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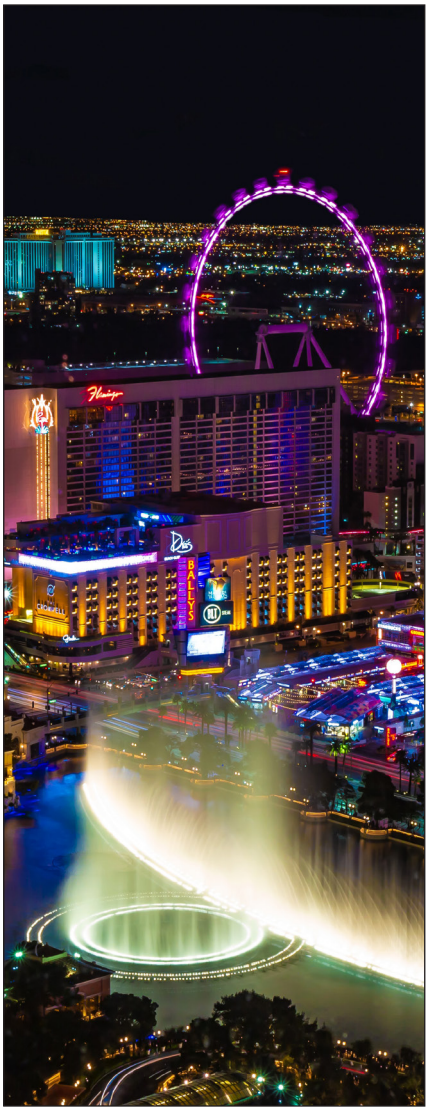

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AI-Validated Decision Tree Improves Success Rates of Live Birth Rates in Euploid Embryo Transfers for Women of Advanced Maternal Age

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Objective: The aim was to utilize a deep learning artificial intelligence approach to investigate the variability in euploid embryo live birth rate (LBR) among women with advanced maternal age (AMA) and to identify the combination of variables that improve LBR.

Design: Retrospective Study in a Preimplantation Genetic Testing Center.

Materials and methods: This multi-site, study was conducted by a private laboratory (Progenesis, Inc.) including 843 patient frozen-thawed embryo treatments (FET) from 22 United States clinics in 2017-2021. All embryos were screened for aneuploidy using whole genome amplification using Next Generation Sequencing. Female patients' age ≥36y with either autologous (n=715, treatment group) or donor oocytes (n=128, control group) created embryos was included. Blastocysts grades were assigned at biopsy using the SART grading. The main outcome measure was live birth rate (LBR). Random Forrest and Evtree supervised learning algorithm and generalized linear model employing logistical regression analysis was used.

RESULTS: The treating clinic was the most significant variable with a probability reduction of 22.1% in LBR and a variable weight of 0.54. Female age <39y had a 66.7% (n=39) probability of LBR when mitosure score is <0.81, TE grade is A or B and embryo is day 5 stage, regardless of source of oocytes (n=39). However, a TE grade of B or C at a high performing clinic (HPC) had LBR of 65.3% (n=101) for female age of <39y in both treatment and control groups. When female age is ≥39y, the probability of LBR is 65.3% with an embryo of <0.96 mitosure score, TE grade of A or B and HPC. Embryo with a mitosure of ≥0.96 had LBR probability of 35.6% (n=59) in the control group and 73.3% (n=150) for treatment group when at a HPC. The final model has a sensitivity of 0.68, specificity of 0.68 and area under the curve (AUC) of 0.68.

Conclusions: AMA at retrieval does not impact LBR for donor or autologous oocyte created euploid embryos independently. Our results highlight the variability of performance and lack of standardization among clinics and laboratories. Variance in stimulation protocols, not included in our evaluation, and laboratory procedures involving technical skill and judgment elucidate the outcome probabilities. Our AI decision tree reveals the intricate connections among actionable variables and delivers a remarkable predictive accuracy of 68%, thereby optimizing live birth rates for FET treatments.

Disclosures: Nothing to Disclose

Funding: None

The Anatomy and Responsiveness to a Culture Medium Recall Event

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Abstract

Objectives: The purpose of this investigation was to evaluate the quality control (QC) measures and practices Embryologists should rely on and apply to detour potentially catastrophic mishaps caused by suboptimal products.

Design: Retrospective validation-verification

Materials & Methods: Standard operating procedures and daily QC practices are applied in our two Pinnacle Fertility IVF Labs staffed with experienced, seasoned Embryologists. Using Global medium (LGGG) + LGPS/hyaluronate, we routinely calibrate the CO2 content of our tri-gas mix to achieve a pH of 7.30 to 7.34 each Lot. While we perform in-house human sperm survival testing (HSST over 5 days) to approve plasticware, we rely on commercial QC testing of media products utilizing endotoxin testing and a mouse embryo bioassay (MEA). Following new Lot pH verification (7.32), a new batch of LGGG began use on 11/13/23. A series of events and prompt actions were taken to mitigate an obvious culture problem where high quality cleaved embryos were failing to compact and form blastocysts (BL), these included: 1) prompt communication between staff and director, with lab affiliates and colleagues, and complaints to distributor representatives; 2) observation and corrective action; 3) experimental validations/data collection for quality assurance; 4) honest transparency to patients; and 5) an open dialog to seek answers and preventative solutions with the distributor. In addition, we conducted comparative HSST in-house and independent, strict MEA testing.

Results: Over a 6d period, normal fertilization and blastomere development was observed. Concerns over the lack of compaction and BL formation, including a 30+ embryo donor cycle on D6 on Monday 11/19, quickly manifest into the emergent use of replacement culture medium (see Table 1). HSST yielded normal +120h motility (<24% motility loss), thus ruling out the absence of Glucose or a toxic residue. MEA testing produced borderline normal BL formation (80%), but hatching and normal cell numbers (mean=150), were significantly reduced ($p<0.05$). Ultimately, only 12 patients were exposed to the New LGGG media, with Table 1 reflecting the mean adverse effects caused by compaction inhibition.

Conclusions: Prompt detection of a lab problem, and identification of the probable source minimized adverse cycle outcomes to 4 patients, whom still produced 1-3 Day 7 BLs. Further validation of the culture media effect and QC measures (HSST/MEA), open communications and updates with the distributor led to an FDA recall and diagnosis, within 1 month, that Mg^{++} had failed to be added to the affected LGGG batch. New Lot release QC measures have been instituted, since batch osmolality and total weight were not sensitive enough to detect the absence of Mg++ which effectively inhibited compaction. Stricter QC standards for culture medium should be required by FDA.

Table 1. Validation of significant (*) BL development alterations caused by Recalled LGGG (New)

	Old LGGG(6d)	New LGGG(6d)	NX_CM (10d)	Gx-TL (6d)	Gx-TL (6 wk)
#MII (pH)	297 (7.31)	146 (7.32)	364 (7.34)	166 (7.33)	797 (7.32)
#2PN	221	114	284	132	656
%FER	74.4%	78.1%	78.0%	79.5%	78.5%
%BUR	55.7%	22.8%*	69.0%	59.1%	67.2%
% of D5	44.7%	15.4%*	51.5%	46.2%	53.4%
% of D6	47.2%	23.1%*	40.3%	35.9%	40.4%
% of D7	8.1%	62.5%*	8.2%	17.9%	7.7%

Study Funding/Competing Interests: None

Trial Registration Number: Not Applicable

Key words: embryo, culture medium, recall, quality control

BlastAssist: A Deep Learning Pipeline to Measure Interpretable Features of Human Embryos

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Objective: To determine the performance of BlastAssist, a deep learning pipeline, in measuring interpretable and clinically relevant features of human embryos.

Design: We conducted detailed comparisons between the BlastAssist pipeline measurements and 1) human experts' manual measurements; 2) embryologists' annotations of clinical features during routine treatments; 3) implantation results of single embryo transfer (SET) cycles; 4) live birth results of cycles where up to four embryos were transferred.

Materials and Methods: The study consists of 67,043,973 EmbryoScope™ image data (n=32,939 embryos), manual embryo annotations (n=18,922 embryos), and treatment outcomes (n=723 SET cycles; n=1801 cycles, n=3421 embryos) recorded in 2012-2017 in the IVF lab of Tel Aviv Sourasky Medical Center. Pearson's χ^2 test was used to evaluate the statistical independence of individual features and implantation success. Bayesian statistics was used to evaluate the association of individual features to the probability of an embryo resulting in live birth.

Results: BlastAssist performs similarly or better than manual measurements by human experts on features including: fertilization status (area under the receiver operating characteristics (AUROC)=0.84−0.94), cell symmetry (2-cell r=0.71±0.06; 4-cell r=0.77±0.07), degree of fragmentation (pipeline acc=69.4%; human experts acc=73.8%), and developmental timing (pipeline acc=90.0%; human experts acc=91.4%).

