2016 AAB Conference and CRB Symposium

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May 12-14
Red Rock Resort
Las Vegas, Nevada
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Characterizing the Microbiome at Embryo Transfer: Next Generation Sequencing of the 16S Ribosomal Gene

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Objective: The vaginal and placental microbiomes are partially characterized and impact obstetric outcomes. The uterine microbiome is largely uncharacterized given the limitations of cultivation-dependent analysis. Culture-free approaches to bacterial identification focus on sequencing of 16S ribosomal gene and allows for increased dynamic range in assessment. Here, we assess two Next Generation Sequencing (NGS) platforms for 16S sequencing, determine their advantages and disadvantages for 16S metagenomics, and study the endometrial microbial environment at the time of embryo transfer (ET).

Design: Method validation and cohort study

Materials and Methods: The method validation of NGS 16S ribosomal gene sequencing was performed on known samples (Escherichia coli, Staphylococcus epidermidis, Cyanobacterium Synechococcus). The Ion 16S metagenomics workflow analyzed seven of nine hypervariable regions (V2,3,4,6,7,8,9) of 16S rRNA gene versus targeted sequences used in Illumina 16S workflow (V3-V4 and V4 only). Customized bioinformatics data analysis was used to improve the taxonomic assignments. Illumina V4 metagenomics workflow was further validated on Microbial Mock Community of 20 bacterial strains. The endometrial microbiome at the time of ET was characterized by analyzing transfer catheter tips with Illumina V4 metagenomics for 70 patients.

Results: The built-in analysis from both Ion PGM and Illumina MiSeq metagenomics failed to generate correct taxonomic classification for genus or species for known single- or poly-microbial samples. The customized bioinformatics improved alignment, however for Ion 16S metagenomics workflow, the majority reads were family level. For both amplicons V3-V4 and V4, Illumina metagenomics workflow identified the genus or species level in the single- and poly-microbial samples. V4 had higher Operational Taxonomic Unit (OTU) counts than V3-V4. Subsequently, Illumina V4 metagenomics workflow with customized bioinformatics detected the genus or species level for all the 20 bacterial strains in the Microbial Mock Community. Finally, the endometrial microbiome of the 70 patients was assessed with Illumina V4 metagenomics and customized bioinformatics. Lactobacillus was detected in all the 70 samples along with other bacteria native to the reproductive tract (Corynebacterium, Bifidobacterium, Staphylococcus, Streptococcus).

Conclusions: The Illumina V4 metagenomics workflow with customized bioinformatics provided a rapid and sensitive method for the identification of bacterial genus or species in single- or poly-microbial samples. Despite the limited starting material when analyzing clinical ET specimens, the Illumina V4 metagenomics approach provided adequate taxonomic identification. This sets the stage for a larger trial analyzing the relationship between endometrium microbial structure and implantation after ET.

Disclosures: Nothing to disclose

Funding: None.
Comparative Evaluation of Accidental Warming Intervals on the Survival of Vitrified Human Blastocysts

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Objective: Current dogma suggests that the viability of vitrified blastocysts (BL) can be compromised by a brief exposure to room temperature. We propose that this critical sensitivity is vitrification (VTF) device/solution dependent. Our experimental aim was to contrast a theoretical threshold of survival for accidentally warming vitrified-BLs in both a closed and open VTF system. We hypothesize that an aseptic, closed, double container device, non-DMSO system may provide extra security to vitrified embryos exposed to suboptimal warming, re-VTF (rVTF) experimental model.

Design: Using a 3 x 2 factorial arrangement of treatments, we evaluated 2 VTF treatments (µS-VTF/I.C.E. vs. Cryolock™/EG-DMSO) and 3 exposure time treatments (0 sec, 10 sec and 1 min).

Materials and Methods: Research consented, Day 5 grade “A” trophectoderm, vitrified-BLs were warmed under ambient conditions (21-22°C) to mimic an accidental exposure at 10 sec, with 1 min serving as a negative treatment (n=10 BL/treatment group). Our positive control group (0 sec)/VTF treatment had no suboptimal warming. The treatment groups were directly re-plunged into LN2, and later warmed (37°C) according to standard laboratory serial sucrose dilution protocols. The BL were assessed for initial survival (0 hr) and continued development (BL expansion/hatching=survival) at 24 hrs. Differences in BL survival were assessed by a χ² test (p<0.05).

Results: Statistical significance* was achieved when comparing blastocysts cryopreserved with µS-VTF/I.C.E compared to Cryolock™/EG-DMSO at 1 min exposure to ambient conditions.

<table>
<thead>
<tr>
<th>VTF</th>
<th>Interval:</th>
<th>0 sec</th>
<th>10 sec</th>
<th>1min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td># Thawed:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>µS-VTF/ I.C.E</td>
<td>0 hr # Survived (%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
<td>9 (90%)*</td>
</tr>
<tr>
<td>Cryolock™/EG-DMSO</td>
<td>0 hr # Survived (%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
<td>0 (0%)*</td>
</tr>
<tr>
<td>µS-VTF/ I.C.E</td>
<td>24 hr # Survived (%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
<td>8 (80%)*</td>
</tr>
<tr>
<td>Cryolock™/EG-DMSO</td>
<td>24 hr # Survived (%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
<td>0 (0%)*</td>
</tr>
</tbody>
</table>

Conclusion: Both closed and open VTF systems appear to be initially insulated to a potentially injurious brief suboptimal temperature exposure (≤10 sec). Yet, BL vitrified by µS-VTF in metastable I.C.E. solutions (>7.9M cryoprotectants) were more resilient to extended suboptimal warming/devitrification events than Cryolock™ VTF in 30% EG-DMSO (<5.0M cryoprotectants) based on assessments of cellular integrity and sustained development. Undoubtedly, the slower initial cooling rates and use of a more concentrated (metastable) VTF solution protected the closed VTF system from injurious recrystallization events. Closed, metastable VTF offers an unexpected level of safety.

Disclosure: None

Funding: None
Detection of A Male Translocation Following an Ovum Donor Preimplantation Genetic Screening Cycle

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**Objective:** It is not standard of care to screen IVF patients for translocations due to their low existence in the general population at ~0.2%. However, the percentage of carriers of balanced translocations may be much higher in the infertile population and they are most likely unaware of their condition. We describe the clinical and laboratory outcomes of an advanced maternal age (AMA) couple seeking infertility care, and the inadvertent discovery of a male partner translocation.

