Noninvasive PGT-A (NIPGT-A)

Promises and Pitfalls

Background

Prenatal genetic testing and preimplantation genetic testing has progressed rapidly over recent decades, a testament to both the scientific endeavor and the development of technologies dedicated to the most important biological function of our species: reproduction. Preimplantation Genetic Testing for Monogenic Disorders (PGT-M) was first reported in 1990 and offered new options for chromosomal testing before an embryo was transferred as part of an assisted reproductive technologies (ART) protocol[1]. Together with advances in embryology, biopsy techniques, vitrification, sequencing, and data analysis, preimplantation genetics now increases the likelihood of pregnancy and decreases the risk of miscarriage and live-birth genetic abnormalities for patients seeking ART services. Preimplantation Genetic Testing for aneuploidy (PGT-A) can identify euploid embryos with greater than 97% accuracy. Currently, DNA for PGT-A typically comes from a small biopsy of only a few cells from the trophectoderm of the implantation-stage embryo, termed a blastocyst. Because the trophectoderm (TE) is extraembryonic and will develop into the placenta without additional genetic contribution to the fetus, cells can be safely removed as a source of embryo genetic material. Although there is no evidence of detrimental effects to the embryo after skilled biopsy, it requires exceptional technical aptitude, relies on sophisticated equipment, and can introduce additional financial burden.

The advent of cell-free DNA (cfDNA) diagnostics is further demonstration of the relentless and determined march of scientific progress. Numerous tests are in development for a variety of diseases or conditions, including those of the heart, liver and many cancers, and of course for noninvasive prenatal testing (NIPT)[2,3,4,5]. Recently, several dozen reports have also suggested that cfDNA in the spent culture media (SCM) can be amplified, sequenced, and used to inform decisions about the genetics of the embryo that grew in the media[6]. Unfortunately, and as is common at the dawn of new technologies, literature and claims have been uneven and have demonstrated current limitations in the ability of NIPGT-A to match the technical performance of biopsy-based PGT.

Given the enormous potential of these technologies and the potential benefit it could afford patients and ART centers, we initiated our own NIPGT-A study. Our results are consistent with the most rigorous and transparent reports[7,8] and suggest that NIPGT-A has tremendous potential. However, for reasons described in this white paper, the evidence suggests that use of NIPGT-A as a clinical test is still in its infancy and continued research and validation are needed. After considerable effort and study, a biopsy-based test with demonstrated benefits (PGT-A with NGS) has been identified. The PGT field would benefit from similar, careful study of NIPGT-A to understand its clinical benefits and future potential.

