Integrating Morphokinetics Parameters with NIPGT-A for Improved Sensitivity

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Disclosure

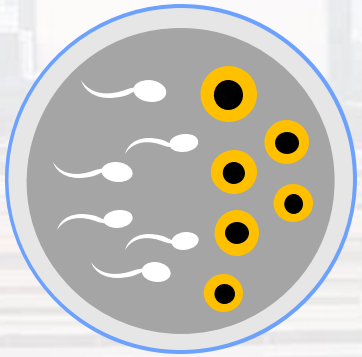
- I have no conflict of interest

PGT and BEYOND...

Background

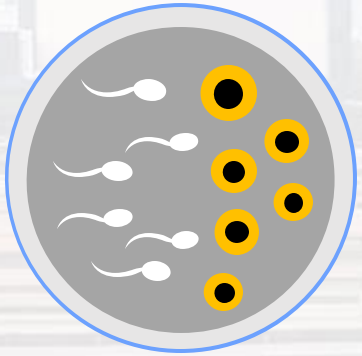
Preimplantation genetic testing for aneuploidy (PGT-A) is a methodology designed to assess the chromosomal complement of embryos generated during in vitro fertilization (IVF) treatments.

A disadvantage of PGT is that it requires biopsy of the preimplantation human embryo, which can limit the clinical applicability of PGT due to invasiveness and complexity of the process. Effort have been made to develop non-invasive approaches for PGT.



Background

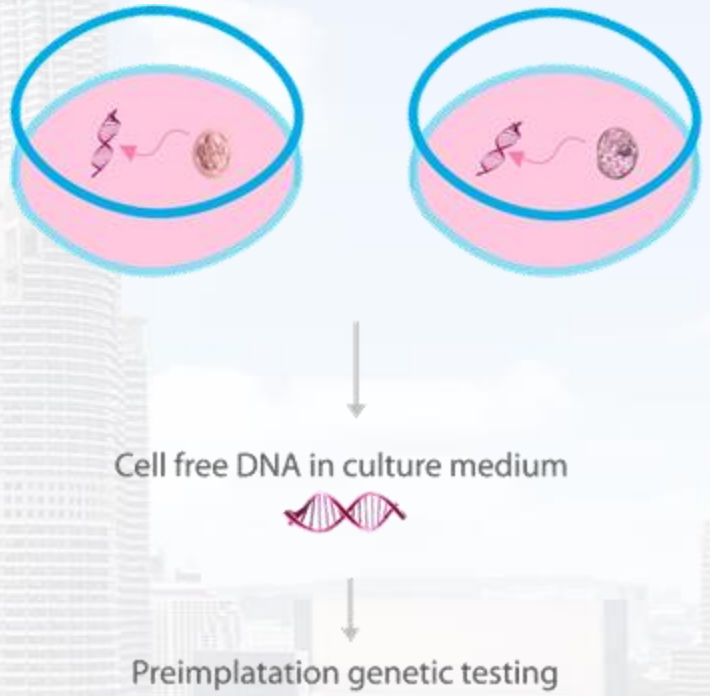
During the last few years, to reduce both laboratory work- load and the potential detrimental effect of suboptimal biopsies, IVF and PGT laboratories have been investigating new sources of embryo-derived DNA.



NIPGT-A

Non-invasive preimplantation genetic testing for aneuploidy (niPGT-A) emerged from the discovery of embryonic DNA in spent embryo culture medium.

Many studies have been develop in order to replace Preimplantation Genetic Testing (PGT) in assisted reproductive clinic, however the results between each studies are varies.



Morphokinetic Time Lapse

- Morphokinetic time-lapse parameters can provide valuable information about embryo development.
- There has been research exploring the potential of using these parameters.
- Using this non-invasive scoring method, several studies have found an association between human embryo ploidy and morphokinetics: slower progression, delayed blastulation, and specific cleavage times, such as t3 or the interval t5–t2, have previously been associated with chromosomal aberrations.