There is strong agreement between BlastAssist and annotations made by embryologists during routine treatments on features including: fertilization status (acc=79.6%, r=0.683), degree of fragmentation (acc=55.4%, r=0.648), pronuclei fade time (r=0.787), and time of blastulation (r=0.887).

For implantation results from SET cycles, 2-cell time ($p<0.01$) and 2-cell symmetry ($p<0.03$) are significantly correlated with implantation success rate, while other features showed correlations without statistical significance.

For live birth results, 2-cell time ($p<5\times10^{-11}$), pronuclei fade time ($p<5\times10^{-10}$), degree of fragmentation on day 3 ($p<5\times10^{-4}$), and 2-cell symmetry ($p<5\times10^{-3}$) showed statistically significant correlation with the probability of the transferred embryo resulting in live birth.

Conclusions: The BlastAssist pipeline provides a comprehensive and holistic means of human embryo evaluation and either performs comparably to or outperforms embryologists and human experts in measuring clinical features. Contrary to "black-box" algorithms, BlastAssist outputs meaningful measurements that can be interpreted and corroborated by embryologists, which is crucial in clinical decision making.

Disclosures: N/A

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The Correlation of Inner Cell Mass Grade and Trophectoderm Grade on Patient Clinical Outcomes Following a Frozen Embryo Transfer

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Objective: To analyze the relationship between embryo morphology grades and the significance on patient pregnancy rates following a single euploid frozen embryo transfer (FET).

Design: Retrospective study.

Materials and Methods: This study included 694 FET cycles of single euploid embryos performed in 2023. Embryos were graded based on the morphology of the inner cell mass (ICM) and trophectoderm cells, at the time of vitrification using the SART grading scale. Transfers were separated into 4 groups based on the embryo grade: Good/Good (n=266), Good/Fair (n=159), Fair/Good (n=95), and Fair/Fair (n=174). Embryo survival, pregnancy and implantation rates were analyzed and compared for each group. Transfers with untested patient embryos, multiple embryos or donor eggs were excluded from the study. Statistical analyses were performed using an N-1 Chi-squared test.

Results: No differences in patient ages or embryo survival rates were noted for any of the embryo grade groups. Embryos graded Fair/Fair showed significantly lower positive β-HCG, clinical, ongoing, and implantation rates compared to embryos that were graded Good/Good or Good/Fair (Table 1).

Table 1. Pregnancy outcomes for different grades of embryos

	Embryo Grade			
	Good/Good	**Good/Fair**	**Fair/Good**	**Fair/Fair**
n	266	159	95	174
Patient age (mean ± SD)	34.9 ± 4.0	35.5 ± 3.6	35.4 ± 4.1	35.3 ± 4.3
Embryos warmed	268	160	98	174
Embryos survived (%)	266 (99.3%)	159 (99.4%)	95 (96.9%)	174 (100%)
Embryos transferred	266	159	95	174
Positive β-HCG rate (%)	66.5[a]	65.4[b]	61.0	54.6[a,b]
Clinical pregnancy (%)	63.9[c]	62.3[d]	54.7	50.0[c,d]
Ongoing pregnancy (%)	58.6[e]	60.4[f]	51.6	45.4[e,f]
Gestational Sacs	171	100	52	87
Implantation rate (%)	64.7[g]	62.9[h]	54.7	50.0[g,h]

Values with the same superscript are significantly different. [a]p=0.012, [b]p=0.045, [c]p=0.003, [d]p=0.024, [e]p=0.006, [f]p=0.006, [8]p=0.002, [h]p=0.018

Conclusions: Embryos graded as Good/Good showed the highest clinical outcomes compared to the other embryo groups. While it has been suggested that embryo grade does not affect the overall clinical outcome if the embryo is euploid, this data suggests grade still plays a role. Embryos graded as Good/Good and Good/Fair showed a significant increase in positive β-HCG, clinical pregnancy, ongoing pregnancy, and implantation rates.

Disclosures: None.

Funding: None.

Evaluation of culture media with antioxidants on blastocyst development

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Objective: Proper culture media is the cornerstone of a good IVF lab as these provide nutrients for proper embryo development. Reactive oxygen species (ROS) have been shown to negatively impact embryo growth, and supplementing media with antioxidants may combat the effects of ROS. The aim of this study was to evaluate the effect of culture media with antioxidants on fertilization and blastocyst development.

Design: Retrospective analysis.

Materials and Methods: A total of 728 patients undergoing IVF with either conventional insemination or intracytoplasmic sperm injection (ICSI) were included in the study. Embryos from patients from January-April 2023 were cultured in Irvine Scientific CSC-NX media and those from August-December 2023 were cultured in Vitrolife GX media. Fertilization rate and good quality blastocyst rates were compared between the two media. Statistical analyses were performed using an N-1 Chi-squared test.

Results: No significant differences were noted in the fertilization rate between the two media for both conventional insemination and ICSI. However, the overall blastocyst rates were significantly improved in the Gx media compared to the CSC-NX media in both conventional insemination (38.3% vs 27.8%, $p<0.0004$) and ICSI (40.6% vs 31.4%, $p<0.0001$) (Table 1).

Table 1. Fertilization and blastocyst development rates of embryos culture in CSC-NX versus GX media following ICSI.

	ICSI						Conventional Insemination					
	CSC-NX media			Gx media			CSC-NX media			Gx media		
Age group	n	Fert %	Blast %	n	Fert %	Blast %	n	Fert %	Blast %	n	Fert %	Blast %
<35	101	75.5	36.3	119	78.2	47.7	17	70.4	33.5	13	69.3	39.3
35-37	81	74.7	33.3	75	75.3	38.9	10	47.7	20.5	15	69.8	43.2
38-40	63	75.3	25.3	70	75.8	36.4	7	75.0	20.6	4	71.9	26.8
41-42	29	78.1	21.4	43	77.1	23.9	2	75.0	18.5	4	62.2	8.7
>42	17	72.6	7.8	26	68.0	28.8	2	28.3	0.0	3	39.6	15.8
Donor	7	86.2	28.6	14	73.4	42.7	4	68.8	30.7	2	57.6	57.9
Total	**298**	**75.7**	**31.4[a]**	**347**	**76.4**	**40.6[a]**	**42**	**63.9**	**27.8[b]**	**41**	**66.9**	**38.3[b]**

[a]P<0.0001; [b]P<0.0004

Conclusions: Gx media with antioxidants has a beneficial effect on blastocyst development that can be applied for both conventional insemination as well as ICSI across all patient age groups. Each laboratory should perform its own quality control to determine the best culture system to provide the best embryo quality.

Disclosures: None

Funding: None

Late matured oocytes – are they "special"? Investigating the morphokinetics and clinical performance of embryos derived from oocytes with delayed maturation

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Objective: To investigate whether morphokinetic pattern of blastocysts formed of oocytes with delayed maturation is similar to sibling embryos acquired from initially mature gametes and evaluate the clinical rationale of their utilization.

Design: A retrospective study included IVF cycles that yielded oocytes converted from MI to MII stage in vitro (MI-MII) at a single academic center in 2017-2023. The morphokinetic parameters were assessed for blastocysts harvested from MI-MII injected oocytes and sibling in vivo matured oocytes in 2022-2023.

Materials & methods: The study included 736 cycles that yielded 1183 late matured oocytes converted from MI to MII stage in vitro before ICSI (study group). All the sibling oocytes that were at the MII stage during denudation (n=5104) were assigned to the control group. Sperm injection was performed 40.5-41.0 h post trigger and MI-MII injected oocytes were cultured separately to track their development.
Fertilization, blastulation, and euploidy rates of the study group were compared with key indicators of in vivo matured gametes from the same cycle. The implantation capacity of blastocysts from the study group was investigated by assessing the outcomes of fresh and frozen single embryo transfers of samples obtained from MI-MII oocytes. Main time parameters annotated for embryos deriving blastocysts in study and control groups were assessed after prolonged culture of samples (n=361) in a time-lapse incubator and included: timing of pronuclei fading (tPNf), cleavage (t2, t3, t4, t5, t6, t7, t8), morulation (tM), initiation of cavitation (tSB) and blastocyst formation (tB).

Main results and the role of chance: The fertilization rate of the late matured oocytes was significantly lower compared to regularly mature ones (55.3% vs 76.3%, $p<0.05$). Also, the blastocyst formation rate was decreased in the study group comprising 29.3%, while the fertilized mature eggs conversion rate reached 50.3% ($p<0.05$). On the contrary, the euploidy rate in tested "MI-MII" blastocysts, n=91, was not statistically different from the sibling mature oocytes cohort, n=318 (32.9% vs 39.3%, $p>0.05$).
Though the fertilization and developmental competence of late matured oocytes seem to be lower compared to mature sibling cells, the implantation potential of MI-MII blastocysts is encouraging. Thus, the implantation rate reached 33.3% for fresh (n=6) and 71.4% for frozen (n=7) single embryo transfers.
Retrieved blastocysts regardless of the initial oocyte maturity demonstrated comparable morphokinetic patterns starting from t3 and further. On the contrary, the timing of earlier events including tPNf and t2 was delayed for embryos formed out of MI-MII oocytes (24,9±4,0 h and

23.9±2.8 h, p=0.04; 27.5±3.6 h and 26.2±2.9 h; p=0.009, for study and control groups respectively).