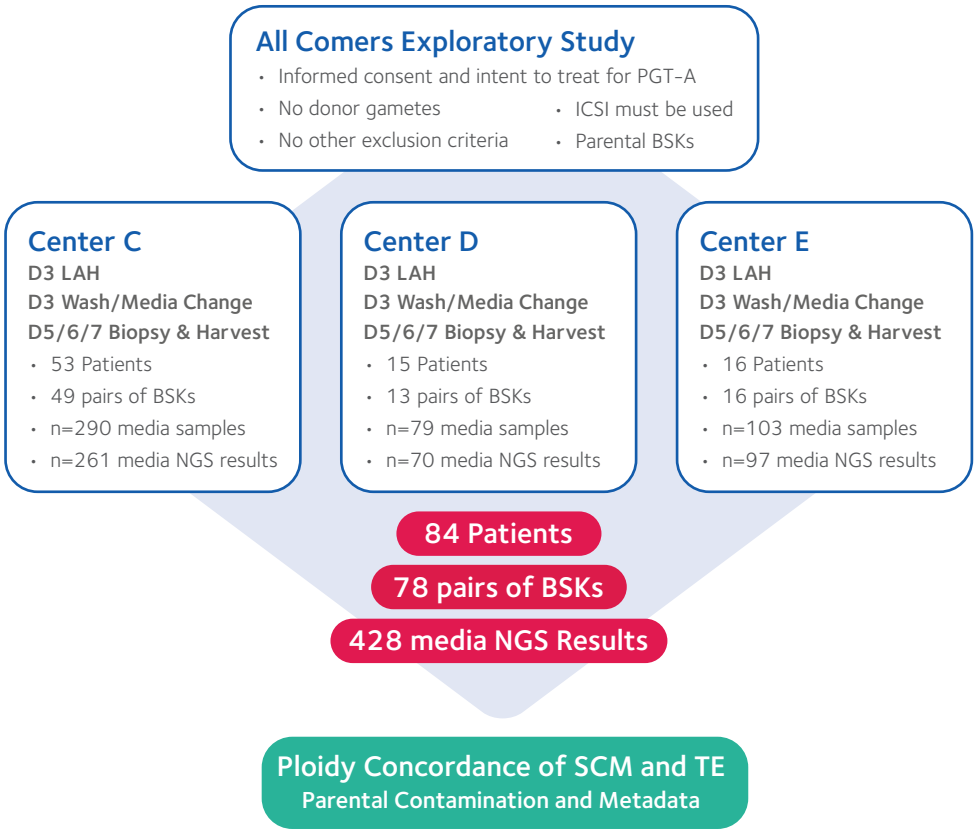
Introduction

CooperGenomics prospective NIPGT-A pilot study (IRB #1271235-CGR-1902)

As stated above, there is considerable and growing community interest in NIPGT-A, but many questions remain unanswered. Therefore, CooperGenomics initiated an IRB-supervised research study to analyze SCM without imposing significant changes to current "best practice" embryology protocols, ensuring this study was open to as many clinics as possible. The sole embryology culture requirement was that the embryos must be grown with an individual culture protocol. Centers generally used their standard protocols with minimal changes, ensuring the research study could build a comprehensive overview of possibilities for NIPGT-A testing (Figure 1). Key inclusion criteria for the study were informed consent and intent to treat for PGT-A; avoidance of donor gametes and fertilization by ICSI were preferred. There were no other exclusion criteria. The presence of DNA in the SCM that does not originate from the embryo is poorly understood, thus Buccal Swab Kits (BSK, buccal swab) were requested of both parents to sequence in parallel. A total of 84 patients, 78 of which provided pairs of BSKs, resulted in 428 informative outcomes. The primary outcomes tested were ploidy and sex concordance between SCM and TE, with secondary outcomes being maternal cell/DNA contamination (MCC), parental dosage, and other incidental findings or correlations from metadata.

Figure 1. Infographic of participating centers, embryology protocols, and sample numbers

The graphic shows the number of patients, corresponding matched buccal swab kits (BSKs), and media samples analyzed in the prospective study. Primary outcome evaluated was ploidy concordance between the spent culture media (SCM) and trophectoderm (TE) biopsy. Secondary outcomes were parental contamination and incidental findings from collected metadata.



Methods

Establishing the DNA concentration in spent culture media

Direct and accurate quantification of cfDNA was complicated by low nucleic acid concentration and the presence of protein, salts, and other media components also present in SCM. We developed a quantification method to directly measure cfDNA from SCM (Pre Amplification), and also used a fluorescent DNA dye assay to measure the whole genome amplification (WGA) product after amplification (Post Amplification).

Maternal DNA / cell contamination (MCC) identified through trio analysis

A major advance of our study is the leverage of parental genetics to perform trio analyses and obtain parental dosage scores for each spent culture media sample. Our sophisticated PGTai^{SM} technology platform uses artificial intelligence to reliably predict karyotype from trophectoderm biopsy NGS results[9]. We retrained this platform with data from NIPGT-A results to incorporate the unique nature of cfDNA in SCM.