Basile N, et al. Fertility and sterility. 2014 Mar 1;101(3):699-704.

Morphokinetic Time Lapse

- In 2014, Basile et al stated that combining the time interval from 2 to 5 cell (t5 – t2) and third cell cycle (cc3) may better predict the embryo aneuploidy status.

- Embryo selection using morphokinetic markers combined with preimplantation genetic screening (PGS) could be the solution.

Basile N, et al. Fertility and sterility. 2014 Mar 1;101(3):699-704.

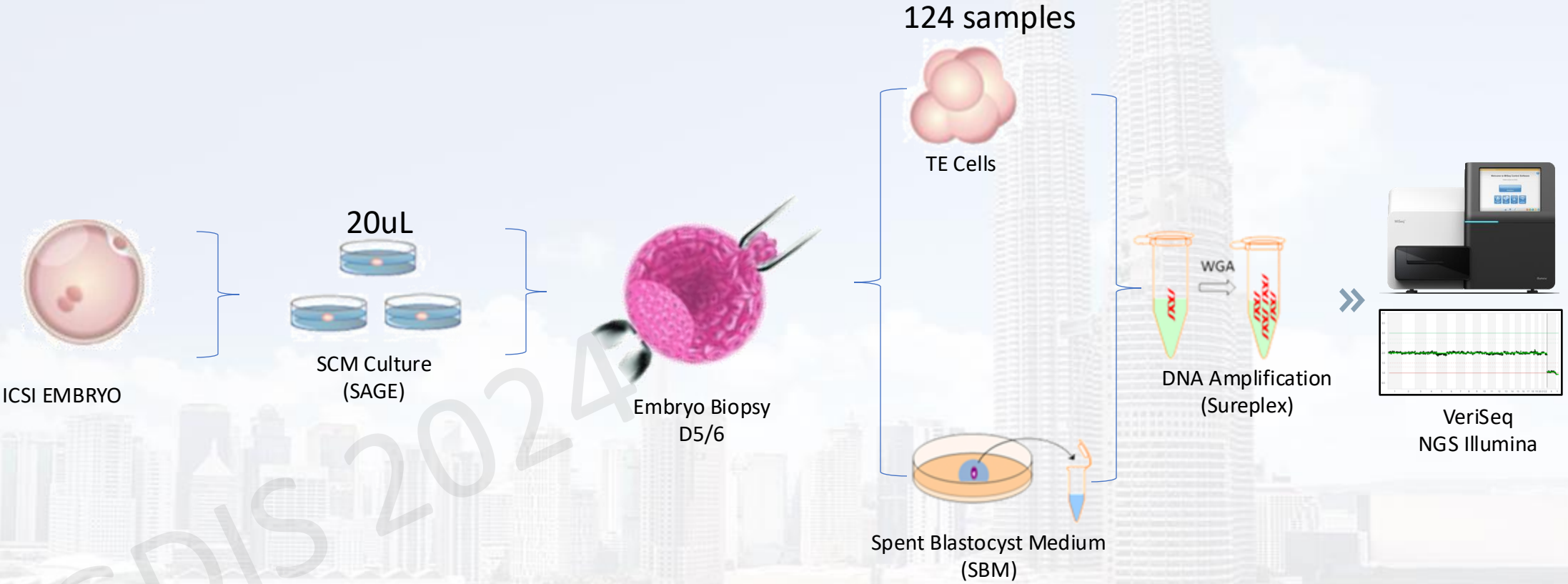
Objective

- Identifying the embryo with the greatest potential for producing an evolutive pregnancy results in better clinical outcomes while minimizing the associated risks.

