Journal of Assisted Reproduction and Genetics (2020) 37:2631–2633
https://doi.org/10.1007/s10815-020-01992-3

COMMENTARY

Closing the circle of reverse genetics in reproductive medicine

David F. Albertini [1]

Published online: 27 October 2020

Linking genotype to phenotype is the singular charge of genetics. And some of the most foundational principles in contemporary human genetics took their origins within the annals of reproductive medicine. But since staking claim in the 1960s and 1970s, momentum in human genetics on the broader scale it now commands has been sustained in recent decades by the exploitation of experimentally tractable animal models lending themselves to observational and functional studies of spontaneous, induced, or engineered mutations whose outcomes could be defined in rigorous terms. While intact animal and cultured cell or organoid models have retained a central role in linking genotypes to phenotypes, the field of human genetics is self-powering forward at an incredible pace thanks in large measure to the remarkable technological advances of the past two decades. Fittingly, we have arrived at a place where a human phenotype can be mapped to a particular gene offering an opportunity to elaborate upon function for gene products perhaps never suspected of being involved in a particular process or behavior at the molecular, cellular, or tissue level.

Human ARTs has contributed to transforming the scenario outlined above into a platform of discovery for reproductive biology. A case in point has materialized over the past few years regarding a specific member of the super family of tubulin genes, TUBB8. The first signs or phenotypes prompting inquiry had to do with clusters of patients who despite exhibiting normal responses to ovarian stimulation, yielded immature oocytes at first associated with meiotic arrest at the germinal vesicle stage [1]. In some ways, the ground work set by careful observation of seemingly penetrant phenotypes in the embryology lab heightened awareness of these and other unusual phenotypes in pre or post-fertilization outcomes giving at least the suggestion of a quasi-penetrant phenotype in the face of an otherwise "normal" appearing IVF cycle. But mapping of the original defect to the TUBB8 gene, upon further investigation, revealed in fact that a family of mutations were involved that phenotypically demonstrated a spectrum of disorders spanning from the failed resumption of meiosis after ovulation triggering to failure to fertilize or cleave [1, 2]. In fact, the range of disturbances observed in the oocytes of patients identified with various mutations in the TUBB8 gene is striking and will command much future research to understand basic processes at play during these initial and critical stages of human development [3].

Recently, in the pages of JARG, the work of Zhao and colleagues not only extended the analysis of the TUBB8 gene products in human oocytes but provided an elegant example of how to close the circle in reproductive genetics when such a robust and glaring phenotype shows up in the clinical embryology laboratory [4] -what some might refer to as the "*bedside-to-bench*" approach. And again in this issue we see the paper by Liu and collaborators extend the range of effects the TUBB8 gene seems to mediate in the human (Identification and rescue of a novel TUBB8 mutation that causes the first mitotic division defects and infertility https://doi.org/10.1007/s10815-020-01945). In the former case, having identified a not-so-surprising phenotype of large polar bodies, they asked if the mutation they recognized in patients could be introduced into a mouse and if so, would it lead to a different or similar phenotype relative to what was originally recognized in their patient population. In this case, a comparable phenotype (phenocopy) resulted and in closing the circle, the stage was set for what we all hope will be an answer to some of the vexing basic questions being faced by the reproductive medicine community.

Among these, few would argue that genetic plasticity at the dawning of human development and our preoccupation with PGT-A reflects a nexus evidencing no signs of slowing down here in this strange year of 2020. In fact, 2020 got off to an auspicious start with the publication of the paper from Munne and associates posing a most fundamental of questions: How would the PGT-A results of in vivo produced human embryos compare to those produced by IVF/ICSI? [5]. More to the point of that puzzling plasticity noted above, since we are working in a medical subspeciality where the pressure to

✉ David F. Albertini
eicjarg@gmail.com

[1] Bedford Research Foundation, Bedford, MA, USA

select (or deselect) embryos for transfer based on the perceived genetic makeup of that embryo (or whatever it takes to make a baby based on the inherent genetic makeup of that conceptus) it would be good to know where we stand as a species when it comes to fecundity (see below) and, most importantly, where we stand with our ART-based tampering at these early stages of human development.

This is hardly a new question. Most can recall the landmark studies (so-called egg hunts) from yesteryear by Hertig and Rock. And for an updated version of where we stand gauging human fecundity, with or without a link to genetic composition, take a close look at the recent analysis of Wilcox and colleagues [6]. Well beyond conversational fodder at your next virtual social gathering, what they conclude, not unlike the original Hertig and Rock estimates from 60 years ago, is that 40–50% of fertilized ova do not implant for mating pairs "….in their prime reproductive years." Again, the phase of development that seems to matter the most in terms of implantation is not the blastocyst-it is the peri- and postfertilization processes that appear to be most at risk of compromising the implantation potential of the conceptus-along with a few other extraembryonic factors such as endometrium.

Returning to the Munne study alluded to above, this work is both symbolic and important for several reasons not the least of which concerns the matter of research integrity and guidelines employed within an ethical context warranting a close investigation. For some, reactions have been negative and yet for others, this work signifies the era of commercialism human ARTs now finds itself in. For a look at two very different perspectives we offer our readership the following: *Medical research and reproductive medicine in an ethical context: a critical commentary on the paper dealing with uterine lavage published by Munné* et al., (https://doi.org/10.1007/s10815-020-01954) and *When pregnancy is a research risk (*https://doi.org/10.1007/s10815-020-01938).