**Design:** We describe a single case study of a rare preimplantation genetic screening (PGS) result, which could have been avoided with an update to genetic pre-screening for any patients initiating expensive IVF cycles.

**Materials and Methods:** A couple’s case files were reviewed and are described to demonstrate the discovery of a male translocation carrier following a donor oocyte IVF/PGS cycle.

**Results:** The couple: 40-year old, G1, P0 (SAB) female with unremarkable hormonal/ultrasonic findings; and a low teratozoospermic, otherwise unremarkable male. The male had a normal genetic carrier screening panel. The couple was counseled about AMA and underwent an autologous IVF cycle with PGS. Two blastocysts developed, they were biopsied and the genetic results were: Monosomy 16/Trisomy 21 and Trisomy 3/Monosomy 16. The couple decided to proceed with donor oocytes for a subsequent cycle with PGS to transfer only a single normal embryo. In the donor cycle, using partner sperm, 20 blastocysts developed, they were biopsied and the PGS results showed 6 normal embryos (30%); 11 embryos (55%) with abnormalities always involving chromosomes 3 and/or 16 (monosomies, trisomies and complex abnormal). Very low numbers of normal embryos from an oocyte donor and repeated abnormalities in chromosomes 3 and 16 from both the autologous and donor cycles resulted in the male being karyotyped, discovering a balanced Reciprocal Translocation: 46 XY; t(3;16)(q22; q22).

**Conclusions:** Although standard pre-screening for an AMA couple seeking infertility treatment was followed, along with completing an autologous IVF cycle with PGS demonstrating findings that would be considered standard in an AMA couple, we discovered a male translocation following a donor oocyte/PGS cycle. The very low cost of a standard karyotype may be warranted prior to initiating donor oocyte cycles. It may even be preferable to change the standard of care to karyotype all couples prior to any infertility treatments to increase the likelihood success.

**Disclosures:** None

**Funding:** None
Effect of Assisted Reproductive Technology (ART) On Babies Born

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Objective: Assisted reproductive technology (ART) has been widely used for the treatment of infertile couples to realize their dream to have a baby. So far, more than ten million IVF/ICSI babies have been born throughout the world. However, many people have worried about ART babies health outcomes. Although many reviews of ART effects on babies born have reported, it is very difficult to find systematic data about ART outcomes on sex ratio and the effect of frozen embryo transfer. The objective of this study is to provide detailed data about the effect of ART on babies born.

Materials and Methods: Based on the reported SART data of Arizona Center for Reproductive Endocrinology and Infertility from 2010 to 2014, all records related to babies births including mother’s age, gestation days, baby sex and birth weight were collected and analyzed according to fresh or frozen embryo transfer procedures. Normal deliver baby data without ART were also collected from an obstetrical hospital as control.

Results: The summary of ART baby birth information is listed as following table.

<table>
<thead>
<tr>
<th></th>
<th>Fresh IVF/ICSI single birth</th>
<th>Frozen Embryo transfer single birth</th>
<th>Multi-baby birth (twin/triplet)</th>
<th>Normal deliver baby birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient No.</td>
<td>235</td>
<td>78</td>
<td>98</td>
<td>194</td>
</tr>
<tr>
<td>Patient age</td>
<td>33.84±4.96</td>
<td>34.59±4.11</td>
<td>32.95±4.60</td>
<td>28.14±4.02</td>
</tr>
<tr>
<td>Gestation days</td>
<td>269.57±13.28</td>
<td>273.00±11.12</td>
<td>239.60±27.46</td>
<td>270.56±15.38</td>
</tr>
<tr>
<td>Male No.*</td>
<td>126</td>
<td>36</td>
<td>100</td>
<td>115</td>
</tr>
<tr>
<td>Female no.</td>
<td>109</td>
<td>42</td>
<td>106</td>
<td>79</td>
</tr>
<tr>
<td>Male birth weight (gram)</td>
<td>3227.71±587.06</td>
<td>3401±479.81</td>
<td>2242.21±598.98</td>
<td>3198.87±604.71</td>
</tr>
<tr>
<td>Female birth weight (gram)</td>
<td>3005.36±427.15</td>
<td>3229±423.34</td>
<td>2046.97±654.98</td>
<td>3217.59±505.88</td>
</tr>
<tr>
<td>Full term birth %</td>
<td>86.81</td>
<td>92.31</td>
<td>27.83</td>
<td>86.09</td>
</tr>
<tr>
<td>Early birth %</td>
<td>13.19</td>
<td>7.69</td>
<td>72.17</td>
<td>13.91</td>
</tr>
</tbody>
</table>

1. ART patient age is significantly older than normal delivering women (P<0.05), but the woman’s age with multi-baby birth is significant younger than normal ART procedures.
2. The gestation following fresh embryo transfer is similar to babies born naturally (P>0.05), but multi-baby birth women have a shorter gestation in days (P<0.01) and frozen embryo transfer women have a longer gestation in days (P<0.05).
3. There is no significant difference on early birth between fresh embryo transfer single babies and normal deliver babies (P>0.05), but multi-babies have a higher early birth rate (72%) and frozen embryo transfer has lower early birth rate (P<0.01).
4. After χ² test, there is no significant difference in the incidence of male or female babies although fresh embryo transfer had a trend of more male babies and frozen embryo transfer had more female babies.
5. There is no significant difference on the baby weight between ART singleton babies and normal deliver babies (P>0.05). However, male baby weight was more than female babies, multi-baby birth weights are significant lower than singletons, while frozen embryo transfer babies have significant heavier birth weight than fresh embryo transfer (P<0.01).

**Conclusion:** The single birth babies with ART treatment do not have any significant differences from normal birth babies in gestation days, early birth rate and baby birth weight, but multi-baby births often results in high early birth rate, lower birth weight and shorter gestation days. The frozen embryo transfer technique may significantly decrease the early birth rate of babies. Thus, frozen embryo transfer may be recommended as a very health strategy in ART.
Growing Zona Pellucida-Free (ZPF) Oocytes from ICSI to Biopsy: It Works

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Objective:
To observe and document the growth of an intact, mature, accidently zona pellucida-free oocyte (ZPF) that underwent ICSI and culture to blastocyst-stage with subsequent trophectoderm biopsy on day six.