Conclusions. Late matured oocytes have compromised developmental competency that manifests in lower fertilization, blastocyst utilization, but comparable euploidy rates. Still, fertilized MI-MII oocytes can progress into euploid blastocysts and implant successfully. Competent embryos derived from oocytes matured in vitro display slightly delayed pronuclei dynamics and first cleavage timing but seem to catch up with sibling embryos at later stages. So despite diminished developmental capacity, late matured oocytes have clinical significance and samples that successfully progress to the blastocyst stage should be considered as valuable specimens for embryo transfer.

Disclosures: None

Funding: None

Remote Monitoring Systems and the Staff that Went Crickets: Percentage of Lab Response to Critical Alarms in USA IVF Laboratories

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Objective: This study aimed to demonstrate the importance of real-time remote monitoring with operator safety net (24/7 human supervision) by assessing the length of time available for addressing a cryotank failure and the percentage of neglected critical alarms in USA IVF Laboratories.

Materials and Methods: Phase 1: Records from pay-per-service clients (n=120) were analyzed to observe the percentage of unattended critical alarms during a period of 3 months. This study included a total of 1229 critical alarms, with specific focus on incubators (n=689) and cryostorage (n=540). Phase 2: A multicenter, controlled cryofailure experiment was performed, including the three most common types of cryo dewars used in IVF, and analysis of length of time available for addressing a cryostorage emergency, after an alert is triggered. Phase 3: A controlled shut off experiment was performed, using 4 commonly used benchtop incubators and the decrease in gas and temperature parameters were controlled once alarm was triggered, in order to determine maximum time available to mitigate risks of sample exposure to adverse culture environment.

Results: Phase 1: Among the observed IVF Laboratories, the range of non-response to critical alarms varied between 13% and 38%, with an average neglect rate of 25% (n=1229). For incubators, the neglect rates ranged from 10% to 90% (n=689), while for cryostorage, the rates varied between 13% and 60% (n=540). Remarkably, in all instances of critical alarms, the safety net operator on call successfully addressed the conditions, preventing any compromise of stored samples due to modified environmental parameters. Phase 2: It has been demonstrated that a time between 2h and 12h is available for mitigation of risks during a cryofailure, depending on its type and severity. Phase 3: It has been demonstrated that the time for addressing an incubator failure is between 20 min and 2h, depending on the type of failure occurring (gas/temperature)

Conclusion: The findings reveal a higher-than-anticipated number of neglected critical alarms in USA IVF Laboratories. Real-time remote monitoring plays a pivotal role in ensuring sample safety due to the immediate alert response to adverse parameters, specially for embryo culture, and the implementation of a safeguard operator action is imperative for the well-being of embryos in the current state of laboratory management. This underscores the urgent need for enhanced monitoring systems overseen by operator responsiveness to critical alarms for the continued success and safety of assisted reproductive technologies.

Disclosures: No conflict of interests to disclose

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With Laser or Without Laser, that is the Question!

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Objective: To assess the potential impact of laser-assisted trophectoderm biopsy on laboratory, clinical, and genetic outcomes.

Design: The thaw survival, implantation, euploidy/aneuploidy, and mosaicism rates in blastocysts in which trophectoderm biopsy was performed with or without laser assistance between January 2015 to December 2023 in Greenwich and Bahceci Fertility Centers were examined retrospectively.

A total of 7252 embryos were analyzed. A subgroup analysis was performed to better understand the genetic outcomes of different blastocyst qualities. Gardner's classification was used to grade blastocysts as good-quality (GQ) (3BB and better) and moderate-quality (MQ) (blastocysts graded with any Cs).

Materials and methods: The study analyzed statistical data of autologous PGT-A cases between 2015-2023. Trophectoderm biopsies were performed using laser assistance (L+) and without laser assistance (L-). The NGS method was used to evaluate the biopsy samples, which enabled the identification of 20% to 80% mosaicism. We assessed whether there were any differences in thaw survival, implantation, mosaicism, euploidy, and aneuploidy rates between L(+) vs. L(-) groups. Moreover, the effects of laser use among the GQ and MQ groups were analyzed in terms of genetic outcomes.

Results: Trophectoderm biopsy was applied on 7252 embryos within the specified period. During the biopsy in 4152 blastocysts laser was used and in 3100 blastocysts laser was not used. Thaw survival rates were analyzed, and there was no statistical significance between groups (98.7% L(+) vs. 99.1% L(-), $p=0.11$). Additionally, the L(-) group's implantation rate is significantly higher than the L(+) group (respectively, 61.8% vs. 59.4%; $p<0.05$).

There were no statistically significance euploidy and aneuploidy rates between L(+) and L(-) groups (respectively, euploidy 47.6% vs 48.2; aneuploidy 39.4 vs 38.6, $p>0.05$). On the other hand, mosaicism rates in the L(+) group were statistically higher compared to the L(-) group (respectively, 14.7% vs 11.2%, $p<0.0001$).

In the GQ, the rate of mosaicism was significantly higher in the L(+) group compared to the L(-) group (respectively, 15.6% vs 12.4%; $p<0.0001$). Interestingly in MQ, there were no

significant differences in mosaicism rates between the groups (respectively, 14.2% vs 14.4%; p>0.05).
Conclusions: This study compared laser-assisted trophectoderm biopsy with non-laser-assisted biopsy in 7252 embryos. The thaw survival rates were similar, but implantation rates were higher in the non-laser group. The laser-assisted group had a significant increase in mosaicism, mainly seen in good-quality blastocysts. The study suggests that laser use should be limited.
Disclosures: Data from Greenwich Fertility Center and Bahceci Health Group Fertility Center are incorporated into this study. Maternal age-related aneuploidy and patient diagnostic histories are factored out from the discussion.
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Assessing the Impact of Cell Phone Emitted Electromagnetic Waves (EMW) on Sperm Genetic Variants

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Objective: Smartphones emit high radiofrequency-electromagnetic radiation (RF-EMR) that the human body can absorb. Previous studies suggest that these radiations, combined with the heat from these devices, might disrupt spermatogenesis by inducing oxidative stress and mitochondrial reactive oxygen species (ROS), causing sperm DNA damage. Our recent in vitro study showed a negative impact of WiFi radiation emitted by smartphones on sperm parameters. However, the molecular pathways through which WiFi radiation affects sperm quality remain unclear. Thus, this study aims to investigate the molecular pathways involved by WiFi radiation emitted from mobile devices on sperm.

Design: Observational-interventional study

Materials and Methods: We recruited six healthy men with normozoospermic semen profiles. Baseline semen parameters were assessed by WHO 2010 standards. Samples were then exposed to WiFi radiation from a smartphone in an active WhatsApp call mode for six hours. Pre- and post-exposure semen samples were subjected to comprehensive whole exome sequencing focusing on single nucleotide polymorphism (SNP) genotyping to identify genomic variations.

Results: Initial semen analysis indicated a statistically significant reduction in progressive sperm motility and viability following RF-EMR exposure ($p<0.05$). Genomic analysis revealed a median increase of four exonic, nonsynonymous SNPs in the post-exposure samples. In total, 20 new exonic, nonsynonymous SNPs were identified post-exposure, with 12 of these variants being expressed in male reproductive tract tissues.

Conclusions: This pilot study showed a decrease in sperm progressive motility and an increase in potentially related genetic variants associated with sperm motility following six hours of exposure to RF-EMR. However, these preliminary results need further research with more comprehensive genotyping mutational analysis to investigate and validate the effects of RF-EMR exposure on semen parameters and to determine the clinical significance of these genetic variants.

Disclosures: None

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Title: Assisted Hatching may improve the Clinical Pregnancy Rates in cases with Conventional IVF cycles but not in ICSI cycles with fresh embryo transfers

OBJECTIVE: The clinical benefits of assisted hatching (AH) remain insufficiently defined despite many previous clinical studies and meta-analyses. This study aimed to compare the impact of AH on clinical outcomes in conventional IVF or ICSI cases with fresh embryo transfers.

