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TECHNOLOGICAL INNOVATION

Shake, rattle and roll: bringing a little rock to the IVF laboratory to improve embryo development

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While the topic of music selection in the IVF laboratory and its potential impact on embryologist performance during various procedures has been discussed in the past [1], a direct impact on developing embryos has not been proven *(anecdotal observations that playing Jerry Garcia and the Grateful Dead lead to embryonic aneuploidy were never proven* [1]*)*. However, emerging dynamic culture platforms have demonstrated a benefit to embryos during the incubation period. These dynamic platforms result in gentle media disruption and embryo movement though tilting, vibration and media flow. Thus, in keeping with the theme of the title, one could say that a little "rock-and-roll" in the IVF lab may be beneficial to in vitro embryo development Table 1.

Recreating the physical forces normally experienced by embryos moving through the reproductive tract in vivo and applying them in vitro has received a fair amount of attention and may further offer a means of improving the culture microenvironment [2–6]. After all, a similar approach of recapitulating the changing chemical environment of the female reproductive tract resulted in formulation of several successful culture media. Though the actions of peristalsis and beating of ciliated epithelium [7, 8], it is estimated that embryos experience a movement and resulting sheer force of approximately 0.1 μm/s and 0-3 dyn/mm^2, respectively [9, 10]. However, caution must be used, as excess sheer forces may compromise embryo development [11, 12]. Systems developed that appear to avoid these excess sheer stresses include a tilting embryo culture system (TECS) that optimized a tilting angle, speed and hold periods to minimize sheer stress (<0.00015 dyn/cm^2) as embryos roll across the dish. This system has been used successfully to culture fresh and frozen/thawed human embryos throughout the entire 5–6 day culture period, improving blastocyst quality, and most recently, increasing positive chemical pregnancy rates [10, 13]. A vibrating embryo culture system use very short burst for 5 s/hr to culture oocytes/zygotes for brief periods to improve subsequent human embryo development, and increases clinical pregnancy and implantation rates in select patients and poor responders [14–16], though parameters of the device may need to be optimized for other species or conditions [17]. Finally, a microfluidic pulsatile microfunnel approach gently flows media over stationary mouse embryos to minimize sheer forces [18, 19]. This approached improved human embryo quality following 2–3 days of culture, and improved pregnancy and implantation rates in the mouse.

Current dynamic culture platforms that involve tilting, vibrating and media flow can result in gentle physical stimulation of oocytes and embryos. This may disrupt media gradients that form around cells, which may or may not be beneficial [2–5]. An alternate explanation for benefit may be the stimulation of mechano-sensitive signaling pathways that can stimulate embryo growth. This "Active Embryo Hypothesis" [2, 6, 15], states that an intermediate level of mechanical stimulation of embryos via gentle movement, media flow or other physical stimuli, is beneficial due to simulation of trophic signaling pathways. This seems plausible, considering prior success with dynamic culture devices and findings that demonstrate excess sheer forces can activate MAP kinase signaling pathways [11, 12]. It should be mentioned, however, that presence of specific mechano-receptors, related signaling pathways or impacts on gene expression remain to be identified in the mammalian oocyte or preimplantation embryo following dynamic culture.

Capsule Emerging dynamic embryo culture platforms may represent a paradigm shift over current static approaches and offer a means to improve embryo development in vitro.

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Table 1 Summarizing recent publications on dynamic embryo culture platforms