Results

DNA concentration and its importance for an informative NIPGT-A result

There was a marked difference between the cfDNA concentrations of SCM samples that failed to return informative NGS results versus those that were informative (Figure 2A, left panel is Pre Amp and right panel is Post Amp). Similarly, SCM samples that returned results discordant with TE biopsy had a lower DNA concentration, on average, than those that were concordant (Figure 2B). Although these trends exist, it was difficult to define a strict cut-off or threshold amount of DNA that would predict NGS success or concordance since samples with very low DNA concentration would sometimes provide informative, concordant results and vice versa. Consistent with previous reports suggesting a model where cfDNA accumulates with time that the embryo is in culture[7], pre-amp and post-amp concentrations of DNA were elevated when SCM was harvested on Day 6 or Day 7 versus those collected on Day 5 (Figure 2C). Metadata of the time of media change and harvest was also recorded, and binning SCM samples by hours-in-culture also demonstrates a progressive increase in cfDNA the longer the embryo grows in the media (Figure 2D).

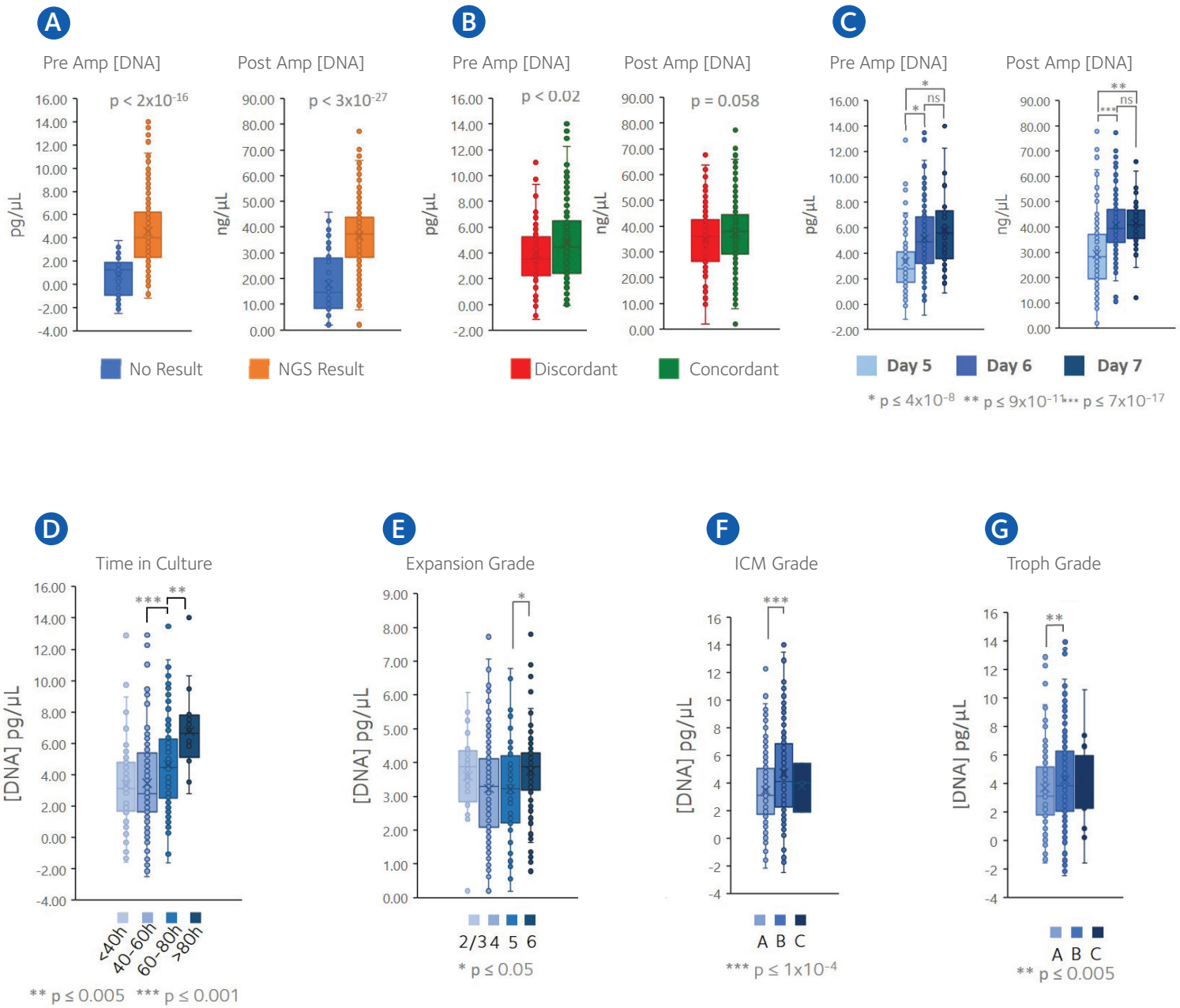


Figure 2. DNA concentration is a determinant of successful WGA, NGS, and concordance, and increases with day of media harvest and time in culture