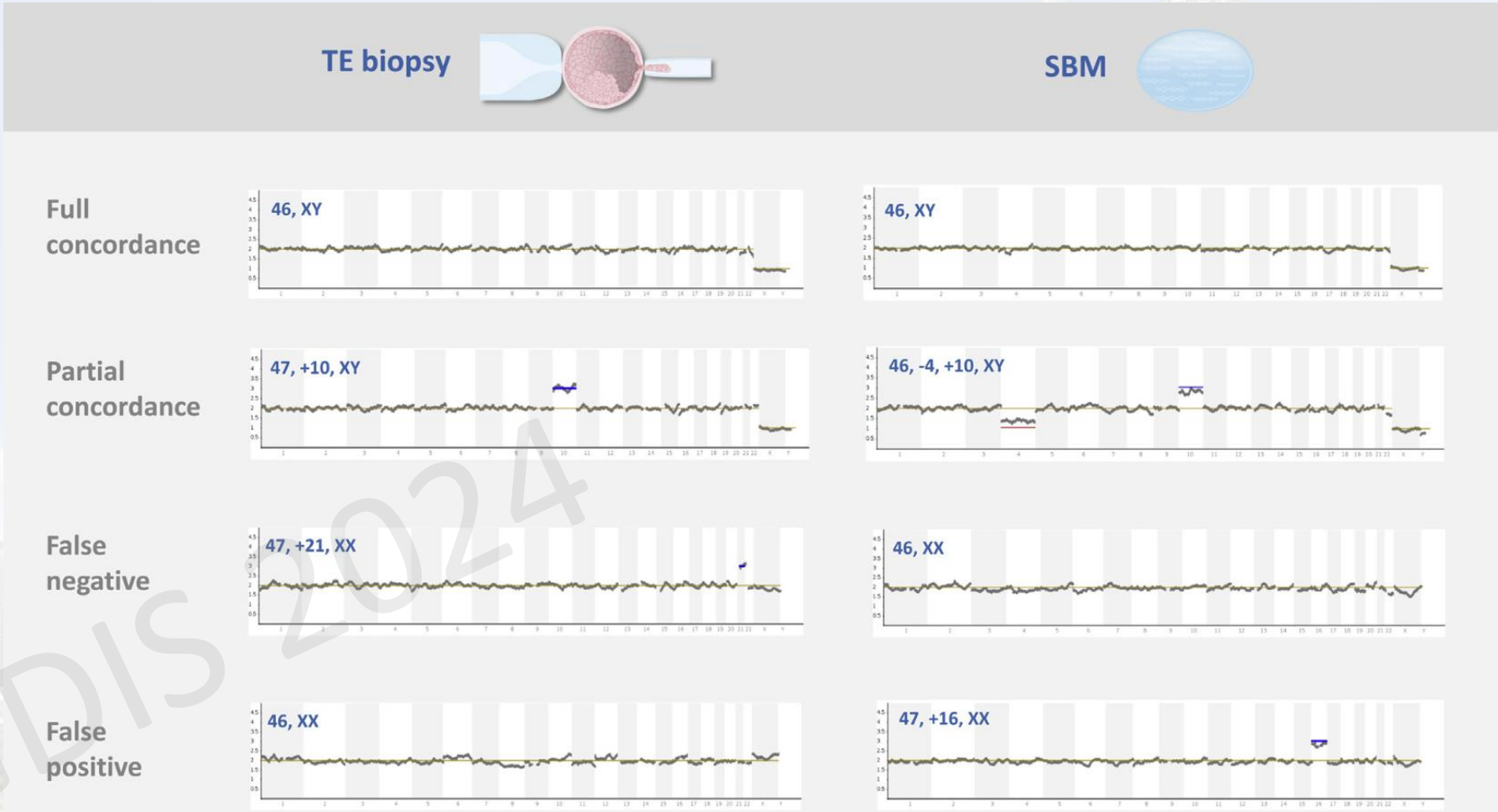
- To compare selected **morphokinetics parameter** combined with **NIPGT-A** versus **conventional PGT-A** for aneuploidy detection.