MATERIALS AND METHODS: This study retrospectively compares the effect of AH on clinical outcomes in conventional IVF and ICSI cycles with fresh embryo transfers. The clinical outcomes of 160 conventional IVF (Group I: IVF-AH; Group II: IVF no AH) and 356 ICSI (Group III: ICSI-AH; Group IV: ICSI no AH) day 3 and day 5 fresh embryo transfer cycles in women under 38 years old from January 2017 and October 2021 in a single clinic setting were included. Decisions for the method of insemination and AH were based on clinician preference and clinical indications. Embryos underwent laser AH on the morning of Day 2 of development in culture at the time of embryo check using an Octax laser (4.0ms). For statistical analysis, the Chi-square, Fisher's exact and Student t-test were used where appropriate and $p<0,05$ was accepted as significant.

RESULTS: Patients within the study groups were matched for female age, BMI, AMH, E2 levels at trigger, number of oocytes retrieved, fertilization rates, mean number of embryos transferred, endometrial thickness and progesterone on the day of trigger. For IVF cases, the clinical pregnancy rate was significantly higher in Group I (66.0% [68/103]) compared to Group II (49.1% [28/57]), ($p=0.004$). Although the implantation rates were favoring the Group I (51.9% [68/131]) compared to Group II (39.4% [28/71]), the results were not statistically significant ($p=0.09$). For ICSI cases, the clinical pregnancy rate in Group III (52.0%, [77/148]) was similar to Group IV (52.9%, [110/208]) ($p=0.87$). The implantation rates were similar in Group III (38.9%, [77/204]) and Group IV (37.7, [110/283]) ($p=0.8$).

CONCLUSIONS: In our clinical setting, AH was associated with improved outcomes in women undergoing conventional IVF cycles and fresh embryo transfer, whereas AH was not associated with a benefit in ICSI cycles. This difference may arise from structural changes in the zona pellucida due to sperm zona interactions, which ICSI bypasses.

IMPACT STATEMENT: Although there is some controversy about the benefits of AH, our preliminary observations indicate that conventional IVF cycles may require AH whereas the ICSI cycles do not in fresh embryo transfers. The reasons and potential mechanisms for these differences must be further investigated.

BLASTOCYST DEVELOPMENTAL POTENTIAL AND EUPLOIDY RATES OF 0PN OOCYTES VERSUS 2PNS

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OBJECTIVE: Oocytes with no indication of pronuclei (0pn) at the time of fertilization assessment are often discarded. While there is literature to support that embryos from 0pn zygotes are viable for transfer, there are few studies that analyze the genetic outcomes of these embryos. The aim of the study was to determine if 0pns are capable of developing into euploid blastocysts compared to 2pns.

DESIGN: Retrospective study

MATERIALS AND METHODS: A total of 55 patients undergoing IVF with ICSI were included in the study. Female patients ages ranged from 23-41, with a mean age of 33. Oocytes were assessed for fertilization between 16-20 hours post insemination. Oocytes that showed 0pn and 2pns were cultured separately until Day 7 and evaluated for blastocyst development. Patients that had no blastocysts derived from either 0pn or 2pn were excluded from the study. Blastocysts were biopsied and tested for euploidy using NGS. The blastocyst development rate and euploidy rates were compared between 0pn and 2pn oocytes using an N-1 Chi-squared test.

RESULTS: The data obtained are depicted in Table 1. A total of 263 0pns and 697 2pns were cultured until day 7. No significant difference was noted in the blastocyst rate between the 0pn and 2pn groups (36.5% vs 39.5%, p=0.39). The blastocysts developed from the 0pn contributed 25.6% of the total blastocyst rate. The 0pn blastocysts showed a 51.7% euploidy rate compared to 61.7% for the 2pn group (p=0.09), which contributed 22.5% of all euploid blastocysts.

Table 1: Blastocyst and euploidy rates of 0pn and 2pn embryos

	0pn	2pn	0pn + 2pn
N	263	697	960
Blastocysts	96	275	371
Blastocyst rate (%)	36.5%[a]	39.5%[a]	38.6%
Embryos biopsied	89	256	345
Euploid (%)	46 (51.7%)[b]	158 (61.7%)[b]	204 (59.1%)
Aneuploid (%)	34 (38.2%)	68 (26.6%)	102 (29.6%)
Mosaic (%)	6 (6.7%)	20 (7.8%)	26 (7.5%)
No result (%)	3 (3.4%)	10 (3.9%)	13 (3.8%)

[a]p=0.39; [b]p=0.09

CONCLUSION: The data shows comparable blastocyst and euploidy rates in blastocysts derived from 0pn and 2pn. The 0pns contributed to the total number of blastocysts available for freezing or biopsy with comparable euploidy rates. This is compelling evidence to support culturing of 0pns to the blastocyst stage. It is possible that the 2pns were not identified correctly or were not visible at time of fertilization check, and therefore should be kept in culture as they have the potential to yield euploid blastocysts.

DISCLOSURES: None.

FUNDING: None.

Continuous Assessment of Competency to Maintain Consistency and Improve Outcomes Across an IVF Lab Network

Objective: To develop and implement a comprehensive internal competency benchmarking system within a network of interconnected IVF laboratories, aimed at ensuring and enhancing quality control and assurance processes. This objective facilitates efficient troubleshooting, and comparison of clinical decision performance to identify areas for improvement. The ultimate goal is to optimize success rates and operational efficiency while mitigating confounding factors through advanced digital tools and methodologies. Internal competency benchmarking is a powerful tool for quality control and assurance. This is especially true when comparing several related labs for external benchmarking within a network with consistent training protocols and standard operating procedures. Conventional competency assessment methods present various limitations when applied across expansive networks, including reliance on paper-based processes, restricted media, and manual tallying and analysis of results.

Design: Quality assurance surveys were conducted across five locations within the CNY IVF Lab network over a two-year period (2022 and 2023). The surveys assessed various aspects of laboratory procedures, equipment maintenance, staff training, and compliance with regulatory standards. Data were collected and statistically analyzed to identify trends and areas for improvement.

Materials and Methods: The ART Compass digital competency assessment, education and training, and survey and research platform (web based, and mobile apps on iOS and Android), descriptive statistics.

Results: Table 1. The CNY Network contains 5 sites with 49 participants. Significant agreement was noted in a number of subjective assessments, including, blastocyst biopsy criteria, SART Blastocyst expansion, and top-quality blastocyst choice. Significant disagreement was noted in the marginal freeze or discard survey, and additional monthly freeze or discard surveys, in line with published results [1].

Table 1. Descriptive statistics and representative examples.

Sites	Participants	Surveys Completed	Significant Disagreement	Significant Agreement
5	49	75	Marginal Freeze or Discard Freeze or Discard? Gardner ICM Grade	Blastocyst Biopsy Criteria SART Blastocyst Expansion Choose Top Quality Blastocyst

Conclusions: In conclusion, our analysis of the CNY Network, comprising five sites and involving 49 participants, revealed notable trends and agreements in various subjective assessments, such as blastocyst biopsy criteria, SART Blastocyst expansion, and top-quality blastocyst selection. These findings underscore the potential for standardized practices and shared protocols across multiple sites to foster consensus and coherence in certain clinical decisions within the IVF industry. However, it is equally important to acknowledge areas of significant disagreement, particularly in surveys regarding marginal freeze or discard decisions and

additional monthly freeze or discard practices. These discrepancies highlight the complexity and nuances inherent in IVF clinical decision-making, suggesting the need for further investigation and refinement of existing protocols. Moving forward, bridging the gap between theoretical insights and clinical practice remains paramount, emphasizing the ongoing need for collaborative efforts and research initiatives to enhance the efficacy of continuous competency assessment on clinical practice.

Disclosures: OP and RK receive salary support from CNY. CLC and DG receive salary support and are shareholders of AIVF.

Funding: AIVF

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Contribution of paternal age on euploidy status of embryo and blastocyst formation rate

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Objective: To identify the effect of paternal age on blastocyst formation rate and euploidy status of embryos created from donor oocytes.

Design: Retrospective study.

Materials and methods: Data was collected from 91 donor oocytes cycles performed between 2016 to 2023. A total of 836 embryos were created from donor oocytes to minimize the bias from oocyte providers through intracytoplasmic sperm injection (ICSI), and those embryos underwent trophectoderm biopsy on day 5, 6 or 7 for preimplantation genetic testing for aneuploidy (PGT-A). All embryos were cultured in single step culture media, and 4BB or greater quality embryos were biopsied. Patients were grouped based on paternal age (<40 y, 40-45 y, >45 y), and the patients with testicular sperm aspiration were excluded. The euploidy status of embryos and blastocyst formation rates were compared among those paternal age groups. One way ANOVA was used to compare the percentage of test variables with the paternal age groups. *P* values less than 0.05 were considered statistically significant.

Results: Average euploidy rates were 60.9, 66.5, 63.4 percent in age groups of <40, 40-45, and >45 respectively. Aneuploidy rates for the age group of <40 was 25.8% while those rates were 20.7% for age group of 40-45 and 22.9% for patients over 45 years old. The percentages of mosaic embryos were 13.2%, 12.7% and 13.6% for the age groups of <40, 40-45, and >45 respectively. There was no statistical difference in euploidy, aneuploidy or mosaic embryo rates among all age groups ($P \geq 0.05$). In addition, blastocyst formation rate of patients younger than 40 was 67.4% whereas those rates were 65.4% in age group of 40-45 and 60.1% in age group >45. However, blastocyst formation rates were not statistically different among paternal age groups ($P \geq 0.05$).