Design:
Retrospective case study where a ZFP oocyte observed after oocyte processing for ICSI, and subsequently grown to day 6 for trophectoderm biopsy.

Materials and Methods:
Anonymous donor oocytes were retrieved and processed according to standard laboratory protocols. After processing, 1 of the 9 oocytes was intact but outside of its zona pellucida. All oocytes were mature and underwent ICSI, including the zona-free oocyte. Oocytes were incubated using the standard continuous culture protocol, under oil with 5% O2, 8%CO2 and balance nitrogen. The ZPF oocyte was cultured individually. Injected ova were evaluated on day 1 (day 0 = day of retrieval) and on day 3 to check fertilization and development respectively. The embryos were checked again on day 5; there were 5 zona-intact blastocysts for biopsy. On day 6, there was 1 zona-intact blastocyst, and the blastocyst from the ZPF oocyte available for biopsy. Immediately post-biopsy, embryos were vitrified and the trophectoderm samples were sent for genetic testing.

Results:
Using routine laboratory protocols the ZPF embryo underwent appropriate development, as visualized by formation of a structured inner cell mass and trophectoderm. No special techniques were utilized, e.g. placing cells into the empty zona, or culturing with increased protein concentration. Genetic testing showed normal embryo. 4 of the 7 biopsied blastocysts were euploid, including the ZPF embryo.

Conclusion:
There have been several case reports describing the successful development of ZPF embryos, and subsequent pregnancy after transfer, however this report further demonstrates that ZPF embryos can no only display expected developmental patterns, but that these embryos can undergo biopsy and have a normal chromosome complement. Embryologists should consider mature ZPF oocytes as available for ICSI, though careful attention to the ICSI process is required.

Disclosures:
Authors have nothing to disclose.

Funding:
No funding was provided nor needed.
Human Blastocyst Toxicity Potential of Different Vitrification Solutions: Experiment II

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Objectives:
We exhibited that human blastocysts are highly resilient to repeated metastable re-vitrification (rVTF), with or without elution, and to 2-6 min exposures to non-DMSO containing VTF solutions (Experiment I). The aim of Experiment II was to comparatively assess the potential toxicity of different VTF solutions to extended exposures.

Design:
360 research consented, discard blastocysts were randomly assigned to a 6x3 factorial design: comparing different exposure intervals post-warming (1,3,5,10,15,20min/20 blastocysts/group) in a commercial EG/DMSO (15/15% v/v, 4.8M; LifeGlobal), EG/PPG (16/16% v/v, 5.1M; Vitrolife) or our control Glycerol/EG (≥7.9M; Innovative Cryo Enterprises) vitrification solutions. Differences in %survival and %24hr development were statistically compared by Chi-square analysis (p<0.05).

Materials & Methods:
Biopsied blastocysts (AA to BB quality) were vitrified using microSecure vitrification (μS-VTF), an aseptic closed system. Embryos approved for research use were randomly assigned a treatment group and discarded within 24hr. Rapid warming was performed on all embryos, with stepwise sucrose elution of blastocysts and 30min isotonic equilibration at 37°C in LG medium+7.5% SS in tri-gas, humidified incubation. All BL were then re-exposed to standard VTF dilution protocols with extended ambient air exposure (20-22°C) in the final VTF solution (up to 20 min), before being returned to 1.0M sucrose and a 4-step serial dilution (3min each), concluding in a 5min exposure to isotonic H-LG medium before being returned to 24 hr in vitro culture. Embryo survival assessments were performed at 0 and 24hr (i.e.,continued expansion) post-treatment.

Results:
24hr development was reduced (p<0.05) by 1-5 min exposures in the EG/DMSO group (83.3%), compared to EG/PPG (93.3%) or Glycerol/EG (96.7%). Interestingly, the developmental integrity of Glycerol/EG treated blastocysts reduced (75%; p<0.05) by 10 min and was significantly lower at 15/20min (30-35%) than either EG/DMSO (55-70%) or EG/PPG (55-60%).

Conclusion:
Our experiments have dramatically demonstrated the plasticity and cryotolerance of blastocyst to rVTF treatments (Expt.1) and extended exposures to 3 different VTF solutions (Expt.2). Since Glycerol/EG has a higher solute concentration solution, it was more toxic after 10min exposure. However, it is also more metastable in its vitrified “glasseous” state without the need for ultra-rapid cooling, as long as sufficiently high warming rates are achieved. The genotoxicity of all treatment solutions to extended exposure remains unknown, especially >5min. Whereas, up to 3 min exposures and 2x VTF in the control group is regarded as safe, having previously yielded proven healthy live births.

Support: Internal funding, involving our Summer Student, Science Training Program*.
Is Nuclear Ploidy Status Associated with Mitochondrial DNA (mtDNA) Load in Human Embryos?

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Reproductive Research Section, Center for Advanced Genetics; Carlsbad, California USA
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Objective: This study sought to assess the association between embryo mitochondrial load and embryo ploidy status.

Materials and methods: This retrospective, multi-cohort investigation measured mtDNA load during early embryogenesis and assessed impact of mitochondrial mass on ploidy status. Data from embryos (n=325) were reviewed for 69 patients who underwent IVF treatment with preimplantation screening at an urban referral fertility unit in 2015. High throughput sequencing of nuclear and mtDNA was performed after whole genome amplification with ion semiconductor technology (ThermoFisher Scientific, Inc). Nuclear and mtDNA ratios were recorded by biopsy time: day 3 (n=90), day 4 (n=145), or day 5 (n=90) and results were stratified by patient age (32-37yrs vs. 38-44yrs).

Results: Successively declining mitochondria quantities were observed at blastomere stage (d3), followed by morula (day-4) and blastocyst stage (day-5), validating a prior observation that mitochondria are not generated during early human embryo development. Among euploid embryos, a relatively high mitochondria load was observed at d3 but successively fewer mitochondria were present at d4 and d5 compared to aneuploid embryos.