Methods

- A cross-sectional study was performed on 148 → 124 embryos and their culture medium who underwent an IVF program in Jakarta, Indonesia.
- Exclude:
  - Do not reach blastocyst stage on day 5
  - Mosaic embryo



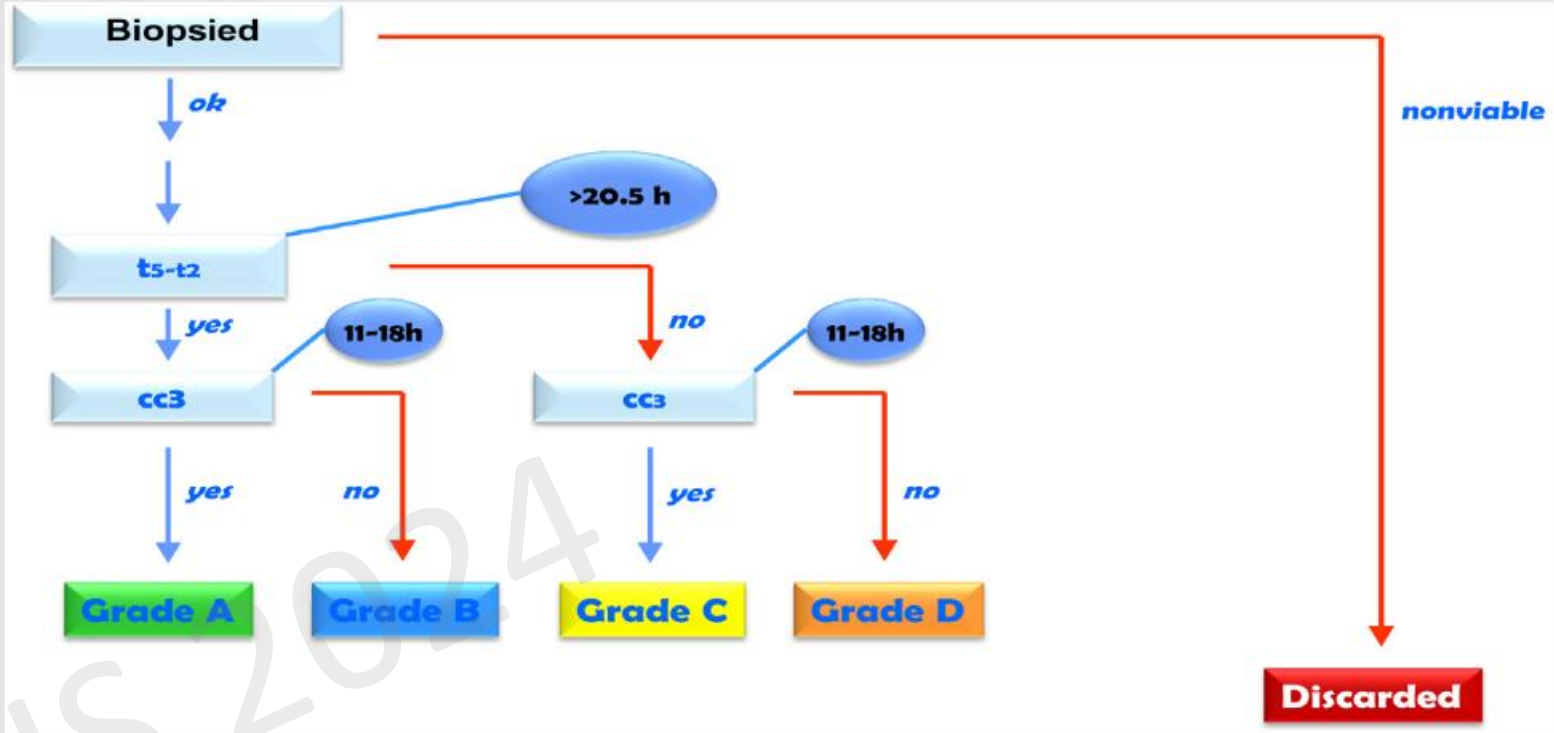
We conducted next-generation sequencing for NIPGT-A from culture media and biopsied trophectoderm cells to determine aneuploidy status.