Conclusions: Advanced paternal age is not associated with euploidy status of embryos and blastocyst formation rate in ICSI-PGTA cycles.

Disclosure: Nothing to disclose

Funding: None

A Deep Learning System for Predicting the Viability of Blastocysts using Gardner Criteria

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Objective: The objective of this work was to investigate the application of deep learning techniques in the field of in vitro fertilization (IVF) for the purpose of creating supportive tools that may aid in determining the optimal transfer order and implantation potential.

Design: Retrospective Study in a Preimplantation Genetic Testing Center.

Materials and methods: The deep learning algorithm was trained on 2000 static two-dimensional embryo images from [1] acquired at 400x magnification using an Olympus IX50 (Vienna, Austria) microscope. The developed blastocysts are scored using a standardized system, such as the Gardner score, which evaluates the blastocyst expansion (EXP) as well as the inner cell mass (ICM) and trophectoderm (TE). We created a novel multi-label multi-class deep learning technique to simultaneously evaluate the EXP, ICM, and TE using a single deep learning architecture. The fundamental architecture for encoders is based on MobileNet v2 [2].

RESULTS: When the deep learning system was assessed on a dataset of 300 images, it achieved accuracy rates of 82.1%, 69.5%, and 65.1% for the EXP, ICM, and TE tasks, respectively. In the evaluation of a blind test set consisting of 110 embryo images, the system exhibited an accuracy of 70.1% in accurately predicting the Gardner score. This outcome highlights the system's robustness and its capacity to generalize beyond the initial development set.

Conclusions: The agreement between AI scores and embryologist quality assessment (Gardner scoring) validates the use of deep learning system for embryo evaluation. The subsequent stages involve the independent assessment of the system by multiple centers on both group and single culture static images.

Disclosures: Nothing to Disclose

Funding: None

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THE EFFECT OF TIMING OF ASSISTED HATCHING ON BLASTULATION, EMBRYO GRADING, AND PLOIDY STATUS

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Objective: To procure a trophectoderm cell sample for preimplantation genetic testing (PGT), the embryo's zona pelucida (ZP) must be breached to access the cells. This laser assisted hatching (AH) can be performed on day 3 of culture or at the time of trophectoderm biopsy. The study's objective is to discover if there is a difference in embryo development, grading, or ploidy status depending on the timing of AH, either on day 3 or the day of biopsy.

Design: Prospective study in a private assisted reproductive technology program.

Materials and Methods: This study examined 50 patients and 923 embryos between May 2023 and November 2023. Each patient had 12 or more embryos at fertilization check which were randomly divided into two groups: AH on day 3 and no AH (AH on day of biopsy). The AH group embryos were assisted hatched on day 3 then were moved into new culture media. The no AH group embryos were not hatched on day 3 but still moved into new culture media. Embryos were evaluated for possible biopsy on days 5-7. The blastocysts were graded using the SART grading scale (Good (G), Fair (F), Poor (P) scale for inner cell mass and trophectoderm). Aggregate data was analyzed and a p-value < 0.05 was defined as statistically significant, with a 95% confidence interval.

Results: Total blastocyst utilization rate (BUR) was not statistically significant between the AH group and no AH group (54.5% vs. 56.6%, p=.4627). No difference was found between the number of embryos biopsied on day 5 (44.3% vs. 51.8%, p=0.1181). No difference was found in euploidy rate between AH group and no AH group (57.1% vs. 64.6%, p=0.1086). A statistically significant difference was found between the number of GG graded embryos between AH group and no AH group (34.8% vs. 48.2%, p=.004).

Conclusion: No difference was observed in BUR, day of blastulation, or ploidy status between the two groups whether the hatching was performed on day 3 or the day of biopsy. There was a significantly larger amount of GG graded embryos in the no AH group compared to the AH on day 3 group. Since we observed higher embryo grading and no impact on development or ploidy status, we no longer hatch embryos for biopsy on day 3 which streamlines lab workflow produces higher quality embryos.

Disclosures: None.

Funding: None.

Evaluation of Three Different Sperm Cryopreservation Media for Human Sperm Freezing and Thawing compared to Traditional TEST-Yolk Buffer

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Objective: Cryopreservation of human sperm is a widely used technique for both, male fertility preservation as well as fertility treatment via assisted reproductive technology (ART). Various sperm cryopreservation media are commercially available. The available media differ in chemical composition and utilization protocols, additionally, no recent data exist on efficiency of various types of cryopreservation media measured by pre- and post-freeze motility rate. The aim of this study is to estimate the post-freeze motility drop using three commercially available sperm cryopreservation media in comparison to that after the traditional sperm freeze using TEST-Yolk Buffer (TYB).

Design: Analyses of post-thaw sperm motility after the use of three, commercially available sperm cryopreservation media compared to traditional TYB at an academic fertility center.

Materials and Methods: After obtaining the institutional review board approval, semen samples that met the inclusion criteria were obtained: volume >1.5 mL; concentration 17 mil/mL; motility 20% or more. Samples were then divided into four equal aliquots. Each aliquot cryopreserved using one of the four different sperm cryopreservation media. Based on the media used the samples were assigned into 4 groups (A, B, C and control TYB). Samples were frozen and thawed according to each vendor's protocol. Motility was evaluated before and after freeze-thaw process. Statistical analyses were performed using ANOVA and paired t-test.

Results: Ten semen samples were allocated into 4 groups. Average sperm motility prior to cryopreservation was 60.2%. As expected, all four sperm cryopreservation media were associated with a drop in sperm motility ($p<0.001$). Control group, TYB showed significantly higher post-thaw motility (35.7%) compared to study groups A, B, and C (26.3%, 24.2% and 25.5% respectively) ($p=0.02$ control versus group A, $p=0.004$ control versus group B, $p=0.006$ control versus group C respectively). There was no significant difference in post-thaw motility drop among the experimental groups A, B and C.

Conclusions: TYB use is generally discouraged due to utilization of animal products and theoretical possibility of viral contamination. Nonetheless TYB is more affordable and results in better post-thaw sperm motility compared to newer human sperm cryopreservation media. In order to promote and further encourage the use of these second generation sperm cryopreservation media, further research and improvements to the media have to take place to have at least comparable, but preferably surpass the post-thaw sperm survival.

Disclosures: Authors have reported no conflict of interests.

Funding: N/A

Hotspots in IVF Incubators: Making a Case for Thermal Imaging

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Objective: To illustrate the usefulness of thermal imaging to identify inconsistent heating and hotspots in IVF incubators.

Design: Retrospective investigation to identify the cause of degeneration of patient material.

Materials and Methods: Site-specific degeneration of ICSI'd eggs was noticed in a planar (BT-37) incubator following an overnight culture. Temperatures were monitored daily using a thermocouple thermometer (Fluke 52 II) via the temperature monitoring ports on the sides of the incubator. After the incident, an IR thermometer (Fluke 62 Mini IR) was used to measure the surface temperature and subsequently, thermal imaging was done using a FLIR-C2 camera to identify the temperature variations across the surface and the extent of the hotspots.

Result: A review of the daily QC showed that the temperature and gasses were within acceptable ranges. The incubator was on contact alarm but did not register any alarms. When the degeneration was specific to a dish, a root cause analysis was performed. After ruling out all other possibilities, it was suspected that the incubator may have had hotspots at that specific location. IR thermometer was used to measure surface temperature and it showed a significant increase (39.2^{o}C) in the spot where the degeneration occurred. Further, imaging with a thermal camera revealed the size, distribution, and gradient of the hotspots in that specific incubator and other incubators. Further measurements of temperature by monitoring through the port, IR, and oil dish showed that temperature monitoring through the port may not be reliable. Thermal imaging appears to be the best method to identify hotspots. Subsequent monitoring also identified the temporal nature of the temperature spike in those spots.

Conclusion: Thermal imaging can be a useful method to identify hotspots in incubators. We identified hot spots that could explain the degeneration of patient material on a specific site within the incubator. Although continuous temperature monitoring and daily measurements by various methods may give good data, they are not designed to identify uneven heat distribution within the incubator chambers. Thermal imaging of incubators could be used to screen for the presence of any hotspots or temperature gradient as part of annual maintenance and could prevent catastrophic outcomes.

Disclosures: None
Funding: None

Improvement in Sperm Recovery Rate and Total Motile Sperm Count Using Alpha-chymotrypsin in Highly Viscous Semen Sample

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Objective: This study addresses the sperm recovery rate and total motile sperm count (TMC) with and without α-chymotrypsin treatment in highly viscous semen for intrauterine insemination (IUI) and vitro fertilization (IVF), with a focus on individuals with severely low sperm count.