Conclusions: While mitochondrial segregation to blastomeres during early embryogenesis is strictly regulated, the distribution of mitochondria during this phase remains poorly understood. Our research shows that mtDNA does vary throughout human embryo development and correlates with nuclear ploidy. Our pilot data also suggest that fewer mitochondria are present in embryos obtained from older IVF patients. We hypothesize that the observed decline in mtDNA at d4 and d5 among euploid embryos occurs secondary to improved metabolic efficiency, where less ATP may be required to execute vital (or perhaps different) cellular processes. A relatively immature respiratory chain at d3 could demand a greater mitochondrial mass, thus explaining higher measured mitochondrial quantities before morulation. Additionally, impaired cell fission in impaired embryos might result in accumulation of mtDNA which would otherwise be dispersed in a “healthy” embryo cellular constituency. NGS is well suited to describe modulation of mitochondrial dynamics during human embryogenesis. However, absolute mitochondria number may not always correlate with mtDNA copy number estimates, so additional research is planned to measure mitochondrial parameters and embryo ploidy status going forward.

Disclosures: Progenesis, Inc. provided support for this study; Progenesis, Inc. is pursuing a commercial test for in vitro mitochondrial assessment.

Funding: None.
Ploidy Variation in Trophectoderm Biopsies from Day 5, 6, and 7 Blastocysts

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Objective: To compare ploidy rates amongst day 5, 6, and 7 blastocysts.

Design: Retrospective study in a private in vitro fertilization laboratory.

Aim: Some data suggests that Day 7 (D7) blastocysts can give rise to nearly 27% ongoing pregnancy success by frozen embryo transfer. We chose to compare the aneuploidy rate observed amongst day 5, 6, and 7 blastocysts in order to determine the efficacy of extended D7 culture in our laboratory.

Materials and Methods: Over the span of 3 years, a total of 1915 blastocyst stage embryos (average maternal age of 37±4.1 years) underwent trophectoderm biopsy for genetic testing of ploidy status. Pre-implantation genetic screening (PGS) results were retrospectively compared to day of trophectoderm biopsy. Mature oocytes were fertilized using ICSI and embryos were group cultured in a continuous single culture medium (Irvine Scientific). On Day 3 of embryo development, all multi-cell embryos (≥6 cells) were artificially hatched by laser ablation of the zona pellucida. Hatched embryos were then cultured to the blastocyst stage. Trophectoderm biopsy was dependent on development of a well-defined inner cell mass and trophectoderm. Hatching or completely hatched blastocysts were biopsied on either Day 5, 6 or 7 and subsequently vitrified for future use in a warmed embryo transfer cycle dependent on ploidy status. Biopsied samples were analyzed with comprehensive chromosomal screening involving the use of array comparative genomic hybridization (aCGH) or next generation sequencing (NGS) technology by a reference laboratory.

Results: 1915 embryos were biopsied yielding a total of 862 (45%) euploid embryos: 485(56%), 328(38%) and 49(5.7%) from D5, D6, and D7, respectively. The incidence of euploidy was 50%, 40%, and 38% among D5, D6, and D7. Chi-square analysis demonstrated that euploid status is dependent on day of biopsy/blastocyst development with chi square statistic calculated to be 17.5847 and a P value < 0.0001.
Conclusion: Embryos biopsied on day 5 and 6 have a significantly higher probability having normal chromosome complements compared to day 7 embryos as a whole. These results contradict previous reports of D5 and D7 blastocysts contributing equal numbers of euploid embryos.\(^2\) Our findings suggest that perhaps a delay in blastulation (until D7) may be associated with a greater likelihood of aneuploidy. Despite this, culturing embryos to D7 appears to be beneficial as a substantial number of blastocysts biopsied on D7 were euploid. Further investigation of ploidy status from D5, D6, and D7 embryos stratified by various age groups, may provide further information regarding the efficacy of D7 blastocyst culture and screening.

Disclosures: None

Funding: None

References:


Timing of Cell Exclusion May Be a Predictor for Blastocyst Ploidy

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Time-lapse imagining for embryo culture provides the ability to observe developmental anomalies. We have observed that some embryos exclude cells early, prior to compaction or late, during the morula and blastulation stage of development. An analysis was done to determine if the timing of cell exclusion correlated with ploidy results from PGS/PGD patient embryos.

Blastocysts (194) biopsied from 38 cycles of 27 patients with an average age of 36.9 years, were divided into 3 categories of development; Controls-no cell exclusion (C, n=101), Early Cell Exclusion (ECE, n=72) and Late Cell Exclusion (LCE, n=21).

The overall aneuploidy rate for this group of patients was 63.4%.

When analyzed by patient age, the outcome was consistent with literature, and showed a significantly higher aneuploidy rate in older patients; 47.4% for <38, 79.4% for ≥38 (n=97 embryos per group; p<0.0001).

There was a higher rate of cell exclusion for the older patients, regardless of whether the exclusion occurred early or late; 36.1% for <38, 59.8% for ≥38 (p<0.001).

Though not statistically different, of embryos with cell extrusion, older patients had a higher percentage occurring early (47.4% vs 26.8%, for ≥38 and <38, respectively).

For all patients, although we see a trend, there was no difference in the ploidy rate between controls (56.4%), ECE (69.4%) and LCE (76.2%) with p=0.09.

In conclusion, it can be theorized that cell exclusion is seen as an effort by the embryo to self-correct by deselecting cells that may be mosaic. Embryos that exclude cells early may be more severely aneuploid and have a more difficult time correcting than those embryos which exhibit later cell exclusion. In the future, time-lapse systems may be programmed to assess the timing and percentage of cell exclusion to more accurately determine an embryo’s clinical potential.
Objective: The success of early pregnancy is dictated by trophoblastic invasion and remodeling of the maternal vasculature. This process is regulated in part by uterine natural killer cells, which contain a variable combination of inhibitory and activating receptors, or KIRs. Activity of these receptors is modulated by interactions with HLA-C ligands present on the trophoblast. Prior studies have documented that specific KIRs are overly represented in recurrent miscarriage patients but they all inferred HLA status from parental genotypes and didn’t have direct embryo data.