Navarro-Sanchez, L et al. RBMO. 2022 44, 5.

Morphokinetics Parameters

Parameters	Definition
2PN	Visibility of two pronuclei
PNF	Pronuclear fading when both pronucleai were no longer visible
t2	The first cell division leading to two cells
t3	The second division leading to 3 cells
t4	The third division (4 cells)
t5	The fourth division (5 cells)
cc2	t3 – t2 (second cell cycle)
s2	t4 – t3 (second synchrony)
cc3	t5 – t3 (third cell cycle)



Hierarchical classification of embryos based on the embryos available for biopsy, interval t5 – t2, and the duration of the third cell cycle (cc3). The algorithm classified embryos into four categories based on the expected percentage of chromosomally normal embryos.

Basile. Embryo kinetics and chromosomal content. Fertil Steril 2014.

We also collected the morphokinetics time-lapse parameter including t5-t2 and cc3 parameters, categorized based on the Basile et al study.

Statistical Analysis

- Sensitivity, Specificity, Area Under Curve
- Comparing 3 groups:
  - NIPGT-A
  - NIPGT-A + cc3 parameter
  - NIPGT-A + cc3 + t5-t2 parameters
- All of the groups were compared to PGT-A results

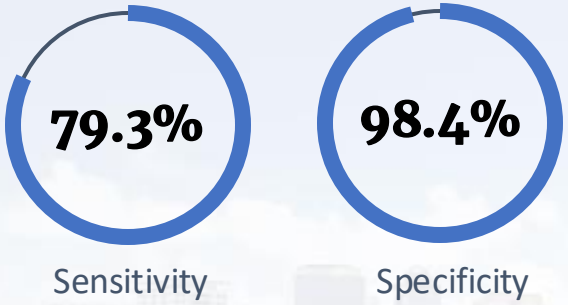
NIPGT-A RESULT	TE BIOPSY RESULT			
	Aneuploid	**Euploid**	**Row Total**	
Aneuploid	A (true positive)	B (false positive)	A + B	PPV A/ (A+B)
Euploid	C (false negative)	D (true negative)	C + D	NPV D/ (C+D)
	A+C	B+D		
	Sensitivity = A/ (A+C)	Specificity = D/ (B+D)		

Results

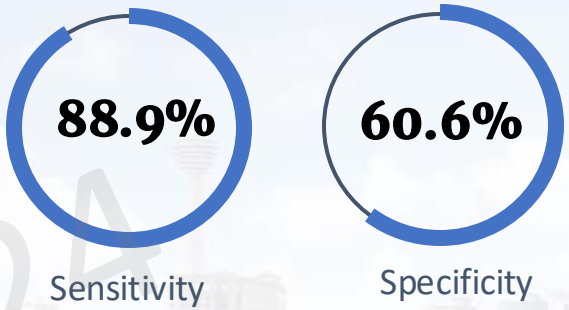
	TE	NIPGT-A
Number of Analyzed sample	124	124
Successful DNA amplification	124 (100%)	124 (100%)
Interpretable NGS result	124 (100%)	124 (100%)
Total Euploidy	61 (49%)	73 (59%)
Aneuploidy	63 (51%)	51 (41%)