A) DNA concentrations before (left) and after (right) amplification are significantly higher in samples that return informative NGS results. B) DNA concentration before and after amplification are higher, on average, in media samples that are concordant with TE biopsy. C) DNA concentrations are elevated in media samples collected on Day 6 or 7 versus media collected on Day 5. D) DNA concentration increases the longer the embryo is in culture. E) DNA concentration in SCM by embryo grade, F) ICM grade, and G) trophectoderm grade.

* represent indicated p values using two-tailed homoscedastic Student's T Test.

Impact of Maternal Contamination and Embryo Management

Because euploid female (46;XX) represents considerable risk of discordant miscalling of spent media results, we first sought to compare results in this group using the new NIPGT-A platform. The pie charts in Figure 3A-B show the breakdown of all SCM samples that returned a 46;XX result. Critically, less than half of 46;XX samples are concordant with TE (Figure 3A) including 20% of SCM actually being euploid male or mosaic in the TE biopsy. NIPGT-A trio analysis pipeline identified MCC and applied a parental dose score for each parent, whereby a threshold was set to flag or gate samples with contamination (Figure 3C). Figure 3B shows improvement in SCM/TE concordance when samples flagged for MCC were removed or manually called. Although more refinement is needed before clinical adaptation, these results suggest an additional layer of confidence in selection of 46;XX embryos for transfer using NIPGT-A. Finally, the number of samples having MCC is dramatically reduced if SCM is harvested on day 6 or 7 versus day 5 (Figure 3D).