Our two objectives were to 1) determine if any KIR genotypes were associated with an increased risk of pregnancy loss following euploid SET, and 2) determine if risk of loss was modified by KIR/HLA-C combinations after directly assessing embryonic HLA status via trophectoderm biopsies (TEBx).

Design: Retrospective descriptive analysis

Materials and Methods: All euploid single embryo transfers (SET) cycles in which stored maternal DNA and residual preamplification DNA from TEBx samples could be genotyped were analyzed. Only the first transfer resulting in a positive pregnancy test for each patient was evaluated. Maternal samples were assessed for 16 possible KIR genotypes. DNA from previous TEBx were genotyped for HLA-C1 or HLA-C2 alleles and patients were separated by biallelic pair (C2/C2, C1/C1, or C2/C1). Known ligand-receptor interactions were specifically evaluated.

Results: 668 euploid SETs were included in the analysis. Overall pregnancy loss rate (including clinical and biochemical losses was 24.7%). Patients who carried KIR2DS1 and KIR3DS1 genotypes were both more likely to experience pregnancy loss than noncarriers (28.8% vs. 21.3%, p=0.01; and 29.4% vs. 20.9%, p=0.01). Among C2/C2 embryos, loss risk was increased when recipients carried KIR2DS1 (35.2% [19/54] vs. 19.3% [11/57], p=0.09) though this effect failed to reach statistical significance.

Conclusions: Carriers of KIR2DS1 and KIR3DS1 were more likely to experience pregnancy loss after euploid SETs than noncarriers. Both of these genotypes are activating genotypes, suggesting that overrepresentation of activating genotypes may predispose patients to pregnancy loss. Furthermore, the presence of KIR2DS1 genotype increased the loss risk among C2/C2 embryos – which is a known ligand receptor combination. This study is unique as it is the first to have direct embryo data on HLA status. Prior studies inferred HLA status by parental genotypes. While further study is needed, these findings have major implications for third party reproduction, where deleterious HLA/KIR combinations may be able to be avoided.

Disclosures: None

Funding: Foundation for Embryonic Competence
Association between Progesterone Levels at Day of Trigger and Oocyte Maturity, Fertilization, and Pregnancy Outcomes After In Vitro Fertilization

*Crawford NM1,2, Young AM1, Boylan C1, Hoff HS1,2, Berger DS1,2,3*

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**Objective:** To determine if progesterone level at time of trigger impacts oocyte maturity, fertilization rate, and clinical pregnancy rate after IVF

**Design:** Retrospective study

**Materials and Methods:** Women ages 21-38 years undergoing autologous fresh IVF cycles from 2013-2015 contributed 336 IVF cycles for this study. Oocyte maturity was determined by the presence of polar bodies at day 0 (ICSI cases) or at fertilization check on day 1 (conventional cases). Percentage of mature oocytes was determined by dividing mature oocytes by total oocytes retrieved. Fertilization was defined as percentage of mature oocytes fertilized normally on day 1. Clinical pregnancy was defined by ultrasound confirmed intrauterine gestation with fetal heart activity. Progesterone levels drawn on the day of trigger were run on an Immulite 1000, with a limit of detection of 0.2ng/mL. Progesterone levels (ng/mL) were categorized into quartiles at day of trigger (<0.72, 0.72-1.04, 1.05-1.43, >1.44). Student's t-test and chi square were used for bivariable analysis to compare continuous and categorical variables. A logistic regression model was used to compare clinical pregnancy rate and progesterone values, both unadjusted and adjusted for age using STATA 13.0.

**Results:** 43% of women in our study achieved clinical pregnancy. Mean progesterone level was 1.17ng/mL. There was no difference in percent of oocyte maturity or fertilization rate between highest and lowest quartiles (71% versus 75%, p=0.30 and 64% versus 70%, p=0.08, respectively). The odds of clinical pregnancy decreased with increasing progesterone levels, as women with in the highest quartile of progesterone levels had approximately half the odds of pregnancy as women in the lowest quartile (Table 1).

**Table 1: Odds of clinical pregnancy by progesterone levels (ng/mL)**

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (P4 0.72-1.04)</td>
<td>0.71 (0.38-1.34)</td>
<td>0.70 (0.37-1.32)</td>
</tr>
<tr>
<td>3 (P4 1.05-1.43)</td>
<td>0.74 (0.39-1.43)</td>
<td>0.72 (0.37-1.40)</td>
</tr>
<tr>
<td>4 (&gt;1.44)</td>
<td>0.47 (0.25-0.89)</td>
<td>0.46 (0.24-0.87)</td>
</tr>
</tbody>
</table>

*based on a logistic regression model using Quartile 1 (P4 <0.72) as the reference group

1adjusted for age

**Conclusion:** Although there was no association seen between progesterone levels and the percentage of mature oocytes or fertilization rate, a higher progesterone level on day of trigger was associated with decreased odds of clinical pregnancy. Thus, the negative impact on pregnancy rates seen with high progesterone levels is likely due to etiologies other than oocyte maturity or function.

**Disclosures:** None

**Funding:** None
Clinical Pregnancy, Implantation and Delivery Rates Following Transfer of Frozen Biopsied and Non-Biopsied Day 7 Embryos.

Popwell J. PhD and J Conaghan, PhD
Pacific Fertility Center, San Francisco CA

Objective:
To define the clinical potential of embryos undergoing extended culture to Day 7 followed by frozen embryo transfer (FET) of both biopsied (BX) and non-biopsied (NBX) embryos.

Design:
A retrospective analysis of FETs conducted with Day 7 BX and NBX embryos.

Materials and Methods:
A database review of cycles identified FETs that occurred with Day 7 embryos from 2009 to 2015. All embryos were vitrified in the blastocyst, expanded blastocyst or hatching blastocyst stages after routine embryo culture or trophectoderm biopsy.