Design: Retrospective cohort study of patients at a university hospital program.

Material and Methods: High viscosity was defined as the absence of a thread over 2cm long from a semen drop after a 30-minute incubation period at 37 C with repeated pipetting. The specimens were treated with 5 mg of α-chymotrypsin for 5-10 min at 37C. Using a 90% gradient solution, 35 patients' sperm was washed with α-chymotrypsin treatment. In the control group, we analyzed the recovery rate and TMC of the same patients from previous sperm preparation not treated with α-Chymotrypsin. As a subset analysis, a severely low sperm count was considered if TMC is less than 10 million. Comparative statistics were performed using ANOVA and paired t-test.

Results: 23 semen samples were treated with α-chymotrypsin and the traditional gradient method. It was noted that the average recovery rate was significantly higher in the α-chymotrypsin treated group (38.9% vs 16.2%, p=0.000022). TMC was significantly increased in the treatment group as well (26.1 million vs 11.6 million, p=0.011108). This was consistent in the subset sample of patients with severely low sperm count for the rate of recovery and TMC (43% vs 10% p=0.02 and 5.89 million vs 1.21million p=0.0004). The change in the recovery rate and TMC was higher in the α-chymotrypsin group with a p-value of 0.0001 (39.65% vs 16.22%) and p= 0.0003 (22.21 million vs 11.58 million) respectively. The use of alpha chymotrypsin consistently yielded a higher recovery rate and TMC in highly viscous semen. To address if sperm function is affected after α-chymotrypsin treatment, we analyzed the outcome of nine IVF cycles (average age of 34.9 years). The fertilization rate using the semen sample treated with α-chymotrypsin was 87.8% (101/115) with a usable blastocyst rate at 56.4% (57/101), similar to the current reported average rate of success with IVF. Four out of five embryo transfer patients are ongoing pregnancies.

Conclusion: Sperm recovery rate and total motile sperm count were significantly increased in highly viscous semen samples treated with α-chymotrypsin compared to traditional gradient medium, especially in males starting with severely low sperm count. Alpha-chymotrypsin treatment does not adversely affect IVF outcomes.

Disclosures: None

Funding: None

Mapping the IVF Industry Terrain: Five Years of Insight through Custom Digital Surveying

Abstract Text:
Objective: Our 5-year investigation was dedicated to pioneering an innovative digital platform (ART Compass) tailored to scrutinize clinical decision-making and competency within the ever changing realm of the IVF industry. Through crafting of customized surveys, we targeted a diverse array of IVF lab networks, clinics, and embryologist education programs, including esteemed entities such as Fertility Associates, Sunfert, CARE Fertility UK, CNY, the Inception Fertility Network, as well as prominent embryologist education programs like EmbryoDirector and W.E.S.T. Our objective was to revolutionize data collection methodologies in the IVF industry and drive profound insights into the multifaceted landscape of assisted reproductive technologies.

Design: We used a mixed-methods approach, including a literature review, expert consultation, and pilot testing, to develop and evaluate the program. The platform was made available to embryologists worldwide from 2018-2023.

Materials and Methods: The ART Compass digital competency assessment, education and training, and survey and research platform (web based, and mobile apps on iOS and Android), descriptive statistics.

Results: Table 1. Over a period of five years (2018-2023) embryologists from 750 IVF labs have been registered, around 2,000 individuals have participated in the surveys, including approximately 1,700 embryologists and 300 IVF lab directors, 96 subtopics have been developed, with over 2,000 images/questions (Table 2). 10 customized approaches have been designed and deployed for five large IVF Lab networks: Dish preparation, Gardner Score (ICM, TE, and expansion), Marginal Blastocyst Freezing, Freeze or Discard?, CMQA 1 and 2 (Caremaps Quality Assurance), Fertilization, Cryopreservation, SART Grading (ICM and TE), and two hybrid digital learning program and research tool, (EmbryoDirector, W.E.S.T.).

Table 1. Descriptive Statistics (2018-2023)

IVF Labs	750
Individuals	1,700 Embryologists, 300 Lab Directors
Subtopics	96
Images/ Questions	>2,000

Table 2. Examples of subjective (denoted with *) and objective surveys

Section	ART Compass Surveys

Andrology	Male Reproductive System
	Sperm Function
	Sperm Cryopreservation
	Is this sperm normal or abnormal?*
Embryology General	All About Media and pH
	Live Cell Imaging and Microscopy
	Dish Preparation*
	QC/ QA In the Embryology Lab
Vitrification	Basic Cryobiology of Embryos
	Cryopreservation
	Liquid Nitrogen Handling
ICSI	Gamete Collection
	Gamete Biology, Fertilization, and Early Embryo Development
	Fertilization*
Biopsy	Genetics of Reproduction
Lab General	FDA Regulations
	Personal Protective Equipment
	Bloodborne Pathogens
	Hand Hygiene
Clinical Decision	Freeze or Discard?*
	Transfer or thaw another?*
	Choose Top Blastocyst*
	Oocyte Grading*
	Marginal Blastocyst Freezing*
	SART / Gardner Blastocyst Grading

Conclusions: Typically, surveys have been conducted in the IVF Field using tools like Survey Monkey, or Google and published surveys and rates of participation are in the order of; 5 questions, 50 embryologists [1], 17 questions, 14 embryologists [2], 7 embryologists (one per center), 12 parameters [3], and can reach to the range 200-300 embryologists [4,5]. To date, ART Compass houses one of the largest free collections of embryologist survey materials and participants, allowing for internal and external benchmarking, quality improvement, and best practices sharing among IVF labs. ART Compass provides tools for mitigating answer bias, embryologist training, and survey customization, among other advanced features that contribute to the advancement of the IVF field. ART Compass hosts one of the most extensive

open source, free repositories of embryologist survey materials, images, and participants, it stands as a pivotal resource empowering the IVF community with invaluable insights and knowledge.

Disclosures: CLC and DG are shareholders of AIVF. KP, ES, TA, are shareholders of ART Compass. AC is a shareholder of CARE Fertility UK. TA is a Shareholder of EmbryoDirector.

Funding: Provided by AIVF

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Non-invasive sex determination of pre-implantation embryos using spent embryo culture medium in ICSI-PGT cycles

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Objective: To explore developing a faster, robust method to identify the y chromosome related genes for fresh embryo transfer.

Design: Prospective study.

Materials and methods: This study collected spent embryo culture medium (SECM) on day 5 or day 6 from a cohort of pre-implantation genetic testing (PGT) cycles. SECM DAN was isolated for detection. The genomic reference DNA was extracted from saliva from males as positive control and females as negative control respectively. The whole genome amplification (WGA) was performed on all SECM samples. Y chromosome specific genes were selected for sex determination, primers and probes of a single-copy gene SRY and a multi-copy gene TSPY1 were designed by the software to target Y chromosome. The specificity of the primers was subsequently validated through comparison against the human Refseq representative genomes database using NCBI BLAST.

Several approaches were investigated in parallel, we compared several gene detection methods including droplet digital PCR, conventional PCR combined with agarose gel electrophoresis (AGE), and real-time PCR (qPCR). Our results were compared side by side with the in house PGT-A results, and the most accurate method was selected and further optimized. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to evaluate the performance of the detection.

Results: A total of 38 spent embryo culture mediums (SECMS) from 7 preimplantation genetic testing (PGT) cycles were collected. We compared the capacity of the conventional PCR, droplet digital PCR, and real-time PCR (qPCR) which are three widely used approaches in cell-free DNA detection.

For droplet digital PCR, two validated commercial assay kits were employed to detect the RPP30 gene (a reference gene located on Chr.17) and the SRY gene (Chr.Y specific gene) respectively. In the reference male genomic DNA, the concentration of RPP30 was twice that of SRY, in accordance with the chromosome number in somatic cells. However, only RPP30 was detectable in SECM samples at a rate of 93.75%, and no positive signals for SRY detection were observed in male embryo culture mediums.

For conventional PCR, the amplification success rates of TSPY1 and SRY were assessed using serially diluted reference genomic DNA. The TSPY1 assay exhibited higher sensitivity compared to the SRY assay. Subsequently, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of TSPY1 in SECM samples were determined to be 40%, 94.1%, 66.7%, and 84.2% respectively.

In comparison to conventional PCR, the qPCR assay demonstrated greater efficiency and sensitivity. The limit of detection (LOD) of the multi-copy sequence in the TSPY1 gene (0.45 genome equivalents) was 10-fold lower than that of the single-copy gene SRY (4.5 genome equivalents) in qPCR assays. With the qPCR assay, an improved detection rate of 60% was achieved, identifying 3 out of 5 male embryos.

