re A, Cencioni C, Bacci L, et al. Metabolic reprogramming by Malat1 depletion in prostate cancer. Cancers (Basel). 2021;13(1):15.
37. Ciccarone F, Di Leo L, Lazzarino G, Maulucci G, Di Giacinto F, Tavazzi B, et al. Aconitase 2 inhibits proliferation of MCF-7 cells. Br J Cancer. 2020;122(2):182–93.
38. Giallongo C, Tibullo D, Camiolo G, Parrinello NL, Romano A, Puglisi F, et al. TLR4 signaling drives stromal cell commitment in multiple myeloma. Cell Death Dis. 2019;10(9):704.
39. Racowsky C, Stern JE, Gibbons WE, Behr B, Pomeroy KO, Biggers JD. Associations among embryo morphology parameters and live birth rate. Fertil Steril. 2011;95(6):1985–9.
40. Kong X, Yang S, Gong F, Lu C, Zhang S, Lu G, et al. Relationship between cell number and developmental potential of cleavage stage embryos. PLoS One. 2016;11(4):e0153697.
41. Lazzarino G, Mangione R, Belli A, Di Pietro V, Nagy Z, Barnes NM, et al. ILB® attenuates oxidative stress and mitochondrial dysfunction in ALS patients. J Pers Med. 2021;11(8):794.
42. Lazzarino G, Amorini AM, Mangione R, Saab MW, Di Stasio E, Di Rosa M, et al. Biochemical discrimination of Down syndrome-related metabolic alterations. Antioxidants. 2022;11(6):1208.