Results:

<table>
<thead>
<tr>
<th>Day 7 Embryos</th>
<th>BX #</th>
<th>NBX #</th>
<th>BX %</th>
<th>NBX %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total # warmed</td>
<td>58</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total # survived</td>
<td>56</td>
<td>20</td>
<td>Survival Rate</td>
<td>97</td>
</tr>
<tr>
<td>Total for transfer</td>
<td>56</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total # of FETs</td>
<td>55</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Betas</td>
<td>24</td>
<td>9</td>
<td>Chemical Preg. Rate</td>
<td>44</td>
</tr>
<tr>
<td>Positive Sacs</td>
<td>16</td>
<td>6</td>
<td>Clinical Preg. Rate</td>
<td>29</td>
</tr>
<tr>
<td># Delivered</td>
<td>3 pending</td>
<td>12</td>
<td>Delivery Rate</td>
<td>22</td>
</tr>
</tbody>
</table>

Conclusions:
Transfer of Day 7 embryos has been shown to be successful in FETs of both BX and NBX embryos. The overall survival rate (97% vs 78.7%) was shown to be higher in our study but similar to the overall implantation rate (30% vs 28.9%) in a previous study (1). The delivery rate (22%) was lower than previously reported (37%) (2). Of the BX patients, 18 of 24 (75%) with positive betas and 14 of 16 (88%) with sacs had only Day 7 embryos to transfer. Of the NBX patients, 3 of 3 with positive betas and 6 of 6 with sacs had only Day 7 embryos to transfer. Blastocysts transferred on day 7 may have a lower, but clinically significant potential thus embryos that do not achieve the blastocyst stage on day 6 should remain in culture an additional day as 29% may result in an ongoing pregnancy.
References:


Disclosures: None

Funding: None
During the past decade, it has been reported that there is a 33% increase in the incidence and prevalence of diabetes in the United States, and this trend is reflected worldwide. The lifetime risk of being diagnosed with diabetes in the United States is one in three for men, and two in five for women. About one in three Americans born in 2000 will develop diabetes.

In a baseline study of a large primary metropolitan clinic, data were extracted using proprietary software (iMorpheus, a middleware owned by Sonic Healthcare USA). The period of time of extraction was 11/12/2014 to 04/01/2016 with a total of 13,881 accessions representing a total of 5,504 patients. Among these, 913 (16.5%) diabetic patients were identified using the Johns Hopkin’s diabetic parameters. Of these patients, 147 did not have a diagnosis documented. Among the 913, 45.6% had not received an HgbA1C for over 12 months. Based on HEDIS guidelines, 864 (94.6%) of these patients were due a follow up visit to their primary care provider. A total of 158 patients had HgbA1C > 9.0 at last visit but no follow up testing.

Further studies will be done to extract the compliance with annual eye examinations. In a preliminary data assessment of potential kidney involvement, 64.6% of the patients had not been tested for Microalbumin.

In summary, there is great opportunity for the data mining to assist primary care providers in identifying diabetics, pre-diabetics and managing patients to be in compliance with HEDIS recommendations for HgbA1c, Lipid studies, kidney assessment and annual eye examinations for retinopathy.
Objective: To determine if better implantation can be achieved by deferring transfer of morula and early blastocyst on Day 5 until developed to advanced stage, vitrified, warmed and transferred in frozen embryo transfer cycles.

Design: Retrospective chart review study.

Materials and Methods: After controlled ovarian hyper-stimulation and fertilization in vitro, zygotes produced were cultured under standard embryo culture conditions using a sequential culture media system. Ultrasound guided embryo transfer was performed either on Day 5 or after preparing uterus in FET cycles. Implantation rates for the following cohorts were calculated and compared:

- Fresh morula transfer
- Fresh early blastocyst transfer
- Fresh advanced blastocyst stage transfer
- Slow Frozen Morula: Frozen transfer of Day 6 advanced blastocysts that were morula on Day 5
- Slow Frozen Early Blastocyst: Frozen transfer of Day 6 advanced stage blastocysts that were early blastocysts on Day 5
- Frozen Advanced Stage Blastocyst: Frozen transfer of Day 5/6 advanced stage blastocysts that were not morula or early blastocyst

Embryos selected for analysis included only those from cycles where the outcome of all embryos transferred could be ascertained. This included single embryo transfers, multiple embryo transfers with all (or no) embryos implanted, and multiple embryo transfers with all embryos of the same type.

We estimated predicted embryo implantation rates for the individual groups by fitting a binomial generalized linear model using Bayesian methods. The software used included R (R Core Team, 2016) and RStanArm (Stan Development Team, 2016)
Results:
Implantation rates:
Fresh Morula: 8.3%
Fresh Early Blastocyst: 24.7%
Fresh Advanced Stage Blastocyst: 53.2%
Slow Frozen Morula: 47.7%
Slow Frozen Early Blastocyst: 46.6%
Frozen Advanced Stage Blastocyst: 47.1%

95% Credible intervals that cross 0 indicate that the difference in implantation rates is consistent with 0, which suggests that by deferring transfer of morula and early blastocyst on Day 5 until developed to advanced stage, vitrified, warmed and transferred in frozen embryo transfer cycles, it may be possible to achieve an increased implantation rate, similar to implantation rates of fresh and frozen advanced staged blastocysts.
Conclusions:

1) Slow developing embryos (morula and early blastocysts) have lower implantation rates.
2) Slow developing embryos may yield higher implantation rates if grown to advanced developmental stage on Day 6, vitrified and transferred in FET cycle.
3) Our data suggests that embryo transfer in patients having only slow developing embryos may benefit by deferring embryo transfer until these embryos are grown to advanced stage, vitrified and transferred in FET cycles.

Disclosures:  Nothing to disclose

Funding:   None
Gender Specific Morphokinetics of Embryos

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Objective: To investigate the gender-specific morphokinetics of embryos.

Design: Retrospective observational study.

Materials and Methods: Data used in this study were collected from our routine IVF-PGS patients during 2013-2015, excluding donor, frozen and PGD cycles. Autologous fresh oocytes from randomly selected patients (n=65; 69 cycles) were incubated in Time-Lapse microscope (EmbryoScope: Unisense Fertilitech, Denmark) following insemination. Following the biopsy either on Day 3 or Day 5 (n=358), ploidy status of embryos was analyzed by Genesis Genetics. According to PGS results, these embryos classified into two groups based on their gender after excluding embryos with complex abnormality on multiple chromosomes (n=96) and no result (n=8). The gender specific morphokinetic parameters and the most common chromosomal abnormalities were determined in XX embryos (n=125) vs. XY embryos (n=129) (Table 1). Data were analyzed using student t-test and χ²-test.