Conclusions: This study shows that a PCR-based method can be a promising strategy for early noninvasive pre-implantation sex determination of D5-D6 embryos under continuous single-culture procedure. The TSPY1 (multi-copy gene) assay had a better performance than the SRY (single-copy gene) assay. However, the fluctuation of the recovery rate of the DNA template and the complicated component in the spent culture medium are the potential causes for the low detection rate. Further experiment is needed for an accurate sex determination assay in SECM to be developed.

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PREMATURE LASER ASSISTED HATCHING FOR PGT IS DETRIMENTAL TO EMBRYO QUALITY AND IS CORRELATED WITH LOWER CYTOKINES LEVELS IN SPENT CULTURE MEDIA

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Introduction: The process of zona pellucida thinning leading to blastocyst hatching is not completely understood. A balance between pro- and anti-inflammatory cytokines causing protease release is implicated in this process. Preimplantation genetic testing during fertility treatments utilizes premature laser assisted hatching (LAH) of the zona pellucida on day 3 to allow for herniation of trophectoderm and ease of biopsy. Studies have shown that this premature hatching resulted in lower numbers of blastocysts available for biopsy compared to embryos left undisturbed in culture till blastocyst stage.

Objective: To identify significant spent culture media (SCM) cytokines and correlations between these cytokines and LAH.

Design: Prospective cohort analysis from a teaching hospital IVF program.

Material and Methods: Luminex Immunobead Assays were performed according to the package insert for LXSAHM 15, R&D Systems®. Zona thickness and embryo diameters were measured using the Zilos® imaging software (version 3.13; Hamilton Thorne Research). SPSS version 26 was used for statistical analysis.

Results LAH on day 3 shows inverse correlation with TNFα, IL-10, MIP1β, IL-4 and GM-CSF cytokine levels in SCM. Hatched embryos showed significantly lower levels of these cytokines in SCM *(Rho = -.27 to -.47, P < 0.05).* Premature LAH of embryos compared to embryos left undisturbed resulted in thicker zone (*Mean difference = 4.46, t (61) = 3.71, p = .001, 95% CI 2.1 to 7.3*) and smaller blastocyst diameter (*Mean difference 8.89, t (61) = 3.1, p = 0.003, 95% CI 3.2 to 14.5*).

Conclusion: Premature hatching of the zona pellucida for PGT on day 3 introduces an artifact that disrupts the process of zona thinning resulting in smaller diameter blastocysts with thicker zonae. In additions lower levels of TNFα, IL-10, MIP1β, IL-4 and GM-CSF were measured in the SCM of prematurely hatched blastocysts.

Previous animal studies have shown that an addition of cytokines in culture media enhances blastocyst development and cytokines are involved in the preimplantation and fetal development. While further studies controlling for removal of the embryos from optimal culture conditions for the purpose of LAH are required, our studies, preliminarily have supported that an intact zona is important for maintaining the cytokine equilibrium necessary for optimal embryo development.

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Progress Toward the Rapid Elution (RE) and Ultra-Fast Vitrification (UFV) of Human Blastocysts and Oocytes

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Abstract

Objectives: To improve the efficacy and reliability of vitrification procedures by reducing the time of exposure to potentially toxic cryoprotective agents (CPA), while maintaining an intracellular metastable glass transition.

Design: Prospective, apriori studies of oocyte UFV/RE have progressed using MII oocytes (GV-matured or irregular Egg Bank research discard materials) contrasting developmental competence to conventional vitrification. As for blastocysts, retrospective analysis of routine RE for all embryos since November 2023 and UFV of D6 blastocysts (BL) since January 1 were investigated.

Materials & Methods: UFV was performed using commercial EG/DMSO solutions and a 1 min ES/ 1 min VS treatment under ambient condition prior to placement onto Cryotop or s-Cryolock open device systems and LN2 storage. For RE, devices were warmed rapidly in 1-3ml of 37ºC thaw solution (TS: 0.5-1.0M Sucrose), isolated in TS for 1 min and Hepes buffered isotonic medium +20% protein for 2-5 min at 37ºC before being pipette into culture dishes. Initial survival was assessed at 0 and +2h, and possibly at +18h in vitro culture. Some of the oocyte studies involved the use of a Polscope to evaluate the integrity and density of meiotic spindles. Phase 3 studies vitrifying MII's used artificial oocyte activation (AOA:2x5µM Ca++ ionophore -5min each + 1µM DMAP- 3h) followed by 7d in vitro culture to assess developmental potential.

Results: Adopting UFV to mature oocytes has supported our GV-modeling efforts with reliably high survival rates (>95%). While GV's matured normally with intact spindle integrity, we have now confirmed intact, reorganized meiotic spindles post-MII vitrification. Furthermore, the developmental competence of UFV/RE mature oocytes is comparable to or better than CV (62-66% activated development), with confirmed BL production post-AOA (19-30%). After validation thawing hundreds of BL, no difference was experienced in post-warming survival (96-100%) applying RE compared to standard dilutions. Clinically, RE has been associated with excellent blastocoel re-expansion and similar implantation/clinical pregnancy rates (65%/59.2%; n=119) compared to CV (63%/57.3%; n=510). Lastly, early clinical assessments of D6 UFV (n=20) reveal 100% survival and good sustained development (67-71.4%), though "C" quality TE experience reduced re-expansion (25%).

Conclusions: Transforming standard vitrification practices to include the rapid dilution of CPA in a single step have proven to be equally effective and more time efficient. In conjunction with UFV, clinical application to BL offers promise to improve pregnancy outcomes. Meanwhile, the experimental UFV of human oocytes has revealed improved reliability (98% survival vs. 80% for CV). Most importantly, oocyte UFV/RE pilot studies have now confirmed BL development to support moving forward with clinical trials.

Study Funding/Competing Interests: Co-authors are affiliated with commercial egg banks and Reproductive Biology associations, but claim no conflicts of interests regarding experimental outcomes.

Trial Registration Number: Not Applicable

Key words: vitrification, oocyte, embryo, dilution

Validation of a Novel External Cryo-Storage Monitoring Platform: The Boreas CryoScout

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Objective
We aimed to evaluate the efficacy, effectiveness and user satisfaction of a weight-based liquid nitrogen tank monitoring/alarm system (CryoScout by Boreas) at two clinical locations over several months.

Design
Routine maintenance conditions and numerous stress tests were performed to assess the overall daily performance of this weight-based monitoring system. Evaporation rate trends were evaluated under varying conditions, aimed to measure intentional acute changes and overall sensitivities of each unit regarding alert notifications.

Materials and Methods
We performed pilot studies with two weight-based platforms supporting small volume liquid nitrogen tanks (MVE 47-11 and Taylor Wharton 35-HC), while additional beta-test units were monitored at corporate locations. Each tank was equipped with an external tank module (TM) which was connected to the weight-platform and a wire temperature surface sensor. The TM is the "brains" allowing for weight calibration and remote cellular LTE-based monitoring with desktop and mobile portal access. We gauged the usefulness of the desktop platform and ease of acquiring and recording real-time evaporation rate information.

Results
The CryoScout proved to have a user friendly and versatile dashboard, granting access to individual tanks at different locations, real-time monitoring of LN_2 volume by weight, user/on-call lists, alarm history and reported activity logs. The alarm systems allowed for setting minimum weight tolerance limits, acceptable ambient temperature surface levels and fine-tuning the detection of rate changes/min. This multi-layered alarm system proved to be comprehensive and alert to induced and unexpected events. Between the rate change per measurement interval (i.e., calculated as /min) and the exterior temperature measure, the alarm response could be nearly instantaneous (within 10-15 min), offering an early warning alert more sensitive than exterior visual cues. Overall, the software provides a noninvasive way to record operational and performance qualification (OQ/PQ) testing of small volume cryo-dewars. While conducting PQ on a retired, aged tank, CryoScout detected a unique failure situation where upon an accelerated evaporation rate was not associated with any detectable exterior visual cues (e.g., condensation or frosting). Instead, a high consumption alarm was detected promptly, several hours before a 20% loss alert would have occurred and long before an internal temperature change (>-170°C) would have warned users to a potentially catastrophic event.

Conclusions

The advent of a versatile, reliable, weight-based remote monitoring system for cryo-dewar tanks makes antiquated monitoring methods (e.g. internal temperature and dip-stick assessments) obsolete. This weight-based system provides an unprecedented safeguard for cryostorage management.

Disclosures
Investigators conducting the validations and writing of the abstract declare no conflicts of interest. Three authors (CJ, WBII, WBIII) have ownership in the Boreas company.

Funding
Product beta-testing was made available by Boreas, but investigators were not paid.

Keywords: cryostorage, tank monitoring, weight, alarm, liquid nitrogen

Vitrified blastocysts successfully survive warming in a medium that has entirely different basal and key components than those contained in the vitrification medium

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Objective: Can blastocysts vitrified in a medium with a Human Tubal Fluid base, MOPS, and sucrose survive warming in a medium with a Continuous Single Culture Medium (CSCM) base, HEPES and MOPS, and trehalose?