Results

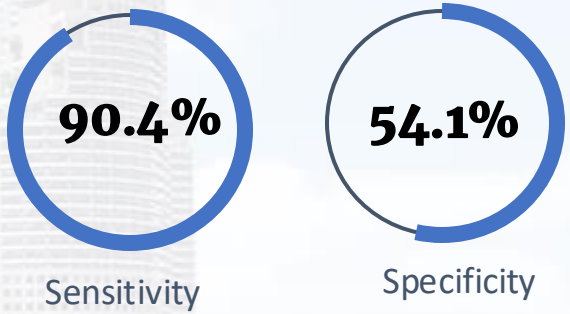
NIPGT-A



NIPGT-A + cc3

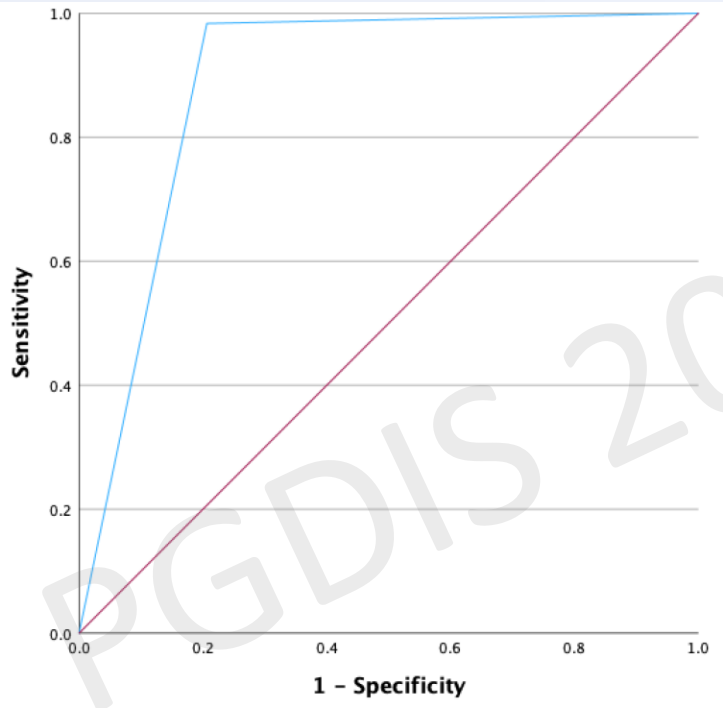


NIPGT-A + (cc3 + t5-t2)