Results: The mean differences of all parameters within groups were tabulated below.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>XX Embryos</th>
<th>XY Embryos</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of embryos</td>
<td>125</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>35.7 ± 5.1</td>
<td>35.8 ± 5.0</td>
<td>0.78</td>
</tr>
<tr>
<td>tPNA (time of PN appearance)</td>
<td>9 ± 2.9</td>
<td>9.5 ± 3.1</td>
<td>0.22</td>
</tr>
<tr>
<td>t2 (time of 2nd blastomere appearance)</td>
<td>27.2 ± 7.6</td>
<td>27.6 ± 6.7</td>
<td>0.74</td>
</tr>
<tr>
<td>t3 (time of 3rd blastomere appearance)</td>
<td>37.7 ± 7.4</td>
<td>37.7 ± 7.1</td>
<td>0.95</td>
</tr>
<tr>
<td>cc2 (time of 2nd cell cycle; t3-t2)</td>
<td>10.4 ± 4.5</td>
<td>10.1 ± 4.2</td>
<td>0.51</td>
</tr>
<tr>
<td>t4 (time of 4th blastomere appearance)</td>
<td>39.8 ± 7.7</td>
<td>40.0 ± 6.9</td>
<td>0.93</td>
</tr>
<tr>
<td>s2 (time of synchrony of 2nd cell cycle; t4-t3)</td>
<td>2.1 ± 4.3</td>
<td>2.3 ± 4.5</td>
<td>0.80</td>
</tr>
<tr>
<td>t5 (time of 5th blastomere appearance)</td>
<td>51.6 ± 8.0</td>
<td>51.8 ± 8.3</td>
<td>0.95</td>
</tr>
<tr>
<td>cc3 (time of 3rd cell cycle; t5-t3)</td>
<td>13.9 ± 5.9</td>
<td>12.9 ± 10.6</td>
<td>0.32</td>
</tr>
<tr>
<td>t8 (time of 8th blastomere appearance)</td>
<td>61.2 ± 9.4</td>
<td>62.7 ± 9.4</td>
<td>0.25</td>
</tr>
<tr>
<td>tM (time from insemination to formation of a morula)</td>
<td>90.2 ± 10.7</td>
<td>90.6 ± 11.3</td>
<td>0.83</td>
</tr>
<tr>
<td>Euploid (%)</td>
<td>62 (49.6%)</td>
<td>72 (55.8%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Aneuploid (%)</td>
<td>63 (50.4%)</td>
<td>57 (44.2%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Monosomy (%)</td>
<td>20 (31.7%)</td>
<td>16 (28.1%)</td>
<td>0.21</td>
</tr>
<tr>
<td>Trisomy (%)</td>
<td>15 (23.8%)</td>
<td>17 (29.8%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Chromosome 16 (%)</td>
<td>9 (14.3%)</td>
<td>2 (3.5%)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Chromosome 21 (%)</td>
<td>4 (6.3%)</td>
<td>6 (10.5%)</td>
<td>0.28</td>
</tr>
</tbody>
</table>
Conclusions: Our findings showed that the morphokinetics of embryos were similar between groups. Chromosome 16 is mostly affected in female embryos compared to their male counterparts. A detailed chromosome screening such as SNP studies might reveal better understanding of gender-specific characteristics of embryos.

Disclosures: Nothing to disclose

Funding: None
Increased Abstinence Period Results in Improved Semen Volume, Sperm Concentration and Total Motile Concentration in Sperm Banking Patients.

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Objective: Semen quality is dependent on highly variable factors, including activity of accessory glands, size of the testes and time since the last sexual activity (WHO, 2010). The extent of these influences have been difficult to determine and are not routinely used in interpretation of the semen analysis. However, quality is of paramount importance when sperm are needed for fertility treatments.

Design: Retrospective database analysis.

Materials and Methods: The study group included all patients seeking sperm cryopreservation for medical or social reasons. A total of 1205 unique sperm banking patients, providing 1877 specimens were analyzed for 1/2004 through 12/2015. Specimens presenting with no sperm, or 0% motility were excluded. Specimens were collected by masturbation at the facility and days abstinence was self-reported. All specimens were evaluated 30 minutes after ejaculation by the same two technologists using quality-controlled Makler chambers. The patient groups were divided based on days abstained: < 2, 2-3, 4-5, 6-7, >7. Data on patient age, days abstinence, volume, concentration, total count, total motile count, and % motility were analyzed using one-way ANOVA and Tukey post-hoc test. Significance was set at p < 0.05.

Results: Semen volume, concentration, and total motile count were significantly improved with an increasing days abstinence up to 6-7 days, beyond which parameters showed some decrease. However, sperm concentration was highest at >7 days. There was no difference between initial % motility in the group with < 2 days abstinence versus 4-5 and 6-7 days, however, > 7 days resulted in a significant decrease in motility.

Conclusions: In this selected patient population, an increase in abstinence appeared to yield a significantly improved sperm count, sperm concentration, total motile count and semen volume. There was no significant change in motility with increased abstinence up to 6-7 days, beyond which motility significantly decreased. Our findings are similar to the results of Levitas E et al. (Fertil.Steril. 83:1680-6, 2005). The data suggest that the role of days abstinence, particularly for men before sperm banking or assisted reproduction procedures, is more important than previously considered. Furthermore, a recommended abstinence of 4-7 days may result in better quality sperm in these groups. Further study of the relationship between days abstinence, sperm parameters and fertility potential is warranted.

Disclosures: None

Funding: None
Practicality and Potential Benefits of Continuous pH Monitoring:
Surveying your IVF System

S Zozula and MC Schiewe
Ovation Fertility, Newport Beach, CA

Objectives: We conducted a trial investigation of a SAFE Sens® continuous pH monitoring device (BCSI, Seattle, WA). The aim of our assessments was to evaluate: 1) the ease of the device set-up and alignment; 2) the practicality of non-invasively and automatically attaining accurate and useful pH measurements; and 3) the clinical relevance of how continuous pH monitoring could improve IVF outcomes.