Dynamic approach	Starting material	Dynamic conditions	Endpoint assessment (dynamic vs. static control)	Ref
Tilting Embryo Culture System (TECS)	Frozen/Thawed cleavage embryos—divided evenly between treatments based on initial quality	10° tilt for 10 min Velocity of 1°/s	Blast formation: 52.9 % vs. 43.5 %, NS Blast quality >3BB: 17.2 % vs. 11.8 %, NS Blast cell #: 43 vs. 34, $p< 0.05$	10
Microvibration	Fresh inseminated oocytes/zygotes—2 per patient assigned alternately to 1 of 2 treatments (no split)	20 Hz[#]for 5 s/hr for 18 h	Normal Fert: 70 % vs. 69 %, NS Day 2, 4–6cell, grade A/B: 91 % vs. 77 %, $p< 0.05$ Day 3, 8cell, grade A/B: 89 % vs. 67 %, $p< 0.01$ Day 4 blast: 11 % vs. 1.1 %, $p< 0.05$ Day 4 morula: 72 % vs. 43 %, $p< 0.01$ Day 5 blast: 71 % vs. 53 %, $p< 0.01$ Preg rate day 3 transfer: 80 % vs. 30 %, $p< 0.01$ Preg rate day 5 transfer: 73 % vs. 36 %, $p< 0.01$	14
Pulsatile Microfluidic Microfunnel	Fresh zygotes randomly divided between treatments	Pulsatile media flow	Day 3 cell #: NS Day 3 Fragmentation: lower in dynamic, $p= 0.002$ Top Quality embryos: higher in dynamic, $p= 0.032$ Good Quality embryos: higher in dynamic, $p= 0.23$ Poor Quality embryos: lower in dynamic, $p< 0.002$	18
Tilting Embryo Culture system (TECS)	Fresh oocytes randomized to treatments	20° tilt Velocity of 1°/s	Normal Fert: 77 % vs. 79 %, NS Day 3 high quality embryo: 37 % vs. 28 %, NS Day 5 total blast: 45 % vs. 32 %, $p= 0.018$ Day 5 blast ≥3BB: 29 % vs. 18 %, $p= 0.018$ Day 6 total blast: 48 % vs. 35 %, $p= 0.023$ Day 6 ≥3BB: 31 % vs. 20 %, $p= 0.019$ Positive serum βhCG: 93 % vs. 67 %, $p= 0.046$*	13
Microvibration	Fresh inseminated oocytes from poor responders—randomized to 1 of 2 treatments (no split)	42 Hz for 5 s/hr for 15–18 h	Normal Fert: 80.0 % vs. 76.1 %, NS Day 3 A Quality: 66.4 % vs. 60.4 %, NS Total Preg rate: 22.9 % vs. 13.2 %$p< 0.05$ Implantation rate: 11.6 % vs. 6.7 %, $p< 0.05$ Day 5 Blast rate: 16.9 % vs. 7.4 %, $p< 0.05$	16

[#] a later review article from the same group indicates 44 Hz was used for additional experiments (15)

*transfers included both fresh and vitrified/thawed embryos from a total of 28 transfers in TECS vs. 9 transfers from static controls

Whether these effects are more prominent at varying development stages is unknown, though the aforementioned human studies suggest that the benefit may be conveyed over various and brief periods of time.

Regardless of the potential of these dynamic culture approaches, widespread clinical implementation has not been achieved due to various limitations. One way to implement dynamic embryo culture on a wider scale could entail combining the approach with emerging real-time cameras and platforms used to collect morphokinetic data. *(After all, with the prevelance of paparazzi and celebrities in popular media today, if we view embryos as "rock stars" in dynamic culture, their filming seems like a perfect fit!)* A simple vibrating device, similar to those found in cell phones, could be adapted to gently vibrate the culture platform or the housing area of portable camera modules or real-time imaging incubators. If vibration was scheduled to occur between image capture sequences, this should have little effect on visualization or cell tracking. However caution may be needed. Though gentle vibration for very brief periods (5 s/hr) has been found to be beneficial in standard culture platforms of larger area/volume [14–16], efficacy within the small confines of the microwells used by many current real-time imaging devices has not been shown. The smaller culture area could result in great sheer stress. Indeed, preliminary data using rotational culture in microwells has been found to be detrimental to mouse embryo development compared to rotational culture on a flat petri dish [20]. Alternatively, with the emergence of small benchtop incubators, applying a tilting or vibrating platform under the outside of the entire incubator unit, rather than inside the incubator under the actual dish, may prove to be an easy alternate approach to provide gentle movement and avoid issues of humidity and electronics, inadequate space and other limitations.

In summary, dynamic platforms offer a potential paradigm shift and hold the promise of revolutionizing in vitro embryo culture. However, as with many new approaches, cost and ease-of-implementation are often immense barriers to overcome. As methods are improved to address these current limitations, perhaps we will see more reports on improved live birth outcomes using dynamic embryo culture. Until then, maybe one could simply take a cue from the title of this commentary. Could it hurt to simply turn up the bass on the lab stereo and point the speakers toward the incubator to produce acoustic waves that may produce gentle stimulation? Others have used acoustic waves for media and somatic cell manipulations on microfluidic platforms [21–24]. A preliminary study even suggests that gentle vibrations from music played from speakers placed inside the culture incubator could influence human oocyte fertilization [25]. Of course, music selection should presumably promote a gentle agitation of embryos *(think "rock-a-bye-baby"… perhaps music conducive to a gentle waltz)* and avoid excess movement *(think "don't shake the baby!"…perhaps best to avoid head-banging heavy metal or fervent swing-dancing music).* However, in this scenario, getting embryologists to agree on music selection may prove to be the more difficult task, but I digress. Dynamic embryo culture may help revolutionize IVF; only time will tell.

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