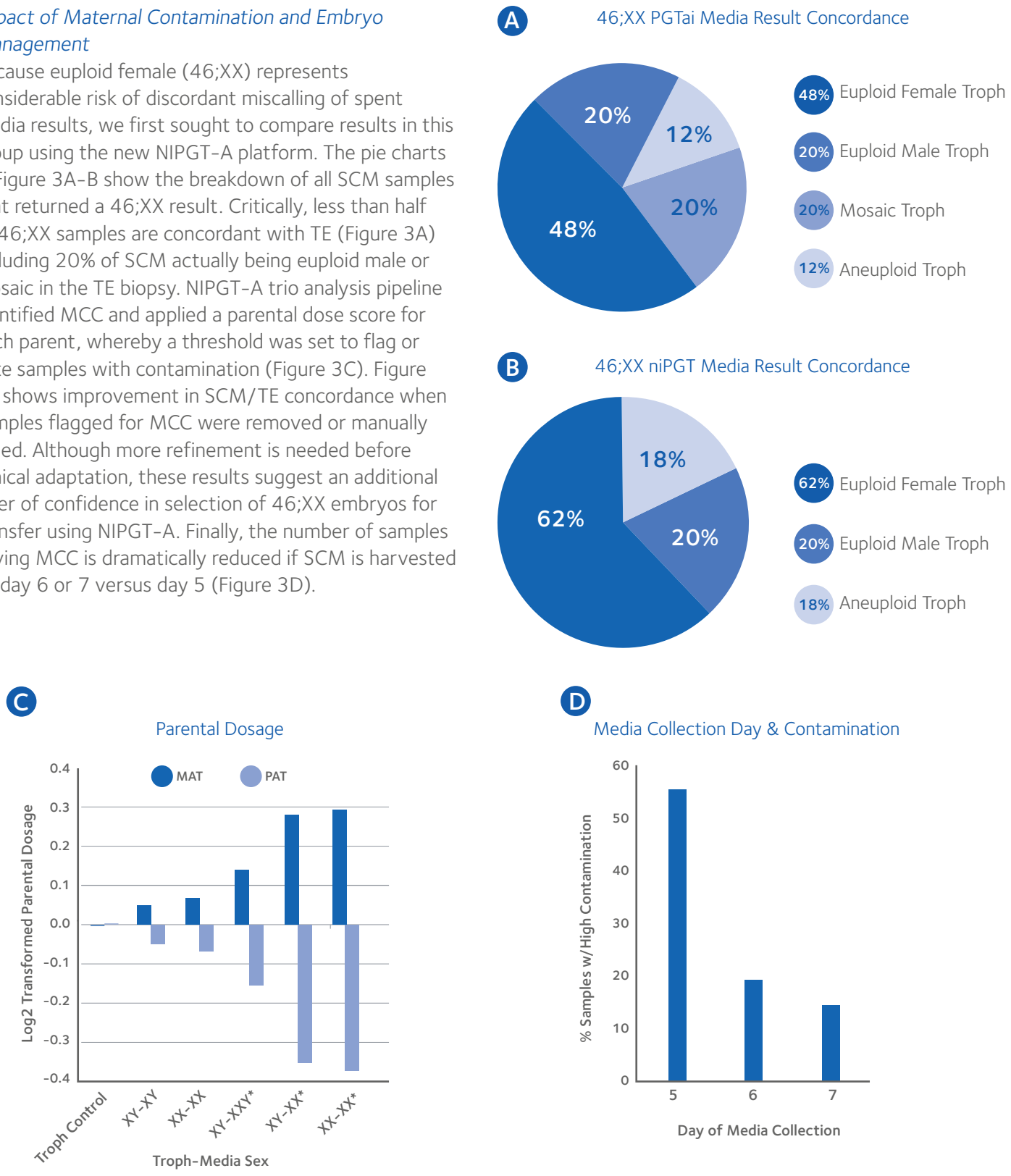


Figure 3. Identification of maternal cell contamination using trio analysis to define parental dosage

A) PGTai 2.0 Plus data analysis platform. Pie graph representing all SCM samples called as female euploid (46;XX) by noninvasive testing. Shaded wedges and corresponding percentages represent the true ploidy status of the embryo as determined by trophectoderm biopsy (PGT-A). B) NIPGT-A data analysis platform. Same as in (A) except using improved NIPGT-A data analysis platform. C) Parental dosage scores identify maternal cell contamination (*) in sex chromosome TE/SCM comparisons. D) Substantial presence of maternal cell contamination in SCM samples harvested on Day 5 relative to Days 6 or 7.

Concordance by day of media harvest and center with advanced analytics

The overall results from the prospective NIPGT-A study with 3 centers are shown in Figure 4. Raw ploidy analysis on all amplified samples by day or center is shown in light blue or light green bars, respectively (Figure 4A-B). Across all days and centers, raw ploidy concordance was approximately 70%, including noninformative NGS results. When noninformative samples were removed from the analysis, as has been done in the majority of prior publications on NIPGT-A, concordance improves to about 77% across all days and centers (Figure 4A-B, middle blue or green bars, respectively). Using the most stringent criteria to identify and remove samples with identified MCC from the analysis results in further improvement of concordance in most cases (Figure 4A-B, dark blue and green bars). A notable exception is Center C, which had a large number of samples harvested on Day 5 and therefore removed many samples initially called concordant euploid female (Figure 4A and Figure 3D). Sex concordance was also evaluated across these same analysis criteria, results are shown by day of SCM harvest (Figure 4C) and center (Figure 4D). Collectively, these results highlight the importance of considering MCC in the context of determining sex using NIPGT.