Prominently on display at the recent ASRM meeting, among the latest trends in PGT-A platforms is the so-called non-invasive version of obtaining samples for analysis (*collect my cell-free DNA please without tampering with my trophectoderm*). That *biopsying* the trophectoderm may not be such a harmless intrusion after all is addressed by Tocci by his contribution this month (The unknown human trophectoderm: implication for biopsy at the blastocyst stage; https://doi.org/10.1007/s10815-020-01925). While a matter of discussion for years now, what distinguishes this paper from all the others is that it comes from someone outside our ART discipline-for a change.

No doubt the clamor over testing embryos PGT-A style (not M please) will continue. And the ends to which our commercially vested colleagues will go to never ceases to bring sufficient pause to the tenor of this much maligned conversation. Take for example the recent scenario that erupted when the good doctor Paulson proffered his latest assessment of the mathematics underscoring the PGT-A utility for patients seeking such testing or not [7]. Within a matter of *nanoseconds*-figuratively speaking of course- comes the response from the gang of three taking to task the claims made in the Paulson commentary [8]. Like it or not, journal editors take seriously claims of substance for or against what they choose to publish in their pages and the commercially vested responders were poised and deliberate in expressing the concerns raised by Paulson's article. But their response then went on to a place uncomfortably personal and beyond the boundaries of discourse we come to expect-professionally speaking. Have a look yourself and decide. And be sure to take note of the end result in this unfortunate turn of events [9]. As the journal editor, how might you have handled this situation?

Journal editors are charged with many tasks in delivering what may or may not qualify as game changing packets of new knowledge, and in our field of ARTs and genetics, we have certainly been witness to examples of the "sea of change" that likely will impact practice and testing patterns [10]. Discretion, hope, excitement and disappointment all emerge from the process of peer review as difficult choices in the end lead to what we all hope will be a "final word," -a kernel of inspiration for we the practitioners and our patients.

The most recent challenge journals and editors have had to manage arrived abruptly in the form of the COVID-19 pandemic. Besides receiving a sometimes eclectic array of papers on various aspects of the SARS CoV-2 impact on reproductive health, notes of caution and deliberation rang clear as we at JARG attempted to do our small part in educating and guiding the reproductive medicine community through this time of crisis. In our efforts to be inclusive and distributive, given the global reach of the pandemic and the varying resources countries have at their disposal, the role of our major societies has become critical to managing and moving forward. To this end we note the letter to the editor published this month from the leadership of ESHRE and ASRM clarifying and extending their roles in guiding us through these protracted times of the pandemic. We thank Cristina Magli, current Chair of ESHRE, and Catherine Racowsky, current President of ASRM for not only recognizing the importance of quality control in the dissemination of information in all forms of media, but bringing into focus the commanding voice that professional societies together enable for the betterment of our stakeholders in these difficult times.

We hope you enjoy this issue and its' humble offerings. Stay well as we fast approach the end of a turbulent year and thank you for your continued support.

References

1. Chen B, Li B, Li D, Yan Z, Mao X, Xu Y, et al. Novel mutations and structural deletions in TUBB8: expanding mutational and

phenotypic spectrum of patients with arrest in oocyte maturation, fertilization or early embryonic development. Hum Reprod. 2017;32(2):457–64.
2. Yuan P, Zheng L, Liang H, Li Y, Zhao H, Li R, et al. A novel mutation in the TUBB8 gene is associated with complete cleavage failure in fertilized eggs. J Assist Reprod Genet. 2018;35(7):1349–56.
3. Chen B, Wang W, Peng X, Jiang H, Zhang S, Li D, et al. The comprehensive mutational and phenotypic spectrum of TUBB8 in female infertility. Eur J Hum Genet. 2019;27(2):300–7.
4. Zhao L, Guan Y, Wang W, Chen B, Xu S, Wu L, et al. Identification novel mutations in TUBB8 in female infertility and a novel phenotype of large polar body in oocytes with TUBB8 mutations. J Assist Reprod Genet. 2020;37:1837–47.
5. Munne S, Nakajima ST, Najmabadi S, Sauer MV, Angle MJ, Rivas JL, et al. First PGT-A using human in vivo blastocysts recovered by uterine lavage: comparison with matched IVF embryo controlsdagger. Hum Reprod. 2020;35(1):70–80.
6. Wilcox AJ, Harmon Q, Doody K, Wolf DP, Adashi EY. Preimplantation loss of fertilized human ova: estimating the unobservable. Hum Reprod. 2020;35(4):743–50.
7. Paulson RJ. Hidden in plain sight: the overstated benefits and underestimated losses of potential implantations associated with advertised PGT-A success rates. Hum Reprod. 2020;35(3):490–3.
8. Capalbo A, Rubio C, Simon C. Testing the mathematical model for PGT-A inefficiency with scientific sources demonstrates the efficacy of PGT-A. Hum Reprod. 2020;35(9):2163–5.
9. Paulson RJ. Reply: the misguided nature of the argument about the current efficiency of PGT-A. Hum Reprod. 2020;35(9):2165–6.
10. Dozortsev DI, Pellicer A, Diamond MP. Term oocyte maturation and term ovarian stimulation: impact on oocyte competence. Fertil Steril. 2020;114(2):221–2.