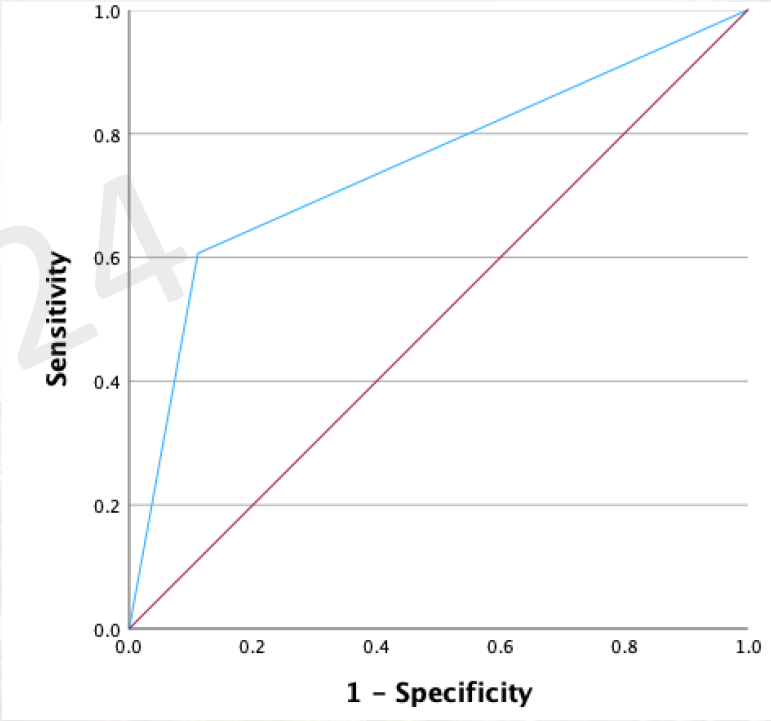
ROC Curve

NIPGT-A



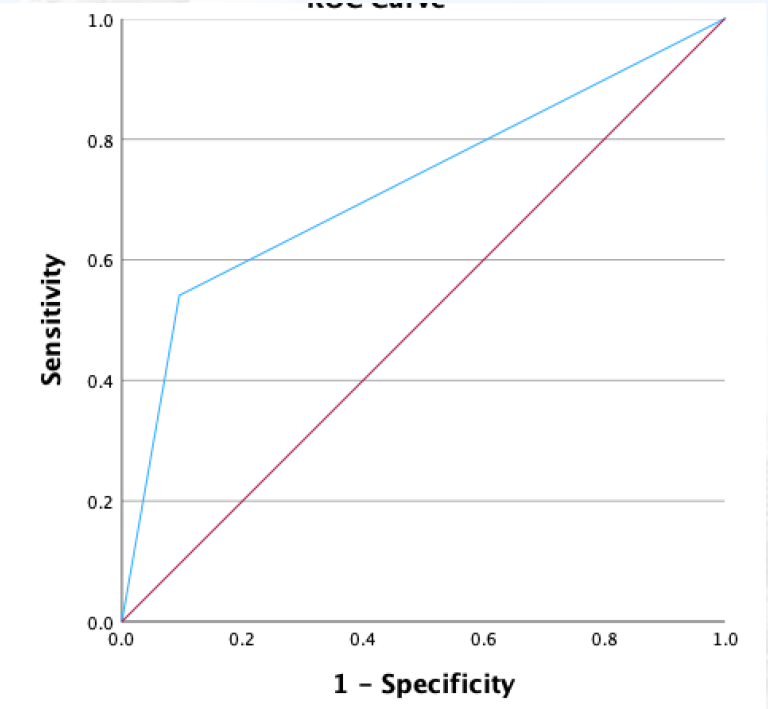
0.889 (95% CI: 0.825-0.952)

NIPGT-A + cc3



0.748 (95% CI: 0.659-0.837)

NIPGT-A + (cc3 + t5-t2)



0.723 (95% CI: 0.631-0.815)

Discussion

- The probability of achieving a successful pregnancy after transferring a euploid embryo **is significantly higher** compared to the results of transferring an embryo without genetic analysis.

- The use of algorithms and grading systems based on morphokinetic parameters to predict ploidy can be of great help when selecting embryos for transfer.

- Previous studies: **synchronized and early cleavages, early blastulation** → being valuable for ploidy prognosis.

Serrano-Novillo, C et al. J Clin Med. 2023, 12, 2983.

Ploidy Concordance Rate in NIPGT-A

	Ploidy Rate
TE and SCM	33.3% - 89.7%
Polar Body and SCM	72.2%
Whole Embryo and SCM	85.7% - 89.8%
TE and SCM+Blastocoel Fluid	70.4% - 100%

Navarro-Sanchez, L et al. RBMO. 2022 44, 5.

Discussion

- **Slower blastocyst formation** is associated with **poorer embryo viability** .
- This delay in development could be explained by the **abnormal activation** of the spindle assembly checkpoint.
- The aneuploid chromosome encounters **difficulties** in aligning with the metaphase plate.
- Defective or lax checkpoint during embryonic development may lead to further development with **chaotic or aberrant divisions.**

Jacobs, K et al. Mol. Hum. Reprod. 2017, 23, 321–329.
Stukenberg, PT et al. Chromosoma 2015, 124, 463-480.

Discussion

Parameter	Odds Ratio	95% IC	*p*-Value
st_2	0.763	1.017–1.936	**0.04**
t_2	1.265	0.887–1.844	ns
t_3	1.061	0.990–1.138	ns
t_4	1.014	0.926–1.110	ns
t_5	1.058	0.017–0.097	**0.005**
t_8	1.008	0.980–10.37	ns
t_{SC}	1.005	0.988–1.023	ns
t_{SB}	1.051	1.021–1.083	**<0.001**
t_B	1.049	1.020–1.081	**<0.001**
t_2–st_2	0.715	0.537–0.899	**<0.0001**
cc2 (t_3–t_2)	1.043	0.976–1.115	ns
cc3 (t_5–t_3)	1.080	1.022–1.146	**0.006**
t_5–t_2	1.053	1.013–1.096	**0.008**
s2 (t_4–t_3)	0.931	0.845–1.023	ns
s3 (t_8–t_5)	0.983	0.955–1.013	ns
t_{SC}–t_8	1.000	0.983–1.017	ns
t_B–t_{SB}	1.006	0.961–1.056	ns
st_2 pattern	0.635	0.468–0.845	**0.001**

ns: not significant.