Design: A prospective interventional study in December 2022 of day-5 hatching blastocysts, vitrified between May 2015 and June 2017, and donated to a private IVF clinic for research. All initial blastocyst warms were performed using a CSCM-based medium warm kit. After a minimum 2-hour recovery in 5mg/mL Human Serum Albumin (HSA), blastocysts were graded and divided into a control and a test group, each with n=60.

Materials and Methods: Blastocysts were individually vitrified either with a CSCM-based medium containing HEPES/MOPS, 10mg/mL HSA, 4mg/mL dextran and 0.5M trehalose (Vitrification Solution (VS) only) (test) or a HTF-based medium with MOPS, 12mg/mL HSA and 0.6M sucrose (VS only) (control). Both vitrification media contain ethylene glycol and DMSO. No earlier than 24-hours post-vitrification, blastocysts from both groups were warmed in a CSCM-based warming kit. Assessed comparative factors included 24-hour survival and 24-hour morphological grade.

Results: Morphological grading was assigned via visual assessment and application of the SART system for blastocyst quality. Chi-square analysis was performed in order to assess statistical significance, where $p < 0.05$ was significant. In the control and test group, the average patient age was 42 years and 38, respectively. Initial "Good" and "Fair" morphological assessment of warmed donated blastocysts at the time of random group allocation was 84.7% (control) and 96.7% (test) ($p < 0.05$). After 24-hour recovery in culture medium (5mg/mL HSA), 83.3% and 91.7% were fully expanded and 6.7% and 5.0% were degenerated, control versus test. Though no statistical significance was seen in the number of fully expanded or degenerated blastocysts per group, those classified as "Good" or "Fair" was significant in the test group, 93.3%, versus the control, 83.3% ($p < 0.05$).

Conclusions: The study results support previous works that demonstrate the success of using entirely different vitrification and warming media solutions effectively together. This potentially allows the IVF laboratory to adjust their processes and procedures to fit their demands and needs without the burden of requiring matching formulations in their cryopreservation systems.

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What is the number of euploid blastocysts produced per ART cycle in various age groups with varying levels of anti-Müllerian hormone (AMH)?

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Objective: To determine the number of euploid blastocysts generated per ART cycle across various age groups and different AMH levels.

Design: A multicenter retrospective data analysis in a referral ART group.

Materials and methods: In this retrospective cohort study, data was collected from 16,417 ICSI cycles with Preimplantation Testing for Aneuploidy (PGT-A) conducted at three referral fertility centers from Jan 2019 to Dec 2023. We excluded cycles without serum AMH, endocrinopathies or recurrent pregnancy loss, males with total sperm count of less than 5 million and total motility less than 30%, and those with parental chromosomal abnormalities.

Patients were stratified into three different categories of AMH (ng/ml), low (< 1.1 ng/mL), intermediate (≥1.1 to < 3.0 ng/mL) and high (≥ 3.0 ng/mL). Each category was subdivided into 6 age sub-categories as follow (< 35), (35 to < 38), (38 to < 40), (40 to < 42), (42 to < 44) and (≥ 44). All of the cycles underwent blastocyst stage biopsy and PGT-A utilising next generation sequencing NGS.

Results: Euploidy rate was insignificant for all AMH levels in the same age group. However, euploid embryo numbers vary significantly. Euploid embryos number shown in low, medium, and high AMH by age group. In the (<35) years was 1.9 ± 1.5, 2.9 ± 2.3, and 4.7 ± 3.5, p<0.0001. In the (35 to < 38) years was 1.3 ± 0.8, 2.1 ± 1.8, and 3.2 ± 2.6, p<0.0001. In the (38 to< 40) years was 0.9 ± 0.8, 1.7 ± 1.4, and 2.6 ± 1.7, p<0.0001. In the (40 to < 42) years was 0.6 ± 0.5, 0.9 ± 0.7, and 1.5 ± 1.4, p<0.0001. In the (42 to < 44) year was 0.26 ± 0.2, 0.49 ± 0.38, and 0.6 ± 53, p<0.0001. In the (≥44) years was 0.10 ± 0.10, 0.23 ± 0.21, and 0.40 ± 0.37, p=0.0170.

Conclusions: The percentage of euploid embryos is insignificant within the same age group and across various levels of AMH. Nevertheless, the absolute number of euploid embryos rises in line with the AMH level within the same age group.

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Addressing Recall Issues in Multiplex PCR Molecular Testing: Enhancing Diagnostic Stewardship for Effective Gastroenteritis Management

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Objectives: The diagnostic methods for the gastroenteritis (GE) often involve molecular techniques besides culture methods. Multiplex gastrointestinal (GI) panels have encountered numerous recalls due to occurrence of false-negative or false-positive outcomes. Presently, our multiplex GI panel is beset by a recall pertaining to false positive Norovirus detections. Therefore, our objective is to address this issue by identifying true Norovirus positive cases, with an emphasis on diagnostic stewardship to curtail supplementary testing, thus averting the necessity for new test validation, minimizing manpower utilization, and mitigating additional costs.

Design: Applications of retrospective study findings in diagnosing the infection

Materials and Methods: Initially, we scrutinized the viral GE patterns, specifically Adenovirus, Astrovirus, Norovirus, Rotavirus, and Sapovirus, over a five-year span to elucidate epidemiological trends and seasonal variations. Whenever feasible, histopathology was also reviewed. At present, we are evaluating the patient's clinical history, stool macroscopy and consistency, concomitant diseases, and medications potentially inducing diarrhea to ascertain true Norovirus infections.

Results: Among the myriad causes of gastroenteritis, infectious and non-infectious alike, our five-year analysis revealed that 25.46% of cases were attributable to viral pathogens. The pediatric patients (72.73%) exhibited a significantly higher prevalence compared to adults (27.07%), with a p-value of 0.015. Norovirus genogroups I and II predominated across all age groups, with a significant prevalence in adults. Gender-based disparities were not evident. Norovirus cases manifested higher incidence during the winter and least during summer months. Histopathological examination revealed inflammatory changes, ulceration, erosion, architectural distortion, and distinctive viral inclusion bodies associated with adenovirus. With this in consideration, we are excluding false Noroviral infections in patients with formed stools, particularly during the off-season, and those lacking signs and symptoms of GE, as well as patients with concomitant diseases or experiencing drug-induced diarrhea. Thus, we have received zero requisitions for sent outs or additional molecular testing.

Conclusions: Our exhaustive examination of viral GE cases, coupled with seasonal analysis, macroscopic examination, and clinical correlation, facilitated circumvention of supplementary confirmatory testing. Consequently, we economized resources in terms of finances, time, and effort typically required for validating alternative molecular tests.

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Exploring Group B *Streptococcus* Colonization in Pregnancy: Impact on Maternal and Neonatal Health Outcomes

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Objectives: In women, Group B *Streptococcus* (GBS) is most commonly found in the vagina and rectum. There are no vaccines to prevent GBS infections. In neonates, GBS infection is the result of vertical transmission from a colonized mother. It is a leading cause of life-threatening neonatal infections. GBS also produces significant maternal morbidity, including bacteremia, postpartum wound infections, endometritis, and chorioamnionitis. This study assesses the frequency of GBS colonization in pregnant women and records complications during pregnancy, childbirth, and the puerperium.

Design: Retrospective study at a university medical center

Methods: As a part of routine antenatal care (ANC), the microbiology and serology laboratories at the University of Mississippi Medical Center (UMMC) screened for GBS according to CDC recommendations. The vaginal and rectal swabs were collected at 36-38 weeks gestation in transport media (Aimes or Stuart's Media without charcoal). They were cultured directly on the blood agar plate as well as after inoculating the selective LIM broth at 35-37ºC for 18-24 hours. The suspected colonies were confirmed by the matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The GBS polymerase chain reaction with appropriate internal and external controls, along with *Bacillus globigii* as a sample processing control was performed for women who have had no prenatal care, or in preterm labor, or unknown GBS test results at the time of delivery. Maternal data spanning five years were collected from UMMC's patient cohort explorer.

Results: The number of GBS carrier state (n= 3044) complicating ANC were significantly higher than other infection carrier states (n= 20). The percentage of African-American GBS carriers was significantly higher in all three complication stages of pregnancy, childbirth and puerperium. Furthermore, the elderly primigravidae carriers (> 35 years) with complications were significantly fewer than their younger counterparts. The complications among the GBS carriers included preterm labor, fetal distress, premature rupture of membranes, abortions and still births.

Conclusions: This study underscores the considerable impact of GBS colonization on maternal and neonatal health, highlighting the need for developing effective preventive strategies. Targeted interventions must be developed to mitigate GBS complications during pregnancy, childbirth, and the postpartum period.

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