Design: Using a SAFE Sens® In Vessel Monitor (IVM) device, we examined the simplicity of the set-up and system alignment needed to initiate pH measurements. Once equilibrated, we assessed the relative accuracy of daily pH changes to 0.5% variations in CO₂ set points (5.0%, 5.5% and 6.0%), and how they correlated to our standard, external pH unit (The Oyster; Grainger). Finally, we speculated as to the relative usefulness of the multi-probe SAFE Sens® Track system in our embryo culture system.

Materials & Methods: Our conventional pH QC monitoring system involves the overnight placement of 3 ml of Life Global culture medium in loosened 10cc snap cap tubes at varying CO₂ levels (5.0%, 5.5% and 6.0%) to create monthly titration curves for new media lots. Tubes were removed in the morning, caps snapped, placed in a warming block and each tube pH determined with an Oyster analyzer. We set up a single SAFE Sens® IVM device on the left side, upper shelf of a Panasonic MCO-5 tri-gas incubator, and took daily measurements at different CO₂ set points over a 4 week period, assessing 2 different media lots (run#1 and runs#2-4). A mean control value at 5.0% was used to create an “intercept” off-set value calibrating the unit to our standards.

Results:

![Graph showing pH measurements for different CO₂ levels and runs]

Conclusions: Sterile, automated fluoroscopic evaluation (SAFE) measurements of real-time, in situ pH was simply achieved using the SAFE Sens® IVM device. The probe and fiber optic cable fit seamlessly into an upright incubator and were easily set-up and aligned. After a bit of a learning curve, the unit was dialed in, automatically generating serial measurements which closely paralleled our prior control curve. We anticipate that the SAFE Sens® Track Station, which can simultaneously monitor and record up to 8 probes throughout each day, will provide us unique insights into understanding our incubation system. Besides facilitating titration curve measurements, we will be able to evaluate and QC incubator recovery times and monitor daily fluctuations in internal gas/pH conditions.

Disclosure: None

Funding: Equipment support provided by Blood Cell Systems, Inc. (BCSI), Seattle, WA.
Quality Improvement:  
How to Make a Suboptimal Andrology Lab into a Winner

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Objective: Laboratory quality assurance, specifically assessment of performance criteria, clearly affect clinical decision-making. Clinical laboratories have focused quality assurance on the analytical phase, but the pre-analytical and post-analytical phases can contribute significantly to laboratory errors. Our objective is to outline quality improvement methods used to significantly improve a failing clinical andrology lab.

Design: Description of laboratory quality improvement methods and results.

Materials and Methods: An internal audit of an established andrology laboratory demonstrated several issues of concern: telephone calls not being answered, clients not being billed for services, lab reports not being promptly issued, no review and update of SOPs, no proficiency testing, minimal quality control efforts and poor record keeping.

Results: The intent of audits was to assess the laboratory operation, including process review and control, administration and regulatory compliance. Outside consultants were hired to oversee the lab, review and update procedures and forms, and to establish a quality improvement system. A structured system was implemented to strengthen the anonymous and directed sperm donor programs, client depositor sperm bank and clinical andrology laboratory. Internal lab management was replaced, some technical staff were replaced, while those who stayed readily took on added responsibility. Employee job descriptions were correctly developed and management insured each employee had a clear understanding of responsibilities. The staff have a renewed enthusiasm for the lab work and for customer satisfaction. A patient billing policy was established and implemented resulting in recovery of significant losses to the laboratory. Efforts continue to establish cost-effective purchasing agreements, reference lab testing for patients and sperm donors, and obtain research grants. Some of these areas still plague FCC, namely billing, data accessioning, and the logistics of specimen discard.

Conclusions: The identification, implementation and monitoring of a quality system are essential in any quality strategy. Shifting the emphasis to total quality centered on patient needs and satisfaction, minimizes the risk of errors and insures quality of laboratory service. Improving the lab is not a one time event, but rather a constantly monitored process. FCC's continued efforts to identify problem areas and to implement cost and time effective solutions now stand as a company core principle. This change in culture and the commitment of the laboratory owner to devote additional resources to improve quality has also resulted in major financial success.

Disclosures: Nothing to disclose

Funding: None
The Utility of Platelet Indices in Differentiating between Hyper-productive and Hypo-productive Thrombocytopenia in Children

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2Directlabconsulting, Houston, Texas

Objective: To assess the sensitivity and specificity of platelet indices and set cutoff values that aid in diagnosis of thrombocytopenia in children.

Design: Prospective study in a teaching hospital and pediatric hematology clinic.

Materials and Methods: Platelet indices were evaluated in thrombocytopenic patients seen in Karbala Teaching Hospital and the Hematology/Oncology Clinic for Children between January 2014 and January 2015. In this study 90 patients were recruited and divided into two groups: Group 1 Hyper-productive thrombocytopenic patients (n=40) included newly diagnosed immune thrombocytopenic purpura (ITP). Group 2 Hypo-productive thrombocytopenic patients (n=50) included cases with aplastic anemia and acute leukemia.

Mean Platelet Volume (MPV) and platelet distribution width (PDW) were derived from an automated cell counter. Receiver Operating Characteristic (ROC) curves were constructed and the accuracy of the tests was measured by the area under the ROC curve. The sensitivity and specificity of MPV and PDW were calculated under various cutoff ranges, and used to differentiate the thrombocytopenia diagnoses between Group 1 and Group 2 patients.

Results: Platelet indices were significantly higher in ITP patients compared to patients with hypoproducive thrombocytopenia. The best cutoff range for MPV was greater than 10.6fL (femtoliter) and for PDW was greater than 16fL; with a sensitivity of (90%) and (95%) and specificity of (86%) and (80%) respectively.

The area under the curve of ROC of platelet indices was large enough to correctly classify, differentiate and diagnose of ITP patients. In addition, PDW had the larger area under the curve (0.938) than MVP where AUC is (0.900), which means that these values are reliable for ITP diagnosis.

Conclusion: Our results indicate that laboratory findings of platelet volume indices can differentiate ITP from hypoproducive thrombocytopenia and be used as a screening tool for the diagnosis of ITP in children. This will help pediatricians to avoid the invasive bone marrow aspiration which requires the expertise of a hematologist for the examination and interpretation of results.