Serrano-Novillo, C et al. J Clin Med. 2023, 12, 2983.

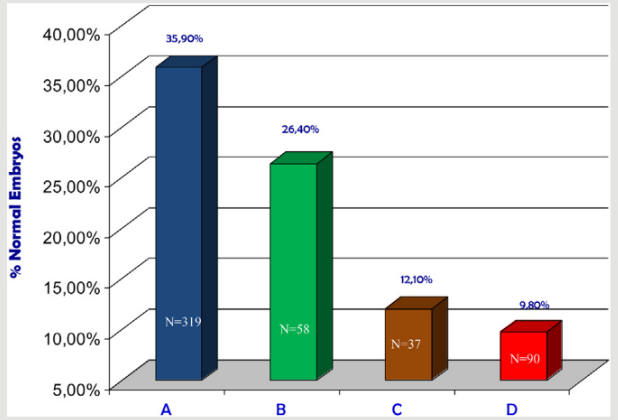
Discussion

Percentage of normal and abnormal embryos in and out of defined optimal ranges.

	N	Abnormal	Normal
t5			
Out	230	79.1% (95% CI, 70.9–84.4) 182/230	20.9% (95% CI, 15.7–26.1) 48/230
47.2–58.2 hours	274	65.3% (95% CI, 57.8–69.2) 179/274	34.7% (95% CI, 29.1–40.3) 95/274
P value			< .001
cc3			
Out	148	83.7% (95% CI, 77.8–89.7) 124/148	16.3% (95% CI, 10.3–22.3) 24/148
11.7–18.2 hours	356	66.6% (95% CI, 61.7–71.5) 237/356	33.4% (95% CI, 28.5–38.3) 119/356
P value			< .001
t5 − t2			
<20.5 hours	126	89.6% (95% CI, 84.3–94.9) 113/126	10.4% (95% CI, 5.1–15.7) 13/126
>20.5 hours	378	65.6% (95% CI, 60.8–70.4) 248/378	34.4% (95% CI, 29.6–39.2) 130/378
P value			< .001

Note: t2: time to 2 cell stage; t3: time to 3 cell stage; t5: time to 5 cell stage; cc3= t5 − t3; t5 − t2: interval between 2 and 5 cells.

Basile. Embryo kinetics and chromosomal content. Fertil Steril 2014.



Percentage of chromosomally normal embryos according to hierarchical classification. N represents the total number of embryos in each category. $P < .001$.

Basile. Embryo kinetics and chromosomal content. Fertil Steril 2014.

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Our Ongoing Research

- **Integrating into machine learning:**
  - **MicroRNA**
  - **Morphology day 3 and 5**
  - **Morphokinetic time lapse**

- **Promising results: AUC 0.95**

Conclusion

- Combining cc3 and t5-t2 with NIPGT-A **enhanced sensitivity but reduced specificity** compared to NIPGT-A alone in detecting aneuploidy. AUC values showed NIPGT-A alone performed best, followed by NIPGT-A with cc3.

- These findings suggest the potential of morphokinetic parameters to improve aneuploidy detection sensitivity but highlight the trade-off with reduced specificity, **necessitating further research for optimal clinical integration.**

Recommendation

- Careful assessment should be performed before generalizing the use of time-dependent variables and algorithms with NIPGT-A for selecting embryos in different clinical settings.

- Embryo development is a dynamic process that can be affected by several extrinsic and intrinsic factors.

- Future studies should integrate AI with multiple contributing factors to better predict aneuploidy.

21ST PGDIS CONFERENCE

PGDIS

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THANK YOU

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KLINIK RSCM Yasmin kencana

PGT and BEYOND...