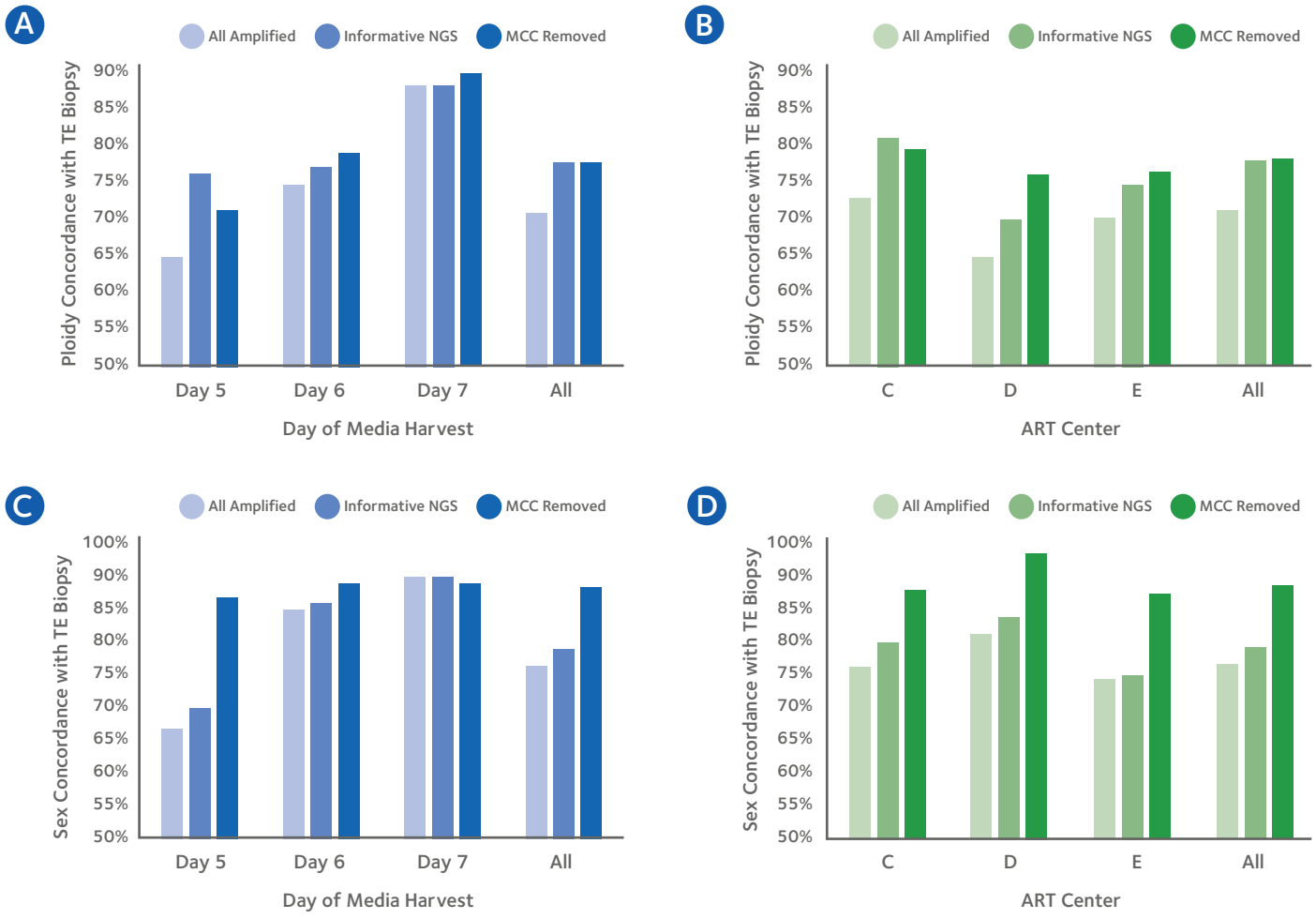


Figure 4. Concordance between SCM and TE biopsy by Day or Center using three analysis methods

A) Ploidy concordance percentages by day of SCM harvest. Light blue bars are results for all amplified samples, blue bars are only those samples that returned informative NGS results (see Figure 1 for n), and dark blue bars are for samples which had no maternal cell contamination. B) Ploidy concordance percentages by ART center. Same as in (A), except light green, green, and dark green bars are used to represent different in silico analyses. C) Sex concordance percentages by day of SCM harvest. Blue bars are same as in (A). D) Sex concordance percentages by ART center. Green bars are same as in (B).

Discussion

It has been reported that DNA concentration in SCM is independent of the ploidy status of the embryo[10]. This was surprising because early thinking about the origin of cfDNA hypothesized that it may be the result of cellular apoptosis or necrosis due to aneuploidy, when in actuality there are no observed differences between the amount of cfDNA in the SCM from euploid and aneuploid embryos[10,11] (data not shown). The origin and mechanism of cfDNA egress from the embryo into the media remains mysterious, although there exist some trends corresponding to embryo grade (Figure 2E-G). The reason for these trends is not immediately obvious and embryo grading is rather subjective; therefore these data should be interpreted with caution. These results show that the amount of cfDNA in SCM samples varies drastically and may be a significant determinant of test success or failure. More research is needed to define the origin, nature, and potential function of cfDNA release from cultured embryos.

The abundance of maternal cell DNA in the spent culture media of embryos, particularly at Day 5, leads to challenges in correctly identifying embryonic DNA vs maternal DNA (Figures 3 and 4). Whilst there may be long term technical and bioinformatic updates that could resolve this issue, for now it is important to understand this as an inherent limitation of NIPGT-A. Confirmation of MCC can be technically challenging, but early reports suggest that upwards of 90% of media samples were likely to have some level of MCC[12]. Here we highlight the utility of parental dosage through obtaining buccal swabs from parents or gamete donors to identify MCC. Ideally, embryology protocols will be employed that reduce or eliminate MCC in SCM samples. Such interventions may include, but are not limited to, aggressive oocyte denuding/stripping of cumulus/follicular cells, sequential media usage with an embryo wash step at Day 3 or 4, and other efforts to reduce or eliminate MCC. Collectively, these results provide evidence that noninvasive sampling of embryo spent culture media is frequently confounded by maternal cell contamination. Utilization of the trio analysis of embryo, maternal, and paternal samples does suggest that having parental buccal swabs should be of benefit in interpreting NIPGT-A results.

To date, most published reports of NIPGT-A are limited to a single clinical center studies that draw conclusions from relatively small cohorts of patients and embryos. To date, multicenter studies have used strict embryology protocols to achieve success, potentially limiting the utility and applicability of NIPGT-A. Moreover, to date, there has been an absence of scientific rigor to measure maternal cell DNA contamination (MCC) in spent culture media (SCM) and its effect on test results. To assess NIPGT-A feasibility without disruptive or cumbersome changes to standard embryology, CooperGenomics continues to recruit centers and their patients to participate in this open research study (Figure 1). A unique facet of our study is the inclusion of parental genetics to assess dosage in the SCM to detect whether DNA from a source other than the embryo has confounded test results. Together with novel assays to determine DNA concentration in the media and state-of-the-art informatics solutions using machine learning, the CooperGenomics NIPGT-A research has significant technical advantages and provides exceptional scientific rigor to fully evaluate this emerging technology.

Summary and Forward-Looking Statement

The prospective study described here, and the efforts to examine metadata parameters and parental dosage, offers more support to the growing corpus of reports that noninvasive genetic testing of embryos is possible. We provide evidence that minimally disruptive embryology protocols may be amendable to noninvasive testing, thus expanding its potential use and applicability to more clinics and centers. CooperGenomics is optimistic and remains committed to offering the most transparent and beneficial tests and services to patients and centers seeking quality reproductive genetics.

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